

DEVELOPMENT AND EVALUATION OF ANTIUROLITHIC HERBOMINERAL FORMULATION

A Thesis

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in

Ayurvedic Pharmacy

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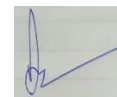
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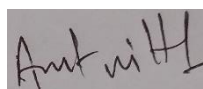


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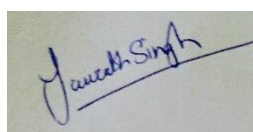
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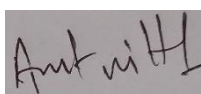
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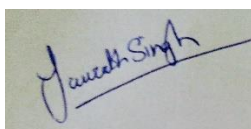
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LIST OF TECHNICAL TERMS

Sr. No.	Abbreviations	Terms
1	g	Gram
2	Lt.	Liter
3	i.e.	That is
4	e.g.	Example
5	Ref. no.	Reference number
6	FM	Foreign matter
7	WSE	Water soluble extractive value
8	ASE	Alcohol soluble extractive value
9	LOD	Loss on drying
10	TA	Total Ash
11	AIA	Acid Insoluble Ash
12	R _f	Retardation factor
13	mg	Milligram
14	ml	Millilitre
15	µl	Micro litre
16	-	Absent
17	+	Present
18	API	The Ayurvedic Pharmacopoeia of India
19	AFI	The Ayurvedic Formulary of India
20	S.No.	Serial number
21	w/w	Weight/ weight
22	w/v	Weight/ volume
23	°C	Degree celsius
24	HPTLC	High Performance Thin Layer Chromatography
25	HPLC	High Performance Liquid Chromatography
26	T. No.	Table Number
27	%	Percentage
28	hr.	Hours
29	NMT	Not more than
30	NLT	Not less than
31	UV	Ultra violet
32	UTI	Urinary tract infection
33	BID	Twice a day
34	TS	Transverse section
35	Tabs	Tablets
36	CaOX	Calcium oxalate crystals
37	FTIR	Fourier-transform infrared spectroscopy

38	DOE	Design of experiments
39	Sr. No.	Serial Number
40	W/W	Weight per weight
41	V/V	Volume per volume
42	W/V	Weight per volume
43	Vol.	Volume
44	No.	Number
45	ROS	Reactive oxygen species
46	BBD	Box Behnken Design
47	RSM	Response surface methodology

ABSTRACT

The urinary system is mainly embedded of kidneys, ureters, urinary bladder, and urethra. Less water intake, electrolyte imbalance, some bacterial i.e. *Escherichia coli* & *streptococci*, viral and parasitic (*Dirofilaria immitis*) infections, autoimmune diseases might be act as causative factor which finally lead to the development of renal calculi. It is an age-old syndrome which possesses not only multifactorial etiological origins but also often associated with high rate of remission-rebound frequency during its management time is kidney stone (termed as urolithiasis). In *Ayurveda*, this syndrome is called as *mutrakricchra* and it is one of the most distressing syndromes among the group of urinary disorder conditions attached to human beings till today. In modern medical practice, plenty of management/treatment options are available which starts from the use of uresis-promoting agent to dietary or nutritional supplement intake. Approaches developed by amalgamating the ayurvedic concept/principle with modern medical practice is a promising strategy and even welcome addition for urolithiasis management. In the present study *surya kshar*, *apamarg kshar*, *sphatika*, aqueous extract of *punarnava* and *gokshur* was chosen as active ingredients. Initially all the raw drugs were subjected for authentication, phytochemical and physicochemical screening to check its identity, purity and strength. Further *surya kshar* and *sphatika* were subjected to *shodhan samsakar* as per the methodology prescribed in *Ayurvedic* literature for addition of properties and separation of impurities. In a next step formulation were optimized with the help of BBD and RSM by using DOE software for identifying the optimized batch. After optimization the *Sphatika* 50mg, *Surya kshar* 50mg, *Apamarg kshar* 50mg, *Gokshur* (aqueous extract) 50mg, *Punarnava* (aqueous extract) 50mg, Acacia powder 45mg and Talc 5mg batch were selected as a final ratio. Which was further used for the preparation of tablets. Pre and post compression studies were performed for the optimized batch. The final formula was subjected for zero day, three-months and six-months stability studies as per ICH Q1A (R2) guideline. Heavy metal analysis, microbial load and aflatoxins determination was performed for individual drugs and prepared tablets. Finally, the prepared formulation was evaluated for its *in-vivo* potential at different dose levels. All the dose reponses of biochemical parameters were compared with each other and after comparing all the doses levels our prepared formulation is showing graded and syngenetic response at dose level of 300 mg/kg and 600 mg/kg which resembles our *in-vitro* studies responses also. During the histopathological studies all the treatment groups showing significant regeneration and repair of renal tissues. Tissues of Group IX and Group X showing maximum restoration and regeneration of tissues architecture.

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CHAPTER 1

INTRODUCTION

Ayurvedic science is based on medication which is derived from therapeutic substances obtained from herbs, metals, minerals, and animals [1-3]. As per *Ayurvedic* principles, the human body is a complex structure composed of *Dosha*, *Dhathu*, and *Mala* [4]. The body's existence is based on many factors; one of which is the proper functioning of *Srotas* (cellular/channels function) while others lead to the formation of various *Malas* (wastes) in the body. *Malas* (wastes) are bi-product produced as a result of the digestion and metabolism of food. Elimination of these from the body is accomplished through their respective excretory channels [5]. *Mutra* (urine) is one of the *Drava mala*. For its excretion from the body *Mutravaha srotas* (urinary system) is responsible [6]. Due to the changes in lifestyle, it has been observed that incidence of urinary problems are on the increase [3, 4, 7].

Water is an essential component that is responsible for digestion, circulation, elimination, and body temperature regulation [8, 9]. In the urinary system, the pivotal function is to maintain the fluid volume and composition in the human body that is executed by tubular re-absorption, tubular secretion of soluble components, glomerular filtration, and filterable elements integrant in plasma [10]. The urinary system, bowels, skin, and lungs constitute the four major excretory systems of the human body [9].

Painful urination is a distressing problem that commonly considered as a sign of urinary tract infections (UTIs). Symptoms of pain at micturition may indicate kidney stone disease or "*Mutrashmari*" or urolithiasis. Whereas the *mutra* or urine means a fluid obtained after the proper functioning of kidneys to infiltrate the metabolic waste materials from the blood and the fluid thus obtained is simply stored in *Basti* or urinary bladder, the *Kricchra* indicates trouble or pain, painful, attended with difficulty and with great exertion or scarcely [7, 11, 12]. Thus, the *Mutrakricchra* simply signifies the discomfort or distress during urination [12, 13]. One more term that is usually mentioned in *Ayurvedic* literature related to *mutra* is "*Mutraghata*". However, the term (*Mutraghata*) indicates drying up or retention of urine [7]. This syndrome holds the accession dating back to the Egyptian mummies kept in the tombs over the period of 4000 B.C or the North American Indian bodies laid down inside the graves during 1500 to 1000 B.C [13].

Intriguingly, the UTIs (Nowadays a major problem of adolescent and geriatric people) confine to an infection involving any of the sub-structures of the urinary tract system which is comprised of ureters, bladder, urethra, and kidneys. The entry and subsequent proliferation of

microorganisms (bacteria, fungi, and virus) initially occur at the anal/rectal region and then, progress with their aetiological activities by making possibly the colonies at the perineum, vaginal introitus, urethra, bladder, pelvic area and kidneys [10, 13-15]. In most cases, the ascending bacterial infection affects only the lower urinary tract (asymptomatic bacteriuria, acute cystitis) but during pregnancy 25-40% of these lower infections ascend to the upper tract and cause acute pyelonephritis. It is to be emphasized that the stage of kidney stone formation poses the greatest medical challenge to manage including its symptoms such as transient pain occurring especially at urine pass-out time. The difficulty to contain/control the pain associated with kidney stone disease is partly related to its multifactorial aetiological. [13, 16-18].

Furthermore, the male sex hormone (testosterone) helps and enhances the chances of kidney stone formation while the female sex hormone (oestrogen) inhibits the process of stone formation. That is why the urolithiasis occurrence rate is comparatively 2-3 times greater in men. It has been shown through clinical estimation that 12% of human beings attain kidney stone disease that too with a possible association of remission-rebound frequency rate of 70-80% in male and only 47-60% in females [13, 19-22].

The kidney stones are composed of different types of compounds/minerals i.e calcium oxalate, calcium phosphate, struvite, urate, cystine & silica. Not only the painful urination is experienced by the patient with kidney stone disease or UTIs but also the urinary calculi (stone) may obstruct the flow of urine, hydro-nephrosis, haemorrhage, and infection in the urinary tract [13, 14]. The calculi thus formed are needed to be removed surgically, lithotripsy and calculus disruption by treating with a high power laser system. Whatever may be the treatment options selected either singly or combinedly, the remission-rebound rate is always high and the treatment options, therefore, become expensive. In today's clinical practice, diversity in management/treatment options exists, starting with the use of ureis-promoting ingredients with the consumption of dietary or nutritional supplements. [10, 13, 15].

Mutravaha srotas (urinary system/renal system) helps in blood filtration and eliminating the waste from the bloodstream in the form of urine. It helps in the maintenance of blood volume, pressure, and pH, regulation of electrolytes and metabolites [23]. Urolithiasis (*Mutrashmari*) was known to mankind from ancient times. *Sushruta Samhita* has explained the *Ashmari roga* classification, symptoms, pathology, etiological factors, complications, and its management scientifically [12].

A renal calculus is a deposition of hard mass which develops in the kidney, consisting primarily of phosphates, oxalates, and urates salts. The process of crystallization expands slowly over a number of months to years[24]. The size might be differing in the case of men and women.

Kidney stone removal through a ureter or the urethra might be trouble-free or might be associated with severe pain depending upon the size of the stone [9, 10]. Medical management of kidney stones is recommended if the stones cannot pass on their own. If it is untreated, it might cause substantial kidney damage and in extreme cases, severe renal impairment or failure occurs. Early incidence recognition is always advantageous in the management of kidney stones [9].

Urolithiasis is ranked third among the most prevalent life-threatening disorders [25]. It is a collective process initiated after nucleation of stone followed by its growth, aggregation or secondary nucleation and in the last attachment of the crystals into the kidneys [16, 26, 27]. The etiology of Urolithiasis is multifactorial and might be based on diet, nutrients, low physical activity, and genetics. Calcium-oxalate and phosphate are the calcium-containing salts that are the commonest type of kidney stones 75-90%. The prevalence of calcium oxalate crystals is more than calcium phosphate crystals, and then struvite 10–15% [28], uric acid 3–10% [29] and cystine 0.5–1% [30-35]. Urolithiasis prevalence depends upon geographical, climatic, ethnic, nutritional dietary, and genetic elements [4, 7]. It is a global problem afflicting humans and present in all geographic areas with an annual incidence of 1 % and lifetime threat of 15-25 %. Once afflicted, urolithiasis tends to be recurrent in the majority of cases. Recurrence chances after the first stone episode are 14% (after 1 year), 35% (after 5 years) and 52% (after 10 years) [13, 32, 36]. *Vedas, Puraṇas, and Samhita* discussed the anatomy, pathophysiology of urolithiasis (*Mutrashmari*) with precautionary measures and remedies for the patients [13]. It is the painful and foremost common urologic disorder related to the excretory organ of the human body [37]. The incidence of kidney stones is rising day by day which leads to a variable degree of pain, bleeding, and secondary infection. Reoccurrence is the major concern for kidney stone disorder [38]. After metabolism calcium, uric acid, phosphatase, and oxalates could also be precipitate and take the form of stone over a period of time. Stones might be varied by size and shape [39].

Kidney stones are classified as primary stones and secondary stones [40]. Primary stones include calcium, oxalate, uric acid, cystine, and xanthine [40, 41]. The secondary stones are developed due to urea splitting organisms i.e. Proteus, Pseudomonas, Klebsiella species and are named as struvite stones [31]. They are composed of magnesium, ammonium, and phosphates [31, 40-42]. The stone formation procedure relies upon urine volume, which comprises calcium, phosphate, oxalate, and sodium ions concentration [43]. High-ion levels [44], low-urine volume, pH, and low citrate levels [45] might be acting as a precursor for the formation of kidney stones [9, 10]. Nephrolithiasis, urolithiasis, and ureterolithiasis terms

indicate the different locations of the stones from where they originated i.e. kidney, urinary tract, and ureter, respectively [26, 30, 34].

In *Ayurveda*, the causes of renal disorders are vitiation of *Mutravahasrotas* (channels carrying urine) the common aetiological factors which are accountable for *mutravahasrotodushti*. The probable causes of Urolithiasis (*Mutrashmari*) are *Ati-vyayam* (excessive exercise), *Tikshna aushadha* and *aahara Seevan* (intake of sharp medicine and dry food), *Rukshamadya prasanga* (excessive consumption of a dry variety of alcohol), *Nitya druta prishthayanat* (riding on the back of fast-moving animals regularly), *Anupa matsya* (intake of the flesh of wetland fish), *Adhyashana & Ajirnat* (eating before the digestion of previous meal & indigestion), *Katiskandha tidharanat* (weight lifting), *Mutravaha srotodushti* (urinary tract infection), *Mutravega nigraha / Mutra vegaavarodha* (suppression of urge) and *Abhikshata* (a person suffering from an injury to the organs of *Mutravaha srotasa*) [7, 12].

The surgical treatment of ailments related to kidney stones has changed in the last decade with the help of technology and advancement in treatment procedures [46-49]. The process of stone formation started when urine is supersaturated started resulting in the precipitation of crystals in the urinary system. Once crystallization occurs it might be flown out through the urine or behold, on kidney resultant in stone formation [50]. The present topic was selected after consideration of prevalence rate, reoccurrence, and side effects of commonly used drugs like headache, dizziness, nausea, vomiting, loss of appetite, stomach/abdominal pain, gas, or diarrhoea, pain or burning during urination, liver problems, feeling of tiredness, dark urine, clay-colored stools, heartburn, acid reflux, skin sores, burning pain in hands or feet, unusual weight loss, eye pain, vision changes etc. In the present formulation drugs of natural origin are chosen as per the *Ayurvedic* principles and combining them for synergistic effect. The developed formulation will be evaluated for its therapeutic efficacy with the help of a suitable animal model.



Fig. no. 1.1 Images of raw material used for experimental study

Herbal and herbomineral combination show pivotal role in human health and the prevention of diseases, including kidney stones. However, the pharmacological evidence of herbs and their phytonutrients in the prevention of kidney stones have not been well-established yet [7, 12]. Research data obtained from *in vitro*, *in vivo* and clinical trials reveal that the phototherapeutic agents could be useful as either an alternative or a complementary therapy in the management of urolithiasis [10, 15]. It has been observed that most of the world's population relies on traditional medicine or natural therapy to treat various disorders. Medicinal plants have a long history of use and are globally safer than synthetic drugs. These are a important source for drug discovery. Medicinal plants are regarded as an acceptable, cheap, easily available and safe source of active compounds for pharmaceutical research and industries.

CHAPTER 2

REVIEW OF LITERATURE

Ayurveda is the first systematic science ever evolved throughout the globe emphasizes on physical and mental fitness with the prevention and preservation of health in a comprehensive manner [5, 51]. This ancient science developed from the extra-sensory logic of our great seers crowned with undoubted knowledge, which is unchallenged till date. But truth has to pass with many tests to prove itself. It's to be scrutinized on every possible parameter to establish its perfectness. This process, rather this bridge between the 'Western Knowledge System' and 'Indian Belief System' is called 'Research in *Ayurveda*'. *Ayurvedic* science incorporates various hidden ideas in it, what we need is to be dug out these deep-seated ideas and use them for the benefit of the globe. This requires a critical mass of different ideas without which it is unrealistic to expect high-quality research. Well-planned research is a very powerful tool that can help this science gain its importance and achieve its generosity all over the globe [52, 53]. As emphasized earlier, *Ayurveda* focuses on preventive as well as curative aspects, and to achieve this, ancient seers tried laudable measures and found that the drugs of different sources are suitable for achieving the same. The art of compounding these drugs into different dosage forms is mentioned in *Rasa Shastra and Bhaisajya Kalpana*. Further, all these compound formulations are categorized into different categories like viz. *Antahparimarjana* (drugs for internal administration) and *Bahirparimarjana* (drugs for topical application) [54].

The ailment has been man's legacy from the start of its existence and the quest for its remedies to battle it, perhaps is equally old. The age-old ancient *Ayurveda* is "an arrow shot by divine bow", through which the confidence of millions of people has been won. This traditional medicine is much popular for managing most of the diseases [51, 52, 55].

The disease *Mutrakricchra* is documented in the classical texts of *Ayurveda*. *Ayurveda* gives guidelines to treat this confidently and increase the quality of life of an individual. Different modalities for the management of *Mutrakricchra* can be correlated to urinary tract infection on the theoretical and clinical symptomatology of diseases [6, 56, 57]. Urinary tract infection is an inflammatory response of the urothelial to bacterial invasion [58]. When this infection is restricted in Lower Urinary Tract i.e., Urethra, Bladder, and Prostrate it is called Lower Urinary Tract Infection (LUTI) [59, 60].

Urolithiasis is the 3rd most common ailment of the urinary tract [61, 62], and its distressing human beings since the earliest days. Still, the recurrence rates [63] continue to be high with 1 out of every 2 patients are developing another stone within 5 years [64, 65].

2.1 HISTORICAL ASPECTS OF STONE FORMATION

Kidney stones (urolithiasis and nephrolithiasis) have progressive antiquity in reverence to their occurrence, diagnosis, and management which was customary for centuries [60, 65].

2.1.1 Egypt: The most ancient evidence about the calculi has been obtained from the mummies of Egypt. In 1901, the English archaeologist Elliot Smith found the earliest known vesicle calculi in a cemetery in Upper Egypt. The date of stone was put as middle or late middle prehistoric period (about 4800 B.C) [66-68].

2.1.2 India: In the 8th century B.C., the first explanation of “cutting for the stone” was mentioned by *Acharya Susruta* with complete information of urinary stones and their anatomy with a suggestive recommendation for surgery of stones [12]. *Acharya Charaka*, mentioned instrumental removal of stone [7]. *Charaka Samhita* was mentioned about the disease under *Tri Marmeeya Chikitsa Adhyaya* under *Mutrakricchra Prakarana* but In *Sushruta Samhita*, *Nidana Sthana*, 3rd chapter dealt with the detailed description of *Mutrakricchra*, its pathogenesis, and types [12, 69, 70].

2.1.3 Mesopotamia: Mesopotamian literature has described the use of turpentine oil, saltpeter for the management of the stone disease. They were able to distinguish between hard and soft calculi. The drugs such as black salt, peter, the shell of an ostrich egg, pine turpentine etc. were used in the treatment of Calculi [65, 71, 72].

2.1.4 Greece: In the 4th century B.C., In the Hippocratic Oath of medical ethics for physicians, Hippocrates was specifically mentioned about stone and the oath attributed “I will not cut for stone, even for the patients in whom the disease is manifest; I will leave this operation to be performed by practitioners” [73-75]. Described the symptoms and management of the ailments related to the kidney and explain the signs and symptoms of bladder stones [76].

2.1.5 Rome: Rufus of Ephesus, (1st century AD) wrote the first monogram on urinary diseases titled “*De Vesical Renumque Affectivus*”, in which he describes nephritis, fairly resembling calculus disease of today [77].

2.1.6 Celsus: He wrote the classical book “*De Medecina*” and described a number of urological procedures. He developed a surgical procedure to remove the stones which later came to be known as “Apparatus Minor” or “Patil Appariel” [77].

In the 16th–18th centuries, Due to variation in nutritional consumption and habits lead to the rise in kidney stone incidence. Intake of a higher amount of alcoholic beverages might be another reason for the rise of kidney stone incidence [59, 65, 71]. During the 17th century, most of the stone elimination became unsuccessful or appeared with various adverse effects [59]. Ancient evidence exhibit the rise in stone incidents, which might be due to changes in nutrients

interactions, vitamin supplements, fluid consumption, and reduction of urinary infections [30, 60, 78-80]

2.2 ETIOLOGY

The causes of renal disorders are vitiation of *Mutravahasrotas* (channels carrying urine) the common etiological factors whichever accountable for *Mutravahasrotodushti*. The probable causes of Urolithiasis (*Mutrashmari*) are *Ati-vyayam* (excessive exercise), *Tikshna aushadha* and *aahara seevan* (intake of sharp medicine and dry food), *Rukshamadya prasanga* (excessive consumption of a dry variety of alcohol), *Nitya druta prishthayanat* (riding on the back of fast-moving animals regularly), *Anupa matsya* (intake of the flesh of wetland fish), *Adhyashana & Ajirnat* (eating before the digestion of previous meal & indigestion), *Katiskandha tidharanat* (weight lifting), *Mutravaha srotodushti* (urinary tract infection), *Mutravega nigraha / Mutra vegaavarodha* (suppression of urge) and *Abhikshata* (a person suffering from an injury to the organs of *Mutravaha srotasa*) [4, 7, 70, 81, 82].

2.3 PATHOLOGY OF CALCULI

2.3.1 According to Ayurvedic literature: The *Samprapti* of *Mutrashmari* can be broadly classified into *Samanya Samprapti* and *Vishishta Samprapti*.

2.3.1.1 Samanya Samprapti :

When *Kapha Dosha* of a person who neglects *Samshodhana* of *Srotas* or intakes an unhealthy diet etc. leads to saturation of *Mutra* (urine) and gives rise to the formation of *Mutrashmari*. Similarly, when *Vayu* dries up *Shukra*, *Mutra*, *Pitta*, and *Kapha*, the *Mutrashmari* gets formed and the *Samprapti Ghatakas* are -

- a) *Dosha: Tridosha*
- b) *Dushya : Mutra, Rasa, Rakta.*
- c) *Srotas : Mutravaha srotas*
- d) *Srotodushti Lakshana: Sanga*
- e) *Adhishtana: Mutravaha srotas, gavini, Basti*
- f) *Udbhavasthana : Pakwashaya*
- g) *Kaala : Chirakaleena Vyadhi* [4, 7, 70]

2.3.1.2 Vishishta Samprapti:

Vataja Mutrashmari, Pittaja Mutrashmari, Kaphaja Mutrashmari, Shukraja Mutrashmari [70].

2.3.2 According to Modern science

Scientists have proposed three main hypotheses for stone formation. They are as follows

- I. Matrix nucleation theory [83].
- II. The precipitation-crystallization theory [84, 85].

III. The inhibitor absence theory[86].

I. Matrix nucleation theory

Urinary calculi as comprising an organic matrix forming 2.5% of the weight of calcium-containing stones, with various amounts of crystalloids deposited within the matrix. The matrix itself is composed of a mucoprotein bounded chemically to a sulphated mucopolysaccharide. It was suggested that the mucoprotein matrix might be responsible for stone emergence by give rise to nucleation and help to crystal growth [83]. It is a coprecipitate which is nonspecific and comes down whenever solid materials precipitate in the urine. This theory states that there are two essential factors responsible for the production of calculi [50].

- a) An organic compound of mucus character in which soluble or poorly soluble material can be deposited.
- b) A fluid supersaturates with putrefying or incrusting substances. Urine has been found to contain a high molecular weight substance which is known as uromucid which promotes the formation of the stones. The increase in the concentration of salt in the urine leads to the aggregation of uromucoids which is the first step in stone formation. This is followed by the precipitation of calcium oxalate or phosphate upon this uromucoid often being passed in the urine, these aggregates might tend to stick high up in the urinary tract and form a nucleus for stones. The crystals stick to the renal tubules not due to their size but through penetration of strands of material through a protein. It is now proposed that this protein might be uromucid. A problem with this theory is that uromucid is not found in large amounts in the stone matrix, instead, it is rich in Sialic acid. It could be that the Sialic acid is transformed into the uromucid at some stages perhaps by removal of Salic acid from uromucid. Ultra-filtration reduced the incidence of samples showing calcium oxalate crystals. The latter showed strong clustering in the whole urine sample but remained dispersed in the ultra filtrates. The changes in the ultra filtrates were almost completely reversed by adding the physiological amount of human uromucid. Uromicd precipitates will increase in urinary concentration within the physiological range. It is therefore postulated that the first step in stone formation is uromucid precipitation. This can trigger the precipitation of calcium salts upon the organic matrix. The whole aggregate may stick high in the renal tract and form the nuclei of stones. When the urinary volume is low, the stone formers show higher uromucid excretion concentration than normal subjects.

II. The precipitation – crystallization theory

III. This theory states that the supersaturation of urinary colloids leads to their precipitation as a crystal and is followed by subsequent crystal growth [85]. Two types of nucleation are described classically i.e. homogenous and heterogeneous.

IV. The inhibitor absence theory

This theory put forth the absence or deficiency of certain protective agents in urine which are considered to restrict one or more of the stone formation process. Magnesium citrate, fluoride, phosphate, and sulphate, trace metals like zinc, cadmium, and cobalt. Macro molecular inhibitors like Glycosaminoglycans, Glycoproteins, Mucopolysaccherides, and polypeptides are also been identified. Calcium stone formers were found to have deficiencies of these natural inhibitors in the urine.

2.4 ANATOMY OF RENAL SYSTEM

The kidney is a bean-shaped organ responsible for disposal of the waste matter produced and help us to maintain the electrolytes balance in the human body [10, 14, 15]. The kidneys are situated at the back of the abdomen at the level of the lowest ribs. Due to liver position, in most of the people right kidney located slightly lower than the left one [10, 14, 15].

2.5 RISK FACTORS

2.5.1 Ayurveda:

1. **Ati-vyayam (Excessive Exercise):** It leads to the vitiation of *vata* & *pitta* which causes *Shrama* (exertion), *Klama* (exhaustion), *Kshaya* (consumption), *Trishna* (thirst), *Raktapitta* (bleeding disorder), *Pratamaka* (dyspnoea), *Kasa* (cough), *Jwara* (fever) and *Chhardi* (vomiting). Moreover, exercise makes urine more acidic due to the production of lactic acid and may also result in haematuria [7].
2. **Tikshna- aushadha (Drugs having sharp properties):** *Tikshna* means sharp or acute and *Aushadha* means drug. According to *Hemadri*, *Tikshnaguna* having *shodhana* property. Hence, the drugs having acute or sharp property if taken in excess causes the *Pitta Prakopa*. This *Pitta* with the influence of *Vata* may accumulate in *Basti* and may cause *Mutrashmari* (Urolithiasis). The intake of excess *Rajika*, *Suranadi* leads to *Mutrashmari* (Urolithiasis). [87].
3. **Ruksha madya prasanga (Continuous use of alcohol in excess):** The excessive *Madhya pana* is responsible for the destruction of *Oja*, as the properties of *Madhya* are just opposite to those of *Oja*, and *Oja kshaya* may result in decreased immunity of the body, resulting in various disorders, especially infections, which may also cause urinary tract infections producing dysuria [7]. *Bhava prakasha* has elaborated on this term by saying that the continuous use of

an excess quantity of *Ruksha* (dry) variety of *Madhya* (Alcohol) leads to *Mutrashmari* (Urolithiasis) [87].

4. ***Nitya druta prishthayanat* (Riding on the back of fast-moving animals, daily):** *Madhukosha* riding on fast-moving animals or vehicles due to *Atigati* and *Kshobha* nowadays, leads to *Vata Prakopa*, mainly of *Apana Vayu* and lead to *Mutrashmari* (Urolithiasis). Regular riding may cause trauma to the introitus of the urethra [11].
5. ***Anupa matsya* (Eating of meat in excess of animals living in *Anupa Desha*):** *Anupa Desha* is one of the three types of habitat, where *Kapha Dosha* is prominent with *Vata*, and the meat of animals living in this area specially the fish is “*Maha Abhishyandi*” in nature [82]. *Maha-Abhishyandi Ahara* is responsible for excessive *Kleda* in *Dosha*, *Dhatu*, *Mala*, and *Srotasa* thus producing favorable conditions in the body for various diseases. According to *Bhavaprakasha* [87] intake of meat of those fish that are living in the region with excessive water. *Madhava* has used the term *Anupa Mamsa* instead of *Anupa Matsya* [11]. Intake of meat of those fish that are living in the region with excessive water. Hence, it can be said that excessive eating of meat of aquatic animals especially fish, is one of the causative factors of *Mutrashmari* (Urolithiasis).
6. ***Adhyashana & Ajirnat* (Eating before the digestion of previous meal & Indigestion):** Bad dietetic habits like eating of food before the digestion of previous meal or indigestion are also the causative factors of *Mutrashmari* (Urolithiasis). Such dietetic habits produce *Ama* in the body, which may lower the body’s resistance, thus making the body and urinary tract more vulnerable to several kinds of infections leading to dysuria [7, 11, 12, 87].
7. ***Katiskandha tidharanat* (Weight lifting):** According to *Acharya Kashyapa* lifting of weight for a long time on *Kati* and *Skandha* directly vitiate the *Pitta Dosha* with *Vata & Kapha*, leading to *Mutrashmari* (Urolithiasis) [88].
8. ***Mutravaha srotodushti* (Urinary tract infection):** It is described by the *Acharya Charaka* [7].
9. ***Mutranigrahat* (Suppression):** A person who is in habit of suppressing his urge for urination may ultimately suffer from *Mutrashmari* (Urolithiasis) [7].

2.5.2 Modern era:

1. **Family history** - The possibility of kidney stones development is observed 2.5times more in those people who have a family history. This extended risk due to genetic composition, surrounding environment, and nutritional elements [89].
2. **Systemic disorders** - It might be acting as a causative factor for kidney stone formation including; primary hyperparathyroidism, renal tubular acidosis, and Crohn’s disease. Interestingly, primary hyperparathyroidism may be found in 5% of stone formers. Increased

body mass index (BMI > 30 kg/m²) and weight gain are now recognized as risk factors for the development of kidney stones [90, 91]. Besides, a history of gout increases the likelihood of forming kidney stones; both uric acid and calcium oxalate (50% more likely to have a history of stones) [92]. Moreover, a history of type II diabetes mellitus increases the risk of stone formation by 30 to 50% in women but not in men [93].

3. **Environmental factors:** If a person works in the hot surrounding, having less fluid consumption may lead to predispose to the formation of renal stones [94].
4. **Dietary Factors:** Nutrients like animal protein, oxalate, sucrose, sodium, calcium, magnesium, and potassium higher or improper intake may predispose to the formation of renal stone [42, 46, 78-80, 89, 95].
5. **Urinary Factors:** Hypercalciuria, hyperoxaluria, hyperuricosuria, and hypocitraturia are related to each other and elevate the risk of stone formation in the kidney [89, 96].
6. **Microorganisms:** A connection in-between Randall's Plaques and the existence of calcifying nano-particles that are look like snow-balls. These are identified in blood and blood products over a decade ago and one of the causing factors for calcifications i.e. kidney stones, prostatic stones, gallbladder stones, atherosclerotic plaques, psammoma bodies of cancer [97, 98]. Calcifying nanoparticles are self-propagating objects. They are morphologically the same in mineral composition to spherical bodies which are recognized in Randall's plaques. Due to the non-existence of its genomic proofs, the calcifying nanoparticle is a controversial agent. Although calcifying nanoparticles produce a specified infection and they are detected in a pathological study of the calcification process. Identification of calcifying nanoparticles includes immunodetection, culture techniques, and electron microscopy [97, 98]. Bacterial urea splitting [99] including; Proteus [100], Pseudomonas, Klebsiella species, and many other bacteria can cause urease-induced stones [101] like Struvite and carbonate-apatite stones [102, 103]. Some viruses have been reported as causes of *Mutrashmari* (Urolithiasis) i.e. Feline Calicivirus (FCV) [104], Feline Syncytium-forming virus (FeFSV) [105], and Cell Associated Herpes Virus (CAHV) [106].

2.6 TYPE OF STONE

2.6.1 Ayurveda

Acharya Sushruta considers *Sharkaraja Mutrakricchra* a different entity, but *sharkara* is a type of *Ashmari*. *Acharya Charaka* says that there is no difference between the pathogenesis of *Ashmari* and *Sharkara*, so there is no need to differentiate these two types of *Mutrakricchra*. Classification of *Mutrakricchra* shown in Table no. 2.1 [4, 7, 12, 81, 82, 87, 107-110].

Table No. 2.1 Mutrakricchra Bhedas

Sr. no.	Prakarar (Types)	Ca	Su	AS	AH	KS	MN	BP	YR	CD	BR	GN	SS
1	Vataja	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	Pittaja	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	Kaphaja	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	Sannipataja	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	Dwandwaja	-	-	-	-	✓	-	-	-	-	-	-	-
6	Rakthaja	✓	-	-	-	✓	-	-	-	✓	✓	-	-
7	Shalyabhighataja	-	✓	-	-	-	✓	✓	✓	-	✓	✓	✓
8	Ashmarija	✓	✓	-	-	-	✓	✓	✓	✓	-	✓	✓
9	Sharkaraja	✓	✓	-	-	-	✓	✓	✓	✓	-	✓	✓
10	Pureeshaja	-	✓	-	-	-	✓	✓	✓	✓	✓	✓	✓
11	Shukraja	✓	-	-	-	-	✓	✓	✓	✓	✓	✓	✓

* Ca = Charaka Samhita, Su = Susruta Samhita, AS = Astanga sangraha, AH = Astanga Hrudaya, KS = Kashyapa Samhita, MN = Madhava Nidana, BP = Bhavaprakasha, CD = Chakradutta, YR =Yogaratnakara, BR = Bhaishajya Ratnavali, GN = Gadani-graha, SS = Sharangdhar Samhita

2.6.2 Modern era

Urinary stones can be categorized according to their size, location, composition, X-ray characteristics, etiology, and risk group for recurrent stone formation [10, 15]. The calculi are divided into Primary, Secondary and Mixed. Primary stones appeared in the urinary tract without any inflammation. The stones are normally found in acid urine and in metabolic diseases. Calcium oxalate, Uric acid, Urate, Cysteine, and Xanthene types of stones fall under this group. Secondary stones are the one which occurs in presence of infection or inflammation. The Urea splitting organism Proteus Mirabilis is the causative organism that converts urine into alkaline by splitting urea into ammonium compounds. Secondary stones are mostly composed of triple phosphates. The majority of secondary stones are phosphate stones and when the phosphate stone occurs as a covering for a primary stone, then such a stone is called as mixed stone. Here the primary stone is usually being in calcium oxalate stone [9, 10, 111].

1. **Calcium stones:** Mostly these are joined with oxalate or phosphate or rarely with uric acid. The calcium oxalate or phosphate stones are either black, grey or white in colour. Small (1cm in diameter), dense and sharply circumscribed on radio-graphs [10, 15, 34, 49].
2. **Uric acid stones:** It is smooth in touch, the shape is round and yellowish-orange in colour. In general, they are radio-graphically transparent unless mixed with calcium crystals or struvite. Diets high in purines, especially those containing meats and fish, result in hyperuricosuria, and, in combination with low urine volume and low urinary pH, can exacerbate uric acid stone formation [10, 15, 34].

3. **Struvite or triple phosphate stones:** They are crystalline in nature and composed of magnesium ammonium phosphate. Radiographs show struvite stones as large, gnarled, and laminated. They are associated with substantial morbidity infection. Signs of struvite stones include urinary pH greater than 7, staghorn calculi, and urease that grow bacteria on culture (proteus, klebsiella, pseudomonas) [10, 15, 37].
4. **Cystine stones:** The cystine stones development is the clinical expression of cystinuria, an autosomal recessive disorder. People who are homozygous for cystinuria excrete more than 600 mg per day of insoluble cystine. The stones are greenish-yellow, flecked with shiny crystallites, and are moderately radio-opaque with a rounded appearance [10, 15, 31].
5. **Protease-related stones:** Use of the protease inhibitor indinavir sulphate [112] are increased due to the increasing numbers of HIV-positive patients. The drug in general well tolerated but it might be associated with *Mutrashmari* (Urolithiasis) the chance of incident is 4–12%. [10, 15].

2.7 TREATMENT

In Ayurvedic science, the treatment (*Chikitsa*) of any disease is prescribed as *Samanya* and *Vishesha Chikitsa*. Moreover, the *Samanya Chikitsa* plays a supportive role only and does not cure the disease completely but might be provides slight relief, whereas the *Vishesha Chikitsa* is advised after knowing about disease type, the involvement of *Doshas*, the status of *Dhatu*s etc. *Mutrakricchra* is *Kapha dosha* driven ailment, hence the expedient of aggravation of *Kapha doshas* to be avoided, and the treatment is focused to control or regulate the *Kapha*. *Aushadhi Chikitsa*, *Basti Karama Chikitsa*, *Kshara Chikitsa* and *Shashtra Chikitsa* [70, 81, 109, 113]

In modern science, the treatment of urolithiasis can be broadly classified as conservative measures, surgical measures, and preventive measures.

In modern science so far as the management of calculi is concerned, it falls into the following categories -

1. Conservative treatment [10, 49]
2. Medical treatment – Hydrotherapy and Chemolysis [13, 47]
3. Non-operative mechanical methods - Extracorporeal shock wave Lithotripsy (ESWL) Ultrasonic Lithotripsy, Electrohydraulic lithotripsy (EHL), Percutaneous nephrolithotomy (PCNL), Ureteric catheterization and Double-J stents, Dormia Basket, Push Band, Litholapaxy, LASER lithotripsy (Light amplification of stimulated emission of radiation), URS (Uretero-rensoscopy) [9]

4. Operative methods – Pyelolithotomy, Subcapsular nephrolithotomy, Nephrolithotomy, Pyelo – nephrolithotomy, Partial nephrectomy, Anatomic nephrolithotomy, Coagulum pyelolithotomy, Bench renal surgery and autotransplantation, Nephrostomy, Nephrectomy [9]
5. Preventive treatment - Depends upon the timely address of stone-forming reasons. Generally, to prevent kidney stone formation by the administration of appropriate nourishment or by using medications. Thus, dietary elements management is the one of best protective strategies against urolithiasis [114-116].

Most of the kidney stones ultimately pass from the kidney through the ureter and bladder lastly through the urethra. However, for the management of the pain treatment is required but consumption of ample liquid will be helpful for the removal of kidney stones from the human body. Some generic drugs tableted in Table 2.2, plant drug is listed in Table no. 2.3 and classical preparation are listed in Table no. 2.4.

Table No. 2.2 Generic Prescriptions and its Pharmacological Action

Sr. No.	Drugs	Category/class	Mechanism of action	References
1	Amiloride	Diuretics	A pyrazine complex impeding sodium reabsorption through sodium channels in nephritic epithelial cells. It used with the combination of diuretics to spare potassium loss	[9, 117]
2	Allopurinol	Xanthine analogue	It reduces the uric acid formation	[9, 118]
3	Cholestyramine	Bile acid Sequestrant	Increase the hepatic LDL receptor and inhibit reductase activity by a statin	[9, 119]
4	Cholic acid	Bile acid derivatives	It facilitates fat absorption and cholesterol excretion	[9, 120]
5	Digoxin	Cardiac glycoside	Inhibit Na ⁺ , K ⁺ ATPase	[9, 121]
6	Etidronate disodium / Etidronic acid	Bisphosphonates	Inhibit calcification	[9, 122]
7	Fluvastatin	Statins / Phenylpyrroles	Decrease the LDL levels. It inhibits HMG-COA reductase	[9, 123]
8	Gemfibrozil	Fibric acid derivatives	Decrease triglycerides through PPARs	[9, 124]
9	Indinavir	Peptidomimetic hydroxy ethylene HIV inhibitors	Binds with HIV protease active site and helps to prevent the processing of polypeptides	[9, 125]
10	Zonisamide	Sulphonamide derivatives	It inhibits the T-type calcium channel or binding allosterically to GABA receptors	[9, 126]

Table No. 2.3 Medicinal plants used as antiurolithiatic agents

Sr. No.	Plants	Part(S)	Functions / Mechanism of action	References
1	<i>Anisotes trisulcus</i>	Leaves infusion	Diuretic, kidney stone, and gallstones with leaves of <i>Pulicaria orientalis</i>	[127, 128]
2	<i>Barleria prionitis</i>	Roots decoction	Diuretic, urinary affections	[129, 130]

3	<i>Dipteracanthus repens</i>	Leaves infusion	Kidney stones	[131]
4	<i>Ecbolium viride</i>	Leaves / roots decoction	Kidney stones, dysuria	[132]
5	<i>Hygrophila auriculata</i>	Roots decoction	Kidney stones	[133]
6	<i>Hygrophila schulli</i>	Root and leaves	Excites urine and moves suppressed urine, weaken stones in the bladder, body affected by fluids, moves urine and swelling of male organs	[132]
7	<i>Thunbergia alata</i>	Leaves decoction	Urinary tract stones	[134]
8	<i>Achyranthes aspera</i>	Roots decoction / infusion	Dysuria, pains of stone, breaks the stones, promotes urination	[132]
9	<i>Aerva lanata</i>	Whole plant	Diuretic, kidney stones	[133, 135]
10	<i>Amaranthus spinosus</i>	Roots decoction	Kidney stone, urinary tract infection, diuretic	[136, 137]
11	<i>Amaranthus tricolor</i>	Whole plant	Kidney stone	[137]
12	<i>Cyathula prostrata</i>	Whole plant, root, and seed	Dysuria, pains of stone	[132]
13	<i>Celosia argentea</i>	Roots infusion	Combine with sugar and given in urinary tract and kidney stone	[134]
14	<i>Leptadenia pyrotechnica</i>	Seeds	The dried plant is used to remove renal stones	[138]
15	<i>Wrightia arborea</i>	Bark, latex, leaves	Urinary stones	[139]
16	<i>Colocasia esculenta</i>	Leaf, rhizome, petiole, tuber	UTI and kidney stones	[140, 141]
17	<i>Parthenium hysterophorus</i>	Whole plant	Kidney stones	[142]
18	<i>Xanthium strumarium</i>	Roots decoction / infusion	Urinary stone	[134]
19	<i>Averrhoa carambola</i>	Fruit	Urinary trouble due to stone	[134]
20	<i>Berberis aristate</i>	Leaves	Leaves decoction UTI and kidney troubles.	[134]
21	<i>Begonia malabarica</i>	Whole plant	Kidney stone	[143]
22	<i>Alyssum maritimum</i>	Aerial part of flower	Kidney (renal lithotripter)	[144]
23	<i>Lobularia maritima</i>	Flowered aerial part	Renal lithotripter	[144]
24	<i>Raphanus sativus / Raphanus raphanistrum</i>	Leaves/roots juice and infusion / seeds powder / fruit	The decoction of roots used to remove the kidney stones in the morning before breakfast	[138]
25	<i>Ananas comosus</i>	Fruit juice	Urinary trouble due to stone	[134]
26	<i>Bryophyllum pinnatum</i>	Leaf juice, whole plant	Kidney stone	[145-147]
27	<i>Benincasa hispida</i>	Whole plant, Fruit juice	Decoction with sugar in UTI and kidney stones	[134]
28	<i>Momordica dioica</i>	Seeds and whole plant	Kidney stones. anti-inflammatory	[148]
29	<i>Mukia maderaspatana</i>	Whole plant and root	Dysuria, breaks stones	[132]
30	<i>Abrus precatorius</i>	Leaves juice	Kidney stone	[134]
31	<i>Bauhinia acuminata</i>	Bark or leaves	Bark or leaf decoction is used for the management of stone in the bladder	[134]
32	<i>Mucuna pruriens</i>	Whole plant	Kidney stones	[149, 150]
33	<i>Aeschynomene indica</i>	Leaves	Kidney stones and urinary disorders	[134, 151]

34	<i>Caesalpinia bonduc</i>	Root, leaves, and fruits	Kidney stones	[132]
35	<i>Indigofera tinctorial</i>	Root and leaves	Nephritic pains of stone resists the powers of poison, the difficulty of urination	[132]
36	<i>Marrubium vulgare</i>	Decoction	Kidney stone	[144]
37	<i>Tectona grandis</i>	Whole plant	Kidney stone	[152]
38	<i>Actinodaphne angustifolia</i>	Plant decoction	Used in kidney diseases due to stone.	[134]
39	<i>Cinnamomum verum</i>	Leaves decoction	Flatulency of kidneys	[132]
40	<i>Asparagus racemosus</i>	Roots decoction	Combine with sugar and given in urinary troubles due to stone	[134]
41	<i>Allium odorosum</i>	Leaves	Painful urination due to UTI and Kidney stone	[134]
42	<i>Abutilon indicum</i>	Whole plant	Urinary troubles	[134]
43	<i>Hibiscus sabdariffa</i>	Leaves decoction	Painful urination due to UTI and Kidney stone	[134]
44	<i>Azadirachta indica</i>	Leaves and flower infusion	Plant powder to remove kidney stones	[138]
45	<i>Glinus lotoides</i>	Whole plant	Kidney stones, urinary disorder	[153]
46	<i>Ensete superbum</i>	Roots, stem, seed, bud, leaves, tuber, fruit	Kidney stones, urinary infections	[154, 155]
47	<i>Musa paradisiaca</i>	Stem/flowers juice/roots/leaves decoction	Kidney stone	[135]
48	<i>Nervilia concolor</i>	Whole plant	Kidney stones	[156]
49	<i>Biophytum candolleianum</i>	Whole plant	Dissolves (breaks) stones	[132]
50	<i>Rhynchostylis retusa</i>	Whole plant, leaves, and stalk	Promote urine, remove the stone	[132]
51	<i>Petalium murex</i>	Whole plant and leaves	Dysuria, pains of stone, removes painful discharge of urine	[132]
52	<i>Cynodon dactylon</i>	Leaves and rhizome decoction	To remove and break the kidney stones	[138]
53	<i>Saccharum arundinaceum</i>	Roots decoction	Kidney stone	[157]
54	<i>Zea mays</i>	Seed decoction / leaves / fruit / flower decoction	The plant's filaments are used to break up the kidney stones with boiling water	[138]
55	<i>Portulaca oleracea</i>	Whole plant	Kidney stones	[136]
56	<i>Spermacoce hispida</i>	Whole plant	Kidney stones	[154]
57	<i>Bonnaya reptans</i>	Whole plant	Decoction in kidney and urinary problem due to stone	[134]
58	<i>Scoparia dulcis</i>	Whole plant	Kidney stones, urinary tract infections	[156]
59	<i>Parietaria officinalis</i>	Aerial parts	Kidney stone	[144]
60	<i>Pouzolzia zeylanica</i>	Aerial parts	Kidney stone, galactagogue	[158]
61	<i>Alpinia nigra</i>	Rhizomes	Stones in bladder or kidney	[132]
62	<i>Curcuma zedoaria</i>	Root and leaves	Cleanses the kidneys	[132]
63	<i>Curcuma longa</i>	Rhizomes	Obstruction on kidneys and the bladder	[132]
64	<i>Tribulus terrestris</i>	Whole plant	Diuretic, kidney stones, urinary disorders	[136, 159]

Table No. 2.4 Ayurvedic classical formulations for the management of Urolithiasis

Sr. No.	Formulation name	Key Ingredients	Therapeutic Actions	Dose	Manufacturer
1	<i>Mutradosh Nashak Vati</i>	<i>Varuna, Punarnava, Gokshur, Pashanbheda, Yavakshar 40mg each, Shuddha Guggul 120mg</i>	Dissolves Calculi, Diuretic	2 Tabs BID after the meal	Sharangdhar Pharma Pvt. Ltd.
2	<i>Vrukka doshantak Vati</i>	<i>Chandanadi Vati and Gokshuradi Guggal 75 mg each, Raswanti 60mg, Triphala Guggal 50mg, Hajrat Ber pisthi 30mg, Kankol Mirch, Kakdi Beej, Nimali, Shwet Parpati and Mulichhar 20mg each, Shilajeet 15mg, Mutra Kruchhantak Ras 10mg, Prawal Pishthi, Udamber Ghan and Jasad Bhasma, 10mg each. Bhawana - Ashmariher Kwath, Varunadi Kwath, Pashanbhedadic Kwath, Mutral Kasay, and Chandandi Kwath 50mg each</i>	Dysuria, hesitancy, renal colic, and urolithiasis	2 Tabs three times a day with water/milk	Vyas Pharmaceuticals Pvt. Ltd., Divya Pharmacy
3	<i>Chandraprabha Bati</i>	<i>Guggul 32mg, Shilajit 32mg, Sharkara 16mg, Karpoor, Ativisha, Haridra, Vacha, Mustak, Amalaki, Haritaki, Bibhitaki, Chavya, Bhunimba, Vidanga, Devdaru, Dhania, Guduchi, Chitraka bark, Shunthi, Darvi, Maricha, Pippali Pippali mool, Gaja pippali, Sarjikshaar, Saindhav Lavan, Suvarchal Lavan, and Vida Lavan 1mg each, Swarnamakshika bhashma, Trivrit, Danti mool, Dalchini, Tejpatra, Ela and Vanksha lochana 4mg each, Lauha Bhashma 8mg</i>	Gonorrhea, renal calculi, vulvitis, fever, burning micturition, and leucorrhoea	2 Tabs BID	Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Unjha Pharmaceuticals Pvt. Ltd., Dabur India Ltd., Shree Dhootapapeshwar Ltd.,
4	<i>Bangeshwar Ras Brihat</i>	<i>Vanga Bhasma, Rajata Bhasma, Karpur and Abhraka Bhasma 19.23mg each, Swarna Bhasma and Mukta Bhasma 4.80mg</i>	Use to treat discomfort, pain or burning when urinating	1 -2 Tabs BID	Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Unjha Pharmaceuticals Pvt. Ltd., Triskand Ayurved Pvt.Ltd., Dabur India Ltd
5	<i>Chandrakala Rasa</i>	<i>S. Parada, Abhraka bhasma, Katuki, Giloya sattva, Pippali, Tamra bhasma, Svet candana, Usira, Parpata, Karpura, Ananta, Gandhaka suddha, Mukta bhasma, Musta, Svet durva, Ketaki, Padma, Satawari, Parpata, Draksha</i>	UTI and kidney stone	1-2 Tabs BID	Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Orient Ayurvedic Pharmacy, Shree Dhootapapeshwar Ltd., Unjha Pharmaceuticals Pvt. Ltd.
6	<i>Trina panchamool</i>	<i>Kusha, Kasha, Shara, Darbha, and Ikshu</i>	Used in the management of Dysuria	10-20 ml after meal	Tatkshana Ayurveda, Sri Navjeevan rasayanshala
7	<i>Ber Patthar/ Hajrul Yahoood Bhasma</i>	<i>Hajrul Yahoood</i>	Diuretic, Lithontriptic, Antipruritic, Renal colic	250 mg to 500 mg BID	Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Unjha Pharmaceuticals Pvt. Ltd., Divya Pharmacy

8	<i>Varuna Mulatwak Kashaya</i>	<i>Varuna mula twak, Shigru mula</i>	Kidney Stones, Diarrhea, Dysuria	45 ml of kwatha BID after meals for 45 days	-
9	<i>Veeratarvadi Kashaya</i>	<i>Gokshura, ashmabheda, sahachara, vasa</i>	Kidney stones/renal calculi	15 ml BID with equal amount of water	Arya Vaidya Kalpashala
10	<i>Ashmarihar Rasa</i>	<i>Yavkshar Hazrul Yahood Bhasma Mulikshar Kalmi Shora Swet Parpati</i>	renal calculi, dysuria, urine retention, burning micturition	1 – 2 tablets BID on an empty stomach with water	Divya Pharmacy
11	<i>Ashmarihar Kwath</i>	<i>Pashanbheda, Sagauna phal, Papaya jadh, Shatavari jadh, Goksura, Varuna twak, Kush jadh, Kasa jadh, Kakdi ke beej, Jatamansi, Punarnava, Guduchi, Apamarga, Khurasani yavani</i>	Prevent and treat kidney and urinary stones	10 – 20 ml, once or twice a day before or after food	Dhanvantari Herbs, Divya Pharmacy, Goodluck Ayurveda
12	<i>Trikantkadi Kwath</i>	<i>Gokhru, Amaltaas, Doob, Javasa, Pakhanabheda, Harad, Kaasmool, Pitpapda</i>	<i>Mutra Roga</i>	48 ml twice a day	Shree Dhootapapeshwar Ltd, Ayursun Pharma
13	<i>Pashanabhedadi Ghrita</i>	<i>Krisnapatala, Sruga, Amalaki, Haritaki, Vibhitaki, citraka, Palasa, Dhava, Simsipa</i>	<i>Mutrashmari</i>	50 ml BID Half an hour before the meal (for 45 days)	-
14	<i>Palasa Kshara</i>	<i>Palasa</i>	<i>Mutrashmari</i>	125-500 mg BID	Manakarnika Aushadhalaya, Arya Vaidya Sala – Kottakkal, Pooja Traders
15	<i>Apamarg Kshara</i>	<i>Apamarg panchang</i>	It has laxative, diuretic, and antacid properties	250 mg to 1 g per day to be taken with water	Ayuverdhanam Pharmaceutical, Bhardwaj Pharmaceutical Works, Shree Baidyanath Ayurved Bhawan Pvt. Ltd.
16	<i>Yava Kshara</i>	<i>Yava</i>	Diuretic, Ashmarihar and antacid properties	125 mg to 500 mg to be taken with water	Ayuverdhanam Pharmaceutical, Bhardwaj Pharmaceutical Works, Shree Baidyanath Ayurved Bhawan Pvt. Ltd.
17	<i>Mooli Kshara</i>	<i>Mooli</i>	Diuretic, Ashmarihar and antacid properties	250 mg to 500 mg to be taken with water	Ayuverdhanam Pharmaceutical, Bhardwaj Pharmaceutical Works, Shree Baidyanath Ayurved Bhawan Pvt. Ltd.

18	<i>Gokshurvarunadi Kwath</i>	<i>Shunthi, Gokshur, Pashanbhed, Varun</i>	Treats urinary infections and keeps kidneys healthy, dysuria, anuria	48 ml BID	Jiva Ayurveda
19	<i>Mutrakrichantak Kwath</i>	<i>Varuna, Apamarga, Gokshur, Shirish, Punarnava</i>	Diuretic, Kidney stone, Kidney failure	48 ml BID	Planet Ayurveda
20	<i>Ashmarihar Kwath</i>	<i>Gokhru, Kulathdaal, Varun, Punarnava, Pashanbhed, Methi</i>	Kidney stone	48 ml BID	Divya Pharmacy
21	<i>Varunshigru Kwath</i>	<i>Varun, shigru</i>	Useful in calculus and Pain due to Calculus.	15 to 30 ml BID	Swasthya Vardhak Pharmacy
22	<i>Varunadi Vati</i>	<i>Punarnava, Varuna, Gokshur, S. Guggulu</i>	Kidney, ureter & bladder stones	2-4 tabs BID with water after meals	Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Planet Ayurveda
23	<i>Eladi kwath</i>	<i>Brihadela, Pippali, Madhuka, Pashanabeda, Kounti/ Renuka, Gokshura, Vasa, Eranda, 3.75g each, Shudda Shilajith 500mg, Sharkara 10g</i>	<i>Mutrashmari</i>	15 to 30 ml BID	-
24	<i>Nagaradi kashaya</i>	<i>Nagara, Varuna, Gokshura, Pashanabeda, Kapotvakraja/ Kakamachi 6g each, Yavakshara 250mg, Guda 10g</i>	<i>Mutrashmari</i>	30 to 60 ml BID	-

2.8 SURYA KSHARA

Surya kshara is mentioned under *Kshara visheshadi vijnaniya* in *Rasatarangni* [113], after explaining the *Ksharatrika vijnaniyam taranga*. It is one of the important *kshara* from which *Soraka dravaka*, *Soraka jalam* etc are to be prepared. It was purified by giving three *Bhavanas* of *Ela toya*, by this process the potency of *Surya kshara* is enhanced as *ela* also having *Mutrala* (diuretic) property. According to *Rasa Jala Nidhi* [160], it possesses both *kshara* as well as *Lavana* because *Ushara* or *sora* and *Sauvarchala* are the same i.e., saltpeter. According to *Rasamritam* [161], it is good for *Mutrakricchra*, *Ashmari Prameha*, *Kamala*, and *Panduroga*. According to modern science, *Surya kshara* has diuretic, diaphoretics, antipyretic, expectorant, and anti-inflammatory properties [162-164].

2.8.1 Synonyms

Sanskrit	- <i>Suryakshara</i>
Hindi	- <i>Sora, Kalmisora</i>
Kannada	- <i>Petluppu</i>
Marathi	- <i>Sorakhara</i>
Arabi	- <i>Abukara</i> [113, 160, 161]

2.8.2 Pharmacological properties (*guna karma*)

Rasa: Katu .Teekshna, Lavana

Guna: Teekshna, Athyushna, Dipaka

Veerya: Ushna

Vipaka: Katu

Karma: Vahni pradeepanam, Ashmarihara, Mutrakricchra nashaka, Panduharam, Pramchasamanam [113, 160, 161]

2.8.3 Matra (Dose)

According to classics '*Dwi gunja*' is the therapeutic dose. This can be increased up to 10 *gunjas* [113, 160, 161]

2.8.4 Pharmacology

It has a cool saline taste. An ordinary dose if taken as a concentrated condition will result in gastroenteritis. In acidity of urine, it helps to protect the alkaline nature. Here KNO_3 acts as follows- Potassium which is secreted by distal tubules is more diuretic. When K^+ is taken additionally, diminishes H^+ exchange with Na^+ due to the common ion effect. It reduces H^+ concentration in urine acidity. If K^+ is not supplied to the exchange for Na^+ more K^+ may be lost. The diuretic action is due to salt, which maintains the water exchange between the blood and lymph and thus promoting the kidney [162-167].

2.8.5 Distribution of Potassium: Potassium salt an important cation of the cells and hence its uniform distribution is related to the proper working of the cell. 70% present in the muscles, 20% in the brain and large viscera while 10% is present in the skin and subcutaneous tissues. As compared to Sodium salt, the presence of Potassium in the bone is small, it is estimated at about 218mEq [162-167]. The mean Serum level of potassium is $4.5\text{mEq}/1 \pm 0.46$ (S.D). [162-167].

2.8.6 Potassium intake and Excretion: The daily potassium intake varies and to be more in vegetarians than in non-vegetarians. The major loss of potassium occurs in urine unlike sodium, potassium is secreted by kidney tubules. It is known that the kidney reabsorbs sodium and in exchange excretes hydrogen and potassium ions in the distal tubules. That why normal urine is acidic in nature. Due to tubular excretion of potassium salt, the urinary loss of potassium is observed and sometimes it is greater than the intake. About 5-10mEq of potassium is excreted through stools. [162-167].

2.9 SPHATIKA

Sphatika or Potash alum or *Phitkari* or *Kankshi* is a mineral origin drug. It has astringent, analgesic, detergent, haemostatic, expulsive for foetus and placenta, antipyretic, corrosive, expectorant, and emetic property [51, 82, 87, 113, 168]. It is colourless, white transparent, odorless crystalline masses or a granular powder with a sweetish astringent taste that contains $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$ [169]. When heated to $200^\circ C$ it melts and loses its water of crystallization with the formation of the anhydrous salt. It is soluble as 1 part in 7.5 parts of water, 1 in 0.3 of boiling water, and 1 in 3 of glycerol [170]. Two types of *Sphatika* have been explained in the classics i.e. *Phataki* and *Phullika*. It is described under *Uparasa varga* in *Rasa ratna samuchaya* [168], *Rasa Hridaya Tantra* [171], *Rasendra Chudamani* [172], *Rasa Prakasha sudhakara* [173].

2.9.1 Synonyms

Sanskrit	<i>Phatikri, Kmakshi, Tuvri, Siithi, Surashtraj</i>
Hindi	<i>Phitkhari</i>
Gujarat	<i>Saurastri</i>
English	Alum, Potash, Sulphate of Alumina, and Ammonium alum
Punjabi	<i>Phitkari</i>
Persian	<i>Shibb-e-Yamani, Zake safed, Zake bilore</i>
Tamil	<i>Patikarm, Adikhrum, Shinacruma</i>
Canra	<i>Phatikara</i>
Telgu	<i>Pattikaramu, Padikharam</i>
Malay	<i>Tawasa</i> [168, 171-173]

2.9.2 Pharmacological properties:

Rasa: *Kashaya, Katu, Amla*

Guna: *Guru, Snigda, Ishtpita, Shubra*

Karma: *Kanthya, Keshya, Vrunagna, Vishanashana, Netrahita, Garadoshavinashini, Lohamarni, Paradajarini, Bijadharini, Vrunashodini, Raktatsambini*

Doshagnata: *Tridoshahara*

Rogagnata: *Svitra, Kushta, Vruna, Garavish, Kaphajaroga, Netraroga, Urahkshata, Kshaya, Shool, Visarpa, Pittajaroga, Tridoshaja Roga, Raktasrava* [87, 113]

2.9.3 Modern view:

Alum (*Phitakari*) is known as alumen (Greeks and Romans). It is a double salt of Aluminum Sulphate, eg: $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$, which easily crystallizes in octahedral. It is prepared by using Alum Shale i.e., Aluminum silicate permeated with pyrites. The potash alum is prepared

by using Alunite (Roman alum) $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 4Al(OH)_3$, by roasting it with fuel, exposing it to air, lixiviating and crystalizing. Alum can be also made by adding the alkali sulphate into an alumina ferric solution. Generally, alum is purified by the process of recrystallization. If the caustic potash and alum solution is mix, then it precipitates the alumina at first and re-dissolves on stirring the mixture. The solution is known as neutral alum, on heating to $40^{\circ}C$ it deposits a precipitate of the same composition as natural alunite [174].

2.9.4 Pharmacology:

2.9.4.1 External: It has not any action on unbroken skin but coagulates the albumin of discharges and tissues and forms a covering on ulcers and sores and arrest bleeding. Hence it is a local astringent and hemostatic. Dilute solutions produce astringent action and concentrated one is irritant. Dried alum is caustic due to the absorption of water [174].

2.9.4.2 Internal: Local astringent to mouth and throat imparting and astringent taste, drying to the throat. In small doses Aluminum has the same astringent action on the stomach and intestine as on raw skin, producing constipation, local hemostatic action. In large doses cause vomiting by direct action on the stomach and excess doses cause gastrointestinal irritation, vomiting, and purgation [174].

2.9.4.3 Absorption and Elimination: An only a small amount is absorbed from the stomach and intestine and is stored in the liver, kidney, muscles, and pancreas. It is chiefly eliminated with feces and partly by skin, bile, and kidneys [174].

2.10 APAMARGA (*Achyranthus aspera*)

Apamarga, is a well-known precious herb globally available as a medicinal weed in India, tropical Asia, Baluchistan, Ceylon, Africa, Australia, and America. Botanically this plant is *Achyranthus aspera* and it belongs to the family Amaranthaceae [175]. The plant is mainly found as a weed near the roadside areas. It is a thorny plant and having spikes. The flowering season is during summer and the colour of the flower is white to red. In *Yajurveda*; it is mentioned that the plant capable to prevent us from all kind of ill actions and bad dreams and in *Atharveda*; it is mentioned that this plant is considered as the lord of all plants as it is capable to increase life span by eliminating all ailments from the humane body. The taxonomical description is listed in Table no. 2.5 and vernacular names are tabulated in Table no. 2.6 [175-178].

Table No. 2.5 Taxonomical classification: [177-179]

Kingdom:	Plantae
Sub-kingdom:	Tracheobinota
Super Division:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliopsida

Subclass: Caryophyllidae
Order: Caryophyllales
Family: Amaranthaceae
Genus: *Achyranthus*
Species: *aspera*

Table No. 2.6 Vernacular names: [176, 180]

Sanskrit	<i>Apamarga, aghata</i>
Hindi	<i>Latrija, chirchira, chirchita</i>
English	Prickly chaff flower, Devil's horsewhip
Bengali	<i>Apaang</i>
Gujarati	<i>Safad Aghedo, Anghadi, Andhedi, Agado</i>
Punjabi	<i>Kutri</i>
Telugu	<i>Uttaraene</i>
Tamil	<i>Shiru-kadaladi, Nayuruvi</i>
Punjabi	<i>Kurti</i>
Malayalam	<i>Kadaladi, Vankadaladi</i>
Assam	<i>Apang</i>
Marathi	<i>Aghada, Pandhara-aghada</i>
Kannada	<i>Uttarane, Utame</i>
Arabian	<i>Atkumah, Mahout, Wazer (Yemen)</i>
French	<i>Achyranth a feuilles rudes, Collant, Gendarme</i>
Indonesia	<i>Jarong</i>
Persian	<i>Khare-vazhun</i>
Philippines (Tagalog)	<i>Hangod</i>
Unani	<i>Chirchitaa</i>

2.10. 1 APAMARG PANCHANG IN AYURVEDIC TEXT

Various opinion regarding *gana*, therapeutic uses, synonyms, and variety mentioned by ancient scholar details of the same is tabulated in Table no. 2.7.

Table No. 2.7 Description of Apamarg Panchang in Ayurvedic text Ayurvedic literature

Sr. No.	Text Name	Gana (group)	Synonyms	Variety	Therapeutic Uses
1	<i>Charka Samhita</i> [7]	<i>Krimighna, Vamanopaga, Sirovirecanopaga</i>	<i>Apamarga, Pratyakpuspi, Mayuraka, Sikhari</i>	<i>Shweta and Rakta</i>	<i>Nasya, Shirovirechana, Bhasmakaroga</i>
2	<i>Sushrut Samhita</i> [12]	<i>Varunadigana, Viratarvadigana, Arkadigana</i>	<i>Kharamanjari, Pratyakpuspi, Mayuraka, Vasir</i>	<i>Shweta and Rakta</i>	<i>Bhasmakaroga,</i>
3	<i>Bhavprakash Nighantu</i> [87]	<i>Guduchyadi varga</i>	<i>Shikhari, Adhahshalya, Mayurak, Markati, Durgraha, Kinihi, Kharmanjari</i>	<i>Shweta and Rakta</i>	<i>Bhasmakaroga, Dipan, Pachan, Pittavirechak, Vamak, Mutrajanan, Kaphaghna, Vishaghna, Kramighna, Amltanashak, Shirovirechan</i>
4	<i>Astanga Sangraha</i> [4]	<i>Sodhanadigana as Sirovirecana dravya, Tikta Skanda drugs</i>	<i>Kinihi, Kharamanjari, Nandi, Mayuraka, Sikhari, Vasir, Pratyakpuspi</i>	<i>Shweta and Rakta</i>	<i>Ashmari</i>
5	<i>Shodhala Nighantu</i> [181]	<i>Guduchyadi varga</i>	<i>Shikhari, Pratyakpuspi, Adhahshalya, Kanti, Markatpippali, Kshav, Adhomarkava, Ghanta, Durabhigraha,</i>	<i>Shweta and Rakta</i>	-

			<i>Pratyakshreni, Vashir, Katu, Kharmanjari</i>		
6	<i>Shaligrama Nighantu</i> [182]	<i>Guduchyadi varga</i>	<i>Shaikharik, Dhamrgava, Mayurak, Pratyakaparni, Kishparni, Kharmanjari</i>	<i>Shweta and Rakta</i>	<i>Vishamajwara, Shiroruj</i>
7	<i>Dhanvantari Nighantu</i> [183]	<i>Guduchyadi varga</i>	<i>Apamarga, Shikhari, Pratyakpuspi, Mayurak, Adhahshalya, Kinihi, Durgaha, Kharmanjari, Shaikharik, Markati, Durabhigraha, Parakpuspi, Vashir, Kanti, Markatpippali.</i>	<i>Shweta and Rakta</i>	<i>Bhasmakaroga, Kandu, Udararoga, Raktatisara</i>
8	<i>Madanpal Nighantu</i> [184]	<i>Abhayadi varga</i>	<i>Shikhari, Kinihi, Kharmanjari, Adhahshalya, Shaikharik, Pratyakpuspi, Mayurak</i>	<i>Shweta and Rakta</i>	<i>Bhasmakaroga, Kandu, Udararoga, Kushta</i>
9	<i>Aadarsh Nighantu</i> [185]	<i>Apamargadi varga</i>	-	<i>Sveta, Rakta, Krishna</i>	-
10	<i>Kaiyadeva Nighantu</i> [186]	<i>Oshadhi varga</i>	<i>Shaikharik, Shikhari, Kharmanjari, Adhahshalya, Ksharmadhya, Durgaha, Durabhigraha, Aaghat, Kinihi, Marga, Mayurak, Pratyakapuspi</i>	<i>Apamarga. Vashira. Ramatha (Jalapamarga)</i>	<i>Udararoga, Kandu, Dadru, Apach, Arshas, Shoola</i>
11	<i>Raja Nighantu/ Nighantu Raja or Abhidhana Cudamani</i> [187]	<i>Shatahwaadi varga</i>	<i>Shikhari, Kinihi, Kharmanjari, Durgaha, Adhahshalya, Pratyakpuspi, Mayurak, Kaandkant, Shaikharik, Kubja, Markati, Durabhigraha, Vashir, Parakpuspi, Kanti, Markatpippali, Katu, Manjari, Nandi, Kshavak, Panktikantaka, Malakant</i>	<i>Apamarga, Raktapamarga, Kshudrapamarga</i>	<i>Bhasmakaroga, Ashmari, Kandu, Udararoga, Adhmana, Raktatisara, Kasa, Shwasa, Pandu, Pradara, Jwara, Krimi, Karnanaada</i>
12	<i>Hridayadipaka Nighantu</i> [188]	<i>Tripaad varga</i>	<i>Pratyakapuspi, Mayurak, Marga, Aaghat, Shikhari, Kharmanjari</i>	<i>Shweta and Rakta</i>	-
13	<i>Priya Nighantu</i> [189]	<i>Shatpuspadi Varga</i>	<i>Pratyakapuspi, Adhahshalya</i>	<i>Shweta and Rakta</i>	-
14	<i>Sausruta Nighantu</i> [190]	<i>Arkadi gana</i>		<i>Shweta and Rakta</i>	-
15	<i>Ashtanga Nighantu</i> [191]	<i>Arkadi Gana</i>	<i>Apamarga, Shaikharik, Pratyakpuspi, Mayurak</i>	<i>Shweta and Rakta</i>	-
16	<i>Madanadi Nighantu</i> [192]	<i>Chaturtha gana</i>	<i>Apamarga, Shaikharik, Pratyakpuspi, Mayurak, Kharmanjari, Adhahshalya, Kshudhaapamarga</i>	<i>Shweta and Rakta</i>	-

2.10.2 Description:

2.10.2.1 Macroscopy:

Root: Taproot is cylindrical and slightly ribbed, the root is thick (0.1-1 cm), gradually tapering roots, the root is rough because scars are present over it. Yellowish-brown is the color of the root and the odour is not distinct [180].

Stem: The colour of the stem is yellowish-brown, the stem is erect, unbranched, and cylindrical. Hairs are present over it. The stem is solid when the stem is dry it becomes hollow from inside [180].

Leaf: Leaves are simple, subsessile, and exstipulate. Leaves are oppositely arranged and their margin is wavy. Apex is slightly acuminate [180].

Flower: Inflorescence of long spikes is present and their color is greenish-white. They are numerous, sessile, bracteate having two bracteoles. Flowers are bisexual, 5 stamens are present and they are oppositely arranged [180].

Fruit: Indehiscent dry urticle which is enclosed [180].

Seed: Seed is subcylindrical, at apex truncate is there. Seeds are round at the base, endospermic and the color is brown [180].

2.10.2.2 Microscopy:

Root: 3-8 layers cork cells showed by the mature roots which are rectangular in shape, thin-walled, and tangentially elongated. 6-9 layers of secondary cortex showed by mature root which are oval to rectangular in shape and thin-walled. Parenchymatous cells are present and showed few scattered single groups of stone cells. Vascular bundles are present, in phloem parenchyma small patches of sieve tubes are distinct, xylem is present which is composed of vessels that are simply pitted, medullary rays are wide (1-3 cells). In the cortical region, the calcium oxalate crystals are present and in medullary rays, they are numerous [180].

Stem: Young stems show ridges (6-10) which are persistent, the single layer of the epidermis is present, the single layer of the epidermis is present which is covered by thick cuticle, 2-5 celled uniseriate covering trichomes are present with glandular and globular heads, stalked is 3-4 celled. 6-10 layers of the cortex contain parenchymatous cells and most of them contain crystals of calcium oxalate (rosette crystals). In mature stem lignified and thin-walled cork cells are present. Fibres are absent, annular vessels, pith consist of an oval to polygonal cells, parenchymatous cells are present, two medullary bundles are present [180].

Leaves:

Petiole: Epidermis is single-layered with a thick cuticle. Parenchymatous cells which are consisting of rosette crystals of calcium oxalate. In mid-region 4-5 vascular bundle is situated [180].

Midrib: Single layered epidermis followed by 4-5 layers of collenchyma on the upper side and 2-3 layers at the lower side. Parenchymatous cells are also present and having a number of vascular bundles which show xylem vessels, thin-layered cambium is followed by the phloem. Non-lignified cells are also present, calcium oxalate crystals are present (rosette crystals) [180].

Lamina: Single layer of tangentially elongated epidermal cells are present having thick cuticle, covering with trichomes, mesophyll are differentiated into palisade and spongy parenchyma. Palisade layers consist of 2-4 layers of thick parenchyma which is slightly elongated on the upper side and smaller and rectangular on the lower side. 3-5 layers of thick parenchyma are present, calcium crystals are present (rosette crystals) in the palisade, and spongy parenchyma cells. Anisocytic and anomocytic stomata are present [180].

Powder: Light yellow in colour which shows the fragments which are elongated, rectangular, and thin-walled epidermal cells. Aseptate fibres are present, vessels are present with annular, spiral, scalariform. Prismatic crystals of calcium oxalate are present [180].

2.10.3 Pharmacological properties: [176, 180, 193]

<i>Rasa</i>	<i>Katu, tikta</i>
<i>Guna</i>	<i>Laghu, sara, tikshana</i>
<i>Veerya</i>	<i>Ushna</i>
<i>Vipaka</i>	<i>Katu</i>
<i>Parbhav</i>	<i>Kaphavata har</i>

2.10.4 Chemical constituents:

Achyranthine, hentriacontane, Betaine, saponins A, B, C and D. In seeds - α -L-rhamnopyranosyl-(1 \rightarrow 4)-(β -Dglucopyranosuluronic acid)-(1 \rightarrow 3)- Oleanolic acid [180, 194-196].

2.10.5 Identity, purity and strength: [180]

Foreign matter:	NMT 2%
Total ash:	NMT 17%
Acid insoluble ash:	NMT 5%
Alcohol soluble extractive:	NMT 2%
Water soluble extractive:	NMT 12%

2.10.6 Pharmacological activities:

- 1) **Blood pressure:** Aqueous and alcoholic extracts of roots show reduction in blood pressure but the extract of the chloroform increases the blood pressure in dogs [175].
- 2) **Anti-cancer activity:** Methanol extraction of leaves has hindering activity against pancreatic cancer cells in humans and able to demonstrate anti-cancer and anti-proliferative properties [197].
- 3) **Hepatoprotective activity:** Methanolic extract of aerial parts is able to reduce the SGPT, SGOT, ALKP, and total bilirubin levels and shown hepatoprotective activity against rifampicin induced model in experimental rats (albino) [198].
- 4) **Anti-inflammatory and anti-arthritis activity:** The alcoholic extract in dose of 100-200mg/kg shown the anti-inflammatory and anti-arthritis [199, 200].
- 5) **Anti-microbial activity:** Alcoholic and aqueous extract of the plant showed antibacterial activity against *Staphylococcus aureus*, *Streptococcus hemolytic*, *Bacillus typhosus* *S. aureus*, and *E. coli* [201, 202].
- 6) **Anti-fertility activity:** Ethanol extract of the root proven anti-fertility activity in female albino rats at 200 mg/kg body weight which is given orally on days 1-7 of pregnancy. It also demonstrated estrogenic activity at a dose of 75mg/kg when tested in immature ovariectomized female albino rats [201, 202].
- 7) **Antidiabetic activity:** Ethanolic and aqueous plant extract showed hypoglycemic activity in experimental rats and diabetic rabbits [203-205].
- 8) **Diuretic activity:** Saponins extracted from the seeds at the dose 10-20 mg/kg intramuscular in experimental rats confirmed the diuretic activity [206, 207].
- 9) **Nephroprotective Activity:** Aqueous extract of roots shows promising results against urolithiasis induced by ethylene glycol and able to decrease the growth rate of calcium oxalate stones. It is also effective in reducing the chance of renal tissue injury, decreasing the size of the formed crystal, and restoring normal kidney function in experimental rats [208].
- 10) **Cardiovascular activity:** Saponins extracted from the seeds at the dose of 1 to 50 µg produce a significant increase in contraction force in the isolated heart of the frog, guinea pig, and rabbit. Water extracts able to decrease blood pressure, heart rate, dilated blood vessels [201, 206, 209-211].
- 11) **Anti-depressant Activity:** Methanolic extract of the leaves demonstrated the anti-depressant effect in mice (forced swimming test model) and rats (tail suspension test model) [212].

- 12) **Bronchoprotective Activity:** Ethanolic extract of plant drug able to produce a bronchoprotective effect on toluene diisocyanate (TDI) induced occupational asthma in experimental Wistar rats [213].
- 13) **Anti-allergic:** Petroleum ether extract revealed the antiallergic activity in mice (milk induced leukocytosis model and milk induced eosinophilia model) [214].
- 14) **Wound Healing and antioxidant activity:** Ethanolic and aqueous extracts of leaves show promising wound healing properties. The study revealed that the antioxidant activity of the aqueous extract is more when compared with the ethanolic extract [215].

2.10.7 Dose:

Swarasa : 10-20 ml, *Kwath* : 50-100 ml, *Churna* (Root) : 2-4 g., *Kshara* : 0.5-2 g.

2.10.8 *Apamarg kshar*

It is an off-white alkaline preparation derived from the water-soluble ash of *Apamarg Panchanga* (*Achyranthes aspera* Linn.) and claimed for the treatment of many pathophysiological conditions i.e. *Gulma* (Abdominal lump); *Udarasula* (Abdomen pain); *Grahani* (Malfunctioning of the duodenum or small intestine); *Visuchka* (Gastro-enteritis with piercing pain); *Alasaka* (Intestinal atony); *Ajirna* (Dyspepsia); *Aruchi* (Tastlessness); *Anaha* (Distention of abdomen due to obstruction to the passage of urine and stool); *Arsha* and *Bhagandara* (Piles and fistula); *Sarkara* (Gravel in urine); *Asmari* (Calculus); *Krmi* (Helminthiasis); *Antarvidradhi* (Hernia); *Swasa* (Asthma); *Karnaroga* (Ear disorder) etc. Different techniques for the preparation of *Kshar* differ by the ratio of water added to prepared ash, soaking time, folds of cloth, and the number of times of filtration described by various authors from time to time [4, 82, 113]. In the present study, *Apamarg kshar* was prepared as per the methodology mentioned in *Rasa Tarangini* [113].

2.11 *PUNARNAVA (Boerhaavia diffusa)*

Punarnava (Boerhaavia diffusa) belongs to the family Nyctaginaceae. It is also named as spreading hogweed and used comprehensively in the *Ayurvedic* system of medicine to cure diseases like *Hridrog* (cardiac disorders), *Pandu* (anaemia), *Vayasthapana/Rasayana* (rejuvenator), *Sotha* (inflammation with swelling), *Mutravahshortogat vikar* (urinary tract disorders), *Jwara* (fever), *yonirog* (vaginal disorders), *sutikarog* (female disorder), *kustharog* (skin diseases) *mrida bhakshana janya rog* (disorders originated due to eating of clay), *Basti karma* (enema), *balarog* (disorders of children's), *madhumeha* (anti-diabetic) etc. Its synonyms, morphology, the therapeutic potential are described in *Ayurvediya Samhitas* and *Nighantus*. *Boerhaavia diffusa (Punarnava)* turned into named in the honor of Dutch physician Hermann Boerhaave in the 18th century [216]. The genus *Boerhaavia* L. (Family:

Nyctaginaceae) consists of 40 tropical and sub-tropical species [217] found growing wild in diverse terrestrial habitats, ranging from managed grasslands, waste-lands, agroecosystems to large forest gaps. *Punarnava* (*Boerhaavia diffusa*); Itself indicates that brings back to life and renews the human body. It is an herbaceous/ Perennial/Seed propagated plant [218]. All parts of the plant are well-acknowledged for their therapeutic potential and have a long history of practice by indigenous and tribal people in India [219]. The therapeutic potential of this plant is able to treat several human ailments which are well described in *Ayurvedic* literature [220].

2.11.1 CLASSICAL REVIEW OF *PUNARNAVA* (*Boerhaavia diffusa*)

The details of various opinions regarding *gana*/varga and synonyms mentioned by ancient scholars are tabulated in Table no. 2.8

Table No. 2.8 *Punarnava gana* (group) classification and synonyms in *Ayurvedic* literature

Sr. No.	Text Name	<i>Gana</i> (group)	Synonyms
1	<i>Charka Samhita</i> [7]	<i>Swedopaga, Anuvasanopaga, Kasahara, Vayasthapana,</i>	<i>Punarnava, Mahavarshabhu, Vrishchiva, Dirghavarshabhu, Shvetamula, Raktavrinta, Vaishakha, Shinati, Varshaketu</i>
2	<i>Sushrut Samhita</i> [221]	<i>Vidaarigandhadi, Vatashanshamana varga, Tikta varga, Shaka varga</i>	<i>Punarnava, Rishabhketu, Mahavarshabhu, Vrishchiva, Dirghavarshabhu, Shvetamula, Raktavrinta, athillaka, Vaishakha, Shinati, Kshudravarshabhu</i>
3	<i>Astanga Sangraha</i> [4]	<i>Viprakirna varga, Vidarigandhadi Gana,</i>	<i>Punarnava, Varshaketu, Vrishchiva, Shvetamulaka, Varshabhu, Dirghapatra, Vikasa, Kathillaka, Sunadik, Raktapushpa, Vishakha, Mandalacchada</i>
4	<i>Bhavprakash Nighantu</i> [87]	<i>Guduchyadi varga</i>	<i>Arunaa, Kshudravarshabhu, Raktapushpaka, Shilatika, Shothaghni, Varshaketu,</i>
5	<i>Shodhala Nighantu</i> [181]	<i>Guduchyaadi varga</i>	<i>Mahavarshabhu, Pravrusaayani, Raktapushpaka, Shivatika, Shophaghni, Varshabhu, Varshaketu</i>
6	<i>Dhanvantari Nighantu</i> [183]	<i>Guduchyaadi varga</i>	<i>Deerghapatraka, Kathillaka, Kshudravarshabhu, Shivatika, Vrushchira</i>
7	<i>Madanpal Nighantu</i> [184]	<i>Abhayadi varga</i>	<i>Arunaa, Kathillaka Kruraka Kshudravarshabhu, Raktapushpaka, Tikta, Shivatika, Varshaketu</i>
8	<i>Aadarsh Nighantu</i> [185]	<i>Punarnavadi Varga</i>	<i>Raktapunarnava, Vishakha, Katilla, Kathila, Shothaghni, Mahavarsabhu</i>
9	<i>Kaiyadeva Nighantu</i> [186]	<i>Aushadhi varga</i>	<i>Deerghapatraka, Shophaghni, Varshabhu, Vrushchiva</i>
10	<i>Raja Nighant/ Nighantu Raja or Abhidhana Cudamani</i> [187]	<i>Parpatadi varga</i>	<i>Raktapunarnava, Krura, Mandalpatrika, Raktakanda, Varshketu, Lohita, Raktapatrika, Vaishakhi, Raktavarshabhu, Shophaghni, Raktapushpika, Viksvara, Vishaghni, Pravrishenya, Sarini, Varshabhav, Shorapatra, Sammlitadruma, Punarnav, Nav, Nachya</i>
11	<i>Hridaya dipaka Nighantu</i> [188]	<i>Dvipadi Varga</i>	<i>Punarnava, Vrishchiva, Varshabhu, Shivatika</i>
12	<i>Priya Nighantu</i> [189]	<i>Shatpushpadi Varga</i>	<i>Punarnava, Varshabhu</i>

2.11.2 MODERN REVIEW OF *PUNARNAVA* (*Boerhaavia diffusa*)

In the modern era, all crude drugs are classified and studied according to their taxonomical classification tabulated in Table no. 2.9 [218, 220, 222, 223].

Table No. 2.9 Taxonomical classification

Domain	:	Eukaryota
Kingdom	:	Plantae
Sub-kingdom	:	Tracheobionta – Vascular plants
Phylum	:	Spermatophyta
Subphylum	:	Angiospermae
Class	:	Dicotyledonae
Sub-class	:	Caryophyllidae
Order	:	Caryophyllales
Family	:	Nyctaginaceae
Genus	:	<i>Boerhaavia</i> .
Species	:	<i>Boerhavia diffusa</i> .
Latin name	:	<i>Boerhaavia diffusa</i> Linn.

2.11.3 ETHNOBOTANICAL USES

The ethnic population at *Purulia* (*West Bengal*) eats that plant as a vegetable. In the Assam region, its leaves are normally available in the market, and people eating them after cooking. Its roots are recommended for the treatment of piles by the residents of the *Garhwal Himalaya* (Uttaranchal) [224]. Root *kalka* is used in bloody dysentery by the *Bhils* of the *Jhabua* (Madhya Pradesh) [224]. The *kwath* is described to treat nodules in the human body. *Sahariya* tribe at *Lalitpur* (Uttar Pradesh) uses it for the treatment of leukorrhea, rheumatism, and stomachache. At *Ambikapur* (Madhya Pradesh) it is described to use for the treatment of elephantiasis [224]. The tribal of *Indo-Nepal Himalayan terai* region harvest that for medicinal purposes like flushing out the renal system, treat seminal weakness and blood pressure [224].

In Brazil it uses in for albuminuria, beriberi, bile insufficiency, cystitis, edema, gallbladder problems, gallstones, guinea worms, hepatitis, hypertension, jaundice, kidney disorders, kidney stones, liver disorders, liver support, nephritis, renal disorders, snakebite, spleen (enlarged) and gonorrhoea [224].

In Iran, it uses in edema, gonorrhoea, hives, intestinal gas, jaundice, joint pain, lumbago, nephritis, and as an appetite stimulant, diuretic, and expectorant.

In Nigeria, it uses in abscesses, asthma, boils, convulsions, epilepsy, fever, guinea worms, as expectorant and laxative [224].

In West Africa, it uses in abortion, guinea worms, menstrual irregularities, and an aphrodisiac.

In Philippines, it uses in fever, purgative, diuretic, and vermifuge.

In Ghana, it uses in Asthma and Boils [135, 138, 148, 159, 216, 218-220, 223, 225-227].

2.11.4 CONTROVERSIAL STATUS

Punarnava has been known to be a controversial plant in *Ayurvedic* literature. In the *Vedic* period, there is no controversy about this drug. But in the *Samhita* period *Punarnava*, *varshabhu*, *Kathillaka*, *Vrishchiva*, *Vrishchira*, and *Vrishchika* have been described together in several places which indicates that these all are separate drugs having similar properties and actions. In some places, we have also found the words like *Punarnave dwe* and *dwi Varshabhu* means two types of *Punarnava* and *Varshabhu* respectively.

The commentators have muddled the subject and created the controversy of *Punanava*. *Chakrapani* considered *Kathillaka* as *Punarnava* (*Ch.Su.27/96*) and *Vrishchira* as *Sweta Punarnava* (*Ch.Su. 4/23*). While *Dalhana* interprets *Varshabhu* as *Punarnava* (*S.S.Su.46*), *Sweta Punarnava* (*S.S.Su.21*) and *Rakta Punarnava* (*S.S.Chi.23*).

In *Nighantu*, all these drugs are described as synonyms to each other *Sodhala nighantu* describes other variety of *Punarnava* i.e. *Vaishakha*, which is having profused branches with red margins around the leaves. Botanically 3 species of *Boerhavia* have been used as *Punarnava* in the different parts of India viz. *B.diffusa*, *B.erecta* and *B.rependa*. *Sharma P.V.* standardized that *Rakta Punarnava* is *B.diffusa* and *sweta Punarnava* is *B. verticillata* based on available literature and their pharmacological activity [7, 12, 82, 181, 228-230].

2.11.5 RASA PANCHAK: [193, 231]

<i>Rasa</i>	<i>Madhur, tikta, Kashaya</i>
<i>Guna</i>	<i>Ruksha</i>
<i>Veerya</i>	<i>Ushna</i>
<i>Vipak</i>	<i>Madhur</i>
<i>Dosha karma</i>	<i>Vata har</i>

2.11.6 DESCRIPTION:

A. Macroscopic

Stem: The colour of the stem is greenish-purple; the stem is stiff and its shape is cylindrical. At the nodes, the stem is swollen. The stem of *punarnava* is slightly globrous, minutely pubescent. It is long more than a meter. Prostrate divaricately branches are there [232].

Root: Is well developed, fairly long, and somewhat tortuous, their shape is cylindrical and 0.2 to 1.5 cm in diameter. The root is of yellowish-brown to brown color. Their surface is soft to touch but it is rough due to the presence of minute longitudinal striations and root scars present over it. The fracture is short and the taste is slightly bitter. No distinct odour is there [232].

Leaves: Are opposite in unequal pairs, larger leaves are 25-37 mm long, and small leaves are 1-18 cm long ovate, oblong or suborbicular. Their apex is oblong or slightly pointed. Base is

subcordate or rounded. The Colour of the leaves is green and the above surface is glabrous and whitish below the surface. Margin is entire or subundulate. The dorsal sides of the leaves are pinkish in certain cases. The texture of the leaves is thick and long petiole [232].

Flowers: Are very small and their color is pink. Flowers are shortly stalked and nearly sessile. 10-15 cm long, present in small umbells. Arrange on slender and long stalks. The shape of the flowers is like funnel and 2-3 stamens are present [232].

Fruit: One seeded nut, the shape is round. 6mm long clavate, seeds are 5 ribbed and viscidly glandular [232].

B. Microscopic

Stem: Stem TS shows an epidermal layer that contains multicellular uniseriate glandular trichomes. It consists of 9-12 stalked cells and it also contains ellipsoidal head. Cortex layer is also present which consists of parenchymatous cells. Endodermis is indistinct. Pericycle is 1-2 layered and consists of thick-walled containing isolated fibers. Stele region is consists of vascular bundles joined together in the form of ring [232, 233].

Root: In a transverse section of roots, cork is present which is composed of thin-walled tangentially elongated cells and the outer few cells are with brown walls. 1-2 layers of thin-walled cells of cork cambium. The secondary cortex is consists of 2-3 layers of parenchymatous cells which are followed by cortex and composed of 5-12 thin-walled layers, oval to polygonal cells. Xylem is composed of vessels, tracheids, and fibers, calcium oxalate crystals, and starch grains are also present in between them [232, 233].

Leaves: In the transverse section anomocytic stomata are present on both sides, they are numerous and have short hairs. On veins and margin, 3-4 celled are present. Only single layer of palisade is present and 2-4 layers of spongy parenchyma are present and small air spaces are present. Calcium oxalate crystals are present and in mesophyll orange-red resinous matter is present. Palisade ratio 3.5-6.5, stomatal index 11-16, vein islet number 9-15 [232, 233].

Powder: The powder shows characters like cork cells in surface view, prismatic and acicular crystals of calcium oxalate. Thin long narrow fibres with sharp pointed ends, oval to rounded starch grains, simple pitted vessels, and few parenchyma with starch grains. The leaf of the plant shows the upper and lower epidermis. In leaf numerous multicellular glandular hairs and anomocytic stomata are present. One layer palisade, spongy parenchyma, cells polyhedral or isodiametric in shape with distinct intercellular spaces [232, 233].

2.11.7 IDENTITY, PURITY AND STRENGTH: [232]

Foreign matter:

NMT 2%

Total ash:	NMT 15%
Acid insoluble ash:	NMT 6%
Alcohol soluble extractive:	NMT 1%
Water soluble extractive:	NMT 4%

2.11.8 PHYTOCHEMISTRY:

Punarnava (Boerhaavia diffusa) contains number of chemical compounds i.e. flavonoids alkaloids, steroids, triterpenoids, lipids, carbohydrates, proteins, and glycoproteins. Punarnavine, boeravinone A-F, hypoxanthine, ursolic acid, punarnavoside, lirodendrin, arachidic acid, α -2-sitosterol, palmitic acid, ester of β -sitosterol, tetracosanoic, hexacosanoic, stearic, hentriacontane, β -Ecdysone [216, 220, 223, 225, 232, 234, 235].

2.11.9 ADULTERANTS AND SUBSTITUTES:

Punarnava (Boerhaavia diffusa) is habitually adulterated with *Varshabhu (Trianthema portulacastrum)*. *Punarnava* and *Varshabhu* probably have similar therapeutic potential but vary widely in their stomatal index and palisade ratios. *Varshabhu (Trianthema portulacastrum)* owning higher values than *Punarnava (Boerhaavia diffusa)* [236, 237].

2.11.10 PHARMACOLOGICAL ACTIVITIES:

- 1) Analgesic and anti-inflammatory activity:** Plant lyophilized decoction and fresh leaf juice use to determine their antinociceptive effect using acetic acid-induced abdominal writhing (chemical) model and hot plate (thermal) analgesic test. *B. diffusa* roots methanol extract was able to inhibit the contractions induced by acetylcholine (ACh) in the isolated guinea-pig ileum [238-240].
- 2) Antibacterial activity:** Experimental studies showed that the aqueous, ethanolic and methanolic extracts of *B. diffusa* (leaf) have substantial antibacterial property against gram-positive and gram-negative pathogenic bacteria [241-243].
- 3) Antistress /Adaptogenic /Immunomodulatory Activity:** Studies confirm the adaptogenic potential of *B. diffusa* root aqueous extract on *Escherichia coli*-induced abdominal sepsis, macrophage phagocytic activity in mice, and cold and forced swimming stress in rats [244-246].
- 4) Hepatoprotective activity:** Extracts of the aerial part and roots of the plant show potential for hepatoprotection against CCl_4 , country made liquor, thioacetamide and acetaminophen-induced hepatotoxicity in rats [247-249].
- 5) Anti-convulsant activity:** *B. diffusa* widely used in epilepsy in Nigerian folk medicine. Isolated compound 'liriodendrin' from the methanolic root extract reported as calcium

channel antagonistic activity which was verified later in male Swiss albino mice model [250-253].

- 6) **Bronchial asthma:** Dried leaves of the *Punarnava* are used as dhoomapana for the treatment of asthma. Leaf decoction is reported for its expectorant properties when combined with ginger juice and black pepper [254].
- 7) **Anticancer Activity:** A dose-dependent *in-vitro* cytotoxic effect of root extract and leaf in HeLa and U-87 tumor cell lines was reported [255].
- 8) **Antifungal activity:** *In-vitro* studies: Ethyl acetate root extract shown mycelial growth inhibition for *Microsporum gypseum* (78.83%), *M. fulvum* (62.33%), and *M. canis* (42.30%) in that order at 1mg/mL. The increase in the concentration of extract also inhibited sporulation [256].
- 9) **Anti-viral activity:** Isolated glycoprotein with a molecular weight of 16–20 kDa when administered by foliar spraying in the field, it protects crops against natural infection by plant viruses [257, 258].
- 10) **Antioxidant activity:** *In-vitro* studies: Ethanolic and methanolic extracts of the dried root showed good antioxidant activities in terms of ferric reduction and hydrogen peroxide quenching in comparison to ascorbic acid [259].
- 11) **Antiuro lithiatic Activity:** Plant extract possess diuretic properties. The extract inhibited CaOx nucleation, aggregation, and crystal formation in the synthetic urine *in-vitro* on the addition of NaOx. The lithogenic treatment caused polyuria, weight loss, hyperoxaluria, and impairment of renal function which was prevented by plant extract [227, 260-265].
- 12) **Antifertility:** Orally administered root extract (50% aqueous and ethanolic) in experimental monkeys able to stop intrauterine contraceptive device (IUCD)-induced bleeding [266].
- 13) **Antidiabetic activity:** The observations indicated that *Boerhaavia diffusa* and its leaf extracts with various solvents revealed the antihyperglycemic activities in alloxan and streptazotocin induced hyperglycemic in experimental rats [267-270].

2.11.11 Dose :

20-30 g of the drug for decoction [232]

3-6 g of drug in *churna* from [193]

2.12 GOKSHUR (*Tribulus terrestris*)

Gokshur (*Tribulus terrestris*) is an annual plant commonly known as puncture vine, goat head, devil's thorn [271, 272]. *Tribulus* genus belongs to the family zygophyllaceae that roughly contains 25 different plant species which grow as hairy herbs in tropical and warm areas [273, 274]. *Goksura* (*Tribulus terrestris*) is a well-recognized and vastly distributed plant of the

genus *Tribulus*. It exists in the Mediterranean, subtropical, and desert areas [275]. *Gokshura* (*Tribulus terrestris*) has been used to treat different diseases due to its bioactive components i.e. saponins, alkaloids, tannins, vitamins, glutamic acid, and aspartic acid [276].

The Chinese use *Gokshur* (*Tribulus terrestris*) for over 400 years. Masai in Southern Africa uses its thorns to pierce the earlobes of children [277]. The Eastern European Olympic athletes claimed that *Gokshur* contributed to their success in the mid of nineties. The research of the Chemical and Pharmaceutical Institute in Sofia, Bulgaria, brought this plant into the limelight in 1982. In India, it is used as an anticonvulsant, anti-inflammatory, aphrodisiac, cardiogenic, diuretic, and litholytic and treated for gleet, gonorrhoeal rheumatism with cystitis, gout, and impotency. Its ash is good for external application in rheumatic arthritis. The taxonomical classification is shown in Table no. 2.10, vernacular name in Table no. 2.11, synonyms in Table no. 2.12, gana at Table no. 2.13, and *Rasa panchak* in Table no. 2.14 [278-280].

Table No. 2.10 Taxonomical classification [275, 281-284]

Classification	<i>Pedaliium murex</i> (<i>Gokshura</i> -big)	<i>Tribulus terrestris</i> (<i>Gokshura</i> -small)
Kingdom	Plantae	Plantae
Subkingdom	Tracheobionta	Tracheobionta
Division	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida
Subclass	Asteridae	Rosidae
Order	Lamiales	Sapindales
Family	Pedaliaceae	Zygophyllaceae
Genus	<i>Pedaliium</i>	<i>Tribulus</i>
Species	<i>Murex</i>	<i>terrestris</i>

Table No. 2.11 Vernacular name [275, 285, 286]

Sanskrit	<i>Gokharuka, Trikata, Svadamshtra, Traikantaka</i>
Hindi	<i>Gokharu, Chotagokhru</i>
Assamese	<i>Gokharu, Gukhorkata</i>
Tamil	<i>Nerunji</i>
Bengali	<i>Gokhri, Gokharu</i>
Marathi	<i>Sarate, Gokaru, Lahangokhru, Sarala</i>
Oriya	<i>Gukhura, Gokhyura</i>
Urdu	<i>Khorkashak, Khar-e-Khasak Khurd, Gokharu</i>
Gujarati	<i>Bethagokhru, Nahanagokhru, Mithagokhru</i>
Telugu	<i>Palleru kayalu, Chinnipalleru</i>
Tamil	<i>Nerinjil, Nerunjeekai</i>
Kannada	<i>Sannaneggilu, Neggilamullu, Neggilu</i>
Kashmiri	<i>Michirkand, Pakhda</i>
Malayalam	<i>Neringil, Nerinnil</i>
Punjabi	<i>Bhakhra, Gokhru, Kurkundai</i>
English	<i>Caltrops fruit</i>
Arabic	<i>Qutuiba</i>
Burma	<i>Charatte</i>
Chinese	<i>Chili, Tsilitse</i>
Sind	<i>Land caltrops</i>
South Africa	<i>Devils thorn</i>
Spanish	<i>Abrojos</i>

Table No. 2.12 Synonyms of Gokshur [4, 7, 12]

Sr. No	Synonyms	C.S	Su.S	A.S	D.N	M.N	R.N	K.N	BP.N	Ma. N	A.R	A.K
1	<i>Bahukantaka</i>	-	-	-	-	-	✓	-	-	-	-	-
2	<i>Bhakshakha</i>	-	-	-	✓	✓	-	-	-	-	-	-
3	<i>Bhakshyaka</i>	-	-	-	✓	-	-	✓	-	-	-	-
4	<i>Bhaksyakanta</i>	-	-	-	-	-	✓	-	-	-	-	-
5	<i>Bhukshura</i>	-	-	-	-	-	-	✓	-	-	-	-
6	<i>Chanadruma</i>	-	-	-	-	-	✓	-	-	-	-	-
7	<i>Gokantaka</i>	-	-	-	✓	✓	-	-	✓	✓	-	✓
8	<i>Gokharu</i>	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
9	<i>Gokharuka</i>	-	-	-	✓	-	✓	-	-	-	-	✓
10	<i>Ikshugandha</i>	-	-	-	-	-	✓	-	✓	✓	-	✓
11	<i>Kantaka</i>	-	-	-	-	-	✓	-	-	-	-	-
12	<i>Kanti</i>	-	-	-	-	-	✓	-	-	-	-	-
13	<i>Kantakashura</i>	-	-	-	-	-	-	-	-	-	-	-
14	<i>Kantakatrika</i>	-	-	-	✓	-	-	-	-	-	-	-
15	<i>Kantaphala</i>	-	-	-	-	✓	-	✓	-	-	✓	-
16	<i>Kshudrakshura</i>	-	-	-	-	-	✓	-	-	-	-	-
17	<i>Kshura</i>	-	-	-	-	✓	✓	✓	✓	-	-	-
18	<i>Kshuraka</i>	-	-	-	-	✓	✓	-	-	-	-	-
19	<i>Kshuranga</i>	-	-	-	-	-	✓	-	-	-	-	-
20	<i>Mahanga</i>	-	-	-	-	-	✓	-	-	-	-	-
21	<i>Palankasha</i>	-	-	-	-	-	✓	-	✓	✓	-	✓
22	<i>Shadanga</i>	-	-	-	✓	✓	-	✓	-	-	-	-
23	<i>Sthalashrunghata</i>	-	-	-	-	✓	✓	✓	-	✓	✓	-
24	<i>Swadamstra</i>	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
25	<i>Swadu kantaka</i>	-	-	-	✓	✓	✓	✓	✓	✓	-	✓
26	<i>Trika</i>	-	-	-	✓	-	-	-	-	-	-	-
27	<i>Trikantaka</i>	-	✓	-	-	✓	✓	✓	✓	✓	✓	-
28	<i>Vanashrunghataka</i>	-	-	-	-	-	✓	-	✓	-	-	✓
29	<i>Vyaladamstraka</i>	-	-	-	-	✓	✓	✓	-	-	✓	-

Table No. 2.13 Classification of Gokshur or Gokshur gana

Sr. no.	Text name	Gana (group)	Therapeutic uses
1	<i>Charak samhita</i> [7]	<i>Anuvasanopaga, mutravirechaniya, shoithahara, krimighana</i>	<i>Sothahara, mutrakricchra, anilhara karmas, mutrashmari</i>
2	<i>Sushruta samhita</i> [12]	<i>Vidharigandhadi gana, veeratarva gana, madhur varga, laghu panch mool gana, kantaka panch mool</i>	<i>Mutrakricchrahara, ashmari</i>
3	<i>Astanga sangraha</i> [4]	<i>Krimighana, laghu panchmoola, mutra virechaniya, sothahara, veeratarvadi, vidaryadi</i>	<i>Sothahara, mutrakricchrahara, anilhara karmas, krimighana</i>
4	<i>Bhavprakash Nighantu</i> [87]	<i>Guduchyadi varga</i>	<i>Asmari, hridya roga, mutral, svasa, kasa, arsha</i>
5	<i>Shodhala Nighantu</i> [181]	<i>Guduchyadi varga</i>	<i>Mutrakricchrahara, ashmari, rasayana</i>
6	<i>Shaligrama Nighantu</i> [182]	<i>Guduchyadi varga</i>	<i>Mutrakricchra, ashmari, prameha, svasa, kasa, vataroga</i>
7	<i>Dhanvantari Nighantu</i> [183]	<i>Guduchyadi varga</i>	<i>Mutrakricchra, hridya roga, prameha, shoola, tridosha shamak, dipak, vrushya, brimhana</i>

8	Madanpal Nighantu [184]	Abhayadi varga	Mutrakricchra, hridya roga, prameha, svasa, kasa, vata roga
9	Aadarsh Nighantu [185]	Laghu Gokharudi varga	Vrishya, mutrakriccha, asmari, hridya roga, prameha, svas, pradar, rasayana
10	Mahaushdha Nighantu [287]	Bilwadi varga	Vrishya, mutrakricchra, asmari, hridya roga
11	Kaiyadeva Nighantu [186]	Oshadadi varga	Mutrakricchra, asmari, hridya roga, prameha, svas, kasa, bastidosha, vrishya, balya
12	Raj nighantu/ nighantu raj or abhidhana cudamani [187]	Shatahvadi varga	Vrisya, Mutrakricchra, asmari, prameha, rasayana
13	Hridayadipaka Nighantu [188]	Doshaghana varga	Tridosha shamak
14	Priya Nighantu [189]	Haritakyadi varga	Tridosha shamak
15	Madava dravyaguna [288]	Vividoushadi varga	Tridosha shamak
16	Amara Kosha [289]	Vanoushadi varga	Tridosha shamak

Table No. 2.14 Rasa panchak (properties and action mentioned in various nighantus)

Text name	Synonyms	Rasa	Guna	Veerya	Vipaka	Prabhav
Bhavprakash Nighantu [87]	Bhakstaka, gokantak, iksugandhika, ksuraka, palamkasa, svdramastra, svadukantaka, trikantaka, vanasrngata	Madhur	-	Sheeta	-	Vata shamak
Dhanvantari Nighantu [183]	Gokantak, swadukantak, gokshur, Gokharuk vakshak, vakshatak, kantakari	-	-	-	-	Tridosha shamak
Madanpal Nighantu [184]	Gokantak, kantaphala, swadukantak, yaladanstra, gokshur, saranga trikantak, trik, khurak, bhaksyantaka, vyaadamstraka, svadamstra, sthulasrngata	Madhur	-	Sheeta	-	Vata shamak
Adarash Nighantu [185]	Swadanstak, gokshur, Gokharuk, saranga, swadamstra, vanasrngata	Madhur, tikta	-	Sheeta	Madhur	Vata shamak
Kaiyadeva Nighantu [186]	Gokantak, kantaphala, bhakstaka, swadukantak, swadanstak, byaladanstra, gokshuru, gokshur, kshur, sarang, trikantak, trik, shalasringat	Madhur	-	Sheeta	-	Kapha vata shamak
Raj nighantu or nighantu raj or abhidhana cudamani [187]	Bhadrakantaka, duscakrama, vyaldantra, mahanga, gokhuraka, antah, bahukantaka, gokantaka, palankasa, bhaksatata, sthalsrangataka, iksu gandha, trikantaka, sadanga, ksura, kantaphala, ksudra-ksura, canadrumqa, van srngataka, kantah	Madhur	-	Sheeta	-	-
Dravyaguna vigyana [193]	Gokshur, trikantaka, Gokharuk	Madhur	Guru, snigdha	Sheeta	Madhur	Tridosha shamak
The Ayurvedic pharmacopeia of India [290]	Mutrakricchra, hridya roga, kasa, arsa, svasa, asmari, prameha	Madhur	Guru, Snigdha	Sheeta	Madhur	-

2.12.1 Etymology of Goksura

The word 'Gokshur' means the spines of the fruit that injures grazing cow or cattle [185].

2.12.2 Substitutes and Adulterants:

Pedaliu murux which is considered as a source for *Bruhat Goksura* is substituted for *Laghu Goksura - Tribulus terrestris*. Pharmaceutical industries use fruits instead of roots for the preparation of various formulations. The fruits of *Acanthospermom hispidum* DC resemble of *Tribulus* and are frequently used in adulteration [55, 291-294].

2.12.3 Description:

a) Macroscopic:

Leaves: leaves are opposite, pinnate, one of each pair and smaller than the other, 3-6 pairs of leaflets, stipules are lanceolate, more or less hairs on the upper surface, base is round oblique, petiole is very short [290].

Flowers: Flowers are axillary of leaf opposed, solitary, 1.2-2cm long pedicle, slender, hairy flowers, petals are 1cm long, oblong or obovate, style is short and stout [290].

Fruit: globose, consisting of 5 hairy or glabrous, woody cocci, each cocci has 2 pairs of hard spines (very sharp), 1 pair if spines are longer than the other, several seeds are present in each coccus with transverse partitions [290].

Root: root is 7-18 cm long and 0.3-0.7 cm in diameter, the root is slender and cylindrical, fibrous, and frequently branched, number of rootlets are present over it. The root is woody and colour of the root is yellow to light brown, surface is rough due to the presence of small nodules. Fracture is fibrous and aromatic odour. Taste is sweetish and astringent [290].

b) Microscopic:

Root: transverse section shows layers of the epidermis (4-5 layers) followed by parenchymatous cortex (thin-walled), endodermis is distinct, in mature root 4-6 layers of cork cells are present, there is an only single layer of cork cambium which is followed by thin-walled parenchymatous cells (6-4 layers) and varying numbers of fibres are present, fibres are found in groups which resembles phloem, there are two zones of secondary phloem, in outer zones numerous phloem fibres are present and also some sieve tubes are present which is slightly collapsed, inner zone consists of parenchymatous, no fibre is there but shows some sieve tubes and companion cells, phloem rays are distinct, in an outer region few cells get converted into fibres, 3-5 layers of cambium, vessels, tracheids, parenchyma and fibres transverse with medullary rays, vessels are scattered, xylem parenchyma is rectangular or slightly elongated, medullary rays are also present. Few prismatic crystals are also present in the xylem ray cells [290].

Fruit: small epidermal cells in each coccus rectangular, trichomes are unicellular, mesocarp (6-10 layers) of large parenchymatous cells, calcium oxalate crystals (rosette) are present, in mesocarp small cells are present which is consist of calcium oxalate crystals (prismatic crystals) [290].

2.12.4 Chemical constituents:

Protodioscin, terrestrosins, glycosides, diosgenin, hecogenin, ruscogenin, quercetin, saponins, alkaloids, Flavonoids, lignn amides etc [275, 290, 292, 295, 296].

2.12.5 Identity, purity, and strength

Root [297]

Foreign matter	NMT 2%
Total ash	NMT 13%
Acid insoluble ash	NMT 3%
Alcohol soluble extractive	NMT 4%
Water soluble extractive	NMT 10%

Fruit [290]

Foreign matter	NMT 1%
Total ash	NMT 15%
Acid insoluble ash	NMT 2%
Alcohol soluble extractive	NMT 6%
Water soluble extractive	NMT 10%

2.12.6 Pharmacological activities:

- 1) **Aphrodisiac activity:** It is used as a traditional medicine for the management of male and female infertility and reported for its aphrodisiac properties [298-300].
- 2) **Antioxidant activity:** It shows antioxidant activity in a concentration-dependent manner by 2,2-di-(4-tert-octylphenol)-1-picrylhydrazyl (DPPH), H₂O₂, and superoxide scavenging activity, as well as the FRAP (Ferric reducing antioxidant power) assay [301]. Diosgenin from the callus of *T. terrestris* was found to have great antioxidant activity [302, 303].
- 3) **Anti-inflammatory activity:** The terrestinones A1/A2 (1a/1b) and N-trans-*p*-caffeoyl tyramine isolated from ethanolic extract of *gokshur* are capable to produce anti-inflammatory activities by inhibiting the productions of nitric oxide [304].
- 4) **Antiuro lithic activity:** It is used for the management of various disorders related to the urinary system including urolithiasis [305-307].
- 5) **Immunomodulatory activity:** Saponins that are isolated from the fruits of *Gokshura* increase phagocytosis which indicates the stimulation of non-specific immune response.
- 6) **Antidiabetic activity:** It shows inhibitory activity against α -glucosidase and postprandial increase in blood glucose which leads to the improvement in insulin-dependent diabetes symptoms [308-310].
- 7) **Protective activity in neuronal cells:** The experimental studies have shown that due to anti-inflammatory and antioxidant effects it able to produce protective effects against neuron injury [296, 311, 312]. The apoptosis of retinal ganglion cells acts as a precursor for glaucoma.

Goksura able to block the optic nerve injury and enhance the survival of the optic nerve to protect the optic nerve [296].

- 8) **Cardiac disorders:** It can play a role in dilating the coronary artery and improving coronary circulation. It was recommended for the treatment of angina pectoris, antihypertensive effect, and dietary intake can able to lower down serum lipid [292, 313-315].
- 9) **Hepatoprotective activity:** The hepatoprotective activity may be due to the antioxidant activity, the influence on metabolism regulation, and the repression of apoptosis of liver cells, which effectively reduces the level of Caspase-3 in liver tissue [316].
- 10) **Anti-cancerous activity:** It has cytotoxic activity. The anti-tumor activity of the drug is well reported with its anti-cancerous effect [317-320].
- 11) **Antimicrobial activity:** Antimicrobial activities depend upon the origin of the plant and the part of the plant used. *Yemeni* plant did not show antibacterial activity whereas the methanolic/ethanolic extracts of different parts i.e. fruits, roots and stems with leaves of *Iranian, Indian, or Turkish* able to inhibit the growth of microorganisms [292, 295, 321-323].
- 12) **Antihelminthic activity:**
Tribulosin and sitosterol glycosides present in 50% methanolic extracts of *tribulus terrestris* were reported to possess antihelminthic properties [324].

2.12.7 Dose:

- Moola (root)/ phala (fruit) *churna*: 3-6 g/day [297]
- Moola (root)/ phala (fruit) *kwath*: 50-100 ml/day or 20-30 g drug for *kwath* preparation [290]

CHAPTER 3

OBJECTIVES/SCOPE OF THE STUDY

3.1 RATIONALE

Urolithiasis is a common disorder and now it is a global challenge. This disorder is because of our life style when stone formation in the kidney takes place in gradual manner. The sequence of events in the forming urinary stone can be saturation of urine followed by supersaturation. Further, it causes the development of nucleation which leads to the growth of crystals and their aggregation. These available crystals in urine persist for long time and become stone. Finally, it leads to kidney failure or nephrectomy in the patients. In modern science, there are a number of medicines indicated in the *Mutrashmari* (Kidney stone), but they are associated with several side effects. Since the traditional system of medicine have several alternatives to modern medicines, one very common example for urolithiasis is the use of *Surya kshar*, *Sphatika*, *Apamarg kshar*, *Punarnava*, and *Gokshur* aqueous extract which has been used since a long time in the *Ayurvedic* system of medicine for the effective management of urolithiasis. After extensive literature search from various databases. It was observed that a single drug cannot solve all associated problems of urolithiasis. Even till date no combination and the synergistic study were reported for the above-mentioned drugs. Hence it was thought worthwhile to work on the combination of *Surya kshar*, *Sphatika*, *Apamarg kshar*, *Punarnava*, and *Gokshur* aqueous extract for the treatment of urolithiasis and also to develop a more palatable quality-controlled dosage form with more patient compliance.

The present project aimed to develop and evaluate of antiurolithic effect of herbomineral formulation in the rat (Male).

3.2 AIM OF THE RESEARCH

Development and evaluation of antiurolithic herbomineral formulation

3.3 OBJECTIVES OF RESEARCH

1. Thorough literature review on the topic
2. Authentication and evaluation of raw materials
3. Development and evaluation of prepared tablets.
4. Stability studies of the developed formulation.
5. *In-vitro* evaluation
6. Safety and efficacy study of formulation through *in-vivo* study

CHAPTER 4

MATERIALS AND METHODS

4.1 PROCUREMENT AND AUTHENTICATION OF RAW MATERIALS

The *surya kshar*, *Spatika*, *Punarnava* (*Boerhaavia diffusa*), *Goksura* (*Tribulus terrestris*) was collected from Jalandhar market and authentication was carried out by Herbal Health Research Consortium Pvt. Ltd., Amritsar. *Apamarga* (*Achyranthus aspera*) was collected from the local market of Jalandhar and authentication was carried out by Guru Nanak Dev University, Amritsar. Cystone was procured from Himalaya Drug Company. Hydrochloric acid, Magnesium stearate, Sodium chloride, Sodium phosphate, Sodium citrate, Magnesium sulphate, Sodium sulphate, Potassium chloride, Calcium chloride, Sodium oxalate, Ammonium hydroxide, Ammonium chloride, Methanol HPLC grade, Acetonitrile HPLC grade, and Ethylene glycol was procured from Loba Chemie Pvt Ltd, Mumbai, India. Ethanol was procured from Changshu Yangyuan Chemicals, China. ERBA Uric acid DEC kit, ERBA calcium OPCP kit, ERBA creatine kit was collected from Transasia Bio-Medicals Ltd. And Coral Uria kit was collected from Coral Clinical Systems, Pantnagar, Uttarakhand.

List of instruments used for the study - Digital pH meter (Labtronics), Digital balance (Contach), Hot plate (Universal), Water bath (Rectangular), Hot air oven (Navyug), Muffle furnace (Navyug), UV spectrophotometer UV 1800 (Shimadzu), Humidity chamber (Remi), Bulk density apparatus (Navyug), Tablet Disintegration Tester DT 1000 (Labindia), Tablet Friability Tester FT 1020 (Labindia), Monsanto hardness tester (Monsanto), Electron microscope (MKOW), Mechanical stirrer (Remi), AAS (Shimadzu), HPTLC (CAMAG), HPLC (Shimadzu, model LC20AD).

4.2 STANDARDIZATION OF PROCURED RAW MATERIALS

4.2.1 PHYTOCHEMICAL SCREENING

Air-dried plant material extracts were prepared and analysed for the presence and absence of phytoconstituents which are responsible to produce therapeutic effects i.e. Tannins, Glycosides, Saponin, Protein, Carbohydrate, Alkaloids, Steroids, Phenols, and Flavonoids. [325, 326].

4.2.1.1 TESTS FOR TANNINS

4.2.1.1.1 Ferric Chloride Test: Drug extract when treated with FeCl_3 [ferric chloride] solution may develop with the intense green, purple, blue or black colour that confirms the presence of tannins [327, 328].

4.2.1.1.2 Lead Acetate Test: Drug extract when treated with few drops of 10% Pb (C₂H₃O₂)₂ [lead acetate]. The precipitate was formed, confirms the presence of tannins [327, 328].

4.2.1.1.3 Bromine water: Drug extract when treated with 10 ml of Br₂ [bromine] water. Decolouration of bromine water confirms the presence of tannins [327, 328].

4.2.1.2 TESTS FOR GLYCOSIDES

4.2.1.2.1 Borntrager's test: Boiled 200 mg of drug with 2 ml of H₂SO₄ [sulphuric acid] for 5 minutes. Afterwards filter it in hot condition. In cooled filtrate add equal volume of CHCl₃ [chloroform]. Separate the chloroform layer and mix with half of its volume with dilute NH₃ [ammonia]. A rose-pink to red colour was developed in the ammoniacal layer confirms the presence of glycoside [327, 328].

4.2.1.2.2 Liebermann's Test: Two ml of CH₃COOH [acetic acid] was added in CHCl₃ [chloroform] and mixed with 2 ml of drug extract. The lower layer of CHCl₃ [chloroform] was separated and shaken. The mixture was then cooled and added a few drops of concentrated H₂SO₄ [sulphuric acid]. The green colour showed the presence of glycoside [327, 328].

4.2.1.2.3 Keller-Kiliani Test: Four ml of CH₃COOH [glacial acetic acid] and 1 drop of 2 % FeCl₃ [Ferric chloride] mixture was mixed with 10 ml of aqueous plant extract and 1 ml concentrated H₂SO₄ [sulphuric acid]. A brown ring formed between the layers which confirms the presence of glycoside [327, 328].

4.2.1.2.4 Salkowski's Test: Two ml concentrated H₂SO₄ [sulphuric acid] was added to the drug extract. A reddish-brown colour formed which confirms the presence of glycoside [327, 328].

4.2.1.3 TEST FOR SAPONIN

4.2.1.3.1 Froth/Foam test: A pinch of the dried powder plant was added to 2-3 ml of distilled water. The mixture was shaken vigorously. The froth was mixed with few drops of olive oil and mixed vigorously. The appearance of foam confirms the presence of saponins [327, 328].

4.2.1.4 TESTS FOR PROTEIN

4.2.1.4.1 Millon's test: Millon's reagent (2 ml) was added to the drug extract. A white precipitate appeared, which turned red upon gentle heating indicating the presence of amino acids [327, 328].

4.2.1.4.2 Biuret test: Biuret reagent (2 ml) was added to drug extract (2 ml). The appearance of violet colour indicated the presence of amino acids [327, 328].

4.2.1.4.3 Ninhydrin test: Amino acid, when boiled with few drops of Ninhydrin solution (5 %). The appearance of violet colour confirms the presence of amino acids.

4.2.1.5 TESTS FOR CARBOHYDRATES

4.2.1.5.1 Benedict's solution test: Benedict's reagent (2 ml) and drug extract (1 ml) mix and heated in boiling water (3 minutes). A colour change from yellowish to bright yellow or bright orange indicated the presence of carbohydrates [327, 328].

4.2.1.5.2 Fehling's test: Fehling's A (10 ml) and Fehling's B (10 ml) reagents were mixed and boiled with drug extract (2 ml). A brick-red precipitate of cuprous oxide indicated the presence of carbohydrates [327, 328].

4.2.1.5.3 Molisch's test: One ml of drug extract was treated with few drops of alcoholic α -naphthol. Concentrated H_2SO_4 [sulphuric acid] was added slowly through the sides of the test tube, purple to violet colour ring appeared at the junction indicated the presence of carbohydrates [327, 328].

4.2.1.6 TESTS FOR ALKALOIDS

4.2.1.6.1 Mayer's test: Mayer's reagent was added to the drug extract. The formation of cream coloured precipitates indicated the presence of alkaloids [327, 328]

4.2.1.6.2 Dragendorff's test: Dragendorff's reagent was added to the drug extract. The formation of reddish-brown precipitate indicated the presence of alkaloids[327, 328].

4.2.1.6.3 Wagner's test: Wagner's reagent was added to drug extract. The formation of reddish-brown precipitate indicated the presence of alkaloids [327, 328].

4.2.1.6.4 Hager's test: Hager's reagent was added to drug extract. The formation of yellow precipitate indicated the presence of alkaloids [327, 328].

4.2.1.7 TESTS FOR STEROIDS

4.2.1.7.1 Libermann-Burchard test: -Two ml of drug extract was treated with few drops of acetic anhydride [$C_4H_6O_3$], boiled, and cool. Then added concentrated sulphuric acid [H_2SO_4] from the sides of the test tube. A brown ring was formed at the junction of two layers and the upper layer turned green which showed the presence of steroids [327, 328].

4.2.1.7.2 Salkowski test: - Two ml drug extract was treated with few drops of concentrated H_2SO_4 [sulphuric acid]. The red colour at the lower layer indicates the presence of steroids [327, 328].

4.2.1.8 TESTS FOR PHENOLS

4.2.1.8.1 Ferric Chloride test: To the drug extract added a few drops of neutral 5 % $FeCl_3$ [ferric chloride] solution. A dark green colour indicated the presence of phenolic compounds [327, 328].

4.2.1.8.2 Liebermann's nitroso reaction: The sample was treated with sodium nitrite [NaNO₂] and concentrated H₂SO₄ [sulphuric acid]. Deep green or blue colour which changed to red on dilution with water indicated the presence of phenolic compounds [327, 328].

4.2.1.8.3 Lead Acetate test: To the test solution, added a few drops of 10% Pb(C₂H₃O₂)₂ [lead acetate]. The formation of white precipitate indicated the presence of phenolic compounds [327, 328].

4.2.1.8.4 Gelatin test: To the test solution, added a few drops of 10% gelatin solution. White precipitates indicated the presence of phenolic compounds [327, 328].

4.2.1.9 TESTS FOR FLAVONOIDS

4.2.1.9.1 Alkaline reagent test: To the drug extract, added a few drops of NaOH [sodium hydroxide] solution. The intense yellow colour was formed which turned to colourless with the addition of a few drops of dilute acid indicated the presence of flavonoids [327, 328].

4.2.1.9.2 Zinc hydrochloride test: To the drug extract, added a mixture of zinc dust and concentrated HCl [hydrochloric acid]. It gave red colour after few minutes indicating the presence of flavonoids [327, 328]

4.2.2 PHYSICOCHEMICAL STANDARDIZATION

4.2.2.1 DETERMINATION OF FOREIGN MATTER (FM)

100 g. of the sample drug was measured with the help of digital balance and then it is spread out in a thin layer. The FM was detected by inspection with the unaided eye and using lens (6X). Weighed the FM and the percentage of FM was calculated concerning the air-dried sample [327-330].

$$\text{Percentage of FM} = \frac{\text{Weight of foreign matter}}{\text{Weight of sample}} \times 100 \% \text{ w/w}$$

4.2.2.2 DETERMINATION OF LOSS ON DRYING (LOD)

Take 5-10 g of powdered drugs in Petri plate and kept it in hot air oven at 105 °C for 5 hours. Afterward kept it in a desiccator for cooling and calculate the weight loss again it was kept in a hot air oven for drying until the variation between 2 progressive observations is less than 0.25 percent [327-330].

$$\text{Percentage of LOD} = \frac{\text{Loss on drying}}{\text{Weight of sample}} \times 100 \% \text{ w/w}$$

4.2.2.3 DETERMINATION OF TOTAL ASH (TA)

Two g accurately weighed sample were incinerated in silica crucible at 450°C until it was carbon-free. Weigh the obtained ash after cooling and calculate the percentage of ash concerning the air-dried sample [327-330].

$$\text{Percentage of TA} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100\% \text{ w/w}$$

4.2.2.4 DETERMINATION OF ACID INSOLUBLE ASH (AIA)

Acquired ash by the above method was boiled with dilute hydrochloric acid (25 ml) for 5 minutes. During the filtration process with the help of ashless filter paper, it was washed with hot water to make it chlorine-free. Collect the insoluble matter and incinerated it in silica crucible to obtain constant weight afterward calculate the percentage of AIA [327-330].

$$\text{Percentage of AIA} = \frac{\text{Weight of AIA}}{\text{Weight of sample}} \times 100\% \text{ w/w}$$

4.2.2.5 DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVE (ASE)

Take coarsely powdered drugs (5 g) and alcohol (100 ml) in a closed conical flask. Now shake it frequently within 6 hrs afterward kept undisturbed for 18 hrs. Filter it, and take 25 ml of filtrate in evaporating dish and kept it for drying. Calculate the percentages of WSE concerning air-dried sample [327-330]

$$\text{Percentage of ASE} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample} \times 25} \times 100\% \text{ w/w}$$

4.2.2.6 DETERMINATION OF WATER SOLUBLE EXTRACTIVE (WSE)

Take coarsely powdered drugs (5 g) and water (50 ml) in a closed conical flask. Now shake it frequently within 6 hrs afterward kept undisturbed for 18 hrs. Filter it, and take 25 ml of filtrate in evaporating dish and kept it for drying. Calculate the percentages of WSE concerning air-dried sample [327-330].

$$\text{Percentage of WSE} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample} \times 25} \times 100\% \text{ w/w}$$

4.2.2.7 DETERMINATION OF pH

The pH denotes the concentration of hydrogen ions in a solution. Before each measurement it is necessary to calibrate the pH meter, the calibration of the pH meter should be done with buffer solution i.e pH 4, pH 7, and pH 9.2 [329, 330]

4.2.2.8 HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

4.2.2.8.1 FOR *GOKSHUR* AQUEOUS EXTRACT AND PREPARED FORMULATION WITH STANDARD

4.2.2.8.1.1 Preparation of Standard Solution: Accurately weigh 10 mg of standard Diosgenin into 10 mL volumetric flask dissolve in Chloroform and make up the volume up to 10 mL with Chloroform. Use the Standard solution thus obtained for HPTLC fingerprinting

4.2.2.8.1.2 Preparation of Test Solution: Accurately weigh 2 g of sample in a conical flask. Reflux for 2 hours with 50 mL of 5 % Methanolic Hydrochloric acid. Allow to cool, add 25 mL of water, and filter with the help of Whatman filter paper No. 1. Transfer the filtrate to separating funnel and partition with 50 mL Chloroform. Collect the Chloroform layer, filter with anhydrous Sodium sulphate and Whatman filter paper No.1. Then, concentrate the filtrate in an evaporating dish on water bath. Thereafter, make up the volume up to 25 mL with Chloroform. Use the Test solution thus obtained for HPTLC fingerprinting.

4.2.2.8.1.3 Chromatographic Conditions

Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Band Length	8 mm
Standard Application Volume	10.0 μ L
Sample Application Volume	20.0 μ L
Distance Between Tracks	15 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene : Ethyl acetate (8 : 1 v/v)
Visualization	@ 254 nm
Quantification Wavelength	@ 200 nm

4.2.2.8.2 FOR PUNARNAVA AQUEOUS EXTRACT AND PREPARED FORMULATION WITH STANDARD

4.2.2.8.2.1 Preparation of Standard Solution: Accurately weigh 10 mg of standard Ursolic acid into 10 mL volumetric flask, dissolve in Methanol and make up the volume up to 10 mL with Methanol. Use the Standard solution thus obtained for HPTLC fingerprinting.

4.2.2.8.2.2 Preparation of Test Solution: Accurately weigh 2 g of sample in a conical flask. Reflux for 30 minutes with 25 mL of Methanol consecutively for 4 times. Filter with the help of Whatman filter paper No. 1 and concentrate the combined extracts in an evaporating dish on water bath. Thereafter, make up the volume up to 25 mL with Methanol. Use the Test solution thus obtained for HPTLC fingerprinting.

4.2.2.8.2.3 Chromatographic Conditions

Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Band Length	8 mm
Standard Application Volume	5.0 μ L
Sample Application Volume	20.0 μ L
Distance Between Tracks	15 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene : Ethyl acetate : Formic acid (7 : 3 : 0.3 v/v)
Visualization	@ 530 nm
Quantification Wavelength	@ 530 nm

4.2.2.9 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

4.2.2.9.1 FOR GOKSHUR IN PREPARED FORMULATION

4.2.2.9.1.1 Experimental conditions and requirements

Instrument	Liquid chromatography (Shimadzu, model LC20AD)
Injector	Rheodyne
Column	Phenomenex
Detector	PDA

Wavelength	203nm
Injection volume	20 μ l
Flow rate	0.5ml/min

Chemicals and reagents

Methanol HPLC grade, Acetonitrile HPLC grade, Millipore water (0.5 mv conductance filtered through Millipore water purification system).

Samples for analysis

Methanolic extract of prepared formulation, Standard Diosgenin

Preparation Sample

The methanolic extract was prepared, concentrated, and dried under reduced pressure. The extract was preserved at 2–4 °C. This extract was used for further investigation.

Preparation of Standard Diosgenin

Standard Diosgenin sample of 1mg/ml was prepared in HPLC grade Methanol

Preparation of Mobile Phase

The mobile phase was prepared with Acetonitrile and Water in the ratio of 90:10 v/v. The mobile phase was filtered by Millipore filtration assembly using nylon membrane filter paper of 0.45 mm diameter. The solvent mixture was degassed and sonicated in an ultrasonic bath for 10min [331, 332].

4.2.2.9.2 FOR PUNARAVA IN PREPARED FORMULATION

Experimental conditions and requirements

Instrument	Liquid chromatography (Shimadzu, model LC-20AD)
Injector	Rheodyne
Column	Phenomenex
Detector	PDA
Wavelength	280nm
Injection volume	20 μ l
Flow rate	0.5ml/min

Chemicals and reagents

Methanol HPLC grade, Acetonitrile HPLC grade, and Millipore water (0.5 mv conductance filtered through Millipore water purification system)

Samples for analysis

Methanolic extract of prepared formulation, Standard Boerhavenone B

Preparation Sample (MeOH Extract)

The methanolic extract was prepared, concentrated, and dried. The extract was preserved at 2–4 °C. This extract was used for further investigation.

Preparation of Standard Beorvenone-B

Standard Beorvenone sample of 1mg/ml was prepared in HPLC grade Methanol.

Preparation of Mobile Phase

The mobile phase was prepared with Acetonitrile and Water in the ratio of 70:30 v/v. The mobile phase was filtered by Millipore filtration assembly using nylon membrane filter paper of 0.45 mm diameter. The solvent mixture was degassed and sonicated in an ultrasonic bath for 10min [331, 332].

4.2.2.10 FTIR ANALYSIS

FT-IR was recorded on Shimazu-made instrument using KBr disc method. IR radiations were passed through a compressed disc to record down the molecular fingerprint (spectrum) of the sample. Potassium bromide (KBr) is the commonest alkali halide used in the pellets. 0.1 to 1.0 % sample is well mixed with potassium bromide (200 to 250 mg) powder. The mixture was now kept into a pellet-forming die and compressed to form a transparent pellet. Degassing is performed to eliminate air and moisture from the KBr powder. Before forming the KBr powder into pellets, pulverize it to 200 mesh max. and then dry at approximately 110 °C for two to three hours [333].

4.3 PREPARATION OF PLANT EXTRACT

4.3.1 GOKSHUR AQUEOUS EXTRACT

Gokshur fruits were collected from the local market of Jalandhar, Punjab, and authentication was done from HHRC, Amritsar. After removal of foreign matter, the plant material cleaned with the help of water and dried properly after that coarsely powdered drug was boiled with 4 parts of water and reduced up to 1/4th of the actual water. Now the contents were filtered through a double-folded muslin cloth. The obtained filtrate was subjected to mild to moderate heat with continuous stirring till it becomes semisolid mass. Then the obtained semisolid contents were subjected to indirect heat and to overcome the chances of burning. After complete removal of a watery portion, the obtained solid mass was kept for further study [82].

4.3.2 PUNARNAVA AQUEOUS EXTRACT

Punarnava roots were collected from the local market of Jalandhar, Punjab, and authentication was done from HHRC, Amritsar. After the removal of foreign matter, the plant material was cleaned with the help of water and dried. After that coarsely powdered drug was boiled with

4 parts of water and reduced up to 1/4th of the actual water. Now the contents were filtered through a double-folded muslin cloth. The obtained filtrate was subjected to mild to moderate heat with continuous stirring till it becomes semisolid mass. Then obtained semisolid contents were subjected to indirect heat to overcome the chances of burning. After complete removal of a watery portion, the obtained solid mass was kept for further study [82].

4.4 PURIFICATION OF RAW MATERIAL (MINERAL ORIGIN)

4.4.1 SURYA KSHAR (SHODHANA)

Surya kshar was collected from the local market of Jalandhar, Punjab, and authentication was done from HHRC, Amritsar. After authentication *Surya kshar* was purified by giving three *bhavanas* of *ela toya* (1:4). By this process, the potency of *Surya kshar* was enhanced as *ela* also having *Mutrala* (diuretic) property [161].

4.4.1 SPHATIKA (SHODHANA)

Sphatika was collected from the local market of Jalandhar, Punjab, and authentication was done from HHRC, Amritsar. Afterward, powdered *Sphatika* were taken in an iron pan/*sharava*. Then it was subjected to *Nirjalikarana* process [161, 334].

4.5 PREPARATION OF APAMARG KSHAR

Apamarga panchanga was collected from the local market of Jalandhar Punjab and authenticated was done from GNDU, Amritsar. After the shade drying of the collected plant material, the foreign matter was removed. The dried *Panchanga* were incinerated to obtain white colour ash and after *swangsheetikaran* (self-cooling) prepared ash was collected. Then water was added into the collected ash in the ratio of (1:6). The contents were properly mashed and kept undisturbed for six hours. Afterward, the clear and supernatant liquid layers were collected and filtered. The residual ash was again mashed by adding freshwater into it and kept undisturbed for the next six hours, followed by a collection of the 2nd and 3rd illustrations as *Ksharajala* was collected. All the collected illustrations were mixed properly and subjected to *Mandagni* to evaporate the watery portion to obtain *Apamarg Kshara*. By following this method three batches of *Apamarg Kshara* were prepared [113].

4.6 PRE-FORMULATION STUDIES

4.6.1 BULK DENSITY (BD)

It is the ratio in-between untapped mass of powder to its bulk volume. The BD is expressed in grams/milliliter although the international unit is kilogram/cubic meter because the measurements are made by using a measuring cylinder. 100 g of the powdered drug was pass through a sieve with apertures size were not greater than 1 mm. Take 250 ml (readable to 2 ml)

measuring cylinder and add 100 g of the test sample. Now gently level the powdered drug without compacting and note down the unsettled volume (V₀) to the nearest graduated unit. The equation for calculate BD (ρ_b) = m/ V_b, where, ρ_b = Bulk density, m = Mass of powder, v_b = Bulk Volume [327, 328, 335, 336].

4.6.2 TAPPED DENSITY (TD)

It is the ratio in-between powder mass to the occupied volume obtained after tapping (100 taps) for a defined period of time in the measuring cylinder. The equation for calculate TD(ρ_t) = m/ V_t, where, ρ_t = Tapped density, m = Mass of powder, v_t = Tapped volume [327, 328, 335, 336].

4.6.3 CARR'S (COMPRESSIBILITY) INDEX (CI)

CI is the capability of the powder dosage form to decrease in volume under pressure. It is indirectly linked with the relative flow rate, distribution of particles, and cohesiveness of the powder. TD (ρ_t) and BD (ρ_b) of the powdered material were used to determine the compressibility of a powder. The equation for calculate CI (%) = ($\rho_t - \rho_b$)/ ρ_t *100, Where, ρ_b = Bulk density, ρ_t = Tapped density [327, 328, 335, 336]

4.6.4 ANGLE OF REPOSE

It is the maximum possible angle in-between the pile surface and horizontal plane of powder. It is determined by the fixed funnel method. Take precisely weighed powder in the funnel but the funnel tip was blocked initially with the help of thumb. Afterward powder blend was permitted to flow freely through funnel tip on a plain surface. The angle of repose is determined by $\theta = \tan^{-1} (h/r)$, Where, θ = Max. the angle between the pile of powder and horizontal plane, h = Height of pile of powder, r = Radius of the base of the conical pile [327, 328, 335, 336]

4.6.5 HAUSNER'S RATIO (HR)

It is the ratio of TD to BD. It is a measure of the compressibility of powder. TD (ρ_t) and BD (ρ_b) of powder material were used to determine Hausner's Ratio [327, 328, 335, 336]

4.7 SCREENING OF TABLET DOSAGE FORM AND OPTIMIZATION OF FORMULATION

4.7.1 SCREENING OF TABLET DOSAGE FORM

Six different combinations were tried for the selection of excipients for the tablet dosage form.

4.7.2 OPTIMIZATION OF FORMULATION BY BOX BEHNKEN DESIGN

Response surface methodology (RSM) investigates the relationship between a number of control variables and one or more response variables. However, the study design requires selecting the required mix of variables and the degree of each factor to be evaluated. Since experimental tests cost time and resources, it is appropriate to reduce the amount of runs

without losing the desired objectives. In general, the design of experiments (DOE) applies to the design of any knowledge collection exercise where variance is present. The Box–Behnken experimental design is a software statistical design expert software-12, which was used to test the impact from the chosen separate variables on the variables to refine the formulation protocol for tablets.

Three formulation factors were found to have significant effects on the flowability, compressibility of granules prepared by wet granulation technique and hence the characters of the compressed tablets. These factors are the percent of Gum acacia (X1), the percent of gildant (X2) and the size of granules (X3) was used in the present study. Preliminary studies also provided a set of the levels for each formulation variable (Table 1). The selected responses were a mean friability (Y1), a disintegration time (Y2), The responses studied and the constraints selected are presented in Table 2.

In this study, the impact of selected separate variables on reactions, to improve flowability and compression, to define the mechanism of the drug release, and to simplify the process were evaluated in a 3³ Box Behnken experimental configuration. This design is perfect for exploring quadratic reaction surfaces and creating polynomial models of second order, which helps to refine the mechanism over a limited number of experimental runs. For the 3³ Box-Behnken experimental design, a total of 17 experimental runs, shown in Table 5.8, are needed. The experimental architecture of Box–Behnken is orthogonal. Therefore, in lower, medium, and high settings the factor levels are equally distributed and coded to 1, 0, and +1 [337-339]. The method was optimized to achieve levels of X1, X2, and X3, which provide optimal values of Y1& Y2, under constraints after generation of the polynomial equations for dependent and independent variables. A new formula has been prepared to validate these values according to the expected X1, X2, and X3 stages. The results obtained (Y1 & Y2) were estimated and compared to the calculated values.

4.8 QUALITY CONTROL TESTS FOR PREPARED TABLET

4.8.1 TABLET APPEARANCE

Appearance is one of the first quality parameters for the acceptance of a tablet. General elegance and its identity play a major role in consumer satisfaction. Acceptance of the appearance of batches of the tablet has been done based on the following factors such as size, colour, shape, odour, taste etc. [329, 330, 336, 340-342].

4.8.2 TABLET DIMENSIONS (Diameter and thickness)

Determined by thickness. The size and shape of tablets play a unique role in patient compliance. Smallest the size of tablets, easy to administer. To determine the thickness of a tablet, a device

known as Micrometer/ Vernier calipers/ Screw gauge can be used to measure the dimensions of tablets [329, 330, 336, 340-342].

4.8.3 WEIGHT VARIATION

According to IP, 20 tablets from each batch should be selected randomly and the weight of the individual tablet was taken and determined their average weight. Any variation in the weight of individual tablets was noted. The acceptable limit of percentage deviation in weight did not exceed the limit of $\pm 10\%$ (for tabs weight 80 mg or < 80 mg), $\pm 7.5\%$ (For tabs >80 mg and < 250 mg) and $\pm 5\%$ (250 mg or >250 mg therefore, they are considered to pass the test [329, 330, 336, 340-342].

4.8.4 HARDNESS TEST

The capability of a tablet to stand against applied load/pressure is known as hardness. It is also known as crushing strength. Randomly take 5-10 tablets from the prepared batch and hardness should be determined by crushing the tablet by hardness tester (Monsanto) and then find out the average and standard deviation. The value of hardness was expressed in kg/cm^2 [329, 330, 336, 340-342].

4.8.5 FRIABILITY

Roche friabilator is used to test the physical strength of tablets when they are subjected to mechanical shock or attrition. The apparatus consists of a transparent synthetic polymer drum which has polished internal surface (diameter of 283-291 mm and a depth of 36-40 mm). The drum has two sides out of which one is removable. A curved projection with an inner radius (75.5-85.5 mm) which extending towards the outer wall of the drum facilitating the movement of tablets inside the drum. The central ring has an outer diameter of 24.5-25.5 mm. The drum is rotating at 25 ± 1 rpm for 4 minutes and tablets are falling at the height of 6'' in each revolution [329, 330, 336, 340-342].

4.8.6 DISINTEGRATION TEST

One tablet is placed in each of the 6 tubes of the basket and operates the apparatus, utilizing water kept up at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ as the immersion fluid unless generally determined in the individual monograph. Toward the end of as far as possible indicated in the monograph lift the basket from the fluid and observe the tablets, every one of the tablets having broken down totally. The disintegration time of uncoated tablets is considered 30 minutes [329, 330, 336, 340-342].

4.9 STABILITY STUDY

The stability studies were carried out for selected tablet batches at Refrigerated condition, Humidity Chamber ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}/ 75\% \text{ R.H.} \pm 5\% \text{ R.H.}$, in stability chamber - Remi electro

technique, Mumbai, India), Room temp. (open area) and Room temp. (close area) for 180 days respectively. The aged samples were analysed and compared with results obtained from freshly prepared tablets. Freshly prepared tablets were considered as time zero and used as a standard to evaluate various parameters of stability studies.

4.10 HEAVY METALS DETERMINATION

Atomic absorption spectrophotometer was used in the determination of heavy metal elements i.e. Lead, Mercury, Arsenic, and Cadmium in *Apamarg kshar*, *surya kshar*, *spkatika*, *gokshur*, *punarnava*, and prepared tablets were evaluated by M/S Ashrivad Pharmaceutical, Varanasi, Uttar Pradesh in Atomic Absorbance Spectrometric (AAS) as it is a suitable method for observing the heavy metals in Ayurvedic pharmaceutical products and provides accurate and rapid determinations [329, 330].

4.11 MICROBIAL LOAD DETERMINATION

Microbial Load determination for *Apamarg kshar*, *surya kshar*, *spkatika*, *gokshur*, *punarnava*, and prepared tablets were done by M/S Ashrivad Pharmaceutical, Varanasi, Uttar Pradesh by adopting the standard method [329, 330]

4.12 IN-VITRO STUDY

4.12.1 PREPARATION OF ARTIFICIAL URINE IN LABORATORY

Artificial urine was composed as per the formula mentioned by Burns and Finlayson [343] with a minor amendment. The formula used for preparation of artificial urine as follows - sodium chloride 105.5mM, sodium phosphate 32.3mM, sodium citrate 3.21mM, magnesium sulphate 3.85mM, sodium sulphate 16.95mM, potassium chloride 63.7mM, calcium chloride 4.5mM, sodium oxalate 0.32mM, ammonium hydroxide 17.9mM and ammonium chloride 0.0028mM. The artificial urine was prepared fresh every time and the pH was adjusted at 6.

4.12.1.1 Study without inhibitor: - For blank reading take artificial urine (2 ml) and distilled water (1 ml) into the cuvette. Then add 0.01M sodium oxalate (1 ml) into previous contents and immediately take the reading.

4.12.1.2 Study with an inhibitor: - Prepare an aqueous extract of the drug and filter it with the help of a membrane filter and prepare different concentration i.e. 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 and 650 µg/ml. Take artificial urine (2 ml) and extract solution (1 ml) in the cuvette. Take blank reading and add 0.01 M Na oxalate (1 ml) and record the absorbance at 620 nm [225, 305, 327, 328, 344, 345].

$$\text{Percentage of inhibition} = [1 - (S_i / S_c)] \times 100$$

S_i = Slope of the graph in presence of inhibitor (extract)

S_c = Slope of the graph without inhibitor (control)

4.12.2 MICROSCOPIC STUDY: Under the 45X objective and 10X eyepiece observed the crystals of calcium oxalate (with and without inhibitors) [327, 328].

4.13 IN-VIVO STUDY

4.13.1 DRUGS AND CHEMICALS

Cystone, *Apamarg kshar*, *Spahitika*, *Surya kshar*, *Gokshur* and *punarnava* aqueous extract
Ethylene glycol, Ammonium chloride.

4.13.2 EXPERIMENTAL ANIMALS

Wistar male rats 7-8 weeks old, weighing 250–275 g were purchased from the National Institute of Pharmaceutical Education and Research (NIPER), Mohali, India. Rats were housed in polypropylene cages lined with husk in standard environmental conditions (temperature $25 \pm 2^\circ\text{C}$, relative humidity $55 \pm 10\%$, and 12:12 light: dark cycle). The animals were given a standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee - School of Pharmaceutical Sciences, Lovely Professional University (LPU/IAEC/2019/55).

4.13.3 STUDY DESIGN

All the 60 healthy animals were divided into 10 groups consisting of six animals in each group. The group of experimental animals was divided into, (I) Normal Control: Healthy rats maintained on regular rat food and distilled water *ad libitum*. [N] (II). Lithiatic Control: Control rats that received urolithiasis inducer for 28 days. They were kept on 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in distilled water for 3 days followed by only 0.75% v/v ethylene glycol for another 25 days, to accelerate lithiasis [LC]. (III) Lithiatic Control rats were treated with *Cystone* (750 mg/Kg) which is a standard positive control [LC+C]. (IV) Lithiatic Control rats treated with *Apamarg Kshar* (950 mg/Kg), orally in distilled water using gum acacia (0.5% w/v), from day 1-28 of calculi induction [LC+AK]. (V) Lithiatic Control rats treated with *Surya Kshar* (1850 mg/Kg), orally in distilled water using gum acacia (0.5% w/v), from day 1-28 of calculi induction [LC+SK], (VI). Lithiatic Control rats treated with *Sphatika* (1850 mg/Kg), orally in distilled water using gum acacia (0.5% w/v), from day 1-28 of calculi induction [LC+S], (VII). Lithiatic Control rats treated with *Gokshur* (aqueous extract) (5000 mg/Kg), orally in distilled water using gum acacia (0.5% w/v), from day 1-28 of calculi induction [LC+G], (VIII). Lithiatic Control rats treated with *Punarnava* (aqueous extract) (1000 mg/Kg), orally in distilled water using gum acacia (0.5% w/v), from day 1-28 of calculi induction [LC+P], (IX) Lithiatic Control rats treated with Prepared tablet formulation (F1, Low

dose) (300 mg/Kg), orally in distilled water using gum acacia (0.5% w/v), from day 1-28 of calculi induction [LC+LD], (X) Lithiatic Control rats treated with Prepared tablet formulation (F2, High dose) (600 mg/Kg), orally in distilled water using gum acacia (0.5% w/v), from day 1-28 of calculi induction [LC+HD]. The Ethylene Glycol orally was given after 2 h of drug administration. The study design is shown in Table 4.1

Table. 4.1 Study design for Anti-urolithic herbomineral drug evaluation

Groups (n=6)	Treatment	Dose (mg/kg, p.o.)
I	N	Normal food/water
II	LC	Vehicle*
III	LC + C (Std)	750
IV	LC + AK	950
V	LC + SK	1850
VI	LC + S	1850
VII	LC + G	5000
VIII	LC + P	1000
IX	LC + F1	300
X	LC + F2	600

N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); LD: Prepared tablet formulation (F1, Low dose) (300 mg/Kg), HD: Prepared tablet formulation (F2, High dose) (600 mg/Kg); C: Cystone (750 mg/Kg). *Contains 0.75% v/v ethylene glycol with 1% w/v ammonium chloride.

Larger animals have lower metabolic rates. The physiological process of larger animals is slower and larger animals required smaller drug doses on a weight basis. Smaller the animal has a higher rate of metabolism, so we can give a higher dose to smaller animal like rats in comparison of humans, for the same we can use allometric scaling which assists us to exchange doses in-between species during research, experiments, and clinical trials. For the conversion of human dose to animal dose, the conversion factor 7.4 was used for converting human dose to animal dose and use body surface area method for dose calculation.

4.13.4 EXPERIMENTAL INDUCTION OF UROLITHIASIS

Ethylene glycol and ammonium chloride induced hyperoxaluria model [62, 260, 346, 347] was used to induce urolithiasis. The composition used was 0.75% v/v ethylene glycol with 1% w/v ammonium chloride [348] in distilled water for 28 days. Healthy adult male rats of Wistar strain weighing 250-275 g were used in the study because the urinary system of male rats resembles that of humans and earlier studies have shown that the amount of stone deposition in female rats was significantly less in comparison to male rats [347].

4.13.5 COLLECTION AND ANALYSIS OF URINE

All animals were kept in individual cages and 24-hour urine samples were collected in a manually prepared metabolic cage on day 0,7,14,21 and 28 of calculi induction treatment [62, 349]. Urine calcium was determined by using ERBA Calcium OCPC Kit. Urine creatinine was determined by

using LIQUIXX Creatinine Kit (Jaffe's Method, Initial Rate). Phosphate and uric acid determination was done by using ERBA Uric acid DES Kit (Modified-Trinder Method, Endpoint).

4.13.6 URINE pH

Uric acid crystals were found to deposit most frequently in the concentrated acid urine. Thus, the nature of the urine was tested by using the pH meter [350, 351].

4.13.7 SERUM ANALYSIS

At the end of the treatment, blood was collected from the retro-orbital puncture and the animal was sacrificed by cervical dislocation under anaesthesia. The serum was separated by centrifugation method at 10,000g for 10 min. The obtained sample was analyzed for creatinine and uric acid with the help of a creatinine kit and uric acid kit. [350, 351].

4.13.8 KIDNEY HOMOGENATE ANALYSIS

At the end of the treatment, the animals were sacrificed by using the cervical dislocation method, and both the kidneys were isolated from each animal after the opening of the abdomen. Isolated kidneys were cleaned properly and rinsed in ice-cold physiological saline. Now the left kidney was weighed and then minced in a beaker. 20% of homogenate was prepared in Tris-HCl buffer solution (0.02 mol/l, pH 7.4). Total kidney homogenate was used for assaying tissue calcium and oxalate [352].

4.13.9 BIOCHEMICAL ESTIMATIONS

4.13.9.1 Estimation of TBARS level

An 0.2 ml of the supernatant material obtained from the homogenate which was prepared in phosphate buffer was pipetted out in a test tube. Add 0.2 ml of 8.1% sodium dodecyl sulphate and 1.5 ml of 30% acetic acid of pH 3.5. Now in the last add 1.5 ml of thiobarbituric acid and make volume up to 4 ml by using distilled water. The test tubes holding the sample were now incubated for the time of 1 hour at 95 °C. Then it was cooled and add 1 ml of distilled water followed by the addition of 5 ml of n-butanol-pyridine mixture (15:1v/v). The tubes containing sample were centrifuged at 4000g for 10 min. The absorbance of the developed pink colour was measured by using UV spectrophotometer at 532 nm. A standard calibration curve was plotted by using 1-10nM 1, 1, 3, 3-tetra methoxy propane. The observed TBARS values were expressed in nanomoles per mg of protein [353].

4.13.9.2 Estimation of Glutathione level

In the liver homogenate, reduced glutathione (GSH) determination was performed as per the methodology prescribed by Ellman [354] The methodology based on the reduction of 5,5'-dithio-bis- (2, nitrobenzoic acid) also known as Ellman's reagent by GSH groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of GSH. The nitro-mercaptobenzoic acid has an intense

yellow color and is measured by using a UV spectrophotometer. 0.5 ml of 10% trichloroacetic acid, 6 ml of disodium ethylene diamine tetraacetic acid, and 0.5 ml of homogenate were taken together and shake gently for the duration of 10-15 minutes. After that, the centrifugation was done at 2,000 rpm for 5 minutes. Take 0.2 ml of the supernatant liquid layer and mix it with 1.7 ml of 0.1M potassium phosphate buffer solution by adjusting the pH 8. Now 0.1 ml of Ellman's reagent will be added to each tube and after 5 minutes the optical density of the sample was determined with the help of UV spectrophotometer at 412 nm against a reagent blank. The observed values are expressed as $\mu\text{mol/g}$ tissue [355].

4.13.9.3 Estimation of Catalase level

The gastric tissue was scraped off and isolated tissue was then homogenized in ice-cold normal saline medium. Then the mixture was centrifuged for 10 mins at 3,000 rpm and the supernatant layer was collected for further estimation. Now take 100 μl of the supernatant layer and add it to a solution containing 3 ml of H_2O_2 -phosphate buffer mixture (50 mM phosphate buffer, pH 7.0 and 30 % H_2O_2). The change in optical density at 240 nm/unit time was taken for the measurement of catalase activity [356, 357].

4.13.10 MITOCHONDRIAL ESTIMATIONS

The mitochondrial enzyme complex activities were performed as per the methodology prescribed by Berman and Hastings [358]. Both the kidneys were homogenized in an isolated buffer and then the homogenates were centrifuged at 13,000g for 5 min at 4 $^{\circ}\text{C}$. Pellets were re-suspended in isolated buffer solution with ethylene glycol tetra-acetic acid and again centrifuged the mixture at 13,000g at 4 $^{\circ}\text{C}$ for 5 min. The obtained supernatants layer was transferred into new tubes and topped off with buffer EGTA and again centrifuged it at 13,000g at 4 $^{\circ}\text{C}$ for 10 min. Now finally pellets containing pure mitochondria were re-suspended in an isolation buffer without EGTA and observation was recorded [358].

4.13.10.1 Complex-I (NADH dehydrogenase activity) estimation

Complex-I was determined by using UV spectrophotometer as per the methodology mentioned by King and Howard [359]. According to the prescribed method, the catalytic oxidation of NADH to NAD^+ with the subsequent reduction in cytochrome C was observed. The reaction mixture was contained 0.2 M glycyl glycine buffer pH 8.5, 6 mM NADH in 2 mM glycyl glycine buffer, and 10.5 mM cytochrome C. The reaction was started after the addition of a required amount of solubilized mitochondrial sample. Finally, determine the changes in absorbance by using UV spectrophotometer at 550 nm for 2 min [359].

4.13.10.2 Complex-II (Succinate dehydrogenase activity) estimation

Complex-II was determined by using UV spectrophotometer as per the methodology mentioned by King and Howard [359]. According to the prescribed method the oxidation of succinate was done by an artificial electron acceptor and potassium ferricyanide. The sample mixture contained 0.2 M phosphate buffer pH 7.8, 1% BSA, 0.6 M succinic acid, and 0.03 M potassium ferricyanide. The reaction was started after the addition of the mitochondrial sample. Finally determine the changes in absorbance by using UV spectrophotometer at 420 nm for 2 min [359, 360].

4.13.10.3 Complex-III (MTT activity) estimation

The MTT assay relies on the reduction of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide also known as MTT. The hydrogenase activity in functionally intact mitochondria and the reduction rate of MTT was used to estimate the activity of the mitochondrial respiratory chain in isolated mitochondria [361, 362]. Take 100 μ l mitochondrial samples and incubated them with 10 μ l MTT for 3 hours at 37 $^{\circ}$ C. The blue formazan crystals were solubilized with di-methyl sulphoxide and determine by using ELISA reader at 580 nm filter [361, 362].

4.13.11 MOLECULAR ESTIMATIONS

Caspase-3 and TNF- α ELISA

The quantitative measurement of Caspase-3 and TNF- α were done by Chemikine and R&D Systems immunoassay kit respectively. The absorbance was recorded on an ELISA plate reader (iMarkTM Microplate absorbance reader, BIO-RAD) and the determination of the concentration of each sample was calculated by plotting with measured absorbance values on the standard curve with the known concentrations.

Caspase-3 colorimetric assay – It is also known as CPP-32 is an intracellular cysteine protease that exists as a proenzyme. Those are activated during the cascade of events associated with apoptosis. The tissue lysates/homogenates were then tested for protease activities with the addition of a caspase specific peptide that is conjugated to the color reporter molecule p-nitroaniline (pNA). The cleavage of the peptide by the caspase releases the chromophore pNA. The quantitative estimation was performed with the help of UV spectrophotometer at 405 nm. The quantitative presence of caspase enzymatic activities in the cell lysate/homogenate is directly proportional to the color reaction. The enzymatic reaction for caspase activity was carried out by using R&D systems caspase-3 colorimetric kit.

4.13.12 HISTOPATHOLOGICAL STUDIES

The isolated right kidney of the rat was fixed in 10% neutral buffered formalin. Now it was processed in a series of graded alcohol and xylene, embedded in paraffin wax and it was sectioned at 5 μ m. Afterward the sample was stained with H and E (Hematoxylin and Eosin) for

histopathological determinations. Tissue slices were photographed by using optical microscopy under polarized light to study the light microscopic architecture of the kidney [363].

4.14 STATISTICAL ANALYSIS

All the experimental data are expressed as mean \pm SEM, respectively. Statistical analysis of obtained data was carried out by one-way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism version 7.0 (GraphPad Software Inc., CA, USA). A value of $P < 0.05$ indicated a significant difference in the obtained results.

CHAPTER 5

RESULT AND DISCUSSION

5.1 PROCUREMENT AND AUTHENTICATION OF RAW MATERIALS

The *surya kshar*, *Spatika*, *Punarnava* (*Boerhaavia diffusa*), *Goksura* (*Tribulus terrestris*) were collected from the local market of Jalandhar, and authentication was carried out by Herbal Health Research Consortium Pvt. Ltd., Amritsar. *Apamarga* (*Achyranthus aspera*) was collected from the local market of Jalandhar and authentication was carried out by Guru Nanak Dev University, Amritsar.

5.2 STANDARDIZATION OF PROCURED RAW MATERIALS

5.2.1 Phytochemical screening of plant origin raw materials

The preliminary phytochemical study of *Apamarg panchanga* (*A. aspera*) apparent the existence of tannins, glycoside, saponin, protein, alkaloids, steroids, phenols, and flavonoids. The preliminary phytochemical study of *Gokshur* (*T. terrestris*) fruit apparent the existence of tannins, glycoside, saponin, alkaloids, phenols, and flavonoids. Similarly; The preliminary phytochemical study of *Punarnava* (*B. diffusa*) root apparent the existence of tannins, saponin, proteins, carbohydrates, alkaloids, steroids, phenols, and flavonoids. The observation was tabulated in Table no. 5.1

Table No. 5.1 Preliminary phytochemical characterization (Qualitative test)

Sr. No.	Qualitative Test	Phytochemical constituents	Observation		
			<i>Apamarg panchang</i>	<i>Gokshur fruit</i>	<i>Punarnava root</i>
1	Ferric chloride test	Tannins	+	+	+
	Lead acetate test		+	+	+
	Bromine water		+	+	+
2	Borntrager's test	Glycoside	+	+	-
	Liebermann's Test		+	+	-
	Keller-Kiliani Test		+	+	-
	Salkowski's Test		+	+	-
3	Foam test	Saponin	+	+	+
4	Millon's test	Protein	+	-	+
	Biuret Reagent test		+	-	+
	Ninhydrin Test		+	-	+
5	Benedict's solution test	Carbohydrates	-	-	+
	Fehling's test		-	-	+
	Molisch's test		-	-	+
6	Mayer's reagent	Alkaloids	+	+	+
	Dragendroff reagent		+	+	+
	Wagner's reagent		+	+	+
	Hager's reagent		+	+	+
7	Liebermann Burchard's reaction	Steroids	+	-	+
	Salkowski test		+	-	+
8	Ferric Chloride Test	Phenols	+	+	+
	Liebermann's nitroso reaction		+	+	+

9	Lead Acetate test	Flavonoids	+	+	+
	Gelatin test		+	+	+
	Alkaline reagent test		+	+	+
	Zinc hydrochloride test		+	+	+

*Present = +, Absent = -

5.2.2 PHYSICOCHEMICAL STANDARDIZATION OF PROCURED RAW MATERIALS

Physicochemical analysis of plant material was done and the observation was compared with the stranded value mentioned by The *Ayurvedic* Pharmacopeia of India. All the plant samples are compliances with the standard value prescribed in the monograph. Three experimental trials were performed for determining the mean value and standard deviation. The observation was tabulated in Table no. 5.2

Table No. 5.2 Physicochemical Test (Herbal Plant)

Parameters	<i>Apamarg Panchang</i>	Standard value as per API (Vol - II)	<i>Gokshur fruit</i>	Standard value as per API (Vol - I)	<i>Punarnava root</i>	Standard value as per API (Vol -IX)
FM % W/W	1 ±0.15	NMT 2 %	0.53 ±0.03	NMT 1 %	2.1 ±0.31	NMT 2 %
TA % W/W	15.2 ±0.20	NMT 17 %	12.3 ±0.21	NMT 15 %	10.3 ±1.20	NMT 15 %
AIA % W/W	3.6 ±0.25	NMT 5 %	1.2 ±0.15	NMT 2 %	2.2 ±0.26	NMT 3 %
ASE % W/W	8.3 ±0.26	NLT 2 %	15.8 ±0.21	NLT 6 %	7.2 ±0.70	NLT 5 %
WSE % W/W	25.8 ±0.15	NLT 12 %	22.8 ±0.26	NLT 10 %	15.2 ±0.045	NLT 8 %
All values are expressed as mean (±) n=3						

5.3 PREPARATION OF PLANT EXTRACT

5.3.1 GOKSHUR AQUEOUS EXTRACT

Gokshur fruits were collected from the local market of Jalandhar, Punjab, and authentication was done from HHRC, Amritsar. Observations were tabulated in Table no. 5.3 which was obtained during the preparation of aqueous extract. The 19.34 % W/W yield was obtained.

Table No. 5.3 Observation and results obtained during the preparation of *gokshur* aqueous extract

Sr.No.	Observation	Result-B I	Result - B II	Result - B III	Total
1	Weight of <i>gokshur</i>	500 g	500 g	500 g	1500 g
2	Water	2000 ml	2000 ml	2000 ml	6000 ml
3	Quantity of <i>gokshur</i> extract obtained after <i>ghankriya</i>	96.5 g	98.4 g	95.2 g	290.1 g ± 1.6

5.3.2 PUNARNAVA AQUEOUS EXTRACT

Punarnava roots were collected from the local market of Jalandhar, Punjab, and authentication was done from HHRC, Amritsar. Observations were tabulated in Table no. 5.4 which was obtained during the preparation of aqueous extract. The 12.37 % W/W yield was obtained.

Table No. 5.4 Observation and results obtained during the preparation of *punarnava* aqueous extract

S.No.	Observation	Result - B I	Result - B II	Result - B III	Total
1	Weight of <i>punarnava</i>	500 g	500 g	500 g	1500 g

2	Water	2000 ml	2000 ml	2000 ml	6000 ml
3	Quantity of <i>punarnava</i> extract obtained after <i>ghankriya</i>	62.8 g	60.3 g	62.5 g	185.6 g ± 1.4

5.3.3 STANDARDIZATION OF PREPARED AQUEOUS EXTRACT

Physicochemical analysis for aqueous extract of *Gokshur* fruit and *Punarnava* root raw was performed in triplicate. No significant variations were observed. As no standard values for the same are mentioned in any official compendium so that the observed values were taken as stranded. Three experimental trials were performed for determining the mean value and standard deviation. The observation was tabulated in Table no. 5.5

Table No. 5.5 Quality Control Test for prepared aqueous extract

Parameters	<i>Gokshur fruit</i>	<i>Punarnava root</i>
LOD % W/W	3.50 ±0.08	3.9 ±0.12
TA % W/W	6.33 ±0.12	7.3 ±0.16
AIA % W/W	0.70 ±0.16	1.2 ±0.12
WSE % W/W	99.17 ±0.25	99.2 ±0.12
pH (10 % solution)	6.27 ±0.17	6.4 ±0.12
Total soluble solids %	99.20 ±0.29	99.3 ±0.16
Bulk density	0.562 ±0.11	0.763 ±0.15
Tap density	0.794 ±0.15	1.087 ±0.13
Residual solvent	Nil	Nil
All values are expressed as mean (±) n=3		

5.3.4 PHYTOCHEMICAL SCREENING OF PREPARED AQUEOUS EXTRACT

Aqueous extract of *Gokshur* fruit and *Punarnava* root was studied for preliminary secondary phytochemicals and it's showed that the samples contain the same phytoconstituents which contains by the original air-dried samples. The observation was tabulated in Table no. 5.6

Table No. 5.6 Preliminary phytochemical characterization of prepared plant extract

Sr. No.	Qualitative Test	Phytochemical constituents	Observation	
			<i>Gokshur fruit aqueous extract</i>	<i>Punarnava root aqueous extract</i>
1	Ferric chloride test	Tannins	+	+
	Lead acetate test		+	+
	Bromine water		+	+
2	Borntrager's test	Glycoside	+	-
	Liebermann's Test		+	-
	Keller-Kiliani Test		+	-
	Salkowski's Test		+	-
3	Foam test	Saponin	+	-
4	Millon's test	Protein	-	+
	Biuret Reagent test		-	+
	Ninhydrin Test		-	+
5	Benedict's solution test	Carbohydrates	-	+
	Fehling's test		-	+
	Molisch's test		-	+
6	Mayer's reagent	Alkaloids	+	+
	Dragendroff reagent		+	+
	Wagner's reagent		+	+
	Hager's reagent		+	+

7	Liebermann Burchard's reaction	Steroids	-	+
	Salkowski test		-	+
8	Ferric Chloride Test	Phenols	+	+
	Liebermann's nitroso reaction		+	+
	Lead Acetate test		+	+
	Gelatin test		+	+
9	Alkaline reagent test	Flavonoids	+	+
	Zinc hydrochloride test		+	+

* Present = +, Absent = -

5.4 PURIFICATION OF RAW MATERIAL (MINERAL ORIGIN)

5.4.1 SURYA KSHAR (SHODHANA)

Surya kshar was collected from the local market of Jalandhar, Punjab, and authentication was done from HHRC, Amritsar. After authentication *Surya kshar* was purified by giving three *bhavanas* of *ela toya* (1:4). By this process, the potency of *Surya kshar* was enhanced as *ela* also having *Mutrala* (diuretic) property. The observation was tabulated in Table no. 5.7. The 95.63 % W/W yield was obtained.

Table No. 5.7 Observation and results obtained during purification of *surya kshar*

S.No.	Observation	Result B I	Result B II	Result B III	Total
1	Weight of <i>Surya kshar</i>	100 g	100 g	100 g	300 g
2	Ela	25 g	25 g	25 g	75 g
3	Water	100 ml	100 ml	100 ml	300 ml
4	Quantity of <i>elajala</i> obtained	95 ml	94.2 ml	95.5 ml	284.7 ml
5	Quantity of <i>surya kshar</i> obtained	96 g	95.2 g	95.7 g	286.9 g ± 0.4

5.4.2 SPHATIKA (SHODHANA)

Sphatika was collected from the local market of Jalandhar, Punjab, and authentication was done from HHRC, Amritsar. Afterward, powdered *Sphatika* were taken in an iron pan/*sharava*. Then it was subjected to *Nirjalikarana* process. The observation was tabulated in Table no. 5.8. The 62.76 % W/W yield was obtained.

Table No. 5.8 Observation and results obtained during purification of *sphatika*

S.No.	Observation	Result B I	Result B II	Result B III	Total
1	Weight of <i>sphatika</i>	150 g	150 g	150 g	450 g
2	Quantity of <i>sphatika</i> obtained after nirjalikaran	94.5 g	94.2 g	93.7 g	282.4 g ± 0.4

5.4.3 PHYSICOCHEMICAL STANDARDIZATION OF MINERAL ORIGIN DRUGS

Physicochemical analysis for mineral raw material was performed before and after the purification of the drug in triplicate. No significant variations were observed. As no standard values for the same are mentioned in any official compendium so that the observed values were taken as stranded. Three experimental trials were performed for determining the mean value and standard deviation. The observation was tabulated in Table no. 5.9

Table No. 5.9 Physicochemical Test (Minerals)

Parameters	<i>Surya Kshar</i> (Before purification)	<i>Surya Kshar</i> (After purification)	<i>Sphatika</i> (Before purification)	<i>Sphatika</i> (After purification)
FM % W/W	Nil	Nil	Nil	Nil
LOD % W/W	1.8 ±0.38	2.1 ±0.20	2.3 ±0.31	2.6 ±0.15
TA % W/W	73.4 ±0.47	69.2 ±0.38	42.3 ±0.20	41.3 ±0.76
AIA % W/W	43.8 ±0.38	39.8 ± 0.21	18.2 ±0.21	17.8 ±0.35
ASE % W/W	48.2 ±0.15	49.8 ± 0.93	45.8 ±0.93	48.8 ±0.81
WSE % W/W	95.6 ±0.26	94.2 ±0.75	98.2 ±1.10	95.2 ±1.27
All values are expressed as mean (±) n=3				

5.5 PREPARATION OF APAMARG KSHAR

Apamarga Panchanga was collected from the local market of Jalandhar Punjab and authenticated was done from GNDU, Amritsar. The *apamarg kshar* was prepared as per the methodology prescribed in *Rastarangini*. Observations were tabulated in Table No. 5.10 which was obtained during the preparation of *apamarg kshar*. The 2.80 % W/W yield was obtained.

Table No. 5.10 Observation and results obtained during the preparation of *apamarg kshar*

S.No.	Observation	Results		
		Batch I	Batch II	Batch III
1	Weight of dried <i>Apamarga panchag</i>	15 kg	15 kg	15 kg
2	Weight of ash obtain	2.5 kg	2.3 kg	2.2 kg
3	<i>Ksharjal</i> collected (1 st illustration)	8 lit.	8 lit.	8 lit.
4	<i>Ksharjal</i> collected (2 nd illustration)	8 lit.	8 lit.	8 lit.
5	<i>Ksharjal</i> collected (3 rd illustration)	8 lit.	8 lit.	8 lit.
6	Total <i>Ksharjal</i> collected	24 lit.	24 lit.	24 lit.
7	Total <i>Apamarga kshar</i> obtained (after <i>ghankriya</i>)	0.435 kg	0.418 kg	0.405 kg
Grand Total		1.258 kg ± 0.02		

5.5.1 PHYSICOCHEMICAL STANDARDIZATION OF APAMARG KSHAR

Physicochemical analysis of prepared *apamarg kshar* was done and the observation was compared with stranded value mentioned of API, Part II, Vol -I. *Apamarg kshar* are compliances with the standard value prescribed in the monograph. Three experimental trials were performed for determining the mean value and standard deviation. The observation was tabulated in Table no. 5.11

Table No. 5.11 Physicochemical Test (*Apamarg Kshar*)

Parameters	Mean value	Standard value as per API part II Vol. I
LOD % W/W	2.37 ±0.12	NMT 4 %
TA % W/W	94.80 ±0.36	-
AIA % W/W	0.52 ±0.04	NMT 1 %
WSE % W/W	95.45 ±0.07	-
pH (10% aqueous solution)	10.4 ±0.09	10 to 11
Sodium (% W/W)	6.29 ±0.03	NLT 4 %
Potassium (% W/W)	34.58 ±0.14	NLT 29 %

5.6 PRE-FORMULATION STUDIES (SINGLE DRUG AND MIXTURE)

Apamarga Kshar, *Sphatika*, *Gokshura* aqueous extract, *Punarnava* aqueous extract, *Surya kshar*, and Mixture of powder flow properties were studied as it is an important parameter of precompression analysis. Flow properties of all the extracts showing good results are tabulated below. The observation was tabulated in Table no. 5.12

Table No. 5.12 Powder flow properties

S.No	Parameters	AK	S	GE	PE	SK	Mixture
1	Bulk density (g/cm ³)	0.676 ±0.14	0.310 ±0.12	0.562 ±0.11	0.763 ±0.15	1.370 ±0.19	0.42±0.05
2	Tapped density (g/cm ³)	1.250 ±0.12	0.408 ±0.10	0.794 ±0.15	1.087 ±0.13	2.273 ±0.11	0.57±0.08
3	Compressibility index	45.9 ±0.24	24.0 ±0.13	29.2 ±0.11	29.8 ±0.17	39.7 ±0.16	26.32 ±0.26
4	Hausner ratio	1.85 ±0.14	1.32 ±0.12	1.41 ±0.11	1.42 ±0.13	1.66 ±0.17	1.36±0.12
5	Angle of repose	38.65 ±0.24	36.12 ±0.27	41.34 ±0.22	39.79 ±0.19	45 ±0.26	28.32±1.37

*AK–*Apamarg Kshar*, S–*Sphatika*, GE–*Gokshur* aqueous extract, PE–*Punarnava* aqueous extract, SK–*Surya kshar*. Weight of sample = 10 g

5.7 SCREENING OF TABLET DOSAGE FORM AND OPTIMIZATION OF FORMULATION

5.7.1 SCREENING OF TABLET DOSAGE FORM

Six different combination were tried for the selection of excipient for the tablet dosage form and the following composition finalised and used for further study. i.e. *Sphatika* 50 mg, *Surya kshar* 50 mg, *Apamarg kshar* 50 mg, *Gokshur* (aqueous extract) 50 mg, *Punarnava* (aqueous extract) 50 mg, Acacia powder – 45 mg and Talc 5 mg. The observation was tabulated in Table no. 5.13

Table No. 5.13 Screening of Tablet dosage form (Excipient)

Sr. No.	Material name	Quantity	Sr. No.	Material name	Quantity
1	<i>Sphatika</i>	50 mg	1	<i>Sphatika</i>	50 mg
2	<i>Surya kshar</i>	50 mg	2	<i>Surya kshar</i>	50 mg
3	<i>Apamarg kshar</i>	50 mg	3	<i>Apamarg kshar</i>	50 mg
4	<i>Gokshur</i> (aqueous extract)	50 mg	4	<i>Gokshur</i> (aqueous extract)	50 mg
5	<i>Punarnava</i> (aqueous extract)	50 mg	5	<i>Punarnava</i> (aqueous extract)	50 mg
6	Acacia Gum	45 mg	6	Starch	45 mg
7	Talc	5 mg	7	Talc	5 mg
Sr. No.	Material name	Quantity	Sr. No.	Material name	Quantity
1	<i>Sphatika</i>	50 mg	1	<i>Sphatika</i>	50 mg
2	<i>Surya kshar</i>	50 mg	2	<i>Surya kshar</i>	50 mg
3	<i>Apamarg kshar</i>	50 mg	3	<i>Apamarg kshar</i>	50 mg
4	<i>Gokshur</i> (aqueous extract)	50 mg	4	<i>Gokshur</i> (aqueous extract)	50 mg
5	<i>Punarnava</i> (aqueous extract)	50 mg	5	<i>Punarnava</i> (aqueous extract)	50 mg

6	Lactose	45 mg	6	Guar Gum	45 mg
7	Talc	5 mg	7	Talc	5 mg
Sr. No.	Material name	Quantity	Sr. No.	Material name	Quantity
1	<i>Sphatika</i>	50 mg	1	<i>Sphatika</i>	50 mg
2	<i>Surya kshar</i>	50 mg	2	<i>Surya kshar</i>	50 mg
3	<i>Apamarg kshar</i>	50 mg	3	<i>Apamarg kshar</i>	50 mg
4	<i>Gokshur</i> (aqueous extract)	50 mg	4	<i>Gokshur</i> (aqueous extract)	50 mg
5	<i>Punarnava</i> (aqueous extract)	50 mg	5	<i>Punarnava</i> (aqueous extract)	50 mg
6	Manitol	45 mg	6	MCC	45 mg
7	Talc	5 mg	7	Talc	5 mg

5.7.2 PREPARATION OF TABLET DOSAGE FROM

All Seventeen formulations (Table no. 5.15) were prepared according to BBD. The basic experiment for the tablet was carried out by *surya kshar*, *apamarg kshar*, *sphatika*, *punarnava*, and *gokshur* aqueous extract. Tablets were prepared by the direct compression method. Drug, polymer, and other excipients were weighed according to proposed formulations generated by Box Behnken design, which is represented in Table no. 5.15 The amount of taken drug was fixed in amount but others were changed according to the generated formula by BBD. Drug and other excipients were blended properly and taken in the hopper and the die and punch were adjusted to get desired weight of the tablet according to the proposed formulation (Table no.5.14).

Table No. 5.14 Proposed formulations generated by Box Behnken design

Factor	Level		
	-1	0	+1
X1:- A: Gum Acacia	30	45	60
X2:- B: Glidant (Talc)	2.5	5	7.5
X3:- C: Size of granules	800	1000	1200

Y1:- Friability Y2:-DT

Table No. 5.15 Optimization of formulation by Box Behnken design

		Factor - 1	Factor - 2	Factor - 3	Response - 1	Response - 2
Std.	Run	X1:- A: Gum Acacia	X2:-B: Glidant (Talc)	X3:- C: Size of granules	Y1:- Friability	Y2:-DT
		mg	mg	micron	%	min
12	1	45	7.5	1200	0.54	20.56
15	2	45	5	1000	0.48	18.32
3	3	30	7.5	1000	0.92	11.2
16	4	45	5	1000	0.43	18.2
11	5	45	2.5	1200	0.52	17.3
17	6	45	5	1000	0.49	19.12
5	7	30	5	800	1.02	9.32
10	8	45	7.5	800	0.51	20.02

1	9	30	2.5	1000	1.01	9.47
7	10	30	5	1200	1.25	8.42
4	11	60	7.5	1000	0.16	43.16
13	12	45	5	1000	0.53	18.12
9	13	45	2.5	800	0.48	21.12
8	14	60	5	1200	0.11	40.38
14	15	45	5	1000	0.5	18.22
2	16	60	2.5	1000	0.1	44.08
6	17	60	5	800	0.12	46.12

Table No. 5.16 ANOVA for Quadratic model - **Response 1: Friability**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.80	9	0.1998	53.69	< 0.0001	significant
A-Gum Acacia	1.72	1	1.72	462.41	< 0.0001	
B-Glidant	0.0000	1	0.0000	0.0134	0.9110	
C-Size of granules	0.0105	1	0.0105	2.83	0.1367	
AB	0.0056	1	0.0056	1.51	0.2586	
AC	0.0144	1	0.0144	3.87	0.0898	
BC	0.0000	1	0.0000	0.0067	0.9370	
A ²	0.0319	1	0.0319	8.57	0.0221	
B ²	0.0027	1	0.0027	0.7359	0.4194	
C ²	0.0114	1	0.0114	3.06	0.1237	
Residual	0.0260	7	0.0037			
Lack of Fit	0.0207	3	0.0069	5.19	0.0727	not significant
Pure Error	0.0053	4	0.0013			
Cor Total	1.82	16				

Factor coding is **Coded**.

The Sum of squares is **Type III - Partial**

The **Model F-value** of 53.69 implies that chosen model for the study was significant. There are only 0.01% chances that an F-value is large that might be due to the noise.

P-values < 0.0500 indicate model conditions chosen are significant. In this case the A, A² are significant model conditions. Values > 0.1000 indicating that the model conditions were chosen are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), the model reduction might be helping to improve your model. The **Lack of Fit F-value** of 5.19 implies there is a 7.27% chance. That a **Lack of Fit F-value** this large that might be due to the noise. Lack of fit is bad - we want the model to fit. This relatively low probability (<10%) is troubling.

Final Equation in Terms of Coded Factors

$$\text{Friability} = 0.486 - 0.46375 * A + 0.0025 * B + 0.03625 * C + 0.0375 * AB - 0.06 * AC - 0.0025 * BC + 0.087 * A^2 - 0.0255 * B^2 + 0.052 * C^2$$

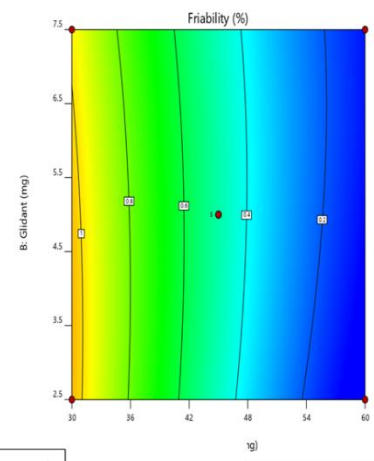
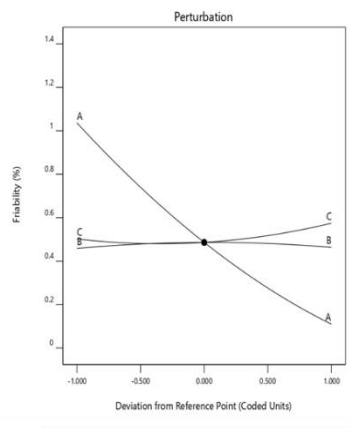
The precondition of coded factors can be utilized to predict the response level-wise mentioned for each of the factors. By default, the high levels of the factors were coded as +1 and the lower one was coded as -1. The coded conditions are useful for recognizing the relative impact of the factors by comparing the factor coefficients.

Table No. 5.17 Comparing the factor coefficients

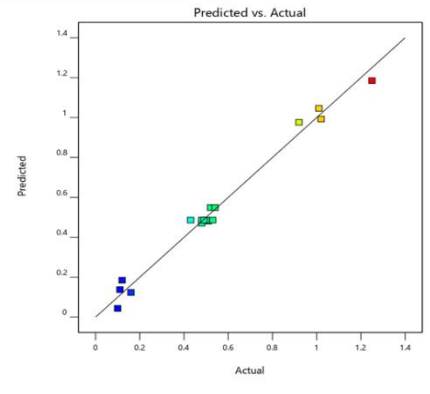
Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	0.5400	0.5487	-0.0087	0.750	-0.287	-0.267	0.025	-0.463	12
2	0.4800	0.4860	-0.0060	0.200	-0.110	-0.102	0.000	-0.051	15
3	0.9200	0.9763	-0.0563	0.750	-1.844	-2.382	1.020 ⁽¹⁾	-4.125 ⁽¹⁾	3
4	0.4300	0.4860	-0.0560	0.200	-1.026	-1.031	0.026	-0.516	16
5	0.5200	0.5487	-0.0287	0.750	-0.943	-0.934	0.267	-1.618	11
6	0.4900	0.4860	0.0040	0.200	0.073	0.068	0.000	0.034	17
7	1.02	0.9925	0.0275	0.750	0.902	0.888	0.244	1.538	5
8	0.5100	0.4812	0.0288	0.750	0.943	0.934	0.267	1.618	10
9	1.01	1.05	-0.0362	0.750	-1.189	-1.232	0.424	-2.133	1
10	1.25	1.18	0.0650	0.750	2.131	3.330	1.363 ⁽¹⁾	5.768 ⁽¹⁾	7
11	0.1600	0.1237	0.0363	0.750	1.189	1.232	0.424	2.133	4
12	0.5300	0.4860	0.0440	0.200	0.806	0.784	0.016	0.392	13
13	0.4800	0.4712	0.0088	0.750	0.287	0.267	0.025	0.463	9
14	0.1100	0.1375	-0.0275	0.750	-0.902	-0.888	0.244	-1.538	8
15	0.5000	0.4860	0.0140	0.200	0.257	0.239	0.002	0.119	14
16	0.1000	0.0438	0.0562	0.750	1.844	2.382	1.020 ⁽¹⁾	4.125 ⁽¹⁾	2
17	0.1200	0.1850	-0.0650	0.750	-2.131	-3.330	1.363 ⁽¹⁾	-5.768 ⁽¹⁾	6

Factor Coding Actual

Actual Factor
 A: Gum Acacia = 45
 B: Glidant = 5
 C: Size of granules = 1000



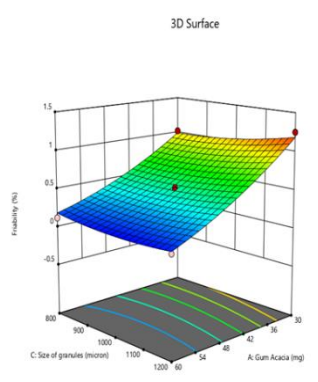
Friability
 Color points by value of
 Friability:
 0.1 1.25



Factor Coding Actual

Design Points:
 ● Above Surface
 ○ Below Surface
 0.1 1.25

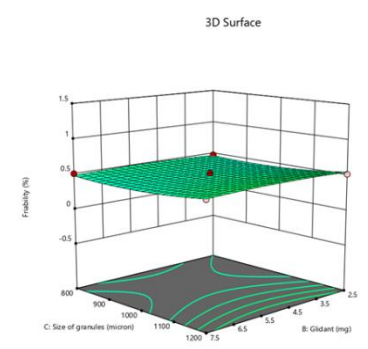
Actual Factor
 B: Glidant = 5



Factor Coding Actual

Design Points:
 ● Above Surface
 ○ Below Surface
 0.1 1.25

Actual Factor
 A: Gum Acacia = 45



Factor Coding: Actual

Design Points:
 ● Above Surface
 ○ Below Surface
 0.1 1.25

Actual Factor
 C: Size of granules = 1000

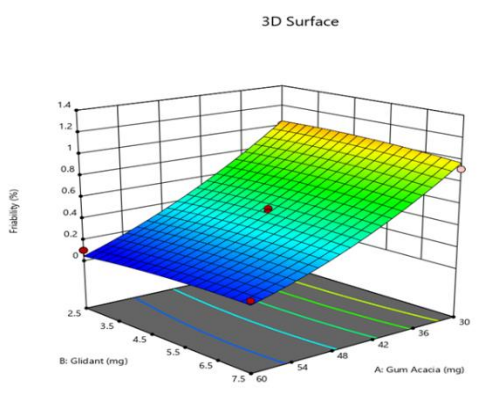


Fig. No. 5.1 Comparing the factor coefficients (Friability)

Table No. 5.18 ANOVA for Quadratic model **Response 1: DT**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2560.37	9	284.49	683.38	< 0.0001	significant
A-Gum Acacia	2289.28	1	2289.28	5499.21	< 0.0001	
B-Glidant	1.10	1	1.10	2.65	0.1477	
C-Size of granules	12.30	1	12.30	29.55	0.0010	
AB	1.76	1	1.76	4.22	0.0791	
AC	5.86	1	5.86	14.07	0.0072	
BC	4.75	1	4.75	11.42	0.0118	
A ²	233.43	1	233.43	560.73	< 0.0001	
B ²	5.43	1	5.43	13.05	0.0086	
C ²	0.2006	1	0.2006	0.4818	0.5100	
Residual	2.91	7	0.4163			
Lack of Fit	2.24	3	0.7462	4.42	0.0926	not significant
Pure Error	0.6755	4	0.1689			
Cor Total	2563.29	16				

The “Model F-value” of 683.38 refers that the model selected is significant. There is only a 0.01% chance that an F-value this large that might be due to the noise.

****P-values**** < 0.0500 indicate that the selected model conditions are significant. In this case, A, C, AC, BC, A², B² are significant model conditions. Values > 0.1000 indicate the model conditions are not significant.

The “Lack of Fit F-value” of 4.42 denotes that there is a 9.26% chance that a Lack of Fit F-value this large that might be due to the noise. Lack of fit is bad - we want the model to fit. This relatively low probability (<10%) is troubling.

Final Equation in Terms of Coded Factors

$$DT = 18.396 + 16.91625 A + 0.37125 B - 1.24C - 0.6625AB - 1.21AC + 1.09BC + 7.44575 A^2 + 1.13575B^2 + 0.21825C^2$$

The precondition of coded factors can be utilized to predict the response level-wise mentioned for each factor. By default, the high levels of the factors were coded as +1 and the lower one was coded as -1. The coded conditions are useful for recognizing the relative impact of the factors by comparing the factor coefficients.

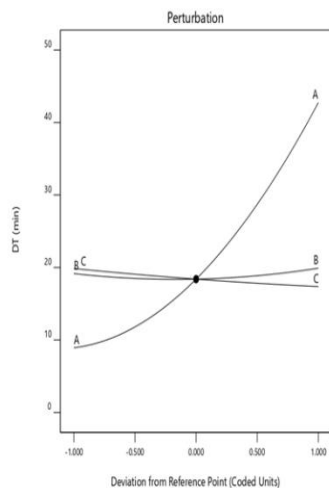
Table No. 5.19 Comparing the factor coefficients

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	20.56	19.97	0.5887	0.750	1.825	2.334	0.999	4.042 ⁽¹⁾	12
2	18.32	18.40	-0.0760	0.200	-0.132	-0.122	0.000	-0.061	15
3	11.20	11.10	0.1050	0.750	0.325	0.304	0.032	0.526	3

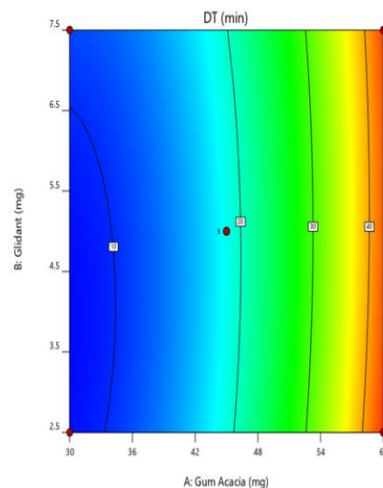
4	18.20	18.40	-0.1960	0.200	-0.340	-0.317	0.003	-0.159	16
5	17.30	17.05	0.2512	0.750	0.779	0.754	0.182	1.307	11
6	19.12	18.40	0.7240	0.200	1.255	1.319	0.039	0.660	17
7	9.32	9.17	0.1462	0.750	0.453	0.426	0.062	0.738	5
8	20.02	20.27	-0.2512	0.750	-0.779	-0.754	0.182	-1.307	10
9	9.47	9.03	0.4425	0.750	1.372	1.485	0.564	2.572 ⁽¹⁾	1
10	8.42	9.11	-0.6938	0.750	-2.150	-3.418	1.387 ⁽¹⁾	-5.920 ⁽¹⁾	7
11	43.16	43.60	-0.4425	0.750	-1.372	-1.485	0.564	-2.572 ⁽¹⁾	4
12	18.12	18.40	-0.2760	0.200	-0.478	-0.450	0.006	-0.225	13
13	21.12	21.71	-0.5887	0.750	-1.825	-2.334	0.999	-4.042 ⁽¹⁾	9
14	40.38	40.53	-0.1462	0.750	-0.453	-0.426	0.062	-0.738	8
15	18.22	18.40	-0.1760	0.200	-0.305	-0.284	0.002	-0.142	14
16	44.08	44.18	-0.1050	0.750	-0.325	-0.304	0.032	-0.526	2
17	46.12	45.43	0.6937	0.750	2.150	3.418	1.387 ⁽¹⁾	5.920 ⁽¹⁾	6

Factor Coding Actual

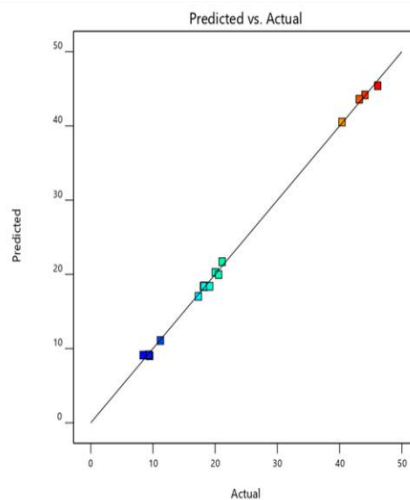
Actual Factors
 A: Gum Acacia = 45
 B: Gildant = 5
 C: Size of granules = 1000



Factor Coding Actual
 Design Points
 8.42 46.12
 X1 = A: Gum Acacia
 X2 = B: Gildant
 Actual Factor
 C: Size of granules = 1000



DT
 Color points by value of DT:
 8.42 46.12



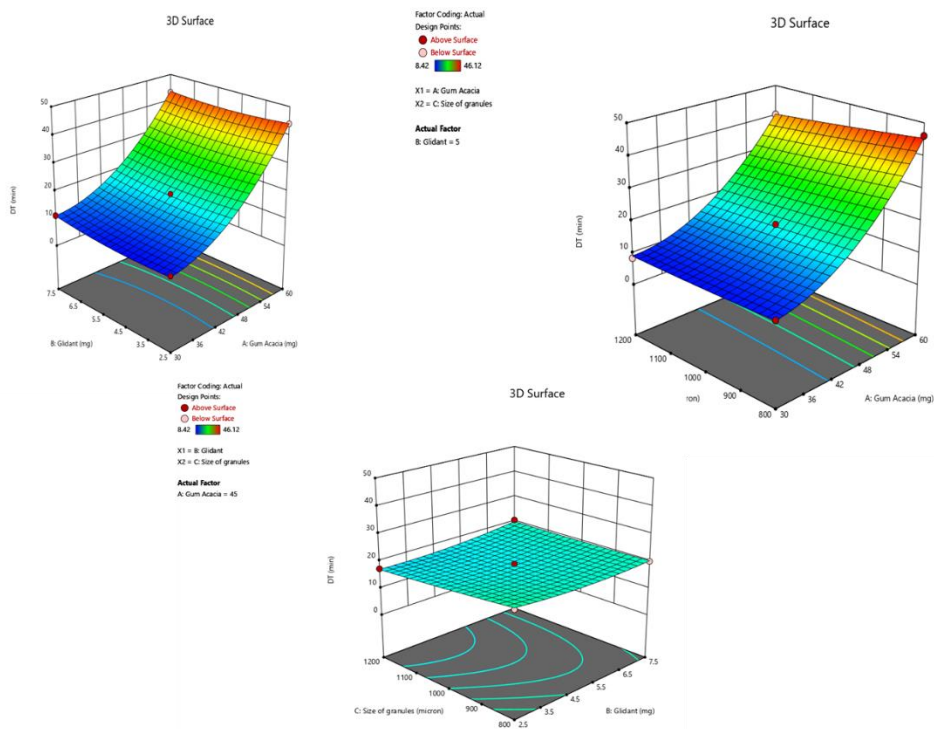


Fig. No. 5.2 Comparing the factor coefficients (Disintegration Time)

OPTIMIZATION OF TABLET FORMULA

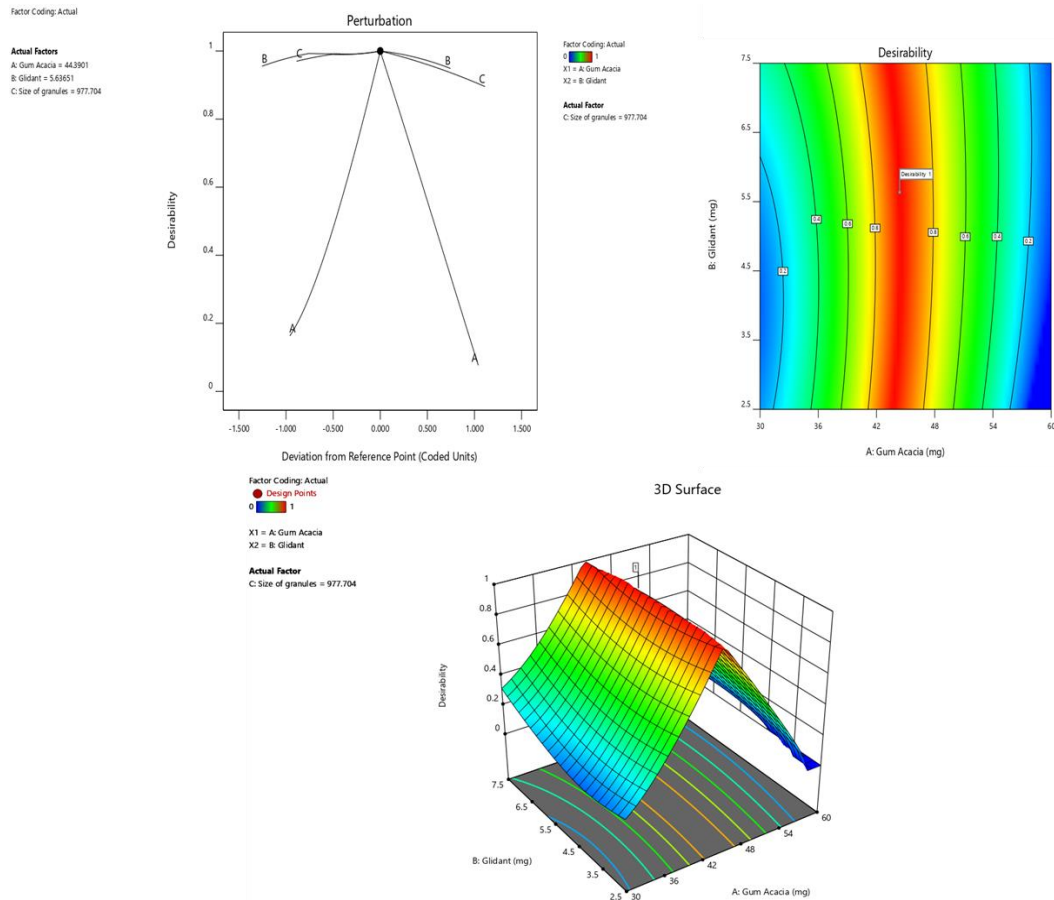


Fig. No. 5.3a Optimization of tablet formula

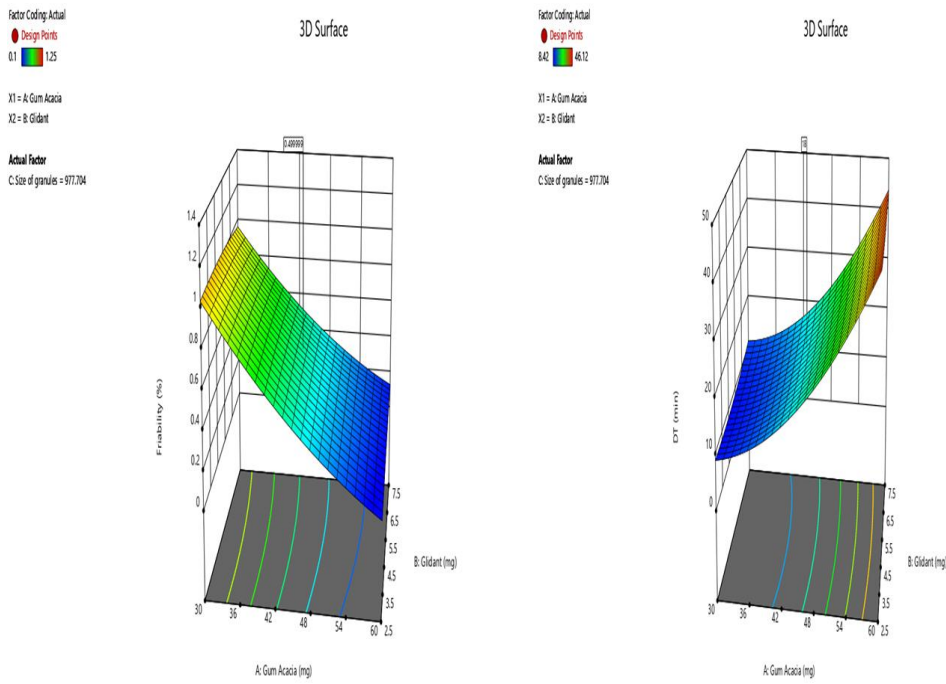


Fig. No. 5.3b Optimization of tablet formula

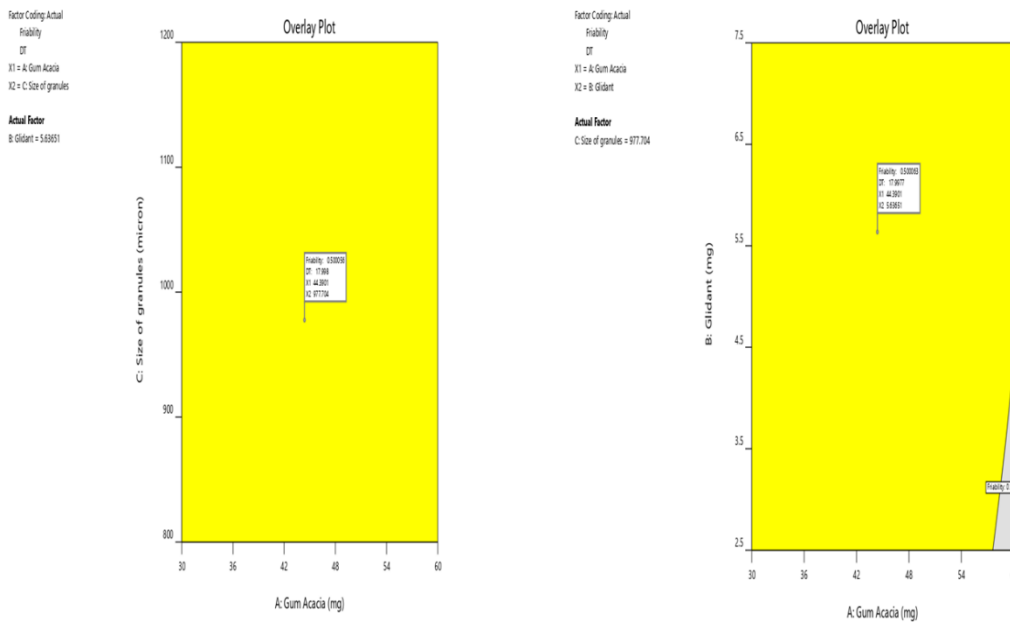


Fig. No. 5.3c Optimization of tablet formula

Table No. 5.20 DOE Solution Response

Solution 1 of Response 83	Predicted Mean	Predicted Median	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Friability	0.499999	0.499999	0.0609977	0.0269584	0.436252	0.563746	0.166557	0.833441

DT	18	18	0.645207	0.285155	17.3257	18.6743	14.473	21.527
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Table No. 5.21 DOE Intercept

	Intercept	A	B	C	AB	AC	BC	A ²	B ²	C ²
Friability	0.486	-0.46375	0.0025	0.03625	0.0375	-0.06	-0.0025	0.087	-0.0255	0.052
p-values		< 0.0001	0.9110	0.1367	0.2586	0.0898	0.9370	0.0221	0.4194	0.1237
DT	18.396	16.9162	0.37125	-1.24	-0.6625	-1.21	1.09	7.44575	1.13575	0.21825
p-values		< 0.0001	0.1477	0.0010	0.0791	0.0072	0.0118	< 0.0001	0.0086	0.5100

5.7.3 Optimized Master Formula

Three prime independent variables that have significant effects on the preparation of tablets have been revealed. These are major factors includes the percent of Gum acacia (X1), the percent of gildant (X2), and the size of granules (X3) Therefore, seventeen formulae of different combinations were prepared, by taking values of the major selective variables X1, X2 and X3 at different levels as shown in Table no. 5.15.

Response data for all 17 experimental runs of Box-Behnken experimental design (F1–F17), performed in accordance with Table no. 5.15 are presented in fig. no. 5.1 and 5.2 Regarding to different combinations of factors and factor levels, a considerable difference between flowability, compressibility, and characters of tablets were obtained.

5.7.3.1 Effect of friability (Y1)

The Y1 response of the design ranged from 0.1 to 1.25% in runs F1 and F17. It revealed that lower the percentage of acacia gum in the formulation (i.e. 30mg) with other concentrations of glidant and size of granules had failed the friability test of tablets. If the concentration of acacia gum is above 30 mg irrespective of other ingredients, all formulation had passed the Friability test. The observation was tabulated in Table no. 5.16 and presented in fig. no. 5.1

5.7.3.2 Effect of disintegration time (Y2),

The Y2 response ranged from 8.42 minutes to 46.12 minutes as shown in Table no. 5.18 and presented in fig. no. 5.2. It was found that X1 has the main effect on determining the disintegration time of the formulations. There was a direct relationship between X1 and Y2. As X1 increased from 30 to 60 mg, Y2 will be amplified from 8.42 minutes to 46.12 minutes. X3 has played role in DT values as more larges size granules the faster disintegration time. From these results, it is obvious that the increase of X1 obviously increases Y2, which may be due to the increase in binding effect as well as the tablet hardness and subsequently lengthening the disintegration time. It can be concluded that an optimized combination of independent factors ensured the desired flowability, compressibility, and DT of the prepared tablet dosage form. Below the optimized formulation given in Table no. 5.22.

Table No. 5.22 Optimized Master Formula

Sr. No.	Material name	Quantity
1	<i>Sphatika</i>	50 mg
2	<i>Surya kshar</i>	50 mg
3	<i>Apamarg kshar</i>	50 mg
4	<i>Gokshur</i> (aqueous extract)	50 mg
5	<i>Punarnava</i> (aqueous extract)	50 mg
6	Acacia powder	45 mg
7	Talc	5 mg

5.8 QUALITY CONTROL TESTS FOR PREPARED TABLETS**5.8.1 QUALITY CONTROL TESTS FOR TABLET (INDIVIDUAL DRUG)**

Disintegration Test was performed at 37.5°C for individual drugs and Water is used as a vehicle. There is no significant variation is observed indicates the improved and good tablet disintegration which will help to better dissolution also. Disintegration time was 3 ± 1 , 4 ± 1 , 8 ± 1 , 4 ± 1 and 6 ± 1 min. The average weight of the tablet was 300 mg, hardness of the tablet was 3 ± 1 , 5 ± 1 , 3 ± 1 , 4 ± 1 , and 7 ± 1 . Friability was 0.85 ± 0.08 , 0.90 ± 0.04 , 0.85 ± 0.02 , 0.83 ± 0.02 and 0.87 ± 0.03 respectively for *Apamarg Kshar*, *Sphatika*, *Gokshur* aqueous extract, *Punarnava* aqueous extract and *Surya kshar* as given in [Table 5.23](#)

Table No. 5.23 Analytical parameters for prepared Tablet (individual drug)

Sr.No	Parameters	AK	S	GE	PE	SK
1	Shape and appearance	Round	Round	Round	Round	Round
2	Diameter (mm)	6.07 ± 0.04	6.07 ± 0.03	6.07 ± 0.04	6.06 ± 0.07	6.06 ± 0.05
3	Thickness (mm)	1.13 ± 0.02	1.13 ± 0.03	1.12 ± 0.02	1.13 ± 0.02	1.13 ± 0.03
4	Hardness (kg/cm ²)	3 ± 1	5 ± 1	3 ± 1	4 ± 1	7 ± 1
5	Friability (% w/w)	0.85 ± 0.08	0.90 ± 0.04	0.85 ± 0.02	0.83 ± 0.02	0.87 ± 0.03
6	Weight variation test (% w/w)	1.8 ± 0.34	1.7 ± 0.32	1.8 ± 0.33	1.8 ± 0.33	1.6 ± 0.34
8	Disintegration time (minutes)	3 ± 1	4 ± 1	8 ± 1	4 ± 1	6 ± 1

*AK–*Apamarg Kshar*, S–*Sphatika*, GE–*Gokshur* aqueous extract, PE–*Punarnava* aqueous extract, SK–*Surya kshar*

5.8.2 QUALITY CONTROL TESTS FOR PREPARED TABLET (FORMULATION)

There is no significant variation is observed in shape, appearance, and diameter but hardness and disintegration time were increased which falls under the limit prescribed by CCRAS for the development of new formulation as Tablets. Disintegration Test was performed at 37.5°C for prepared formulation and Water is used as a vehicle. Friability decreased indicates the improved strength of the prepared tablets as tabulated in Table No. 5.24

Table No. 5.24 Analytical parameters for prepared Tablet

S. No.	Parameters	I	II	III	IV
1	Shape and appearance	Round	Round	Round	Round
2	Diameter (mm)	6.06 ± 0.04	6.07 ± 0.02	6.07 ± 0.04	6.06 ± 0.04
3	Thickness (mm)	1.12 ± 0.02	1.13 ± 0.02	1.12 ± 0.02	1.12 ± 0.02

4	Hardness (kg/cm ²)	8 ± 1	8 ± 1	8 ± 1	8 ± 1
5	Friability (%w/w)	0.49 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.49 ± 0.02
6	Weight variation test (%w/w)	1.8 ± 0.32	1.8 ± 0.32	1.8 ± 0.32	1.8 ± 0.32
7	Disintegration time (minutes)	18 ± 1	18 ± 1	18 ± 1	18 ± 1

5.8.2 PHYSICOCHEMICAL ANALYSIS OF PREPARED TABLET (FORMULATION)

Physicochemical analysis of prepared tablet (formulation) was done. Three experimental trials were performed for determining the mean value and standard deviation. There is no significant variation is observed in values which were tabulated in Table no. 5.25

Table No. 5.25 Physicochemical Test (Prepared Tablet)

Sr. No.	Parameters	Zero Day	After 3 months	After 6 months
1	FM % W/W	Nil	Nil	Nil
2	LOD % W/W	0.53 ± 0.05	0.50 ± 0.47	0.52 ± 0.03
3	TA % W/W	23.95 ± 0.44	23.38 ± 0.33	24.01 ± 0.42
4	AIA % W/W	10.53 ± 0.26	10.40 ± 0.13	10.60 ± 0.23
5	ASE % W/W	56.83 ± 0.35	57.10 ± 0.22	56.62 ± 0.45
6	WSE % W/W	97.06 ± 0.51	97.26 ± 0.32	97.34 ± 0.43
7	pH (5 %)	8.05 ± 0.04	8.07 ± 0.43	8.04 ± 0.03

5.9 STABILITY STUDY

Physicochemical parameters, post-compression parameters, and stability studies Q1A (R2) were performed for the prepared tablet as per ICH guidelines No significant changes were observed which are tabulated in Table no. 5.26 and 5.27.

Table No. 5.26 Stability study - Analytical parameters for prepared Tablet (After 3 Month)

Sr. No.	Parameters	I	II	III	IV
1.	Shape and appearance	Round	Round	Round	Round
2.	Diameter (mm)	6.06 ± 0.04	6.07 ± 0.02	6.07 ± 0.02	6.06 ± 0.04
3.	Thickness (mm)	1.12 ± 0.02	1.13 ± 0.02	1.12 ± 0.02	1.12 ± 0.02
4	Hardness (kg/cm ²)	6 ± 1	6 ± 1	7 ± 1	8 ± 1
5.	Friability (%w/w)	0.55 ± 0.14	0.57 ± 0.22	0.49 ± 0.12	0.50 ± 0.33
6.	Weight variation test (%w/w)	1.7 ± 0.31	1.9 ± 0.32	1.8 ± 0.32	1.8 ± 0.34
7.	Disintegration time (minutes)	17 ± 1	15 ± 1	16 ± 1	16 ± 1

Q1A (R2)

Batch I – Refrigerated condition, Batch II – Humidity Chamber, Batch III – Room temp. (open area), Batch IV – Room temp. (close area)

Table No. 5.27 Stability study - Analytical parameters for prepared Tablet (After 6 Month)

S. No.	Parameters	I	II	III	IV
1	Shape and appearance	Round	Round	Round	Round
2	Diameter (mm)	6.06 ± 0.04	6.07 ± 0.02	6.07 ± 0.02	6.06 ± 0.04
3	Thickness (mm)	1.12 ± 0.02	1.13 ± 0.02	1.12 ± 0.02	1.12 ± 0.02
4	Hardness (kg/cm ²)	7 ± 1	7 ± 1	8 ± 1	8 ± 1
5	Friability (%w/w)	0.50 ± 0.04	0.51 ± 0.02	0.51 ± 0.02	0.54 ± 0.03
6	Weight variation test (%w/w)	1.7 ± 0.31	1.9 ± 0.32	1.8 ± 0.32	1.8 ± 0.34
7	Disintegration time (minutes)	18 ± 1	15 ± 1	16 ± 1	16 ± 1

5.9.1 HPTLC STUDY FOR *GOKSHUR* AQUEOUS EXTRACT AND PREPARED FORMULATION WITH STANDARD

The HPTLC method was employed in the estimation of Diosgenin in MeOH Extract of *Gokshur* aqueous extract. The quantification of Diosgenin was carried out @ 200 nm; 0.074 % of yield was measured in *Gokshur* aqueous extract and 0.039 % of yield was measured in formulation mixture. Visualization were performed @ 254 nm and obtained R_f values are tabulated in Table no. 5.28. The component separated at R_f value 0.28 was observed identical to standard Diosgenin spot.

Table No. 5.28 R_f value for *Gokshur* aqueous extract and prepared formulation with standard

Spot No.	T1	T2	T3
1	0.13	--	0.13
2	0.21	--	--
3 (Diosgenin)	0.28	0.28	0.28
4	0.32	--	0.32
5	0.39	--	0.39
6	0.44	--	--
7	0.61	--	--
8	0.81	--	--
9	0.87	--	0.87

*Track T1: *Gokshur* Aqueous Extract, Track T2: Diosgenin Standard, Track T3: Mixture

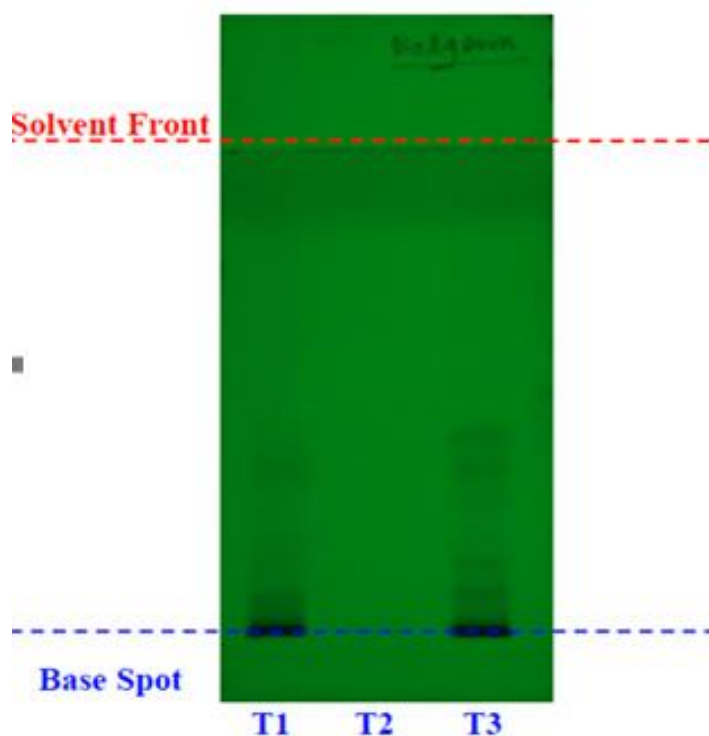


Fig. No. 5.4 HPTLC Plate for *Gokshur* Aqueous Extract, Diosgenin Standard and Mixture

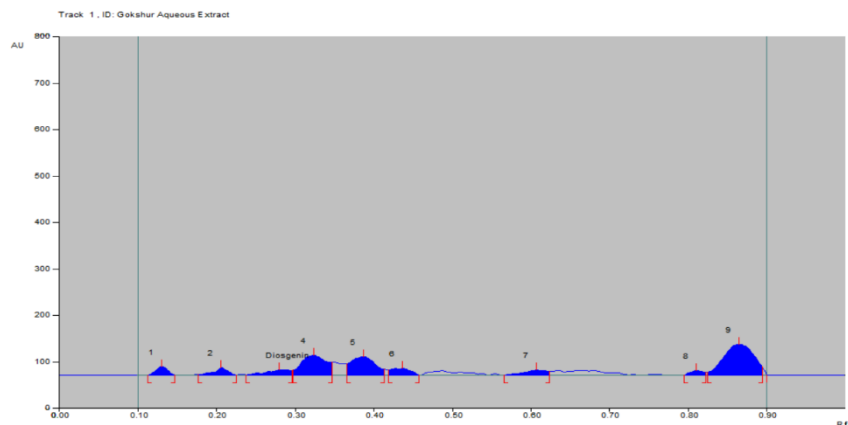


Fig. no. 5.5a 2D Chromatogram of Gokshur Aqueous Extract

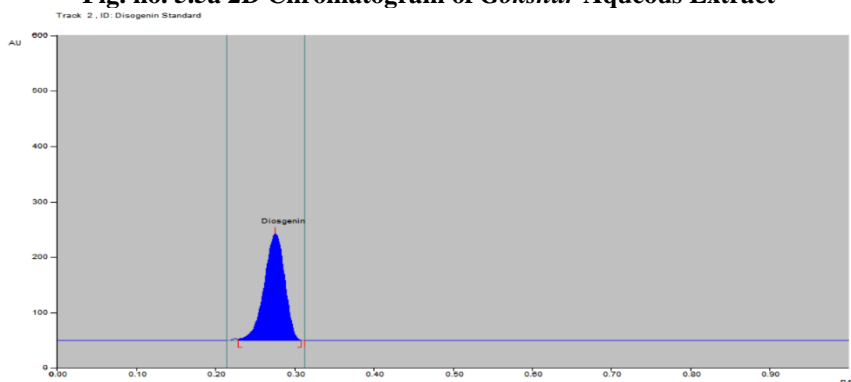


Fig. no. 5.5b 2D Chromatogram of Standard Diosgenin

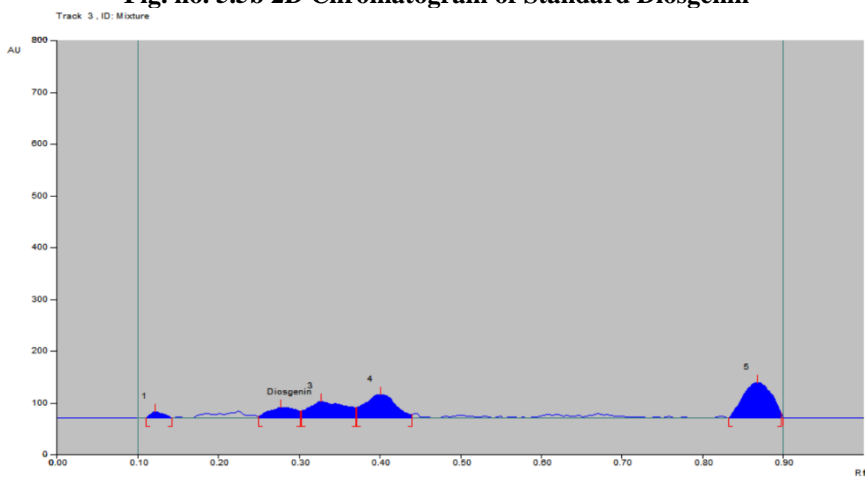


Fig. no. 5.5c 2D Chromatogram of Mixture

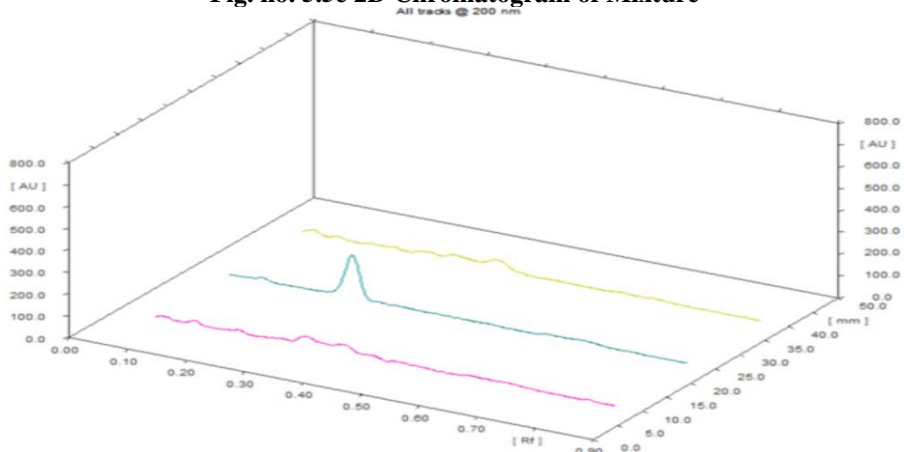


Fig. no. 5.5d 3D HPTLC Chromatogram @ 200 nm

5.9.2 HPTLC STUDY FOR *PUNARNAVA* AQUEOUS EXTRACT AND PREPARED FORMULATION WITH STANDARD

The HPTLC method was employed in the estimation of Ursolic acid in MeOH Extract of *Punarnava* aqueous extract. The quantification of Ursolic acid was carried out @ 530 nm; 0.099 % of yield was measured in *Punarnava* aqueous extract and 0.051 % of yield was measured in formulation mixture. Visualization and scanning were performed @ 530 nm and obtained R_f values are tabulated in Table no. 5.29. Toluene: Ethyl acetate: Formic acid (7:3:0.3 v/v) was used as solvent system. The component separated at R_f value 0.58 was observed identical to standard Ursolic acid spot.

Table no. 5.29 R_f value for *Punarnava* aqueous extract and prepared formulation with standard

Spot No.	T1	T2	T3	
1	--	--	0.16	
2	0.19	--	0.19	
3	0.28	--	--	
4	0.31	--	0.31	
5	0.37	--	--	
6	0.43	--	0.42	
7 (Ursolic acid)	0.58	0.58	0.58	
8	0.64	--	0.64	
9	0.72	--	0.73	
10	0.80	--	--	

*Track T1: *Punarnava* Aqueous Extract, Track T2: Ursolic acid Standard, Track T3: Mixture

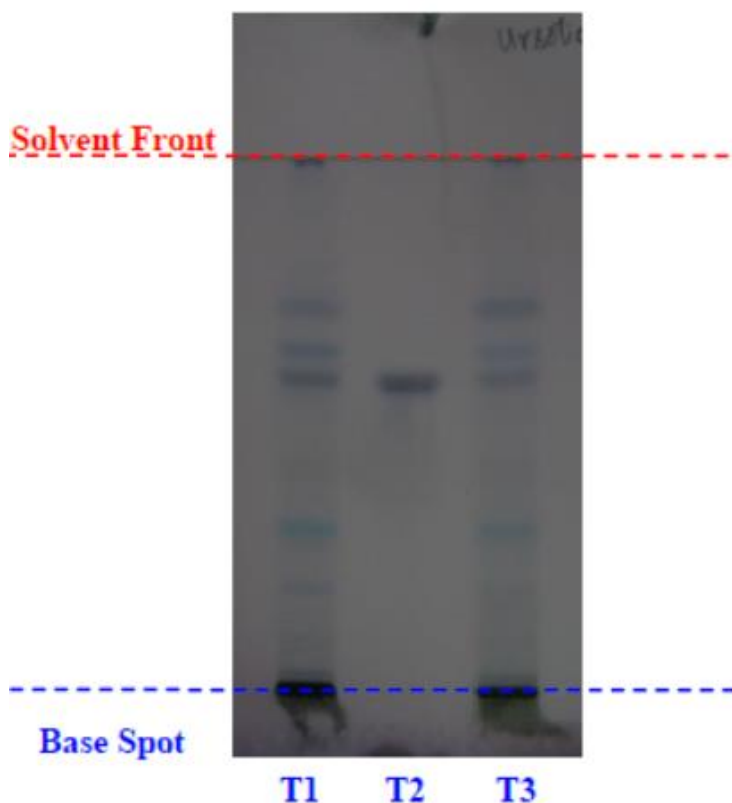


Fig. No. 5.6 HPTLC Plate for *Punarnava* Aqueous Extract, Ursolic acid Standard and Mixture

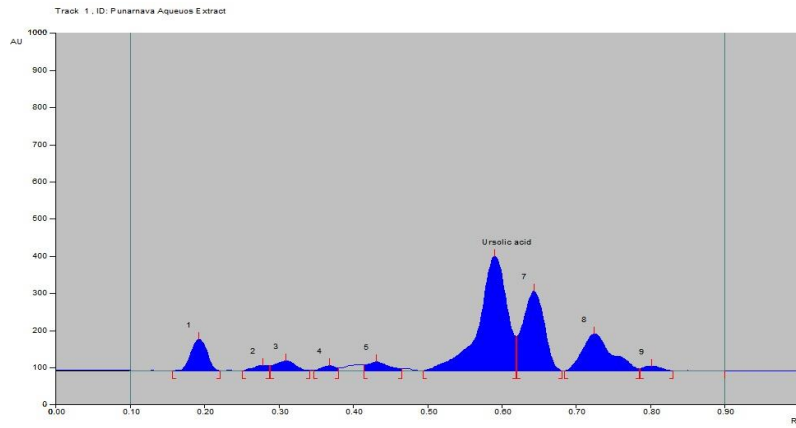


Fig. no. 5.7a 2D Chromatogram of Punarnava Aqueous Extract

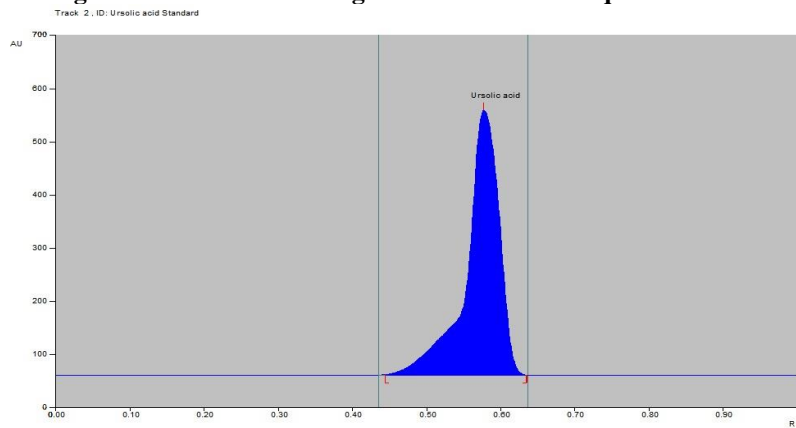


Fig. no. 5.7b 2D Chromatogram of Standard Ursolic acid

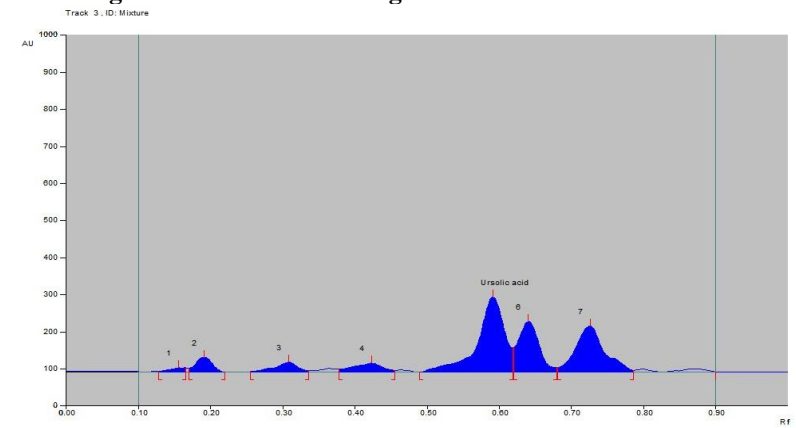


Fig. no. 5.7c 2D Chromatogram of Mixture

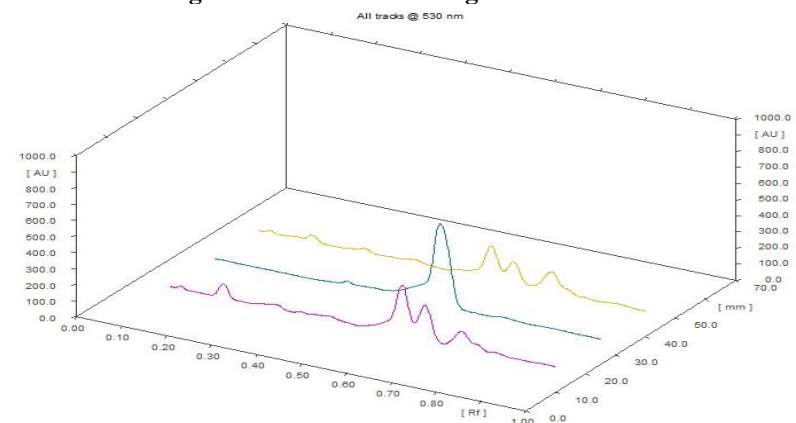


Fig. no. 5.7d 3D HPTLC Chromatogram @ 530 nm

5.9.7 HPLC ANALYSIS FOR GOKSHUR IN PREPARED FORMULATION

HPLC analysis was carried in methanolic extract of prepared formulation for *gokshur* standard Diosgenin. For the desired purpose ACN: Water in the ratio of 90:10 v/v was used as mobile phase. A well-resolved and sharp peak was obtained in this solvent system. The detection wavelength was 203 nm and the flow rate was maintained at 0.5ml/min.

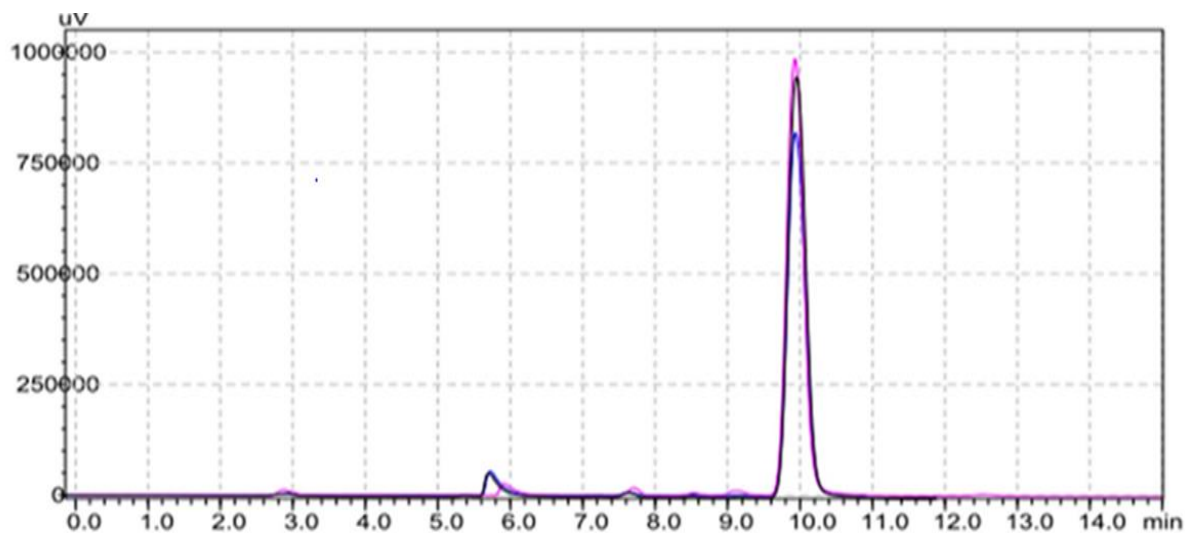


Fig. no. 5.8 Comparative chromatogram for *Gokshur* and Diosgenin standard in the prepared formulation

5.9.8 HPLC ANALYSIS FOR PUNARAVA IN PREPARED FORMULATION

HPLC analysis was carried out in prepared formulation for *Punarnava* sample standard Boeravinone B. For the desired purpose ACN: Water in the ratio of 70:30 v/v was used as mobile phase. A well-resolved and sharp peak was obtained in this solvent system. The detection wavelength was 280 nm and the flow rate was maintained at 0.5ml/min.

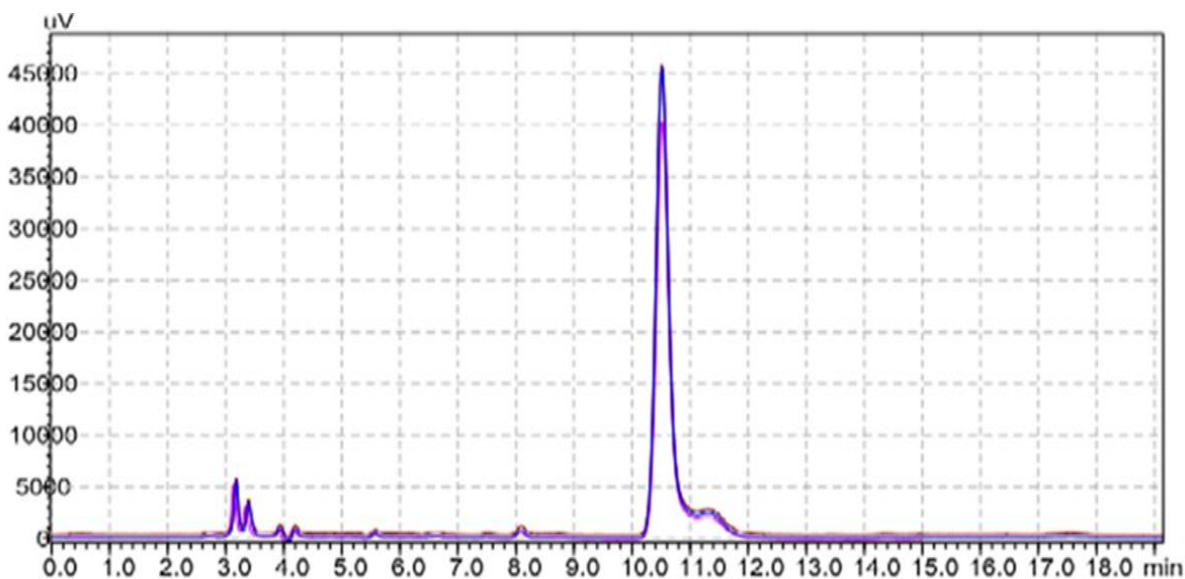


Fig. no. 5.9 Comparative chromatogram for *Punarnava* and Boeravinone B standard in prepared formulation

5.9.9 INFRARED SPECTROSCOPY

Surya kshar raw sample and purified sample compared with standard Potassium Nitrate the characteristic pick at 1363.72 both the test sample confirms the presence of potassium nitrate in the sample.

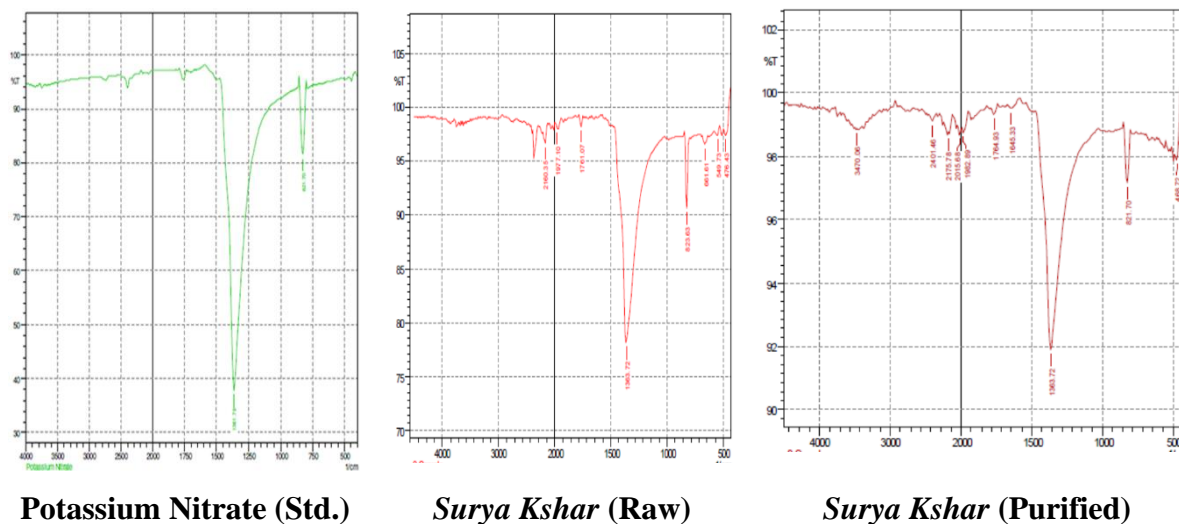


Fig. No. 5.10 FTIR study of *Surya kshar*

5.10 HEAVY METAL DETERMINATION

Apamarga Kshar, *Sphatika*, *Goksura* aqueous extract, *Punarnava* aqueous extract, *Surya kshar*, and a prepared formulation were analysed for the presence of heavy metal. All the analysed samples were found within the limit. Though the value of lead (Pb) in *Suryakshara* and prepared formulation is within the maximum permissible limit but a little higher which can be taken care of during the selection of raw materials. Observation are tabulated in Table no.

5.30

Table No. 5.30 Heavy metal analysis

S.No.	Metals	Lead	Mercury	Arsenic	Cadmium
1	Limit as per API	10 ppm	1 ppm	3 ppm	0.3 ppm
2	Observed values (<i>Apamarga Kshar</i>)	1.1 ppm	Not detected	2.1 ppm	Not detected
3	Observed values (<i>Sphatika</i>)	Not detected	0.22 ppm	2.8 ppm	Not detected
4	Observed values (<i>Goksura</i> aqueous extract)	1.2 ppm	0.04 ppm	1.8 ppm	0.11 ppm
5	Observed values (<i>Punarnava</i> aqueous extract)	1 ppm	Not detected	1.8 ppm	Not detected
6	Observed values (<i>Surya kshar</i>)	6.7	0.5 ppm	2.8 ppm	Not detected
7	Formulation (Tablet)	6.9	0.09 ppm	2.7 ppm	0.22

5.11 MICROBIAL LOAD DETERMINATION

Apamarga Kshar, *Sphatika*, *Goksura* aqueous extract, *Punarnava* aqueous extract, *Surya kshar*, and prepared formulation were analysed for the presence of microbial load, and test for specific pathogen was also performed. Specified microbial load and specific pathogen in all samples were found within the API acceptable range. Observation are tabulated in Table no.

5.31

Table No. 5.31 Microbial load and Test for specific Pathogen

Microbial analysis	Total bacterial count	Total yeast and mould	<i>Escherichia coli</i>	<i>Salmonella spp.</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas Aeruginosa</i>
Limit as per API	NMT 10 ⁵ CFU/ml	NMT 10 ³ CFU/ml	-	-	-	-
Observed values (<i>Apamarga Kshar</i>)	800 CFU/ml	250 CFU/ml	-	-	-	-
Observed values (<i>Sphatika</i>)	Nil	Nil	-	-	-	-
Observed values (<i>Goksura</i> aqueous extract)	87000 CFU/ml	600 CFU/ml	-	-	-	-
Observed values (<i>Punarnava</i> aqueous extract)	69000 CFU/ml	500 CFU/ml	-	-	-	-
Observed values (<i>Surya kshar</i>)	1200 CFU/ml	600 CFU/ml	-	-	-	-
Formulation (Tablet)	1600 CFU/ml	400 CFU/ml	-	-	-	-

* Absent = -

5.11.1 AFLATOXINS DETERMINATION

Apamarga Kshar, *Sphatika*, *Goksura aqueous extract*, *Punarnava aqueous extract*, *Surya kshar*, and prepared formulation were analysed for the presence aflatoxins. Specified aflatoxins in all samples were found within the API acceptable range. Observation are tabulated in Table no. 5.32

Table No. 5.32 Aflatoxins

S.No.	Aflatoxins	Observation			
		B ₁	B ₂	G ₁	G ₂
1	<i>Apamarga Kshar</i>	-	-	-	-
2	<i>Sphatika</i>	-	-	-	-
3	<i>Goksura</i> aqueous extract	-	-	-	-
4	<i>Punarnava</i> aqueous extract	-	-	-	-
5	<i>Surya kshar</i>	-	-	-	-
6	Tablets	-	-	-	-

* Absent = -

5.12 IN-VITRO ANTIUROLITHIATIC ACTIVITY**5.12.1 *Apamarg Kshar***

CaOx crystal growth inhibition started at a drug concentration of 50 µg/ml but 550 µg/ml of drug concentration showed maximum crystal inhibition of 82%. Dose depended activity observed and results were tabulated in Table 5.33

Table No. 5.33 *In-vitro* inhibitory activity of CaOx crystals growth by UV spectrophotometer (*Apamarg Kshar*)

S.No.	Drug Conc. (µg/ml)	Absorbance (UV spectrophotometer)	Percentage inhibition
1	50	0.342	2.29
2	100	0.316	9.71
3	150	0.285	18.57
4	200	0.272	22.29
5	250	0.253	27.71
6	300	0.238	32.00

7	350	0.223	36.29
8	400	0.190	45.71
9	450	0.140	60.00
10	500	0.075	78.57
11	550	0.063	82.00

* Control sample Absorbance without drug **0.350**

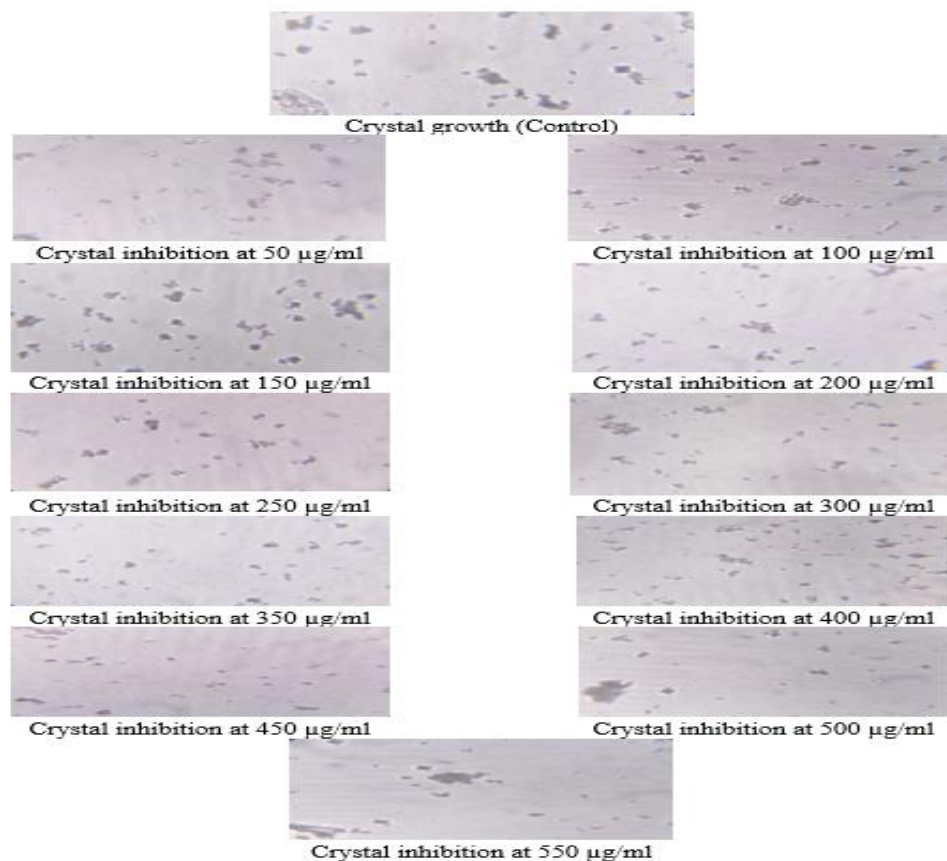


Fig. No. 5.11 *In-vitro* inhibitory activity of CaOx crystals growth by microscopic study (*Apamarg Kshar*)

5.12.2 *Sphatika*

CaOx crystal growth inhibition started at a drug concentration of 50 µg/ml but 650 µg/ml of drug concentration showed maximum crystal inhibition of 53.90%. Dose depended activity observed and results were tabulated in Table 5.34

Table No. 5.34 *In-vitro* inhibitory activity of CaOx crystals growth by UV spectrophotometer (*Sphatika*)

S. No.	Drug Conc. (µg/ml)	Absorbance (UV spectrophotometer)	Percentage inhibition
1	50	0.288	2.37
2	100	0.276	6.44
3	150	0.269	8.81
4	200	0.255	13.56
5	250	0.239	18.98
6	300	0.220	25.42
7	350	0.215	27.12
8	400	0.212	28.14
9	450	0.203	31.19
10	500	0.195	33.90

11	550	0.176	40.34
12	600	0.150	49.15
13	650	0.136	53.90

* Control sample Absorbance without drug **0.295**

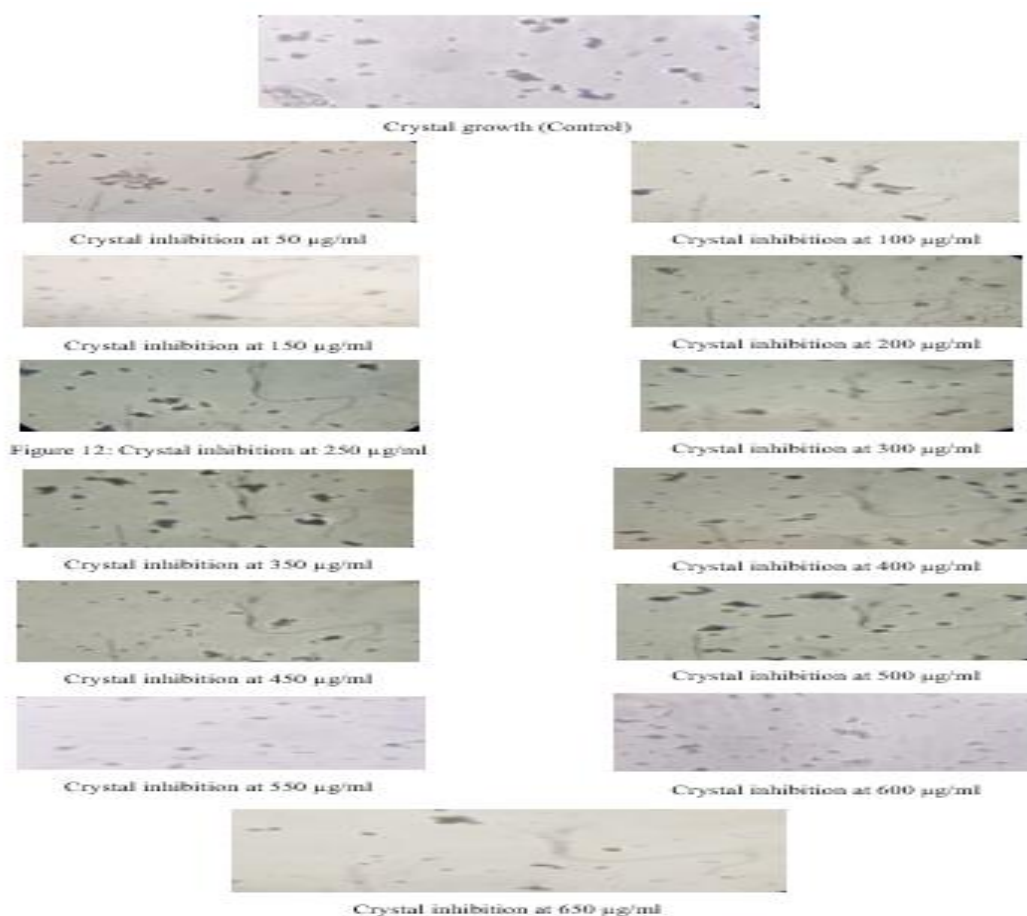


Fig. No. 5.12 *In-vitro* inhibitory activity of CaOx crystals growth by microscopic study (*Sphatika*)

5.12.3 Gokhshur aqueous extract

CaOx crystal growth inhibition started at a drug concentration of 50 µg/ml but 650 µg/ml of drug concentration showed maximum crystal inhibition of 58.24%. Dose depended activity observed and results were tabulated in Table 5.35

Table No. 5.35 *In-vitro* inhibitory activity of CaOx crystals growth by UV spectrophotometer (*Gokhshur* aqueous extract)

S. No.	Drug Conc. (µg/ml)	Absorbance (UV spectrophotometer)	Percentage inhibition
1	50	0.510	2.30
2	100	0.460	11.88
3	150	0.441	15.52
4	200	0.435	16.67
5	250	0.396	24.14
6	300	0.359	31.23
7	350	0.346	33.72
8	400	0.334	36.02
9	450	0.304	41.76
10	500	0.282	45.98
11	550	0.260	50.19

12	600	0.242	53.64
13	650	0.218	58.24

* Control sample Absorbance without drug **0.522**

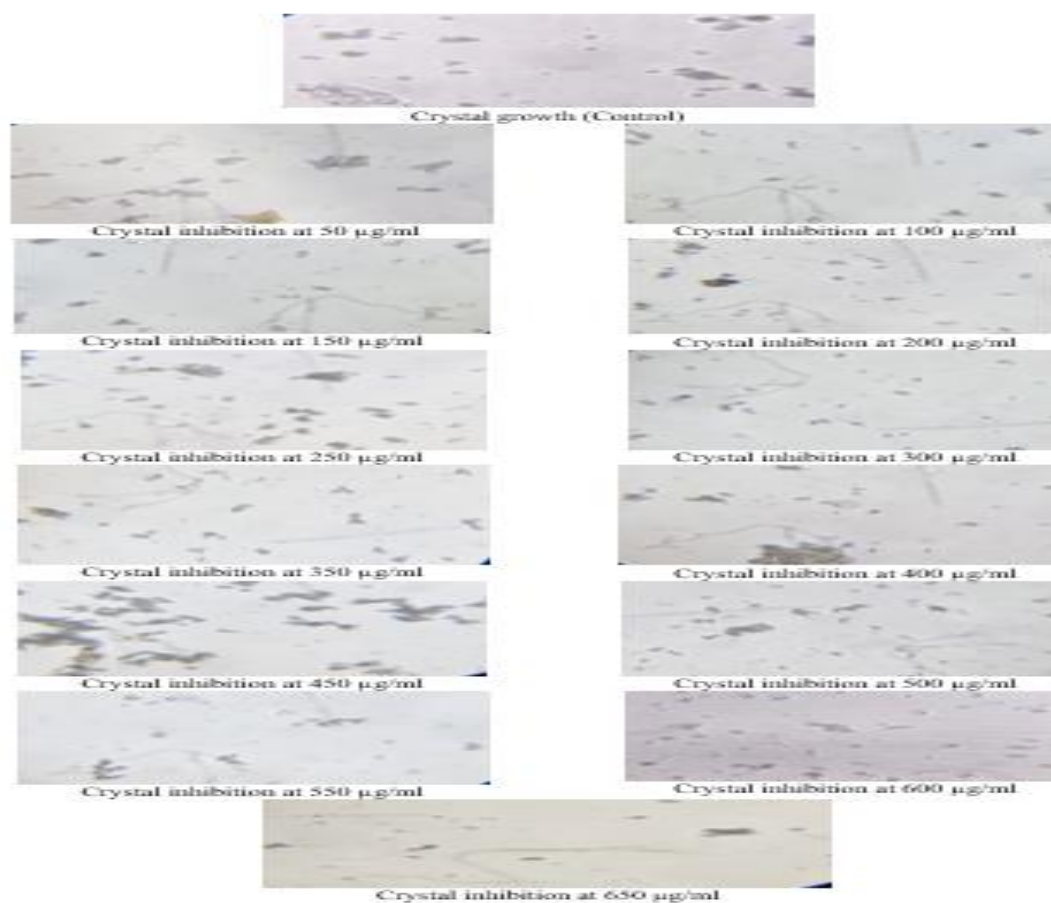


Fig. no. 5.13 *In-vitro* inhibitory activity of CaOx crystals growth by microscopic study (*Gokhshur* aqueous extract)

5.12.4 *Punarnava* aqueous extract

CaOx crystal growth inhibition started at a drug concentration of 50 µg/ml but 650 µg/ml of drug concentration showed maximum crystal inhibition of 50 %. Dose depended activity observed and results were tabulated in Table 5.36

Table No. 5.36 *In-vitro* inhibitory activity of CaOx crystals growth by UV spectrophotometer (*Punarnava* aqueous extract)

Sr. No.	Drug Conc. (µg/ml)	Absorbance (UV spectrophotometer)	Percentage inhibition
1	50	0.454	4.62
2	100	0.435	8.61
3	150	0.415	12.82
4	200	0.397	16.60
5	250	0.385	19.12
6	300	0.357	25.00
7	350	0.332	30.25
8	400	0.323	32.14
9	450	0.308	35.29
10	500	0.293	38.45
11	550	0.287	39.71

12	600	0.258	45.80
13	650	0.238	50.00

* Control sample Absorbance without drug **0.476**

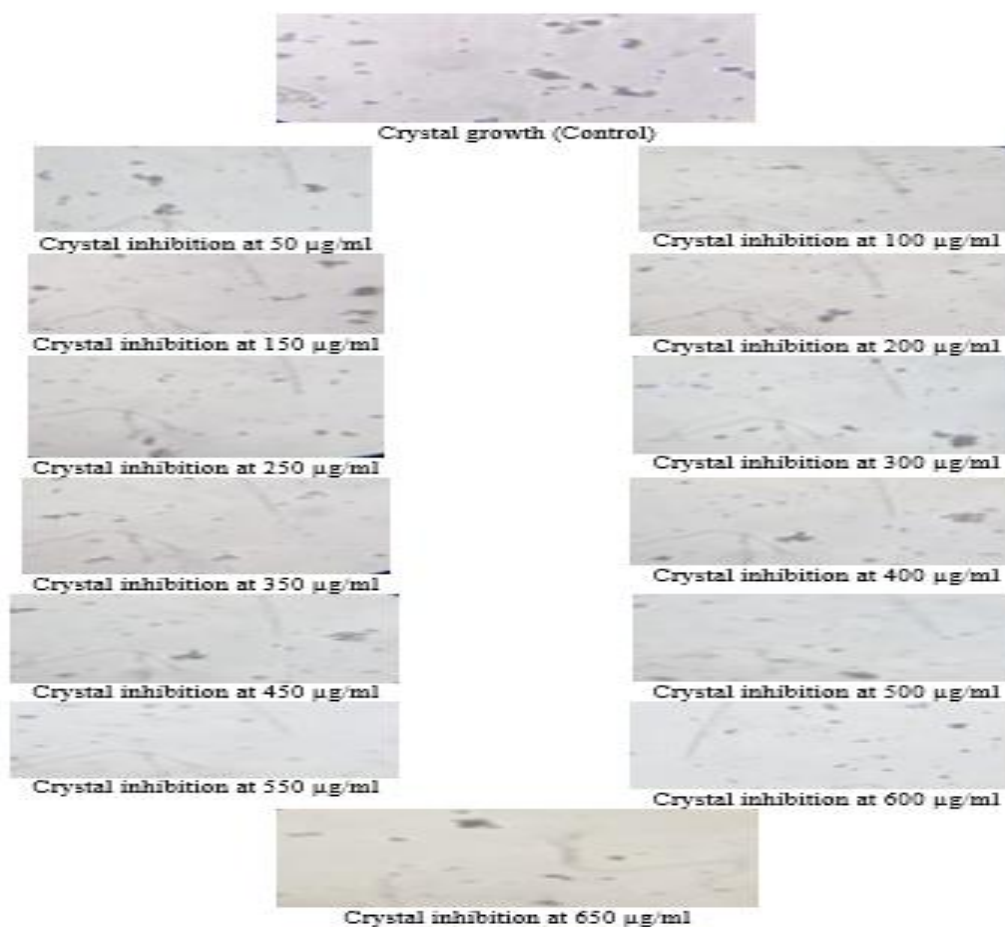


Fig. no. 5.14 *In-vitro* inhibitory activity of CaOx crystals growth by microscopic study (*Punarnava* aqueous extract)

5.12.5 *Surya Kshar*

CaOx crystal growth inhibition started at a drug concentration of 50 µg/ml but 650 µg/ml of drug concentration showed maximum crystal inhibition of 54.26%. Dose depended activity observed and results were tabulated in Table 5.37

Table No. 5.37 *In-vitro* inhibitory activity of CaOx crystals growth by UV spectrophotometer (*Surya Kshar*)

Sr. No.	Drug Conc. (µg/ml)	Absorbance (UV spectrophotometer)	Percentage inhibition
1	50	0.253	1.94
2	100	0.235	8.91
3	150	0.217	15.89
4	200	0.209	18.99
5	250	0.201	22.09
6	300	0.194	24.81
7	350	0.182	29.46
8	400	0.174	32.56
9	450	0.161	37.60
10	500	0.153	40.70

11	550	0.147	43.02
12	600	0.133	48.45
13	650	0.118	54.26

* Control sample Absorbance without drug **0.258**

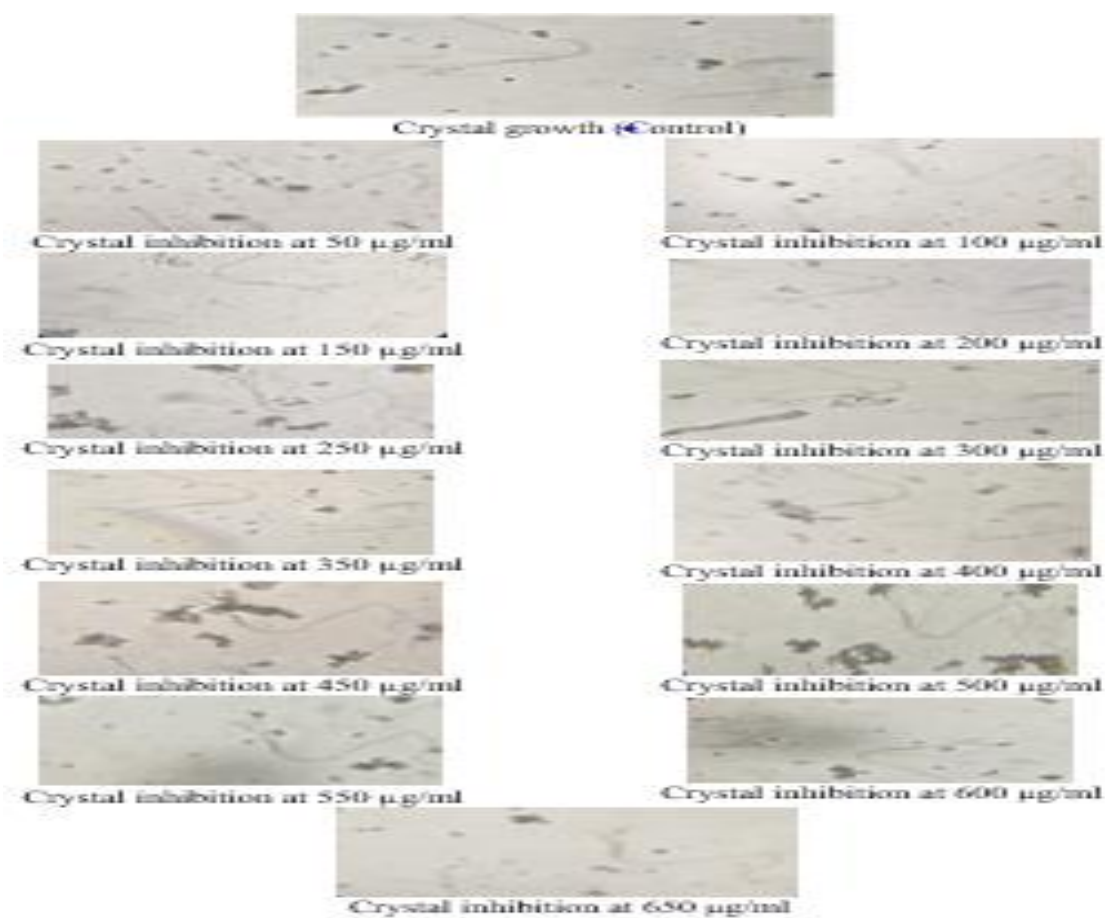


Fig. no. 5.15 *In-vitro* inhibitory activity of CaOx crystals growth by microscopic study (Surya Kshar)

5.12.6 Prepared formulation – Tablet

CaOx crystal growth inhibition started at a drug concentration of 50 µg/ml but 650 µg/ml of drug concentration showed maximum crystal inhibition of 87.16%. Dose depended activity observed and results were tabulated in Table 5.38

Table No. 5.38 *In-vitro* inhibitory activity of CaOx crystals growth by UV spectrophotometer (Prepared formulation – Tablet)

S. No.	Drug Conc. (µg/ml)	Absorbance (UV spectrophotometer)	Percentage inhibition
1	50	0.331	1.19
2	100	0.288	14.03
3	150	0.272	18.81
4	200	0.265	20.90
5	250	0.198	40.90
6	300	0.180	46.27
7	350	0.156	53.43
8	400	0.141	57.91
9	450	0.127	62.09
10	500	0.106	68.36
11	550	0.083	75.22

12	600	0.063	81.19
13	650	0.043	87.16

* Control sample Absorbance without drug **0.335**

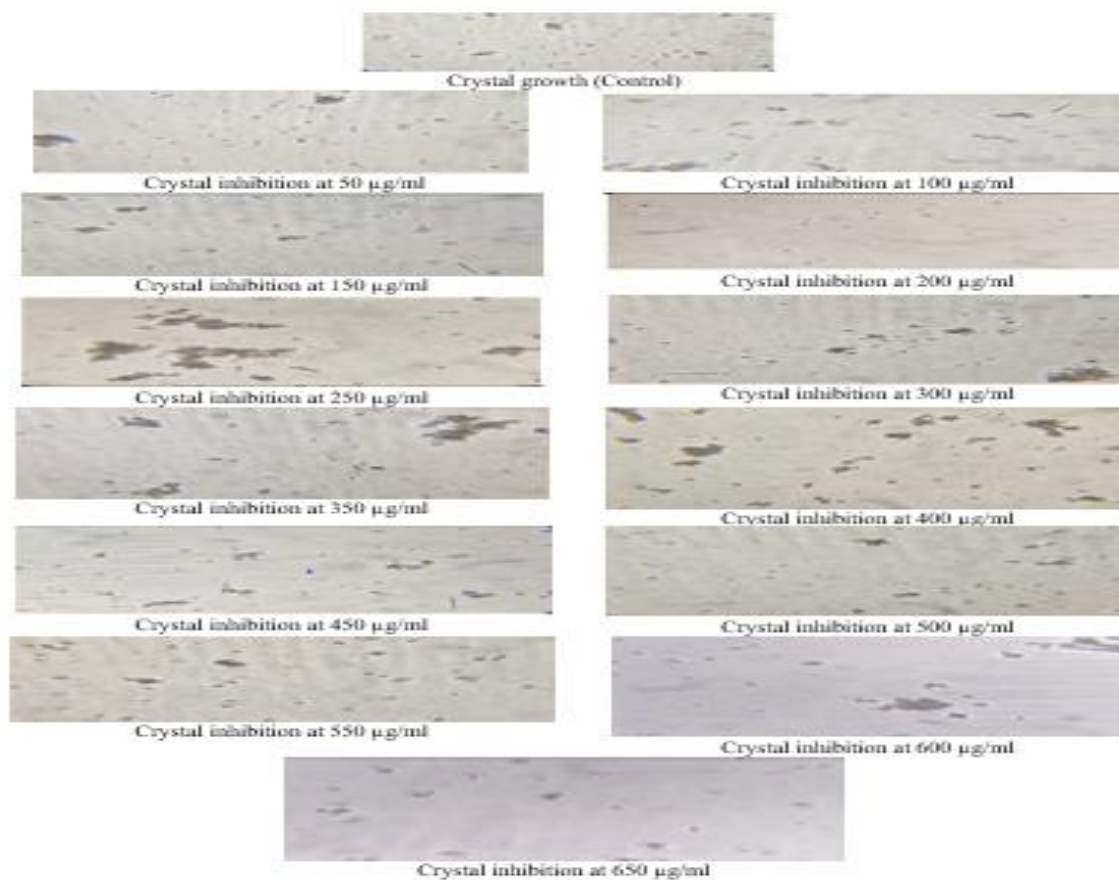


Fig. no. 5.16 *In-vitro* inhibitory activity of CaOx crystals growth by microscopic study (Prepared formulation – Tablets)

GRAPHICAL REPRESENTATION OF *IN-VITRO* STUDY

The graphical representation shows that the percentage of CaOx crystals growth inhibition of *Apamarg kshar* (AP), *Sphatika* (SP), *Gokshur* aqueous extract (GW), *Punarnava* aqueous extract (PW), *Surya kshar* (SK), and prepared tablet by combining all the drugs (Tab). *In-vitro* antiurolithic activity of formulation showed a 3-fold increase in protection with respect to the sum of individual response however the final response of formulation will be concluded after animal studies.

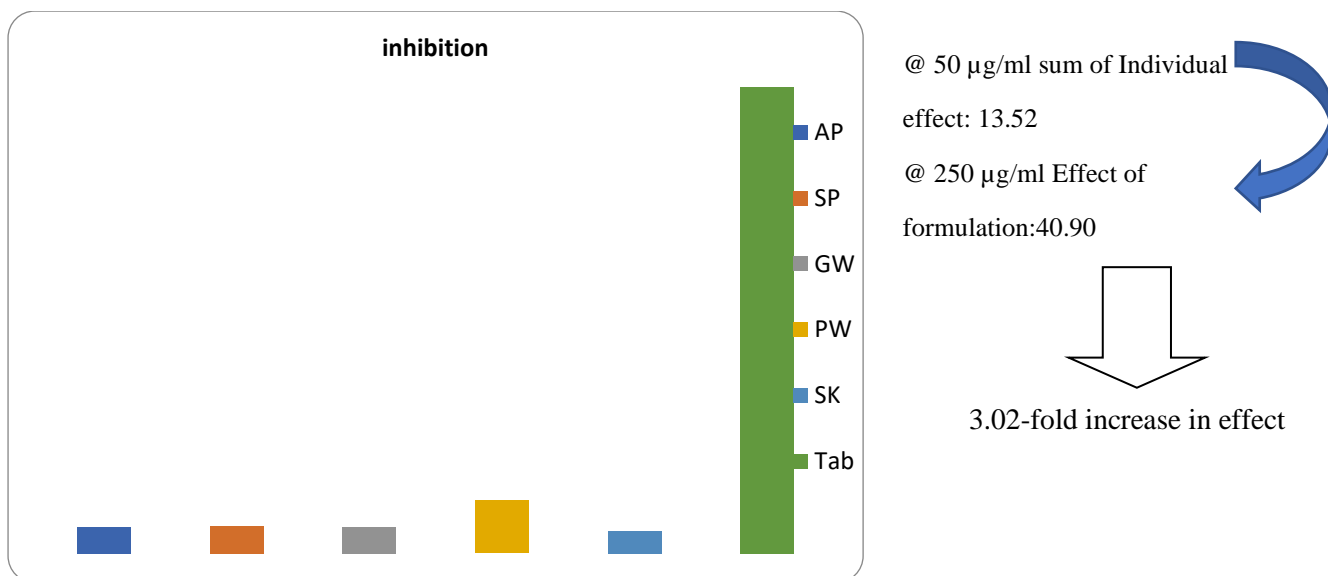


Fig. no. 5.17 Graphical representation of *in-vitro* study

5.13 *IN-VIVO* ANTIUROLITHIATIC ACTIVITY

5.13.1 EFFECTS OF TREATMENT ON CHANGES IN BODY WEIGHT IN A RAT MODEL OF UROLITHIASIS

Initially rats body weight were observed to be statistically similar ($P > 0.05$) in the different experimental groups. The average gain in body weight was calculated as the difference in body weight in-between day 0 to day 28. The average body weight of rats was decreased in the groups of II, IV and V on day 28. In the first 7 days of experimental treatment decrease in body weights in groups III to X was observed. From the 14th day onwards till the 28th day, a significant improvement ($P < 0.05$) in body weight was noted in the case of rats of these groups when compared to rats of group II. This improvement was observed to be less ($P < 0.05$) in rats of group IV and V when compared with rats of group VI, VII, VIII, IX and X. On day 28, the rats of groups III and X exhibited body weight gain close to that of normal control group I. The body weight changes are shown in **Table 5.39**.

Table No. 5.39 Effects of treatment on change in body weight in a rat model of urolithiasis

Day	N (Group - I)	LC (Group - II)	LC + C (Group - III)	LC + AK (Group - IV)	LC + SK (Group - V)	LC + S (Group - VI)	LC + G (Group - VII)	LC + P (Group - VIII)	LC + F1 (Group - IX)	LC + F2 (Group - X)
0	254.64±5.50	262.49±12.12	253.30±11.60	275.53±8.16	234.23±10.27	237.45±4.23	235.41±4.23	233.45±10.42	232.38±6.4	272.98±8.44
7	270.37±8.16	238.43±9.39	260.20±5.62	257.39±10.16 ^{a,c}	258.39±9.88 ^{a,b}	233.44±11.25	223.44±11.25	223.43±6.26 ^a	240.41±4.5 ^a	260.41±4.52
14	286.59±4.23	208.65±12.11 ^a	286.39±12.09 ^b	241.32±10.13 ^{b,c}	249.32±9.82 ^{b,c}	216.43±9.12 ^b	227.20±9.12 ^b	235.24±10.03 ^{b,c}	250.61±7.9 ^{b,c}	280.61±7.98 ^{b,c}
21	298.38±5.86	167.31±8.23 ^a	294.36±3.04 ^b	204.36±8.12 ^{a,b,c}	216.36±6.54 ^{a,b,c}	208.31±7.78 ^{b,c}	203.44±7.7 ^{a,b,c}	230.52±4.56 ^{b,c}	274.74±6.6 ^{b,c,d}	294.74±6.3 ^{a,b,c,d,e}
28	311.28±7.66	131.33±9.30 ^a	305.30±2.11 ^b	170.05±3.23 ^{a,b,c}	200.05±4.76 ^{a,b,c}	211.91±2.09 ^{a,b,c}	212.41±2.1 ^{a,b,c}	235.87±6.78 ^{a,b,c}	282.48±5.3 ^{a,b,c,d}	302.48±5.3 ^{a,b,c,d,e}
Wt. gain (g)	28.55	-	29.45	-	-	3.46	18.45	22.42	30.10	34.55
Wt. loss (g)	-	184.15	-	21.46	15.46	-	-	-	-	-

- Values are measured and expressed as mean ±SEM, n=6 experimental animals/group. Observed data was considered as significant if P<0.05 a. when it compared with normal control; b. when it compared with Lithiatic Control; c. when it compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer multiple comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

5.13.2 EFFECTS OF TREATMENT ON URINARY OUTPUT IN A RAT MODEL OF UROLITHIASIS

The urinary output was found increased in drug-treated groups IV to X in comparison lithiatic control group (Fig. no. 5.18) which shows the diuretic effects of the drugs. A low volume of urine output will be considered as one of the risk factors for the onset of renal calculi higher urine volume help to eliminate the calcium oxalate crystals also preventing the recurrence of the renal stone.

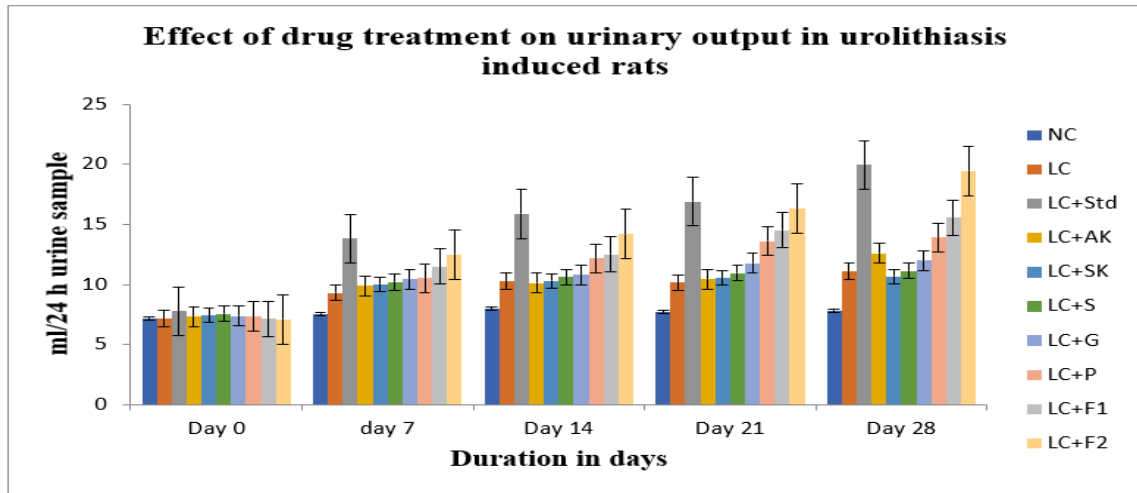


Fig. no. 5.18 Effects of treatment on urinary output in a rat model of urolithiasis

5.13.3 EFFECTS OF TREATMENT ON CALCIUM EXCRETION IN A RAT MODEL OF UROLITHIASIS

Urinary calcium excretion was observed to decrease in Lithiatic control group. However, drug treatment groups IV to X shows the prevision and helps to normalizes the calcium level (Fig. no. 5.19). This might be happening due to the increased bioavailability of nitric oxide leads to activation of cyclic guanosine monophosphate which regulates the level of calcium.

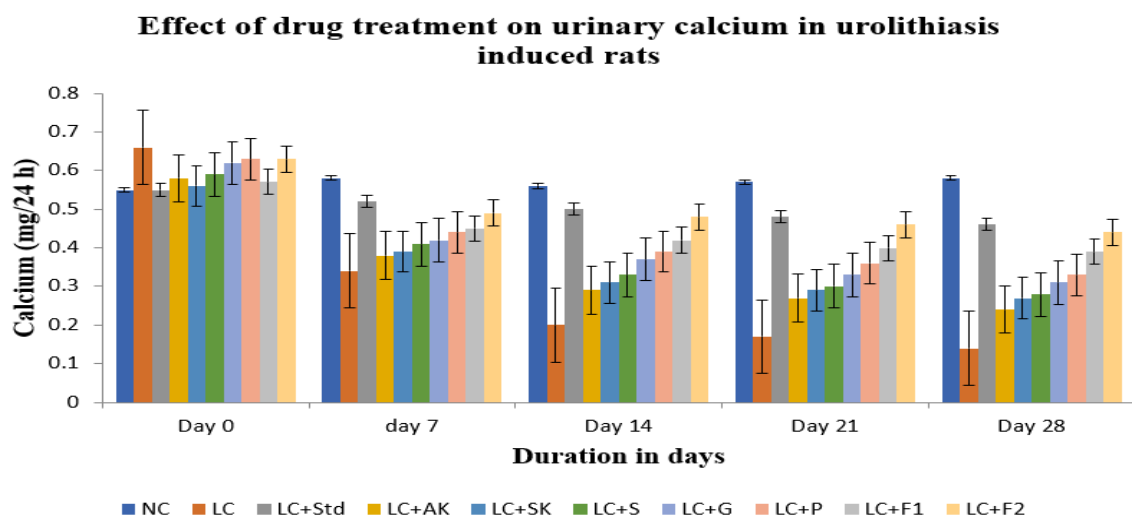


Fig. no. 5.19 Effects of treatment on calcium excretion in a rat model of urolithiasis

5.13.4 EFFECTS OF TREATMENT ON OXALATE AND URIC ACID EXCRETION IN A RAT MODEL OF UROLITHIASIS

Increased oxalate (Fig. no. 5.20) and uric acid (Fig. no. 5.21) excretion were observed in Lithiatic control group. However, that was significantly prevented in drug-treated groups IV to X. These changes observed in urinary oxalate and uric acid excretion shows dose-dependent effects. The Uric acid hinders the solubility of calcium oxalate and reduces the inhibitory activity of glycosaminoglycans by binding with crystals and modulate its crystallization which leads to stone formation. The drug treatments are able to lower down the excretion of uric acid and helps to reduce the risk of stone formation.

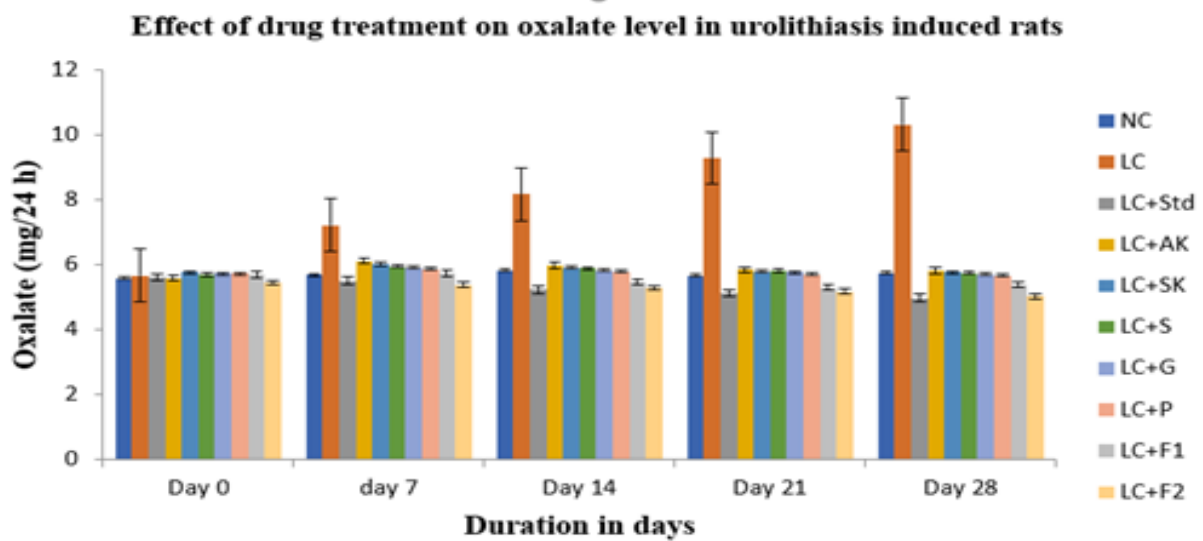


Fig. no. 5.20 Effects of treatment on oxalate excretion in a rat model of urolithiasis

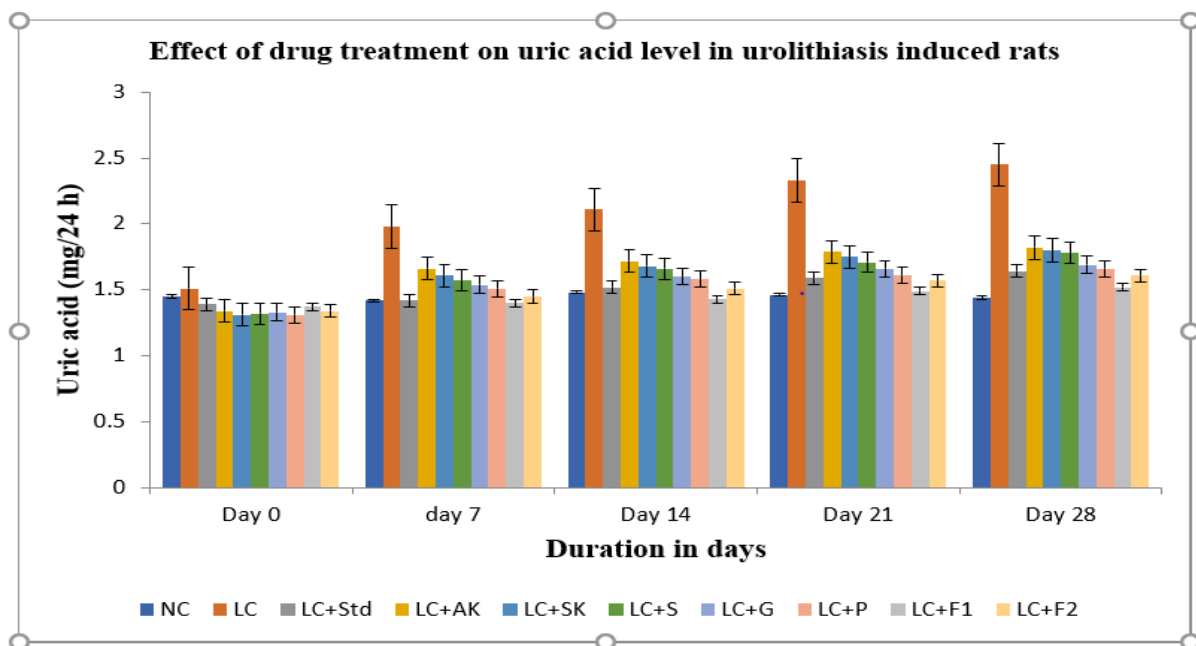


Fig. no. 5.21 Effects of treatment on uric acid excretion in a rat model of urolithiasis

5.13.5 EFFECTS OF TREATMENT ON RELATIVE KIDNEY WEIGHT, SPECIFIC GRAVITY AND pH IN A RAT MODEL OF UROLITHIASIS

On day 28 the kidney relative weight (Table: 5.40), specific gravity (Table: 5.40), and Urine pH (Table: 5.40) were found significantly increased ($P < 0.05$) in rats of group II that received ethylene glycol only as compared to rats of group I. This increase in the level of relative weight, specific gravity, and decrease in urine pH in group II indicated damage of kidney cells could be due to increasing in the level of ROS during chronic hyperglycaemia. The relative kidney weight, specific gravity, and pH changes are shown in Table no. 5.40

Table No. 5.40 Effect of treatments on changes in kidney weight (g), specific gravity, and pH in a rat model of urolithiasis

Group	Treatment	Relative weight of kidney (g/100 g)	Specific gravity	Urine pH
I	N	1.47+0.088	1.007+0.004	7.6+0.35
II	LC	1.92+0.012 ^a	1.067+0.032 ^a	6.2+0.22 ^a
III	LC + C (Standard)	1.37+0.066 ^b	1.003+0.093 ^b	7.8+0.65 ^b
IV	LC + AK	0.97+0.009 ^{a,b}	1.012+0.022 ^{a,b}	6.6+0.32 ^{a,b}
V	LC + SK	1.07+0.066 ^{a,b}	1.017+0.007 ^{a,b}	6.5+0.37 ^{a,b}
VI	LC + S	1.17+0.055 ^{a,b,c}	1.027+0.002 ^{a,b,c}	6.8+0.90 ^{a,b,c}
VII	LC + G	1.17+0.008 ^{a,b,c}	1.037+0.008 ^{a,b,c}	6.6+0.85 ^{a,b,c}
VIII	LC + P	1.27+0.005 ^{a,b,c}	1.047+0.099 ^{a,b,c}	6.6+0.39 ^{a,b,c}
IX	LC + F1	1.37+0.003 ^{a,b,c,d}	1.057+0.033 ^{a,b,c,d}	7.0+0.55 ^{a,b,c,d}
X	LC + F2	1.47+0.00 ^{a,b,c,d,e}	1.077+0.05 ^{a,b,c,d,e}	7.5+0.95 ^{a,b,c,d,e}

N: Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data was considered significant when the $P < 0.05$ a. when it is compared with normal control group; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The observed data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

5.13.6 EFFECT ON HAEMATOLOGICAL PROFILE

On day 28 the kidney parameters like Urea, Uric acid, Creatinine, and Calcium Table No. 5.41 were found significantly increased ($P < 0.05$) in rats of group II (control) that given ethylene glycol as compared with experimental animal group I. This increase in the level of Urea, Uric acid, Creatinine and Calcium in rats of group II indicated damage of kidney stones due to increasing in the level of serum urea. In groups VIII, IX, and X, a significant reduction (Table 5.41) in the level of Urea, Creatinine was observed when compared to group II. In groups IV to VI, treated with low and high doses of herbomineral drugs significant increase in these parameters was observed as that of rats of group I and III (Table no. 5.41).

Table. 5.41 Effect of treatments on serum biochemical parameters in rat model of urolithiasis

Group	Treatment	Serum Urea (mg/dL)	Serum Creatinin (mg/dL)	Serum Uric acid (mg/dL)	Serum Calcium (mg/dL)
I	N	46.57+0.120	0.52+0.014	1.16+0.34	7.8+0.12

II	LC	81.96+0.090 ^a	0.96+0.002 ^a	1.66+0.45 ^a	8.6+0.33 ^a
III	LC + C (Std)	48.92+0.060 ^b	0.55+0.012 ^b	1.12+0.67 ^b	7.6+0.56 ^b
IV	LC + AK	80.77+0.070 ^{a,b}	0.90+0.023 ^{a,b}	1.65+0.22 ^{a,b}	8.6+0.77 ^{a,b}
V	LC + SK	75.97+0.050 ^{a,b,c}	0.85+0.056 ^{a,b,c}	1.60+0.45 ^{a,b,c}	8.4+0.8 ^{a,b,c}
VI	LC + S	70.07+0.019 ^{a,b,c}	0.80+0.067 ^{a,b,c}	1.55+0.67 ^{a,b,c}	8.2+0.24 ^{a,b,c}
VII	LC + G	65.47+0.059 ^{a,b,c}	0.75+0.034 ^{a,b,c}	1.52+0.55 ^{a,b,c}	8.0+0.35 ^{a,b,c}
VIII	LC + P	60.88+0.033 ^{a,b,c}	0.70+0.045 ^{a,b,c}	1.44+0.67 ^{a,b,c}	8.1+0.22 ^{a,b,c}
IX	LC + F1	55.44+0.054 ^{a,b,c,d}	0.60+0.045 ^{a,b,c,d}	1.32+0.77 ^{a,b,c,d}	7.9+0.11 ^{a,b,c,d}
X	LC + F2	47.12+0.019 ^{a,b,c,d,e}	0.53+0.011 ^{a,b,c,d,e}	1.19+0.33 ^{a,b,c,d,e}	7.4+0.67 ^{a,b,c,d,e}

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. The observed data was considered significant when P<0.05 a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The data was analysed using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

5.13.7 ANTIOXIDANT PROFILE

The kidney of the urolithiatic rats (group II) after treatment with Ethylene glycol for 28 days revealed a substantial reduction in the levels of serum catalase (CAT), Reduced Glutathione (GSH), and Total proteins (Figure: 5.22.4a-d) whereas there was a significant increase in the level of serum TBARS. On the other hand, the increased oxidative stress markers were found to have reversed in rats of group V-X that received Ethylene glycol and herbal/mineral therapy. However, there was no significant difference in antioxidants levels in group IV to VI (Figure: 5.22a-d) while compared with experimental rats of group II. In scavenging the toxic intermediates, CAT and GSH play an important role which is also known as free radicals. The treatment with high dose formulation (F2) (600 mg/Kg) of herbomineral combination (group X) increased significantly the level of antioxidant enzymes, thereby helped to control the free radicals. Further, the reduction in the amount of total protein content was observed in urolithiatic animals (group II) that may be because of progressive proteinuria. However, treatment of different herbal/mineral drugs to animal group V to VIII. The total protein content of urolithiatic rats was significantly normalised which implying that the treatment shows a curative effect on kidney function. The maximum positive effect was observed in the case of experimental rats of group X which was treated with a high dose of herbomineral combination (F1) (Fig. no. 5.22a-d).

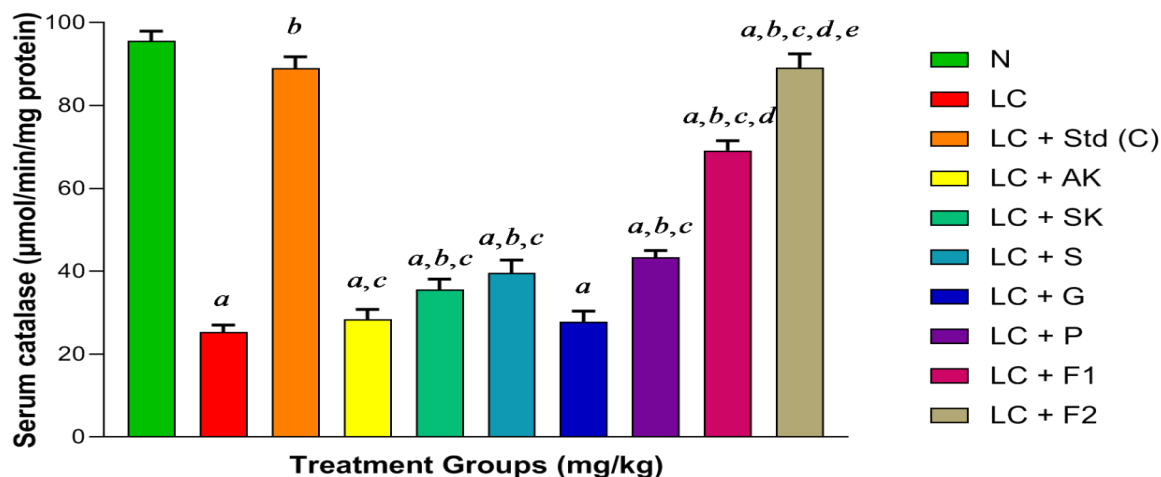


Fig. no. 5.22a: Effect of treatments on serum Catalase in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data were considered significant when $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

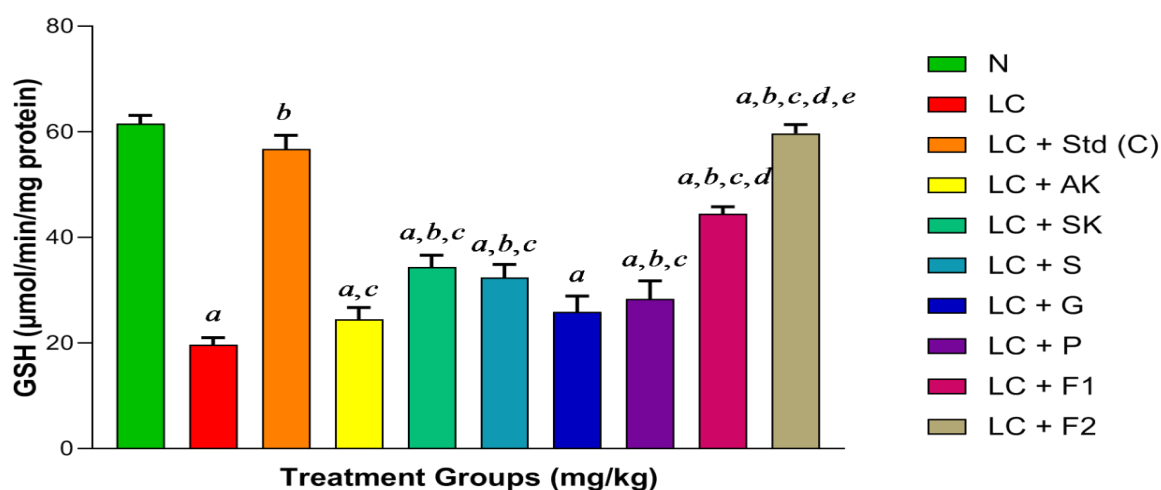


Fig. no. 5.22b: Effect of treatments on serum GSH in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data were considered significant when $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

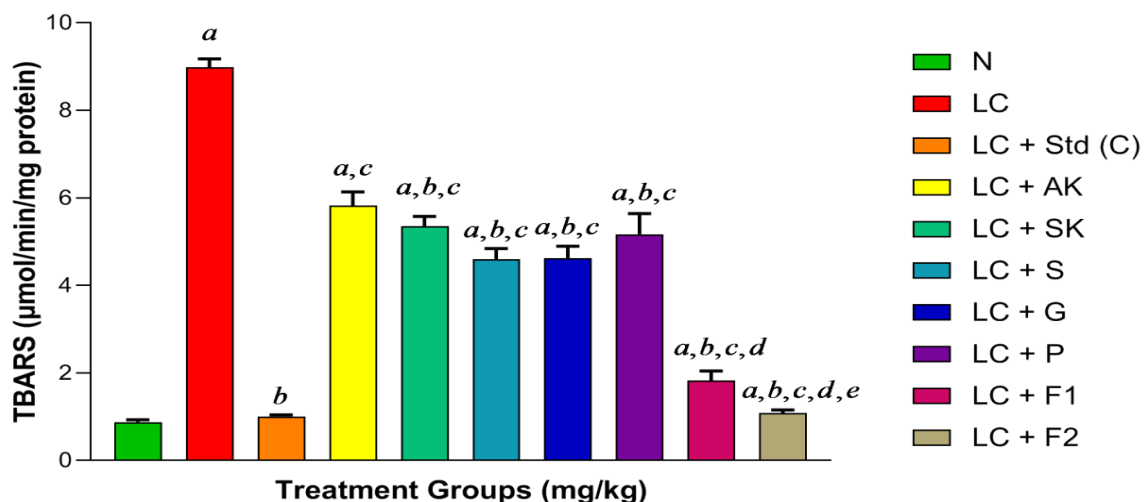


Fig. no. 5.22c: Effect of treatments on serum TBARS in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data was considered significant when $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

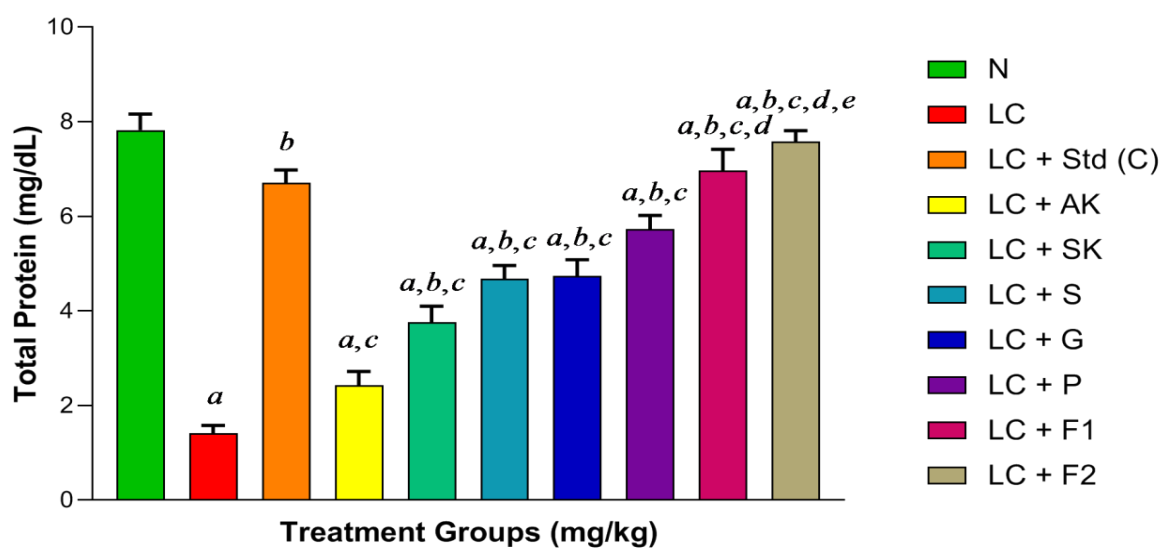


Fig. no. 5.22d: Effect of treatments on serum total proteins in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data was considered significant if $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg)

5.13.8 MITOCHONDRIAL ESTIMATIONS

Mitochondrial oxidative damage is caused by the mitochondrial respiratory chain, which is one of the most important sources of superoxide anion (O₂⁻). The production of energy in mitochondria is catalysed by the protein complexes, named NADH - ubiquinone oxidoreductase (complex-I), succinate-ubiquinol oxidoreductase (complex-II), and ubiquinol cytochrome c oxidoreductase (complex-III). Ethylene glycol treatment for 28 days reduced mitochondrial enzyme complex activity, as demonstrated by decreased NADH dehydrogenase, succinate dehydrogenase activity, and MTT potential in the current study (Fig. no. 5.23a-c). Furthermore, mitochondrial dysfunction can result in an excess of nitric oxide (NO), which can lead to oxidative damage. Furthermore, evidence indicates that the ROS causes mitochondria to become weakened and lose their functional integrity over time, increasing oxidative harm. This indicates that in the rat model of urolithic kidney stones, mitochondrial dysfunction is a key factor in the development of ROS, which contributes to oxidative damage. On the other hand, the increased mitochondrial-oxidative stress markers were found to have reversed in rats of group VI-X that received Ethylene glycol and herbal/mineral/herbomineral therapy. However, there was no significant difference in antioxidants levels in group IV to V (Fig. no. 5.23a-c) as compared to rats of group II. The treatment with high dose formulation (F2) (600 mg/Kg) of herbomineral combination (group X) Significantly increased the level of mitochondrial enzymes, thereby it helped to control the free radicals. However, treatment of different herbal/mineral/herbomineral drugs to animal group VI to X considerably normalized the increased mitochondrial stress markers in urolithiatic rats suggesting their curative functions in the experimental animals kidney. The maximum positive response was recorded in the case of experimental rats of group X, which were treated with a high dose of herbomineral combination (F1) (Fig. no. 5.23a-c).

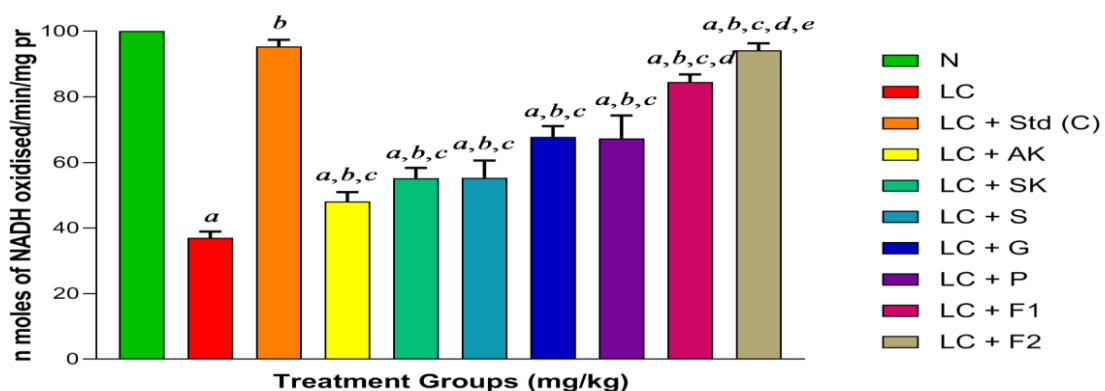


Fig. no. 5.23a: Effect of treatments on mitochondrial enzyme complex I activities in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data was considered significant if $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

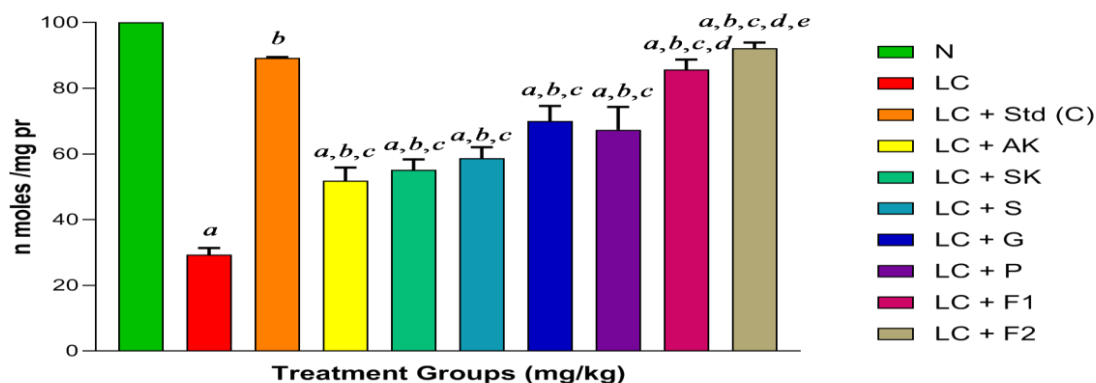


Figure. 5.23b: Effect of treatments on mitochondrial enzyme complex II activities in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data was considered significant if $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

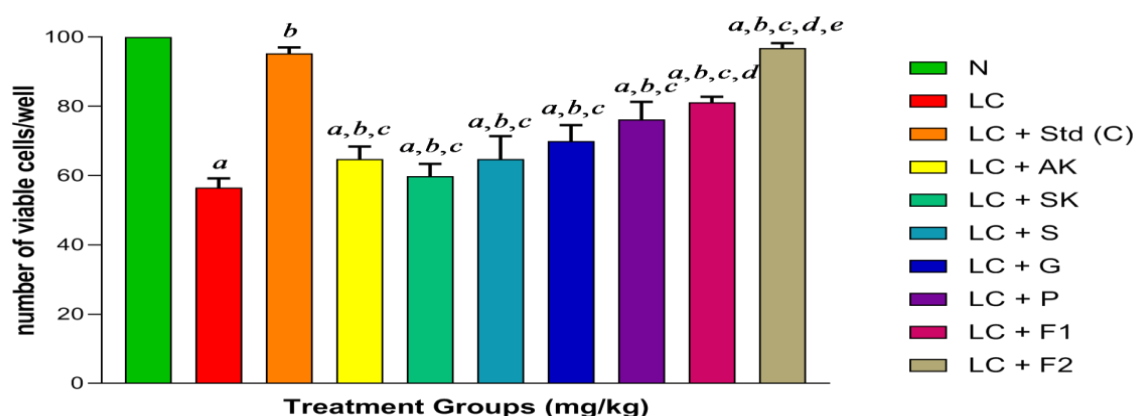


Figure No. 5.23c: Effect of treatments on mitochondrial MTT assay in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data was considered significant if $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

5.13.9 MOLECULAR ESTIMATIONS

Caspase-3 and TNF- α ELISA - Ethylene glycol-induced urolithiasis is related to the generation of inflammatory cytokines like TNF- α , in addition to oxidative and mitochondrial stress (Figure 5.24a). In the present investigation, it was observed that the elevated levels of TNF- α in urolithiasis resulting an increased inflammatory reaction. This was later controlled by using herbomineral combination with a higher dose which attributes to its potent anti-inflammatory properties (Figure 5.24a). We also found a significant enhancement in the levels of an apoptotic factor of caspase-3 which advocating its role in apoptotic pathway in Ethylene glycol induced urolithiasis (Figure 5.24b). Ethylene glycol treatment for 28 days caused increased inflammation and apoptotic cell death, as indicated by an increase in the Caspase-3 and TNF alpha in kidney homogenates (Figure 5.24a-b). while the herbal/mineral/herbomineral treatments were found to have reversed in rats of group V-X that received Ethylene glycol and herbal/mineral/herbomineral therapy (Figure 5.24a-b). However, there was no significant difference in inflammation and apoptosis level in group IV to V (Figure: 5.24a-b) as compared to rats of group II. The treatment with high dose formulation (F2) (600 mg/Kg) of herbomineral combination (group X) significantly reduced the level of inflammation, thereby helped to control the free radicals. However, treatment of different herbal/mineral drugs to animal group VI to X considerably normalized the increased non- inflammation and non-apoptosis effects in urolithiatic rats suggesting its curative functions in the kidney. The maximum positive effect was observed in the case of experimental rats of group X which was treated with a high dose of herbomineral combination (F1).

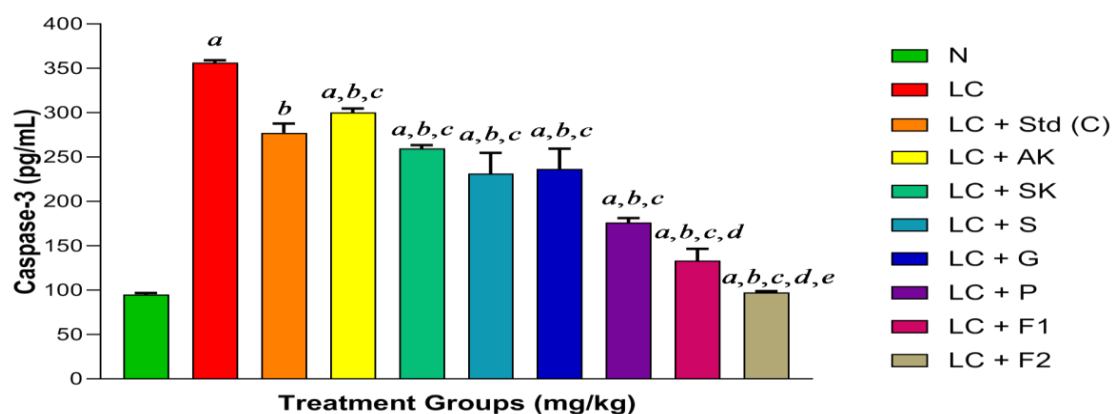


Figure. 5.24a: Effect of treatments on Caspase-3 activity in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data was considered significant if $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic

activity Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

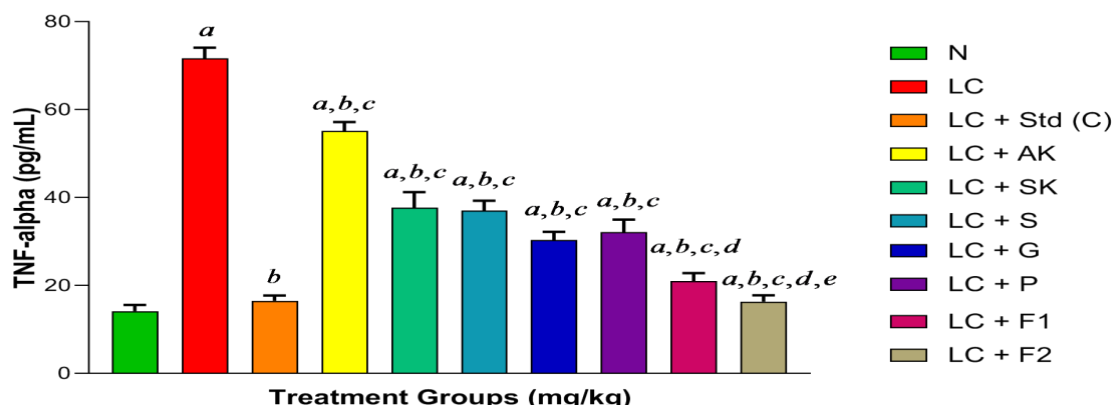


Figure. 5.24b: Effect of treatments on TNF alpha activity in a rat model of urolithiasis

Values are measured and expressed as mean \pm SEM, n=6 experimental animals/group. Observed data was considered significant if $P < 0.05$ a. when it is compared with normal control; b. when it is compared with Lithiatic Control; c. when it is compared with standard control (Cystone); d. when it is compared with LC+P; e. when it is compared with LC+F1. The obtained data was analysed by using One-Way ANOVA followed by Turkey-Kramer Multiple Comparisons test. N: Normal Control; LC: Lithiatic activity Control; AK: Apamarg Kshar; SK: Surya Kshar, S: Sphatika, G: Gokshur (aqueous extract), P: Punarnava (aqueous extract); F1: Prepared tablet formulation (Low dose) (300 mg/Kg), F2: Prepared tablet formulation (High dose) (600 mg/Kg); C: Cystone (750 mg/Kg).

5.13.10 HISTOPATHOLOGICAL STUDIES

The histopathological report shows that the kidney damage (Table 5.42) that occurred due to ethylene glycol induced urolithiasis was found to be successfully treated in groups IV-X. However, an improvement was found a little less in the case of rats treated mono-mineral / herbal/mineral drugs (group IV-VIII) as compared to prepared herbomineral formulation (group IX and X). The in-vivo antiurolithiatic activity of the prepared herbomineral formulation seems to be working as antiurolithic in male wistar rats. Histopathological findings of the kidney under a light microscope of different animal groups were shown in Fig. no. 5.25

Table No. 5.42 Histological report for Anti-urolithic herbomineral drug evaluation

Group	Remark
I: Normal control	No signs of deposition of calcium oxalate in the nephron It represents normal morphological architecture of nephron
II: Lithiatic control	Shrunk glomeruli and severe tubular damage observed. More calcification on surface of the renal parenchyma and the papillary tip of kidney was recorded. Intratubular and interstitial crystals were observed on the cortex Widespread interstitial inflammation and dilatation of proximal tubules was also observed. Presence of large number of crystal material.
III: Cystone (standard control)	Section revealed much clearer evidences of restoration of tissue damage in all aspects No crystal material appears. Architecture of the tissues appears normal .
IV: Apamarg Kshar	Reduced renal damage, inflammation was recorded. Glomerular development was observed.
V: Surya kshar	Lesser calcification on surface of the renal parenchyma as well as papillary tip of kidney was recorded.

	Intratubular and interstitial crystals were observed on the cortex
VI: Sphatika	Widespread interstitial inflammation and dilatation of proximal tubules was also observed
VII: Gokshur (aqueous extract)	Decreased renal epithelial damage, inflammation. Restored normal morphology of glomerular morphology
VIII: Punarnava (aqueous extract)	Reduced renal injuries and normalized renal architecture observed. Crystal deposits were also visibly small and less abundant compared to those in the untreated kidneys.
IX: Prepared tablet formulation (Low dose)	Restoration of tissue structure looks lesser than group IX. Reduction in interstitial inflammation and dilatation of proximal tubules was observed. It looks closer to normal architecture of nephron
X Prepared tablet formulation (High Dose)	More restoration of tissue structure was observed. There was no congestion in blood vessels, recovery of distended tubules, and increased cellularity between tubules was observed. It looks very closer to normal architecture of nephron.

5.10.1 HISTOPATHOLOGY STUDY IMAGES

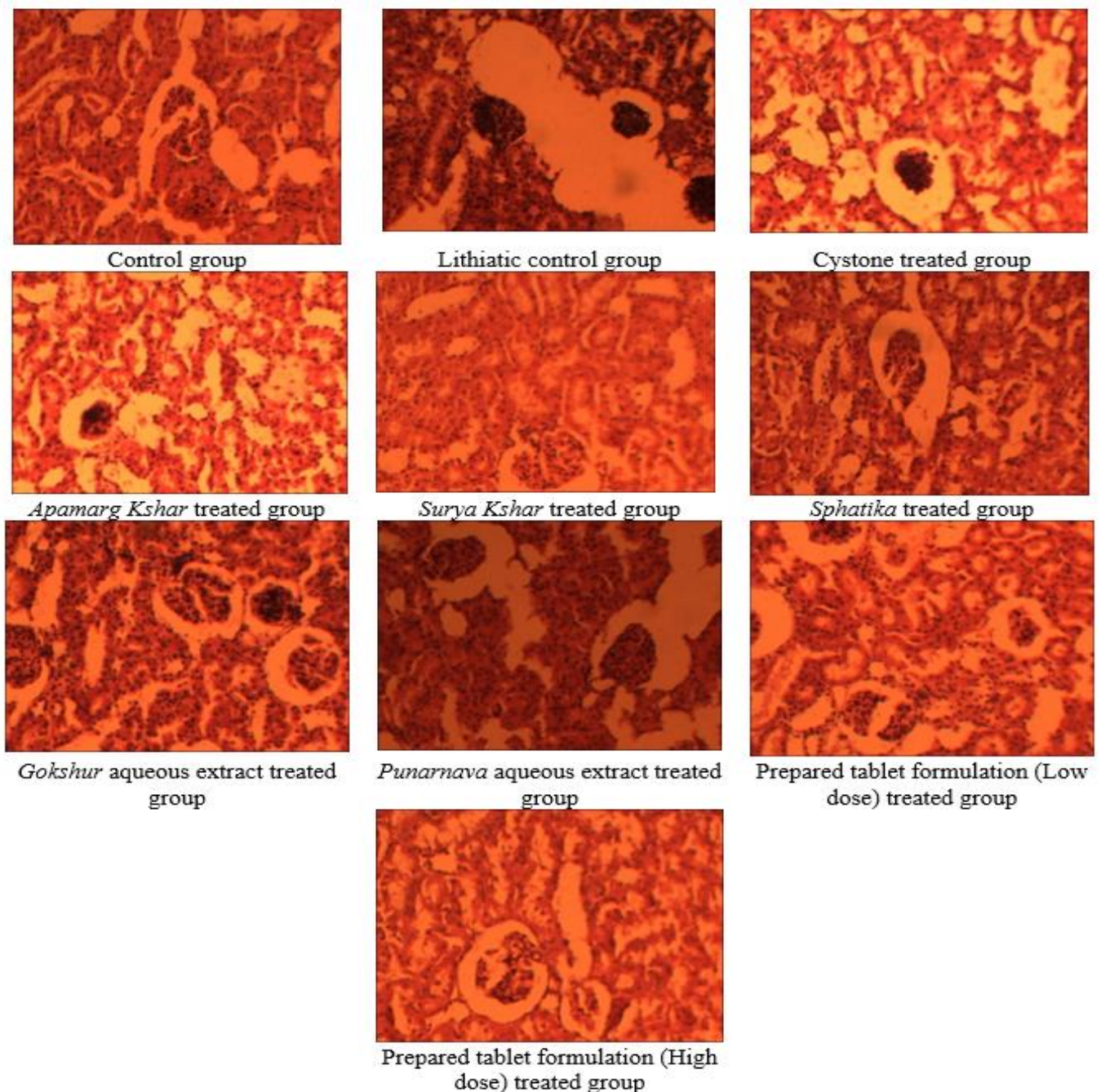


Fig. no. 5.25 Effect of drug treatment on kidney of urolithiatic rats

Calcium oxalate stone buildout in kidneys with a multifactorial process and in it various etiological factors are involved. Calcium oxalate crystal deposition leads to the cellular injury mediated by lipid peroxidation through free oxygen radical generation. Studies revealed that these cellular injuries favour the events of calcium oxalate retention in renal tubules which is significant for further stone development. Antioxidant property of prepared formulation was found protective against such oxidative injury due to calcium oxalate crystal deposition. The prepared formulation altered the urine output in a dose dependant manner and maximum diuresis observed at 600 mg/kg when compared with normal control group the hypocalciuric and diuretic effects of prepared formulation might be help to reduce urinary super saturation of calcium salts and thus it prevents the further stone formation. After 28 days, administration of EG + AC showed larger and aggregated crystals in urolithic control animals compared to the prepared formulation and Cystone-treated groups. Prepared formulation reduced the polyuria associated with lithogenic treatment as compared to urolithic control groups. As per the earlier reports, stone induction treatment leads an increase in oxalate and decrease in Calcium ion excretion. This is confirmed in the present study in which urolithic control animals showed the similar effect, and prepared formulation and Cysteine treated groups are able to reverse this effect. The present investigation thus reports the significant antiurolithic potential of prepared formulation at 300 and 600 mg/kg doses by the combination of antioxidant, diuretic, and hypocalciuric effects. The results of the formulation were found significant as compared with Cystone which is a standard drug available in the market used in the treatment of urolithiasis.

CHAPTER 6

SUMMARY AND CONCLUSION

6.1 SUMMARY

Urolithiasis is the 3rd most common ailment of the urinary tract and it is distressing human beings since ancient times. The recurrence rates of urolithiasis are continuing to be high and it was observed in trends that one out of every two patients is developing such stone within 5 years of treatment. Urolithiasis (*Mutrashmari*) is an age-old syndrome that possesses not only multifactorial etiological origins but also often associated with a high rate of remission-rebound frequency during its management time is kidney stone (termed as urolithiasis). In Ayurveda, this syndrome is called as *Mutrashmari* and it is one of the most distressing syndromes among the group of urinary disorder conditions attached to human beings till today. Even *Acharya Sushruta*, a pioneer in the art of surgery, described the root causes and management of urolithiasis. In modern medical practice, plenty of management/treatment options are available which starts from the use of uresis-promoting agent to dietary or nutritional supplement intake. Approaches developed by amalgamating the ayurvedic concept/principle with modern medical practice is a promising strategy and even welcome addition for urolithiasis management.

Urolithiasis (*Mutrashmari*) is documented in classical texts of *Ayurveda* as one of the resultant product of mechanical life style of modern day's human beings. Primarily any abnormalities in *vyana vayu*, *samana vaya*, *pachaka pitta*, *ranjaka pitta* and *apana vayu* observed in human body caused due to dietary, habitual, injury and bacterial factors result in Urolithiasis (*Mutrashmari*). Although plenty of treatment options are available in modern medical practice, the drugs used might usually have either side effects or addict promoting activities. On the other hand, the *Ayurvedic* drug principle gives guidelines or directions to treat this syndrome in a confidential manner and at the same time, it increases the quality of life of an individual by adapting the modalities such as amalgamating the *Ayurvedic* concept with modern medical/pharmaceutical practice. Moreover, non-availability of specific targeting approaches for drugs to treat against urolithiasis in conjunction with the possible remission-rebound frequency rates during treatment always creates uncertainty in the minds of infected human populations. These incidents or scenario creates not only panic situations among the patient population but also puts pressure on clinicians who prescribe medicines to manage kidney stone conditions. It looks highly positive to see the promising results of mono-or poly-herbomineral formulation (developed using the principles of modern medical or pharmaceutical practice) in treating kidney stones and thus the UTIs. However, an in-depth understanding about the

anatomical and physiological visions of ayurvedic principles and their subsequent amalgamation or sandwiching with the modern medical/pharmaceutical practice should unequivocally provide a new insight or way for the management of Urolithiasis (*Mutrashmari*). The causes of renal disorders are vitiation of *Mutravahasrotas* (channels carrying urine). The probable causes of Urolithiasis are *Ati-vyayam* (excessive exercise), *Tikshna aushadha* and *aahara seevan* (intake of sharp medicine and dry food), *Rukshamadya prasanga* (excessive consumption of a dry variety of alcohol), *Nitya druta prishthayanat* (riding on the back of fast-moving animals regularly), *Anupa matsya* (intake of the flesh of wetland fish), *Adhyashana & Ajirnat* (eating before the digestion of previous meal & indigestion), *Katiskandha tidharanat* (weight lifting), *Mutravaha srotodushti* (urinary tract infection), *Mutravega nigraha / Mutra vegaavarodha* (suppression of urge) and *Abhikshata* (a person suffering from an injury to the organs of *Mutravaha srotasa*). There are various theory for this disease but as per modern science, urolithiasis is going to be occur by matrix nucleation, precipitation-crystallization and due to the absence of inhibitors.

The preliminary phytochemical study of *Apamarg*, *Punarnava* and *Gokshur* revealed the presence of tannins, glycoside, saponin, protein, alkaloids, steroids, phenols and flavonoid. The drug sample was subjected to physicochemical evaluation parameters like foreign matter, total ash, moisture content, alcohol and water-soluble extractives and the results were found to be within the limits. The ingredients and excipients were thoroughly mixed and subjected to preformulation studies. Carr's index, Hausner's ratio and angle of repose were found to be satisfactory. The compressed tablets were evaluated for post-compression parameters like shape, thickness, hardness, friability, weight variation, and disintegration time. Prepared tablets were able to comply with the pharmacopoeial standards and no significant changes were observed. FTIR study of *surya kshar* raw sample and purified sample compared with standard Potassium Nitrate showed the characteristic peak at 1363.72 confirming the presence of potassium nitrate in the sample. Crystal growth inhibition started at a concentration of 50 µg/ml but in 550 µg/ml of apamarg kshar showed maximum inhibition of 82%. Similarly, at 650 µg/ml concentration *Sphatika*, *Gokshur* water extract, *Punarnava* water extract, *Surya Kshar* showed maximum inhibition of 53.90%, 58.24%, 50%, 54.26% respectively. The prepared tablets show the maximum effect at 650 µg/ml concentration 87.16%. as compared to individual drugs which may be considered as synergism based on in-vitro investigation however the final synergistic response will be concluded after animal studies. *Apamarga Kshar*, *Sphatika*, *Goksura* aqueous extract, *Punarnava* aqueous extract, *Surya kshar* and prepared formulation were analysed for the heavy metal, Aflatoxins, Microbial load and Test

for specific Pathogen. All the analysed samples were found within the limits prescribed by The Ayurvedic Pharmacopoeia of India. Tablets were stable over a period of 6 months when exposed to accelerated stability studies.

An increase in the weight of the kidney was observed in the diet control group of experimental animals when it is compared with a normal control group. We found that feeding with ethylene glycol leads to a significant decrease in urine pH when it is compared with the normal control group. The feeding of an ethylene glycol-rich diet resultant a statistically and significant rise in blood urea and serum creatinine levels. Treatment with monotherapy with herbal/herbomineral drugs failed to attenuate these factors to a significant extent. Feeding of ethylene glycol diet for 28 days lead to a significant increase in lipid peroxidation and nitric oxide levels in kidney tissue homogenate in comparison to the normal control group. Treatment with herbomineral drug (F1 and F2) significantly attenuated lipid peroxidation. Further, administration of a higher dose (F2) significantly enhanced the level of total glutathione and glutathione peroxidase activity. Microscopic examination of kidney sections from the normal control group showed normal cytoarchitecture. Sections from diet control group showed the presence of a large number of crystal material containing tubules, especially in the cortical region; dilatation of tubules (due to stones) along with necrosis of the tubular epithelium. Sections from VC treated group showed a moderate decrease in the size and number of crystals containing tubules and mild tubular epithelial necrosis, while the marked decrease of these features are observed in F2 treated group.

The feeding of ethylene glycol leads to hypercalciuria and increased phosphate renal excretion. Supplementation with various experimental dose able to prevent the change in urine calcium, oxalate, and phosphate level excretion through urine with dose-dependent manner. It was observed during the treatment that increased number of calcium oxalate crystals deposition leads the formation of kidney stones in the experimental rats which was significantly reverted by the various experimental dose. The herbomineral supplementation are able to prevent the impairment of renal functions. In this study novelty lies in the choice and combination of herbal/herbomineral formulation i.e. *Apamarg kshar*, *Spahitika*, *Surya kshar*, aqueous extract of *Gokshur* and *punarnava*.

Prepared tablet formulation at high dose (600 mg/kg) treated animals showed its effective management of urolithiasis while compared with other experimental groups. A part of that the prepared formulation also shows the remarkable antioxidant properties which support the effective management of urolithiasis. Moreover, a further study is required to identify and

explore the various active principles which are responsible for this and to know the exact mechanism of action of formulation involved in the observed activity profile.

6.2 CONCLUSION

- On the basis of DOE optimized batch was composed of *Sphatika* 50mg, *Surya kshar* 50mg, *Apamarg kshar* 50mg, *Gokshur* (aqueous extract) 50mg, *Punarnava* (aqueous extract) 50mg, *Acacia* powder 45mg and Talc 5mg. Further, Prepared tablet were evaluated for all the all the characteristic parameters and observed under the prescribed range of Ayurvedic compendial. The formulation was found stable till 6 months when it was subjected to stability studies.
- *In-vitro* antiurolithic activity of formulation showed 3-fold increase in a protection with respect to sum of individual response. In *In-vivo* studies dose dependent activity was recorded at dose level of 300 and 600 mg/kg of formulation. Various biochemical, oxidative biomarkers and inflammatory mediator were also improved by administration of 300 and 600 mg/kg of formulation. The response for the treatment of urolithiasis was finally confirmed by histopathological studies as the treatment groups showed significant regeneration and repair of renal tissues. Hence, the formulation exhibit antiurolithiatic effect probably due to its antioxidant and diuretic property.

CHAPTER 7

REFERENCES

1. Sen, S. and R. Chakraborty, *Herbal Medicine in India: Indigenous Knowledge, Practice, Innovation and its Value* . Springer Nature. 2019.
2. Abhishek Chatterjee, et al., *In-Vitro Anti-inflammatory and Anti-oxidant Activities of Hinguleswara Rasa-Based Herbomineral Formulations*. Asian J Pharm Clin Res, 2018. 11 (Special issue 2): p. 24-27.
3. Mishra, L.C., *Scientific basis for Ayurvedic therapies* . CRC press. 2003.
4. Vidyanath, R., *A Hand Book of Astanga Sangraha*. Chaukhamba Surbharati Prahashan, Varanasi, India, 2006.
5. Samal, J., *The concept of public health in Ayurveda*. International Ayurvedic Medical Journal, 2013. 1(2): p. 1-5.
6. Savitha D Bhat, B.A., Rabinarayan Acharya, *Critical analysis of herbs acting on Mutravaha srotas*. AYU, 2010. 31(2): p. 167-169.
7. Sastri, K., *Charak Samhita by Agnivesa*. Chaukhambha Bharti Academy, Varansi, 2013.
8. Nilore, P., *The role of inorganic elements in the human body*. Nucleus, 1984. 21(4): p. 3-23.
9. Brunton, L.L., B.A. Chabner, and B.C. Knollmann, *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12e*. Pharmacotherapy of the Epilepsies, Valproic Acid, 2011.
10. Satoskar, R.S., N. Rege, and S. Bhandarkar, *Pharmacology and Pharmacotherapeutics-E-Book* . Elsevier Health Sciences. 2015.
11. Tripathi, B., *Madhava nidana by madhavakara*. Chaukhambha Surbharati Prakashan, Varanasi, 1996.
12. Yadavji, A.T., *Sushruta Samhita by Sushruta*. Krishnada Academy, Varanasi, 1980.
13. Baghel, D., et al., *Amalgamation of Ayurvedic concept with modern medical practice to manage kidney stone (Urolithiasis): An abbreviated review*. Indian Drugs, 2018. 55 (11): p. 7-18.
14. Brunton, L., B. Knollmann, and R. Hilal-Dandan, *organizadores. Goodman & Gilman's the pharmacological basis of therapeutics* . McGraw Hill Medical . New York. 2018.
15. Tripathi, K., *Essentials of medical pharmacology* . JP Medical Ltd. London. 2013.
16. Ferraro, P.M., et al., *History of kidney stones and the risk of coronary heart disease*. Jama, 2013. 310(4): p. 408-415.
17. Kamel, K.S., et al., *Studies to identify the basis for an alkaline urine pH in patients with calcium hydrogen phosphate kidney stones*. Nephrology Dialysis Transplantation, 2007. 22(2): p. 424-431.
18. Worcester, E.M., et al., *Renal function in patients with nephrolithiasis*. The Journal of urology, 2006. 176(2): p. 600-603.
19. Harsh, M., *Textbook of pathology* . Jaypee Brothers Medical Publishers (P) Ltd . New Delhi. 2010.
20. Soundararajan, P., et al., *Effect of Aerva lanata on calcium oxalate urolithiasis in rats*. Indian Journal of Experimental Biology, 2006. 44(12): p. 981-986.
21. Mazdak, H., M.M. Nikkar, and L. Ghanea, *Evaluation of the Raphanus sativus effect on urinary pH*. Journal of Research in Medical Sciences 2007. 12(2): p. 58-61.
22. Hiatt, R.A. and G.D. Friedman, *The frequency of kidney and urinary tract diseases in a defined population*. Kidney international, 1982. 22(1): p. 63-68.

23. Jones, T.C., G.C. Hard, and U. Mohr, *Urinary system* . Springer Science & Business Media . Berlin. 2013.
24. Shanmugapriya, J. and S. Kumar, *A prospective study on quality of life in patients with urinary calculi*. Asian Journal of Pharmaceutical and Clinical Research, 2017: p. 191-193.
25. A.I. Nouri, M.A.H., *Assessment of kidney stone disease prevalence in a teaching hospital*. African Journal of Urology, 2018. 24(3): p. 180-185.
26. Fredric L Coe, A.E., Elaine Worcester, *Kidney Stone disease*. The Journal of clinical investigation, 2005. 115(10): p. 2598-2608.
27. Manjula, K., et al., *Herbal remedy for urinary stones : Vegetables and human health*. Scientific publishers, 2015: p. 454-468.
28. Manzoor, M.A., et al., *Investigation on growth and morphology of in vitro generated struvite crystals*. Biocatalysis and Agricultural Biotechnology, 2019. 17: p. 566-570.
29. Ringertz, H., *The molecular and crystal structure of uric acid*. Acta Crystallographica, 1966. 20(3): p. 397-403.
30. Romero, V., H. Akpınar, and D.G. Assimos, *Kidney stones: a global picture of prevalence, incidence, and associated risk factors*. Reviews in urology, 2010. 12(2-3): p. e86.
31. Moe, O.W., *Kidney stones: pathophysiology and medical management*. The lancet, 2006. 367(9507): p. 333-344.
32. Stamatelou, K.K., et al., *Time trends in reported prevalence of kidney stones in the United States: 1976–1994*. Kidney international, 2003. 63(5): p. 1817-1823.
33. Pearle, M.S., et al., *Medical management of kidney stones: AUA guideline*. The Journal of urology, 2014. 192(2): p. 316-324.
34. Khan, F., M. Haider, and M. Singh, *A comprehensive review on kidney stones, its diagnosis and treatment with allopathic and ayurvedic medicines*. Urology & Nephrology Open Access Journal, 2019. 7(4): p. 69-74.
35. Thongprayoon, C., A.E. Krambeck, and A.D. Rule, *Determining the true burden of kidney stone disease*. Nature Reviews Nephrology, 2020. 16(12): p. 736-746.
36. Asplin, J., et al., *Supersaturation and stone composition in a network of dispersed treatment sites*. The Journal of urology, 1998. 159(6): p. 1821-1825.
37. Khan, S.R., et al., *Kidney stones*. Nature Reviews Disease Primers, 2016. 2(1): p. 1-23.
38. Selvam, R., et al., *Effect of A. lanata leaf extract and VEDIUPPU CHUNNAM on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria*. Pharmacological Research, 2001. 43(1): p. 89-93.
39. Moe, O.W., *Uric acid nephrolithiasis: proton titration of an essential molecule?* Current opinion in nephrology and hypertension, 2006. 15(4): p. 366-373.
40. Bhasin, B., H.M. Ürekli, and M.G. Atta, *Primary and secondary hyperoxaluria: understanding the enigma*. World journal of nephrology, 2015. 4(2): p. 235.
41. Hoppe, B., et al., *Diagnostic and therapeutic approaches in patients with secondary hyperoxaluria*. Front Biosci, 2003. 8(1-3): p. e437-e443.
42. Awasthi, M., S. Malhotra, and R. Modgil, *Dietary habits of kidney stone patients of Kangra district, Himachal Pradesh, North India*. Journal of Human Ecology, 2011. 34(3): p. 163-169.
43. Mandel, N.S. and G.S. Mandel, *Urinary tract stone disease in the United States veteran population. II. Geographical analysis of variations in composition*. The Journal of urology, 1989. 142(6): p. 1516-1521.
44. Silverio, A.A., et al., *The potential of at-home prediction of the formation of urolithiasis by simple multi-frequency electrical conductivity of the urine and the comparison of its*

- performance with urine ion-related indices, color and specific gravity. *Urolithiasis*, 2016. 44(2): p. 127-134.
45. Tiselius, H.-G., *A hypothesis of calcium stone formation: an interpretation of stone research during the past decades*. *Urological research*, 2011. 39(4): p. 231-243.
 46. Jung, H., et al., *Urolithiasis: evaluation, dietary factors, and medical management: an update of the 2014 SIU-ICUD international consultation on stone disease*. *World journal of urology*, 2017. 35(9): p. 1331-1340.
 47. Skolarikos, A., *Medical treatment of urinary stones*. *Current opinion in urology*, 2018. 28(5): p. 403-407.
 48. Brisbane, W., M.R. Bailey, and M.D. Sorensen, *An overview of kidney stone imaging techniques*. *Nature Reviews Urology*, 2016. 13(11): p. 654.
 49. Hanna, L. and M. Bultitude, *The assessment and management of renal and ureteric stones*. *Trends in Urology & Men's Health*, 2019. 10(5): p. 8-12.
 50. Vermeulen, C., E. Lyon, and F. Fried, *On the nature of the stone-forming process*. *The Journal of urology*, 1965. 94(2): p. 176-186.
 51. Sivananda, S., *Practice of Ayurveda*. Divine Life Society. Rishikesh. 2006.
 52. Krishna, S., K. Dinesh, and P. Nazeema, *Globalizing Ayurveda-Opportunities and Challenges*. *International Journal of Health Sciences and Research*, 2020. 10(3): p. 55-68.
 53. Patwardhan, K. and S. Kumar, *A proposal for the revival and advancement of Ayurveda education*. *Journal of Research and Education in Indian Medicine*, 2014. 20(4): p. 137-140.
 54. Murthy, A. and R. Singh, *The concept of psychotherapy in ayurveda with special reference to satvavajaya*. *Ancient science of life*, 1987. 6(4): p. 255.
 55. Shea, B., *Handbook of Chinese Medicine and Ayurveda: An Integrated Practice of Ancient Healing Traditions*. Simon and Schuster. New York. 2018.
 56. Paul, S. and A.K. Jain, *An Epidemiological Study on Mutrakricchra WSR to Etiological Consideration*. *Journal of Drug Delivery and Therapeutics*, 2019. 9(4-A): p. 305-309.
 57. ACHARYA, R., *A Pharmacotherapeutic Analysis of Herbal Drugs Indicated in Mutrakricchra (Dysuria)*. *J. Res. Educ. Indian Med*, 2010. 16(1-2): p. 7-17.
 58. Klein, R.D. and S.J. Hultgren, *Urinary tract infections: microbial pathogenesis, host-pathogen interactions and new treatment strategies*. *Nature Reviews Microbiology*, 2020: p. 1-16.
 59. Eknoyan, G., *History of urolithiasis*. *Clinical reviews in bone and mineral metabolism*, 2004. 2(3): p. 177-185.
 60. Lopez, M. and B. Hoppe, *History, epidemiology and regional diversities of urolithiasis*. *Pediatric nephrology*, 2010. 25(1): p. 49.
 61. Smith, L.H., *The medical aspects of urolithiasis: an overview*. *The Journal of urology*, 1989. 141(3): p. 707-710.
 62. Kalyani Divakar, A.P., SB Chandrasekhar, SB Dighe, Goli Divakar, *Protective effect of the hydro-alcoholic extract of Rubia cordifolia roots against ethylene glycol induced urolithiasis in rats*. *Food and Chemical Toxicology*, 2010. 48(4): p. 1013-1018.
 63. Kavouras, S.A., et al., *Urine osmolality predicts calcium-oxalate crystallization risk in patients with recurrent urolithiasis*. *Urolithiasis*, 2021: p. 1-7.
 64. Singh, K.B. and S. Sailo, *Understanding epidemiology and etiologic factors of urolithiasis: an overview*. *Scientific Visualization*, 2013. 13(4): p. 169-174.
 65. Shah, J. and H. Whitfield, *Urolithiasis through the ages*. *BJU international*, 2002. 89(8): p. 801-810.
 66. Badr, M., *The history of urology in ancient Egypt*. *The Journal of the International College of Surgeons*, 1963. 39: p. 404-413.

67. Salem, M.E. and G. Eknoyan, *The kidney in ancient Egyptian medicine: where does it stand?* American journal of nephrology, 1999. 19(2): p. 140-147.
68. Ghazwan Butrous, B.M., Magdi Yacoub, *The lamp of medicine of Ancient Egypt is still burning*. Global Cardiology Science and Practice, 2020. 2020(1): p. 1-6.
69. Acharya, Y., *Sushruta Samhita of Sushruta, Sutra Sthana*. Ch, 2008. 45: p. 207.
70. Bhishagratna, K.L., *An English Translation of the Sushruta Samhita*. 1963: Chowkhamba Sanskrit Series Office . Varanasi.
71. Eknoyan, G. *Beginnings—The Kidney and Nephrology in Ancient Mesopotamian Culture* . Wiley Online Library . New Jersey. in *In Seminars in dialysis*. 2016.
72. Powell, M.A., *Drugs and pharmaceuticals in ancient Mesopotamia*, in *The healing past*. 1993, Brill. p. 47-67.
73. Saitoh, H., *Descriptions of urinary stone in the Hippocratic collection*. Nihon Hinyokika Gakkai zasshi. The Japanese journal of urology, 2005. 96(6): p. 632-639.
74. Marketos, S.G., *Hippocratic medicine and nephrology*. American journal of nephrology, 1994. 14(4-6): p. 264-269.
75. Modlin, M., *A history of urinary stone*. South African Medical Journal, 1980. 58(16): p. 652-655.
76. Dimopoulos, C., et al., *Hippocrates: founder and pioneer of urology*. British Journal of Urology, 1980. 52(2): p. 73-74.
77. Riches, E., *The history of lithotomy and lithotripsy*. Annals of the Royal College of Surgeons of England, 1968. 43(4): p. 185.
78. Curhan, G.C., et al., *Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women*. Annals of internal medicine, 1997. 126(7): p. 497-504.
79. Curhan, G.C., et al., *Beverage use and risk for kidney stones in women*. Annals of internal medicine, 1998. 128(7): p. 534-540.
80. Curhan, G.C., et al., *Prospective study of beverage use and the risk of kidney stones*. American journal of epidemiology, 1996. 143(3): p. 240-247.
81. Sen, G., *Bhaishajya ratnavali, Hindi Commentary by Ambikadutta Shastri*. Chaukhamba Sanskrit Sansthan, Varanasi 2002.
82. Tripathi, B., *Sharangadhara samhita*. Chaukhamba Surabharati Prakashan, Varanasi, 1994.
83. Boyce, W.H. and N.M. Sulkin, *Biocolloids of urine in health and in calculous disease. III. The mucoprotein matrix of urinary calculi*. The Journal of clinical investigation, 1956. 35(10): p. 1067-1079.
84. Butt, A.J., *Role of protective urinary colloids in prevention of renal lithiasis*. The Journal of urology, 1952. 67(4): p. 450-459.
85. Tanihata, I., et al., *Measurements of interaction cross sections and nuclear radii in the light p-shell region*. Physical Review Letters, 1985. 55(24): p. 2676.
86. Francis, M.D., et al., *Diphosphonates inhibit formation of calcium phosphate crystals in vitro and pathological calcification in vivo*. Science, 1969. 165(3899): p. 1264-1266.
87. Chuneekar, K.C. and G. Pandey, *Bhavprakash nighantu*. Chukhamba bharti academy, Varanasi, 2004.
88. Teewari, P., *Kashyapa samhita*. Chaukhamba Sanskrit samsthan, Varanasi, 2007.
89. Curhan, G.C., *Epidemiology of stone disease*. Urologic Clinics of North America, 2007. 34(3): p. 287-293.
90. Pak, C.Y., M.I. Resnick, and G.M. Preminger, *Ethnic and geographic diversity of stone disease*. Urology, 1997. 50(4): p. 504-507.
91. Taylor, E.N., M.J. Stampfer, and G.C. Curhan, *Obesity, weight gain, and the risk of kidney stones*. Jama, 2005. 293(4): p. 455-462.

92. Kramer, H.J., et al., *The association between gout and nephrolithiasis in men: The Health Professionals' Follow-Up Study*. *Kidney international*, 2003. 64(3): p. 1022-1026.
93. Taylor, E.N., M.J. Stampfer, and G.C. Curhan, *Diabetes mellitus and the risk of nephrolithiasis*. *Kidney international*, 2005. 68(3): p. 1230-1235.
94. Atan, L., et al., *High kidney stone risk in men working in steel industry at hot temperatures*. *Urology*, 2005. 65(5): p. 858-861.
95. Taylor, E.N., M.J. Stampfer, and G.C. Curhan, *Dietary factors and the risk of incident kidney stones in men: new insights after 14 years of follow-up*. *Journal of the American Society of Nephrology*, 2004. 15(12): p. 3225-3232.
96. Ziemba, J.B. and B.R. Matlaga, *Epidemiology and economics of nephrolithiasis*. *Investigative and clinical urology*, 2017. 58(5): p. 299-306.
97. Ciftcioglu, N., et al., *Nanobacteria: an infectious cause for kidney stone formation*. *Kidney international*, 1999. 56(5): p. 1893-1898.
98. Ciftcioglu, N., et al., *Association between Randall's plaque and calcifying nanoparticles*. *International journal of nanomedicine*, 2008. 3(1): p. 105.
99. Chan, J.Y., et al., *Predictors of urosepsis in struvite stone patients after percutaneous nephrolithotomy*. *Investigative and clinical urology*, 2021. 62(2): p. 201.
100. Milo, S., et al., *A small-molecular inhibitor against Proteus mirabilis urease to treat catheter-associated urinary tract infections*. *Scientific reports*, 2021. 11(1): p. 1-15.
101. Griffith, D.P., *Urease stones*. *Urological research*, 1979. 7(3): p. 215-221.
102. Kosikowska, P. and Ł. Berlicki, *Urease inhibitors as potential drugs for gastric and urinary tract infections: a patent review*. *Expert opinion on therapeutic patents*, 2011. 21(6): p. 945-957.
103. Samuell, C. and G. Kasidas, *Biochemical investigations in renal stone formers*. *Annals of clinical biochemistry*, 1995. 32(2): p. 112-122.
104. Radford, A.D., et al., *Feline calicivirus*. *Veterinary research*, 2007. 38(2): p. 319-335.
105. Osborne, C.A., M.K. John, and P.L. Jody, *Feline lower urinary tract disorders: definition of terms and concepts*. *Veterinary Clinics: Small Animal Practice*, 1996. 26(2): p. 169-179.
106. Fabricant, C.G., *Herpesvirus induced feline urolithiasis—A review*. *Comparative immunology, microbiology and infectious diseases*, 1979. 1(3): p. 121-134.
107. Tripathi, I., *Gada nigraha*. Prayogakhanda Asavaadhikara. Verse, 2005: p. 196-201.
108. Hridaya, A., *of Vagbhata*. Edited with the Vidyotini Hindi commentary, by Kaviraja Atrideva Gupta, Chaukambha Sanskrit Sansthan, Varanasi, 2000.
109. Shastri, B., *Yogaratanakara*. Vidyotini Hindi Tika by Vd Laxmipati Shastri Ayurvedacharya, Nityaprakvrutti prakara, verse, 2005(104).
110. Tripathi, J. and B. Shastri, *Chakra Dutta*. 1983, Chaukhambha Sanskrit Series, Varanasi.
111. Hyun, J.S., *Clinical significance of prostatic calculi: a review*. *The world journal of men's health*, 2018. 36(1): p. 15-21.
112. Bruce, R.G., et al., *Urolithiasis associated with the protease inhibitor indinavir*. *Urology*, 1997. 50(4): p. 513-518.
113. Sharma, S., *Rasatarangini*. 11 ed. 2004: Motilal Banarsidas, Delhi.
114. Mattle, D. and B. Hess, *Preventive treatment of nephrolithiasis with alkali citrate—a critical review*. *Urological research*, 2005. 33(2): p. 73-79.
115. Paterson, R., et al., *Evaluation and medical management of the kidney stone patient*. *Canadian Urological Association Journal*, 2010. 4(6): p. 375.
116. Alelign, T. and B. Petros, *Kidney stone disease: an update on current concepts*. *Advances in urology*, 2018. 2018.

117. Bank, D. *Adjunctive treatment with thiazide diuretics*. 2019 [cited 2019 15-11-2019]; Available from: <https://www.drugbank.ca/drugs/DB00594>
118. Bank, D. *Uric acid lithiasis, and/or nephropathy*. 2019 [cited 2019 15-11-2019]; Available from: www.drugbank.ca/drugs/DB00437.
119. Bank, D. *Kidney diseases*. 2019 [cited 2019 15-11-2019]; Available from: www.drugbank.ca/drugs/DB01432.
120. Bank, D. *Gall stone diseases*. 2019 [cited 2019 15-11-2019]; Available from: www.drugbank.ca/drugs/DB02659
121. Bank, D. *Kidney diseases*. 2019 [cited 2019 14-11-2019]; Available from: www.drugbank.ca/drugs/DB00390.
122. Bank, D. *Kidney stones*. 2019 [cited 2019 15-11-2019]; Available from: www.drugbank.ca/drugs/DB01077.
123. Bank, D. *Gall stone diseases*. 2019 [cited 2019 14-11-2019]; Available from: www.drugbank.ca/drugs/DB01095.
124. Bank, D. *Gall bladder diseases*. 2019 [cited 2019 15-11-2019]; Available from: www.drugbank.ca/drugs/DB01241.
125. Bank, D. *HIV diseases, Kidney diseases*. 2019 [cited 2019 15-11-2019]; Available from: www.drugbank.ca/drugs/DB00224
126. Bank, D. *Ailments of stone diseases*. 2019 [cited 2019 15-11-2019]; Available from: www.drugbank.ca/drugs/DB00909
127. Um, N., *Foreign Doctors at the Imam's Court: Medical Diplomacy in Yemen's Coffee Era*. Genre: Forms of Discourse and Culture, 2015. 48(2): p. 261-288.
128. El-Shanawany, M.A., et al., *Chemical constituents, anti-inflammatory, and antioxidant activities of Anisotes trisulcus*. Bulletin of Faculty of Pharmacy, Cairo University, 2014. 52(1): p. 9-14.
129. Khare, C., *Indian Medicinal Plants-An Illustrated Dictionary. 1st Indian Reprint Springer (India) Pvt. Ltd. New Delhi, 2007. 28.*
130. Shukla, P., et al., *In vitro propagation of Barleria prionitis Linn and its antibacterial activity*. International Journal of Research in Pharmaceutical Sciences, 2011. 2: p. 198-200.
131. Samuel, A.J.S.J., et al., *Ethnomedical survey of plants used by the Orang Asli in Kampung Bawong, Perak, West Malaysia*. Journal of ethnobiology and ethnomedicine, 2010. 6(1): p. 5.
132. Manilal, K. and M. Remesh, *An Analysis of the Data on the Medicinal Plants Recorded in Hortus Malabaricus . Centre for Research in Indigenous Knowledge Science and Culture*. Vol. 5-6. 2009: SAMAGRA.
133. Pushpakarani, R. and S. Natarajan, *Ethnomedicines used by Kaniyakaran tribes in Kaniyakumari district-Southern Western Ghats of Tamil Nadu, India*. Journal of Applied Pharmaceutical Science, 2014. 4(2): p. 56.
134. Lokendrajit, N., et al., *Herbal folk medicines used for urinary and calculi/stone cases complaints in Manipur*. NeBIO, 2011. 2(3): p. 1-5.
135. Samy, R.P., et al., *Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamilnadu, India*. Journal of Ethnopharmacology, 2008. 115(2): p. 302-312.
136. Prachitha, J. and K. Shanmugam, *Efficiency of raising health outcomes in the Indian States . Madras School of Economics, Chennai. 2012.*
137. Shanmugam, S., K. Rajendran, and K. Suresh, *Traditional uses of medicinal plants among the rural people in Sivagangai district of Tamil Nadu, Southern India*. Asian Pacific Journal of Tropical Biomedicine, 2012. 2(1): p. S429-S434.

138. Hussein, S. and A. Dhabe, *Ethnobotanical study of folk medicinal plants used by villagers in the Hajjah district-Republic of Yemen*. Journal of Medicinal Plants Studies, 2018. 6(5): p. 24-30.
139. Prasad, P.R.C., et al., *Folklore medicinal plants of North Andaman Islands, India*. Fitoterapia, 2008. 79(6): p. 458-464.
140. Dansi, A., et al., *Traditional leafy vegetables and their use in the Benin Republic*. Genetic Resources and Crop Evolution, 2008. 55(8): p. 1239-1256.
141. Rajendran, A., K. Ravikumar, and A. Henry, *Plant genetic resources and knowledge of traditional medicine in Tamil Nadu*. Ancient science of life, 2000. 20(1-2): p. 25.
142. Pradheeps, M. and G. Poyyamoli, *Ethnobotany and utilization of plant resources in Irula villages (Sigur plateau, Nilgiri Biosphere Reserve, India)*. Journal of medicinal plants research, 2013. 7(6): p. 267-276.
143. Pandikumar, P., N.P. Babu, and S. Ignacimuthu, *Hypoglycemic and antihyperglycemic effect of Begonia malabarica Lam. in normal and streptozotocin induced diabetic rats*. Journal of ethnopharmacology, 2009. 124(1): p. 111-115.
144. Parada, M., et al., *Ethnobotany of the Alt Emporda region (Catalonia, Iberian Peninsula): plants used in human traditional medicine*. Journal of Ethnopharmacology, 2009. 124(3): p. 609-618.
145. Gairola, S., et al., *Plants used for treatment of dysentery and diarrhoea by the Bhoja community of district Dehradun, Uttarakhand, India*. Journal of ethnopharmacology, 2013. 150(3): p. 989-1006.
146. Manikandan, P.A., *Folk herbal medicine: A survey on the paniya tribes of Mundakunnu village of the Nilgiri hills, South India*. Ancient science of life, 2005. 25(1): p. 21.
147. Agnivesa, C.S., *chapter no 7, sloka 33*. 1994, Varanasi: Chaukhambha Sanskrit Sansthan.
148. Choudhary, K., M. Singh, and U. Pillai, *Ethnobotanical survey of Rajasthan-An update*. American-Eurasian Journal of Botany, 2008. 1(2): p. 38-45.
149. Gayakvad, P., et al., *Ethno-veterinary medicinal plants of mahal village of dang district, Gujarat, India*. Research in Environment and Life Sciences, 2014. 7(2): p. 99-100.
150. Bhaskar, A. and L.R. Samant, *Traditional medication of Pachamalai Hills, Tamilnadu, India*. Global Journal of Pharmacology, 2012. 6(1): p. 47-51.
151. Dey, A. and J.N. De, *Traditional use of plants against snakebite in Indian subcontinent: a review of the recent literature*. African Journal of Traditional, Complementary and Alternative Medicines, 2012. 9(1): p. 153-174.
152. Sreeramulu, N., et al., *Ethno-botanico-medicine for common human ailments in Nalgonda and Warangal districts of Telangana, Andhra Pradesh, India*. Annals of Plant Sciences, 2013. 2(7): p. 220-229.
153. Bhardwaj, M., et al., *Insecticidal and wormicidal plants from Aravalli hill range of India*. Journal of ethnopharmacology, 2011. 136(1): p. 103-110.
154. Wagh, V.V. and A.K. Jain, *Inventory of ethnobotanicals and other systematic procedures for regional conservation of medicinal and sacred plants*. Environment Systems and Decisions, 2015. 35(1): p. 143-156.
155. Kosalge, S. and R. Fursule, *Investigation of ethnomedicinal claims of some plants used by tribals of Satpuda Hills in India*. Journal of Ethnopharmacology, 2009. 121(3): p. 456-461.
156. Udayan, P., et al., *Medicinal Plants used by the Kaadar tribes of Sholayar forest Thrissur district, Kerala*. 2005.

157. Dileep, P. and G.G. Nair, *Taxonomic and ethnobotanical studies of grasses used by tribals of Wayanad District, Kerala, South Western Ghats of India*. Journal of Global Biosciences, 2015. 4(5): p. 2212-2235.
158. Ratnam, K.V. and R. Raju, *Folk remedies for insect bites from Gundlabrahmeswaram wild life sanctuary, Andhra Pradesh*. 2008.
159. Ayyanar, M., K. Sankarasivaraman, and S. Ignacimuthu, *Traditional healing potential of Paliyars in southern India*. Ethnobotanical Leaflets, 2008. 2008(1): p. 37.
160. Mookerji, B., *Rasa Jala Nidhi . Avani Prakashan, Jhunjhunu* Vol. 5. 1984.
161. Damodar Joshi and G.P. Rao, *Rasamritam of Vaidya Jadavji Trikamji Acharya*. Chaukhamba Sanskrit Bhavan, Varanasi, 1998.
162. Demigné, C., et al., *Protective effects of high dietary potassium: nutritional and metabolic aspects*. The Journal of nutrition, 2004. 134(11): p. 2903-2906.
163. Ann Crawford, H.H., *Balancing act: Na⁺ sodium K⁺ potassium*. Nursing2019, 2011. 41(7): p. 44-50.
164. Hoon Young Choi, S.K.H., *Potassium balances in maintenance hemodialysis*. Electrolytes & Blood Pressure, 2013. 11(1): p. 9-16.
165. KEITH, N.M. and M.W. BINGER, *Diuretic action of potassium salts*. Journal of the American Medical Association, 1935. 105(20): p. 1584-1591.
166. Young, D.B., H. Lin, and R.D. McCabe, *Potassium's cardiovascular protective mechanisms*. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1995. 268(4): p. R825-R837.
167. KEITH, N.M., A.E. OSTERBERG, and H.B. BURCHELL, *Some effects of potassium salts in man*. Annals of Internal Medicine, 1942. 16(5): p. 879-892.
168. Vagbhata, A., *Rasaratna Samuccaya*. 1961: Chowkhamba Sanskrit Series, Varanasi.
169. Roqaiya, M. and W. Begum, *A review on medicinal aspect of alum in unani medicine and scientific studies*. World Journal of Pharmaceutical Research, 2015. 4(6): p. 929-940.
170. ALtaei, T.S. and R.H. Al-Jubouri, *Evaluation of the efficacy of alum suspension in treatment of recurrent ulcerative ulceration*. Journal of baghdad college of dentistry, 2005. 17(2): p. 45-48.
171. Govinda, B., *Rasa Hridaya Tantra*. 1998, Krishnadas Academy, Varanasi.
172. Vidhyalankar, S.c.b.J., *Rasendra Chudamani . Banarasidas Motilal, Delhi*. 1932.
173. Mishra, S., *Rasa Prakasha Sudhakara of Acharya Yashodhara . Chaukhambha Surbharati Prakashan, Varanasi*. 2004.
174. Dileep Singh Baghel, A.M., Saurabh Singh, Anand Kumar Chaudhary, Amit Bhatia, Shruti Chopra, *IN-Vitro Potential of Sphatika Tablet in the Management of Urolithiasis (Mutrakrichra)*. Plant Archives, 2020. 20(2): p. 1210-1216.
175. KM, V., M. Khan, and P. Sharma, *Review on Achyranthes aspera*. Journal of Pharmacy Research, 2010. 3(4): p. 714-717.
176. Prasad, P. and P. Subhaktha, *Apamarga (Achyranthes aspera Linn.) A Medico-Historical Review*. Bull. Ind. Hist. Med, 2001. 31: p. 11-24.
177. Tripathy, S., P. Seth, and R.S. Kushtwar, *Achyranthes aspera one of important medicinal plant of Indian Flora*. Innovat International Journal Of Medical & Pharmaceutical Sciences, 2017. 2(3).
178. Sharma, V. and U. Chaudhary, *An overview on indigenous knowledge of Achyranthes aspera*. Journal of Critical Reviews, 2015. 2(1): p. 7-19.
179. Shekokar, A.V. and V.P. Ukhalkar, *Critical Review on Ksharaplota and its Therapeutic Aspects*. Journal of Drug Delivery and Therapeutics, 2019. 9(1-s): p. 510-515.

180. Anonymus, *The Ayurvedic Pharmacopoeia of India*. Department of Ayush, Ministry of Health and Family Welfare, Government of India, New Delhi, Part I, 1999. Volume II: p. 20-24.
181. Sharma, P., *Shodhala Nighantu*. Chaukamba Orientalia, Varnasi, 1978.
182. Shaligrama, S., *Shaligrama nighantu*. Khemaraj Shri Krishna das Prakashan, Mumbbi, 2002.
183. Kamat, S., *Dhanvantari nighantu*. Chaukhambha Sanskrit Pratisthan, Delhi, 2002.
184. Pandey, G., *Madanpal Nighantu*. Varanasi: Chowkhambha orientalia, 2012.
185. Bapalal, V., *Nighantu Adarsh* Chaukhambha Bharati Academy, Varanasi, 1999.
186. Sharma, P.V. and G.P. Sharma, *Kaiyadeva nighantu*. Chaukhambha Orientalia, Varanasi, 1979.
187. Tripathi, I. and V. Dwivedi, *Raj Nighantu*. Chaukhamba Krishnadas Academy, Varanasi, 2006.
188. Sharma, P., *Hrdya Dipaka Nighantu and Siddhamantra*. Chaukhambha Amarabharati Prakashan, Varanasi, 1977.
189. PV, S., *Priya nighantu*. Chaukhamba Surabharati Prakashana, Varanasi, 2004.
190. Kashiraj Sharma, N.T., *Saushrut Nighantu*. Mahendra Sanskrit University, Nepal, 2001.
191. Anonymous, *Ashtanga Nighantu, e Nighantu*. Collection of Ayurvedic Lexicons, CCRAS, New Delhi, 2012.
192. Anonymous, *Madanadi Nighantu, e Nighantu*. Collection of Ayurvedic Lexicons, CCRAS, New Delhi, 2012.
193. Sharma, P., *Dravyaguna Vigyan Part-II*. Chaukhamba Bharati Academy Varanasi, 1998.
194. Dayal, R., *Fatty acid composition of Achyranthes aspera seed oil*. Journal-Oil Technologists Association of India, 2003. 35: p. 53-54.
195. Anand, M., et al., *Phytoconstituents from the Roots of Achyranthes aspera and their anticancer activity*. Chemistry of Natural Compounds, 2017. 53(1): p. 189-191.
196. Talreja, T., A. Goswami, and T. Sharma, *Preliminary phytochemical analysis of Achyranthes aspera and Cissus quadrangularis*. Journal of Pharmacognosy and Phytochemistry, 2016. 5(5): p. 362.
197. Dey, A., *Achyranthes aspera L: phytochemical and pharmacological aspects*. International journal of pharmaceutical sciences review and research, 2011. 9(2): p. 72-82.
198. Nazir, A.B., et al., *Achyranthes aspera: A Medicinal Plant of the Himalayas*. Journal of Indian Research, 2018. 6(1): p. 49-56.
199. Vetrichelvan, T. and M. Jegadeesan, *Effect of alcohol extract of Achyranthes aspera Linn. on acute and subacute inflammation*. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 2003. 17(1): p. 77-79.
200. Gokhale, A., et al., *Preliminary evaluation of anti-inflammatory and anti-arthritic activity of S. lappa, A. speciosa and A. aspera*. Phytomedicine, 2002. 9(5): p. 433-437.
201. Basu, N., N. Neogi, and V. Srivastava, *Biological investigation of Achyranthes aspera Linn. and its constituent achyranthine*. J Proc Inst Chem, 1957. 29: p. 161-165.
202. Saravanan, P., V. Ramasamy, and T. Shivakumar, *Antimicrobial activity of leaf extracts of Achyranthes aspera Linn*. Asian Journal of Chemistry, 2008. 20(1): p. 823.
203. Dhar, M., et al., *Screening of Indian plants for biological activity: I*. Indian journal of experimental biology, 1968. 6(4): p. 232.

204. Akhtar, M.S. and J. Iqbal, *Evaluation of the hypoglycaemic effect of Achyranthes aspera in normal and alloxan-diabetic rabbits*. Journal of ethnopharmacology, 1991. 31(1): p. 49-57.
205. Vidhya, R., et al., *Evaluation of antidiabetic potential of Achyranthes aspera Linn. on alloxan induced diabetic animals*. International Journal of Pharmacy and Pharmaceutical Sciences, 2012. 4(5): p. 577-580.
206. Gupta, S., et al., *Diuretic effect of the saponin of Achyranthes aspera (Apamarga)*. Indian Journal of Pharmacology, 1972. 4(4): p. 208.
207. Dutta, K.N., et al., *Herbal plants used as diuretics: a comprehensive review*. Journal of Pharmaceutical, Chemical and Biological Sciences, 2014. 2(1): p. 27-32.
208. Jayakumar, T., et al., *Experimental studies of Achyranthes aspera (L) preventing nephrotoxicity induced by lead in albino rats*. Journal of Health Science, 2009. 55(5): p. 701-708.
209. Kapoor, V. and H. Singh, *Investigation of achyranthes aspera Linn*. The Indian Journal of Pharmacology, 1967. 29: p. 285-288.
210. Gambhir, S., A. Sanyal, and N. Chowdhury, *Pharmacological study of Achyranthes aspera Linn. A preliminary report*. Indian journal of physiology and pharmacology, 1965. 9(4): p. 185-188.
211. Neogi, N., R. Garg, and R. Rathor, *Preliminary pharmacological studies on achyranthine*. Indian Journal of Pharmacy, 1970. 32(2): p. 43-46.
212. Barua, C., et al., *Antidepressant-like effects of the methanolic extract of Achyranthes aspera Linn. in animal models of depression*. Pharmacologyonline, 2009. 2: p. 587-94.
213. Hasan, S., *Pharmacological and medicinal uses of Achyranthes aspera*. International Journal of Science, Environment and Technology, 2014. 3(1): p. 123-129.
214. Zalavadiya, V., et al., *Achyranthes aspera-Plant with high Medicinal Important*. Research Journal of Pharmacology and Pharmacodynamics, 2013. 5(4): p. 266-272.
215. Edwin, S., et al., *Wound healing and antioxidant activity of Achyranthes aspera*. Pharmaceutical biology, 2008. 46(12): p. 824-828.
216. Banjare, L., A.K. Prasad, and M. Naik, *Boerhaavia diffusa from traditional use to scientific assessment-a review*. International Journal of Pharmaceutical and Biological Archive, 2012. 3(6): p. 1346-1354.
217. L. Thamizharasi, V.B., S. Venkatesan, G. Ambedkar, S. Saravanan, A. Sabaridasan, *In-Vitro Biological Activities and Characterization of Aerial Part of Boerhaavia diffusa*. World Journal of Pharmaceutical and Life Sciences, 2017. 3(9): p. 168-173.
218. Rajpoot, K. and R. Mishra, *Boerhaavia diffusa roots (Punarnava mool)–review as rasayan (rejuvenator/antiaging)*. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011. 2(4): p. 1451-1460.
219. Shikha Mishra, V.A., Praveen Kumar Gaur, Sanjay M Jachak, *Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: Boerhavia diffusa Linn*. BioMed research international, 2014. 2014.
220. Sahu, A., et al., *Phytopharmacological review of Boerhaavia diffusa Linn.(Punarnava)*. Pharmacognosy Reviews, 2008. 2(4): p. 14.
221. Bhishagratna, K.L., *An English translation of The Sushruta Samhita: based on original Sanskrit text*. 1911.
222. Mayur Chandranshu Mishra, S.P.S., Virendra Rajak, Manoj Tripathi, *Pharmacognostic Evaluation of Stem of Punarnawa (Boerhavia diffusa Linn.)*. International Journal of Recent Biotechnology, 2020. 8(4): p. 1-7.
223. Bhowmik, D., et al., *Traditional Indian herbs Punarnava and its medicinal importance*. Journal of Pharmacognosy and Phytochemistry, 2012. 1(1): p. 52-58.

224. Jaswinder Kaur, S.S., Amit Mittal, Anand Kumar Chaudhary, and D.S. Baghel, *A Synoptic Overview on Boerhavia diffusa for Its Medicinal Importance*. Plant Archives, 2020. 20(2): p. 1217-1223.
225. Apu, A.S., et al., *Phytochemical screening and in vitro bioactivities of the extracts of aerial part of Boerhavia diffusa Linn.* Asian Pacific journal of tropical biomedicine, 2012. 2(9): p. 673-678.
226. Nayak, P. and M. Thirunavoukkarasu, *A review of the plant Boerhaavia diffusa: Its chemistry, pharmacology and therapeutical potential*. The Journal of Phytopharmacology, 2016. 5(2): p. 83-92.
227. Kapil S. Patil, S.R.B., *Ethnomedicinal uses, phytochemistry and pharmacological properties of the genus Boerhavia*. Journal of ethnopharmacology, 2016. 182: p. 200-220.
228. Nair, R.V., *Controversial drug plants*. 2004: Universities Press (India) Private Limited, Hyderabad.
229. Sereena, K., I. Balachandran, and A. Shree, *Comparative Root Anatomy of Boerhavia Species*. Journal of Tropical Medicinal Plants, 2011. 12(2).
230. Saroya, A.S., *Controversial Herbal Drugs of Ayurveda*. 2013: Scientific Publishers.
231. Vaidya Gogte, V., *Ayurvedic Pharmacology and Therapeutic Uses of Medicinal Plants Dravyaguna Vigyan*. Chaukhamba Publication. New Delhi, 2009.
232. Anonymus, *The Ayurvedic Pharmacopoeia of India*. Department of Ayush, Ministry of Health and Family Welfare, Government of India, New Delhi, Part I, 1986. Volume I: p. 163-166.
233. Meena, A., et al., *A quality assessment of Boerhaavia diffusa Linn. commonly known as 'Punarnava' plant*. International Journal of Pharmacognosy and Phytochemical Research, 2010. 2(1): p. 25-8.
234. Bhope, S., et al., *RP-HPLC method for the simultaneous quantitation of boeravinone E and boeravinone B in Boerhaavia diffusa extract and its formulation*. Natural product research, 2013. 27(6): p. 588-591.
235. Pradhan, D., et al., *Isolation of major Phytoconstituents and Standardization of different extracts of Boerhavia diffusa by RP-HPLC*. Research Journal of Pharmacy and Technology, 2020. 13(1): p. 297-302.
236. Mahesh, A., et al., *Detail Study on Boerhaavia diffusa plant for its medicinal importance-A Review*. Research Journal of Pharmaceutical Sciences, 2012. 1(1): p. 28-36.
237. Goyal, B., et al., *Pharmacological potential of Boerhaavia diffusa: an overview*. International Journal of Pharmaceutical Sciences and Drug Research, 2010. 2(1): p. 17-22.
238. Hiruma-Lima, C., et al., *The juice of fresh leaves of Boerhaavia diffusa L.(Nyctaginaceae) markedly reduces pain in mice*. Journal of Ethnopharmacology, 2000. 71(1-2): p. 267-274.
239. Borrelli, F., et al., *Spasmolytic Effects of Nonprenylated Rotenoid Constituents of Boerhaavia d iffusa Roots*. Journal of natural products, 2006. 69(6): p. 903-906.
240. Murti, K., M.A. Panchal, and V. Lambole, *Pharmacological properties of Boerhaavia diffusa-a review*. International Journal of Pharmaceutical Sciences Review and Research, 2010. 5(2): p. 107-110.
241. Desai, S.K., et al., *Antistress activity of Boerhaavia diffusa root extract and a polyherbal formulation containing Boerhaavia diffusa using cold restraint stress model*. International Journal of Pharmacy and Pharmaceutical Sciences, 2011. 3(1): p. 130-132.

242. Akinnibosun, F., H. Akinnibosun, and D. Ogedegbe, *Investigation on the antibacterial activity of the aqueous and ethanolic extracts of the leaves of Boerhavia diffusa L.* Science World Journal, 2009. 4(2): p. 15-18.
243. Agrawal, A., et al., *Inhibitory effect of the plant Boerhavia diffusa l. against the dermatophytic fungus Microsporum fulvum.* Journal of environmental biology, 2004. 25(3): p. 307-311.
244. Mungantiwar, A., et al., *Adaptogenic Activity of Aqueous Extract of the roots of Boerhaavia diffusa linn.* Indian drugs, 1997. 34(4): p. 184-189.
245. Meera Sumanth, S.S.M., *Antistress, adoptogenic and immunopotentiating activity roots of Boerhaavia diffusa in mice.* International Journal of Pharmacology, 2007. 3(5): p. 416-420.
246. Mungantiwar, A., et al., *Studies on the immunomodulatory effects of Boerhaavia diffusa alkaloidal fraction.* Journal of ethnopharmacology, 1999. 65(2): p. 125-131.
247. Rawat, A., et al., *Hepatoprotective activity of Boerhaavia diffusa L. roots—a popular Indian ethnomedicine.* Journal of Ethnopharmacology, 1997. 56(1): p. 61-66.
248. Chandan, B., A. Sharma, and K. Anand, *Boerhaavia diffusa: a study of its hepatoprotective activity.* Journal of Ethnopharmacology, 1991. 31(3): p. 299-307.
249. Rajkumari Gulati, S.A., SS Agarwal, *Hepatoprotective activity of Boerhaavia diffusa Linn. against country made liquor induced hepatotoxicity in albino rats fed on controlled calorie diet.* Indian Journal of Pharmacology, 1991. 23(4): p. 264-267.
250. Mandeep Kaur, R.K.G., *Anti-convulsant activity of Boerhaavia diffusa: plausible role of calcium channel antagonism.* Evidence-Based Complementary and Alternative Medicine, 2011. 2011: p. 1-7.
251. Adesina, S.K., *Anticonvulsant properties of the roots of Boerhaavia diffusa Linnaeus.* Quarterly Journal of Crude Drug Research, 1979. 17(2): p. 84-86.
252. Akah, P. and A. Nwambie, *Nigerian plants with anti-convulsant property.* Fitoterapia, 1993. 64(1): p. 42-44.
253. Lami, N., et al., *Constituents of the roots of Boerhaavia diffusa L. III. Identification of Ca²⁺ channel antagonistic compound from the methanol extract.* Chemical and Pharmaceutical Bulletin, 1991. 39(6): p. 1551-1555.
254. M Sasi Kala, S.V.K., K Gauthaman, *Relevance of the use of Alternative Medicine for Bronchial Asthma: A review.* Journal of Young Pharmacists, 2009. 1(2): p. 184-189.
255. Srivastava, R., D. Saluja, and M. Chopra, *Isolation and screening of anticancer metabolites from Boerhavia diffusa.* Indian Journal of Medical Research, 2005. 151(supplement 1): p. S19.
256. Agrawal, A., S. Srivastava, and M. Srivastava, *Antifungal activity of Boerhavia diffusa against some dermatophytic species of Microsporum.* Hindustan antibiotics bulletin, 2003. 45(1-4): p. 1-4.
257. Awasthi, L. and H. Verma, *Boerhaavia diffusa—A wild herb with potent biological and antimicrobial properties.* Asian Agri-History, 2006. 10(1): p. 55-68.
258. Seemi Lohani, A.J., H.N. Verma, *In vivo and in vitro resistance induction in tobacco by Boerhaavia diffusa systemic resistance inducing protein and transfer of induced resistance in in vitro tobacco plants.* Biotechnology, 2007. 6(3): p. 389-392.
259. Rachh PR, R.M., Modi DC, Shah BN, Bhargava AS, Patel NM, Rupareliya MT, *In vitro evaluation of antioxidant activity of punarnava (Boerhaavia diffusa Linn.).* International Journal of Pharmaceutical Research, 2009. 1: p. 36-40.
260. Pareta, S.K., et al., *Prophylactic role of Boerhaavia diffusa in ethylene glycol induced calcium oxalate urolithiasis.* African Journal of Urology, 2011. 17(2): p. 28-36.

261. Balaji, L., D. Banji, and O.J. Banji, *Evaluation of antiurolithiatic activity of the aqueous and alcoholic extracts of roots of Boerhaavia diffusa*. Indo American Journal of Pharmaceutical Research, 2015. 5(1): p. 525-530.
262. Sawardekar, S.B. and T.C. Patel, *Evaluation of the effect of Boerhavia diffusa on gentamicin-induced nephrotoxicity in rats*. Journal of Ayurveda and integrative medicine, 2015. 6(2): p. 95.
263. Chowdhury A, S.P., *Studies on Boerhaavia diffusa Linn. Effect on diuresis and some renal enzymes*. Ann Biochem Exp Med, 1955. 15: p. 119-126.
264. Gujral, M., P. Saxena, and S. Mishra, *An experimental study of the comparative activity of indigenous diuretics*. Journal of the Indian Medical Association, 1955. 25(2): p. 49-51.
265. Haewook Han, W.P.M., Samer Nasser, *Nutritional and Medical Management of Kidney Stones*. 2019: Humana Cham, Springer Nature, Switzerland.
266. Seth RK, K.M., Chaudhary M, Singh S, Sarin JPS, *Estimation of punarnavosides, a new antifibrinolytic compound from Boerhaavia diffusa*. Indian Drugs, 1986. 23(10): p. 583-584.
267. Gholap, S. and A. Kar, *Hypoglycaemic effects of some plant extracts are possibly mediated through inhibition in corticosteroid concentration*. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2004. 59(11): p. 876-878.
268. Nalamolu, R.K., K.M. Boini, and S. Nammi, *Effect of chronic administration of Boerhaavia diffusa Linn. leaf extract on experimental diabetes in rats*. Tropical Journal of Pharmaceutical Research, 2004. 3(1): p. 305-309.
269. Bhatia, V., et al., *Antidiabetic activity of the alcoholic extract of the arial part of Boerhaavia diffusa in rats*. Recent research in Science and Technology, 2011. 3(7): p. 4-7.
270. Pari, L. and M.A. Satheesh, *Antidiabetic activity of Boerhaavia diffusa L.: effect on hepatic key enzymes in experimental diabetes*. Journal of Ethnopharmacology, 2004. 91(1): p. 109-113.
271. Evstatieva, L. and B. Tchorbanov, *Complex investigations of Tribulus terrestris L. for sustainable use by pharmaceutical industry*. Biotechnology & Biotechnological Equipment, 2011. 25(2): p. 2341-2347.
272. Thomas Gaskell Tutin, V.H.H., Norman Alan Burges, David H Valentine, *Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae)*. Vol. 4. 2006: Cambridge University Press, Cambridge, United Kingdom.
273. Mamdouh N. Samy, M.M.B., Ahmed A. Ahmed, Hanaa M. Sayed, Mohamed S. Kamel, *Pharmacognostical Studies on Flower of Tribulus terrestris L.* Journal of pharmacognosy and phytochemistry, 2012. 1(5): p. 19-23.
274. Varghese, M., S. Yadav, and J. Thomas, *Taxonomic status of some of the Tribulus species in the Indian sub-continent*. Saudi Journal of Biological Sciences, 2006. 13(1): p. 7-12.
275. Suresh Reddy Yanala, D.S., K. Kannan, *A recent phytochemical review-fruits of Tribulus terrestris Linn.* Journal of Pharmaceutical Sciences and Research, 2016. 8(3): p. 132-140.
276. Lubna Fatima, A.S., Saad Ahmed, Shabiya Sultana, *Pharmacological Activities of Tribulus terrestris Linn: A Systemic Review*. World Journal of Pharmacy and Pharmaceutical Sciences, 2014. 4(2): p. 136-150.
277. Saima Hashim, T.B., Khan Bahadar Marwat, Asad Jan, *Medicinal Properties, Phytochemistry and Pharmacology of Tribulus terrestris L. (Zygophyllaceae)* Pakistan Journal of Botany, 2014. 46(1): p. 399-404.

278. Khare, C., *Indian medicinal plants: an illustrated dictionary*. 2008: Springer Science & Business Media.
279. RP Rastogi, B.M., *Compendium of Indian medicinal plants, vol. 1*. CSIR, New Delhi. 1990.
280. Warriar, P.K., *Indian medicinal plants: a compendium of 500 species*. Vol. 5 (311). 2002: Orient Longman Private Limited, Anna Salai, Chennai.
281. NRCS, U., *The Plants Database. National Plant Data Center*, in *Natural Resources Conservation Service, United States Department of Agriculture, Baton Rouge, LA.[Online]*. Available from <http://plants.usda.gov> [11 July 2015]. 2015.
282. Saurabh Chhatre, T.N., Gauresh Somani, Divya Kanchan, Sadhana Sathaye, *Phytopharmacological overview of Tribulus terrestris*. *Pharmacognosy Reviews*, 2014. 8(15): p. 45-51.
283. Dinesh Kumar Patel, D.L., Rajesh Kumar, Siva Hemalatha, *Pedaliium murex Linn.: an overview of its phytopharmacological aspects*. *Asian Pacific journal of tropical medicine*, 2011. 4(9): p. 748-755.
284. V Rajashekar, E.U.R., P Srinivas, *Biological activities and medicinal properties of Gokhru (Pedaliium murex L.)*. *Asian Pacific journal of tropical biomedicine*, 2012. 2(7): p. 581-585.
285. Anonymus, *The Ayurvedic Pharmacopoeia of India*. Department of Ayush, Ministry of Health and Family Welfare, Govt. of India, New Delhi, Part I, 1986. I: p. 49-51.
286. Muneer Al-Ali, S.W., Husni Twaij, Ahmad Al-Badr, *Tribulus terrestris: preliminary study of its diuretic and contractile effects and comparison with Zea mays*. *Journal of Ethnopharmacology*, 2003. 85(2-3): p. 257-260.
287. Singha, A.K., *Mahaushdha nighantu with 'VIDYOTINI' Hindi commentary and notes by shri Indradeva Tripathi*. Chaukhambha Vidyabhavwan, Varanasi, 1971.
288. Sharma, P.V., *Madava dravyaguna*. Chaukhamba Vidyabhawana, Varansi, 1973.
289. Simha, A., *Amarakosha*. Choukamba Sanskrit series, Varanasi, 1957.
290. Anonymus, *The Ayurvedic Pharmacopoeia of India*. Department of Ayush, Ministry of Health and Family Welfare, Govt. of India, New Delhi, Part I, 1986. I: p. 67-69.
291. Joshi, M.C., *Hand book of Indian medicinal plants*. 2019: Scientific Publishers, Jodhpur.
292. Priyanka Meena, A.A., Vishal Kumar, *A comprehensive overview of Gokshura (Tribulus terrestris Linn.)*. *Journal of Ayurveda and Integrated Medical Sciences*, 2020. 4(6): p. 205-211.
293. Agrawal, K., *Review of drugs under Laghupanchmula*. *Journal of Pharmacognosy and Phytochemistry*, 2018. 7(3): p. 3363-3369.
294. Neetika Kundailia, V.S., Smita Johar, *Medicinal Plants with Special Focus on Adulterants and Substitutes*. *International Journal of Scientific and Technical Development*, 2015. 1: p. 52-63.
295. S. Abubakar, B.O.A., V.A. Etim, O. Segun, J.C. Ogbu, *Comparative study of phytochemical and synergistic anti-bacterial activity of Tribulus terrestris (L.) and Pandiaka heudelotii (Moq.) Hien on some clinical bacterial isolates*. *PHARMACEUTICAL AND BIOLOGICAL EVALUATIONS*, 2016. 3(1): p. 83-91.
296. Wenyi Zhu, Y.D., Hong Meng, Yinmao Dong, Li Li, *A review of traditional pharmacological uses, phytochemistry, and pharmacological activities of Tribulus terrestris*. *Chemistry Central Journal*, 2017. 11(1): p. 60 (1-16).
297. Anonymus, *The Ayurvedic Pharmacopoeia of India*. Department of Ayush, Ministry of Health and Family Welfare, Govt. of India, New Delhi, Part I, 1986. I: p. 64-66.
298. K Gauthaman , P.A., RNV Prasad, *Aphrodisiac properties of Tribulus Terrestris extract (Protodioscin) in normal and castrated rats*. *Life sciences*, 2002. 71(12): p. 1385-1396.

299. M Protich, D.T., B Nalbanski, R Stanislavov, M Katsarova, *Clinical trial of the preparation Tribestan in infertile men*. Akush Ginekol, 1983. 22(2): p. 326-329.
300. Gregory A. Brown, M.D.V., Emily R. Martini, Marian L. Kohut, Warren D. Franke, David A. Jackson, Douglas S. King, *Endocrine and lipid responses to chronic androstenediol-herbal supplementation in 30 to 58 year old men*. Journal of the American College of Nutrition, 2001. 20(5): p. 520-528.
301. Sushama Bhuvad, K.N., *Assessment of free radical scavenging activity of ten madhuraskandha drugs through UV spectroscopic and chromatographic technique*. International Journal of Pharmacy and Pharmaceutical Sciences, 2016. 8(3): p. 92-96.
302. Yogendra Kumar Gautam, V.Y., *Antioxidant activity and RP-HPLC analysis of diosgenin from the callus of Tribulus terrestris Linn*. International Journal of Research in Ayurveda and Pharmacy, 2014. 5(3): p. 343-346.
303. S Hemalatha, R.H., *Comparative antioxidant activities of crude ethanolic and saponin rich butanol extracts of Tribulus terrestris fruits*. International Journal of Pharma and Bio Sciences, 2013. 4(4): p. 784-793.
304. Han-Jik Ko, E.K.A., Joa Sub Oh, *N-trans- ρ -caffeoyl tyramine isolated from Tribulus terrestris exerts anti-inflammatory effects in lipopolysaccharide-stimulated RAW 264.7 cells*. International journal of molecular medicine, 2015. 36(4): p. 1042-1048.
305. Ikshit Sharma, W.K., Sayeed Ahmad, *In vitro and ex vivo approach for anti-urolithiatic potential of bioactive fractions of gokhru with simultaneous HPLC analysis of six major metabolites and their exploration in rat plasma*. Pharmaceutical biology, 2017. 55(1): p. 701-711.
306. Anshu Aggarwal, S.T., Surinder Kumar Singla, Chanderdeep Tandon, *A novel antilithiatic protein from Tribulus terrestris having cytoprotective potency*. Protein and peptide letters, 2012. 19(8): p. 812-819.
307. Vasanthy Arasaratnam, S.B., A. Senthuran, R. Rajendraprasad, *A study of Tribulus terrestris extract on risk factors for urinary stone in normal subjects and urolithic patients*. Journal of the National Science Foundation of Sri Lanka, 2010. 38(3): p. 187-191.
308. Pinar Ercan, S.N.E., *Inhibitory effects of chickpea and Tribulus terrestris on lipase, α -amylase and α -glucosidase*. Food chemistry, 2016. 205: p. 163-169.
309. SJ Zhang, S.F., *Effects of saponins from Tribulus terrestris on postprandial blood glucose levels in normal and type 2 diabetic rats*. Pract Pharm Clin Remed, 2012. 15(1): p. 1-3.
310. Nasrin Babadai Samani, A.J., Mahmood Soveid, Mojtaba Heydari, Seyed Hamdollah Mosavat, *Efficacy of Tribulus terrestris extract on the serum glucose and lipids of women with diabetes mellitus*. Iranian journal of medical sciences, 2016. 41(3 Suppl): p. S5.
311. F Zhai, H.L., FB Zhou, F Lin, LX Guan, *Effects of saponins of Tribulus terrestris on PPAR γ and NF- κ B signaling pathways expression in rat brain following cerebral ischemic injury*. Med Recapitulate, 2015. 21(24): p. 4539-4540.
312. Li LB, L.J., Li H, *Protective effects of gross saponins of Tribulus terrestris on experimental intracerebral hemorrhage in rats*. Journal of Harbin Medical University, 2006. 40(2): p. 99-102.
313. Li M, Q.W., Wang Y, Wan H, Tian C, *Hypoglycemic effect of saponin from Tribulus terrestris*. Zhong yao cai= Zhongyaoacai= Journal of Chinese medicinal materials, 2002. 25(6): p. 420-422.
314. AR Murthy, S.D., K Tripathi, *Anti-hypertensive effect of Gokshura (Tribulus terrestris Linn.)-A clinical study*. Ancient science of life, 2000. 19(3-4): p. 139-145.

315. Muhammad Asif Hanif, S.Y., Rafia Rehman, Asma Hanif, Raziya Nadeem, *Puncture Vine*, in *Medicinal Plants of South Asia*. 2020, Elsevier Ltd., Amsterdam, Netherlands. p. 571-585.
316. Fatemeh Almasi, M.K., Shima chehrei, Ali Ghanbari, *Effect of gross saponins from Tribulus terrestris on hepatic apoptosis in mice's acute hepatic injury induced by tripterygium glycosides*. International Journal of Morphology, 2017. 35(1): p. 345-350.
317. Apurva Patel, A.S., Nikhat J. Siddiqi, Preeti Sharma, *An insight into the anticancer mechanism of Tribulus terrestris extracts on human breast cancer cells*. 3 Biotech, 2019. 9(2): p. 58 (1-10).
318. Svetla Angelova, Z.G., Maria Krasteva, Georgi Antov, Valentin Lozanov, Tsanko Markov, Stefan Bozhanov, Elena Georgieva, and V. Mitev, *Antitumor activity of Bulgarian herb Tribulus terrestris L. on human breast cancer cells*. Journal of BioScience & Biotechnology, 2013. 2(1): p. 25-32.
319. Sun B, Q.W., Bai Z, *The inhibitory effect of saponins from Tribulus terrestris on Bcap-37 breast cancer cell line in vitro*. Zhong yao cai= Zhongyaocai= Journal of Chinese medicinal materials, 2003. 26(2): p. 104-106.
320. TE Goranova, S.B., VS Lozanov, VI Mitev, RP Kaneva, EI Georgieva, *Changes in gene expression of CXCR4, CCR7 and BCL2 after treatment of breast cancer cells with saponin extract from Tribulus terrestris*. Neoplasma, 2015. 62(1): p. 27-33.
321. Sara Batoei, M.M., Reza Yari, *Antibacterial activity of Tribulus terrestris methanol extract against clinical isolates of Escherichia coli*. Herba Polonica, 2016. 62(2): p. 57-66.
322. Seyed Amir Razavi Satvati, M.S., Mansour Amin, Farid Shiezadeh,, *Evaluation of the Antimicrobial activity of Tribulus terrestris, Allium sativum, Salvia officinalis, and Allium hirtifolium Boiss against Enterococcus faecalis*. International Journal of Enteric Pathogens, 2017. 5(2): p. 63-67.
323. Farooq Azam, S.M., Maliha Batool, Bashir Ahmad, Ghazanfar Abbas,, *A review on advancements in ethnomedicine and phytochemistry of Tribulus terrestris—a plant with multiple health benefits*. International Journal of Biosciences, 2019. 14(1): p. 21-37.
324. Deepak M, D.G., Prashanth D, Asha MK, Amit A, Venkataraman BV,, *Tribulosin and β -sitosterol-D-glucoside, the anthelmintic principles of Tribulus terrestris*. Phytomedicine, 2002. 9(8): p. 753-756.
325. William Charles Evans, *Trease and Evans Pharmacognosy (16th edn) - E-Book*. Elsevier Limited, United States, 2009.
326. Khandelwal, K., *Practical pharmacognosy*. 2008: Pragati Books Pvt. Ltd., Delhi.
327. Dileep Singh Baghel, A.M., Saurabh Singh, Rajesh Kumar, Anand Kumar Chaudhary, Amit Bhatia, *Formulation and Evaluation of In vitro Potential of Punarnava ghan Tablet against Urolithiasis (Mutrakrichra)*. Research Journal of Pharmacy and Technology, 2021. 14(3): p. 1469-1476.
328. Dileep Singh Baghel, A.M., Saurabh Singh, Anand Kumar Chaudhary, Amit Bhatia, Shruti Chopra, *Formulation, Evaluation and Assessment of In Vitro Potential of Gokshur Ghan Tablet against Urolithiasis (Mutrakrichra)*. Research Journal of Pharmacy and Technology, 2021. 14(4): p. 1945-1952.
329. Central Council for Research in Ayurvedic Sciences. *General Guidelines for Drug Development of Ayurvedic Formulations, Volume - I*. 2018 [cited 2018 15-11-2018]; Available from:
http://ccras.nic.in/sites/default/files/viewpdf/Publication/CCRAS_Guideline%20of%20Drug%20Development.pdf.

330. Lohar, D., *Protocol for testing of Ayurvedic, Siddha and Unani medicines*. Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines, Ghaziabad, 2007: p. 36-145.
331. Lough, W.J. and I.W. Wainer, *High performance liquid chromatography: fundamental principles and practice*. 1995: CRC press.
332. Meyer, V.R., *Practical high-performance liquid chromatography*. 2013: John Wiley & Sons.
333. DL Pavia, G.L., GS Kriz, JA Vyvyan, *Introduction to spectroscopy*. 2014: Nelson Education.
334. Krisnanadaji Maharaj, *Rasa Tantra Sara Va Siddha Prayoga Sangraha*. Krishna Gopal Ayurveda Bhavan, Rajasthan, 2010.
335. Parikh, D.M., *Handbook of pharmaceutical granulation technology*. Taylor & Francis Group. Abingdon. 2005.
336. L Lachman, H.L., JL Kanig, *The theory and practice of industrial pharmacy*. Lea & Febiger. 1986.
337. Shailesh K Singh, J.D., Manzer J Durrani, Mansoor A. Khan, *Optimization and characterization of controlled release pellets coated with an experimental latex: I. Anionic drug*. International journal of pharmaceutics, 1995. 125(2): p. 243-255.
338. Praveen, R., et al., *Cross linked alginate gel beads as floating drug delivery system for cefdinir: Optimization using Box- Behnken design*. Journal of Pharmaceutical Investigation, 2015. 45(2): p. 187-199.
339. Anees A Karnachi, M.A.K., *Box-Behnken design for the optimization of formulation variables of indomethacin coprecipitates with polymer mixtures*. International journal of Pharmaceutics, 1996. 131(1): p. 9-17.
340. Smith, F.D. and G. Lyndall, *Apparatus for testing pharmaceutical tablets*. Google Patents. 1936.
341. Jain, K. and M. Bharkatiya, *Process validation of tablet dosage form: A comprehensive review*. The Pharma Innovation Journal, 2018. 7(3): p. 433-438.
342. Haritha, B., *A review on evaluation of tablets*. Journal of Formulation Science & Bioavailability, 2017. 1(107): p. 2.
343. Arumugam, N., A. Ramaswamy, and S.V. Prabhu, *In Vitro Antilithiasis Activity and Cytoprotective Properties of Acalypha indica Extracts*. CURRENT APPLIED SCIENCE AND TECHNOLOGY, 2021: p. 240-254.
344. Srinivasa, A.K.B., et al., *Antiuro lithiatic activity of Gokhsuradi churan, an ayurvedic formulation by in vitro method*. Advanced pharmaceutical bulletin, 2013. 3(2): p. 477.
345. Ahmed, S., M.M. Hasan, and Z.A. Mahmood, *In vitro urolithiasis models: An evaluation of prophylactic management against kidney stones*. Journal of Pharmacognosy and Phytochemistry, 2016. 5(3): p. 28.
346. Atul Makasana, V.R., Dishant Desai, Jaymin Mendpara, Vivek Parekh, *Evaluation for the anti-urolithiatic activity of Launaea procumbens against ethylene glycol-induced renal calculi in rats*. Toxicology Reports, 2014. 1: p. 46-52.
347. Ravindra V Karadi, N.B.G., KR Alagawadi, Rudraprabhu V Savadi, *Effect of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in rats*. Journal of ethnopharmacology, 2006. 105(1-2): p. 306-311.
348. Jie Fan, M.A.G., Paramjit S Chandhoke, *Impact of ammonium chloride administration on a rat ethylene glycol urolithiasis model*. Scanning Microsc, 1999. 13(2-3): p. 299-306.
349. Waqar Ahmed Siddiqui, M.S., Arham Shabbir, Ali Ahmad, *Evaluation of anti-urolithiatic and diuretic activities of watermelon (Citrullus lanatus) using in vivo and in vitro experiments*. Biomedicine & Pharmacotherapy, 2018. 97: p. 1212-1221.

350. Siddiqui, W.A., et al., *Evaluation of anti-urolithiatic and diuretic activities of watermelon (Citrullus lanatus) using in vivo and in vitro experiments*. Biomedicine & Pharmacotherapy, 2018. 97: p. 1212-1221.
351. Meher, S.K., et al., *Experimental studies on the Renal Protective effect of Gokshura (Tribulus terrestris Linn) and Varuna (Crataeva nurvala Buch-Ham)*. Research Journal of Pharmacology and Pharmacodynamics, 2016. 8(2): p. 75-82.
352. Hodgkinson, A., *A combined qualitative and quantitative procedure for the chemical analysis of urinary calculi*. Journal of clinical Pathology, 1971. 24(2): p. 147-151.
353. Ohkawa, H., N. Ohishi, and K. Yagi, *Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction*. Analytical biochemistry, 1979. 95(2): p. 351-358.
354. Ellman, G.L., *Tissue sulfhydryl groups*. Archives of biochemistry and biophysics, 1959. 82(1): p. 70-77.
355. Ozkan, O.V., et al., *Effects of β -glucan pretreatment on acetylsalicylic acid-induced gastric damage: an experimental study in rats*. Current therapeutic research, 2010. 71(6): p. 369-383.
356. Yang, T. and B. Poovaiah, *Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin*. Proceedings of the National Academy of Sciences, 2002. 99(6): p. 4097-4102.
357. Kate, I.E. and O.O. Lucky, *Effects of Vernonia amygdalina Del. Extract on Cholesterol Level and Lipid Peroxidation Status in Rats Given Red Dye Adulterated Palm Oil Diets*. Journal of Pharmaceutical Research International, 2012: p. 98-107.
358. Berman, S.B. and T.G. Hastings, *Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease*. Journal of neurochemistry, 1999. 73(3): p. 1127-1137.
359. King, T.E. and R.L. Howard, *Preparations and properties of soluble NADH dehydrogenases from cardiac muscle*, in *Methods in enzymology*. 1967, Elsevier. p. 275-294.
360. King, T.E., *Preparation of succinate dehydrogenase and reconstitution of succinate oxidase*, in *Methods in enzymology*. 1967, Elsevier. p. 322-331.
361. Liu, Y., et al., *Mechanism of cellular 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction*. Journal of neurochemistry, 1997. 69(2): p. 581-593.
362. Wang, H., et al., *An improved 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) reduction assay for evaluating the viability of Escherichia coli cells*. Journal of microbiological methods, 2010. 82(3): p. 330-333.
363. Touhami, M., et al., *Lemon juice has protective activity in a rat urolithiasis model*. BMC urology, 2007. 7(1): p. 18.

CHAPTER 8
APPENDIX

8.1 LETTER OF CANDIDATURE (OLD)



Division of Academic Affairs

LPU/DAA/EC/160204/036

Dated: 4th Feb 2016.

Dileep Singh Baghel
H. No 26/782, Pokhari Tola
Gangajali Niwas, Chhatrapati Nagar
Huzur, Rewa, Madhya Pradesh.

Subject: Letter of Candidacy for Ph.D.

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. degree on 12th Oct 2015 by accepting your thesis research proposal titled:

"Development and Evaluation of Antirolithic Poly Herbal Formulation", supervised by Dr. Amit Bhatia, Associate Professor, at Lovely Professional University, Phagwara, Punjab, and Co-supervised by Dr. Shruti Chopra, Assistant Professor, at Lovely Professional University, Phagwara, Punjab.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. degree students of the University, and amendments, if any, made from time to time.

We wish you the very best in completing your thesis research requirements in the near future. Please do not hesitate to contact us in case you have questions about the rules and regulations of the University.


Signature

8.2 LETTER OF CANDIDATURE (NEW)



Centre for Research Degree Programmes

LPU/CRDP/EC/050620/00332

Dated: Friday 05, June 2020

Dileep Singh Baghel
Registration Number: 41400104
Programme Name: Ph.D. - (Ayurvedic Pharmacy) (Part Time)

Subject: Letter of Candidacy for Ph.D.

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on December 06, 2019 by accepting your research proposal entitled: "Development and Evaluation of Antiurolithic Herbomineral Formulation" under the supervision of Dr. Amit Mittal.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your programme, please contact Centre of Research Degree Programmes.

A handwritten signature in blue ink, appearing to read 'Raw', followed by a horizontal line.

HOS
Centre for Research Degree Programmes

8.3 CERTIFICATE BY IAEC FOR ANIMAL STUDY

CENTRAL ANIMAL HOUSE FACILITY (CAHF)

Lovely School of Pharmaceutical Sciences, Lovely Professional University

Ludhiana- Jalandhar G.T. Road, Phagwara (Punjab), 144402

Registration Number -954/PO/Re/S/06/CPCSEA

CERTIFICATE

This is to certify that the project titled "*Development and Evaluation of Antiuro lithic Herbomineral Formulation*" has been approved by the IAEC.


Name of Principal Investigator: Dr. Amit Mittal

IAEC approval number: LPU/IAEC/2019/55

Date of Approval: 16.11.2019

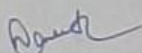
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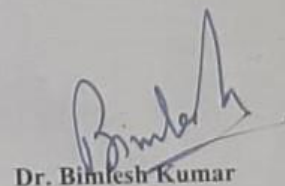
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Dr. Navneet Khurana

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IAEC



Dr. Bimlesh Kumar

Scientist In-Charge of Animal House,
Member Secretary IAEC

8.3 PUBLICATION DETAILS

8.3.1

REVIEW ARTICLE

AMALGAMATION OF AYURVEDIC CONCEPT WITH MODERN MEDICAL PRACTICE TO MANAGE KIDNEY STONE DISEASE (UROLITHIASIS): AN ABBREVIATED REVIEW

Baghel D. S.^a, Chopra S.^b, Bhatia A.^b and Tamilvanan S.^{a,c*}

(Received 14 December 2017) (Accepted 02 November 2018)

ABSTRACT

An age-old syndrome which possesses not only multifactorial etiological origins but also often associated with high rate of remission-rebound frequency during its management time is kidney stone (termed as urolithiasis). In Ayurveda, this syndrome is called as *mutrakricchra* and it is one of the most distressing syndromes among the group of urinary disorder conditions attached to human beings till today. Even Acharya Sushruta, a pioneer in the art of surgery, described the root causes and management of urolithiasis. In modern medical practice, plenty of management/treatment options are available which starts from the use of uresis-promoting agent to dietary or nutritional supplement intake. Approaches developed by amalgamating the ayurvedic concept/principle with modern medical practice is a promising strategy and even welcome addition for urolithiasis management. This review provides a comprehensive insight on how the amalgamation of ayurvedic concept with modern medical practice helps in urolithiasis management.

Keywords: Kidney stone, mutrakricchra, urolithiasis, polyherbal formulations, ayurvedic concept

INTRODUCTION

Painful urination (dysuria) is the most distressing problems of human beings which alarms the arrival of urinary tract infections (UTIs). One such syndrome that

holds the accession dating back to the Egyptian mummies kept in the tombs over the period of 4000 B.C or the North American Indian bodies laid down inside the graves during 1500 to 1000 B.C.

Intriguingly, the UTIs (a major problem of adolescent and geriatric people nowadays) confine to an infection involving any of the sub-structures of the urinary tract

8.3.2

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IN-VITRO POTENTIAL OF SPHATIKA TABLET IN THE MANAGEMENT OF UROLITHIASIS (MUTRAKRICHRA)

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Abstract

The urinary system is mainly embedded of kidneys, ureters, urinary bladder, and urethra. Less water intake, electrolyte imbalance, some bacterial i.e. *Escherichia coli* & *streptococci*, viral and parasitic (*Dirofilaria immitis*) infections, autoimmune diseases might be act as causative factor which finally lead to the development of renal calculi. *Sphatika* (potash alum) is consider as *mutrakrichraghan dravya* which helps to break down the calculi and remove them through the urine. In the present work tablets of *Sphatika* were prepared by using direct compression technique. Crystal growth inhibition started at a concentration of 50 µg/ml but 650 µg/ml of drug showed maximum inhibition of 53.89%. The microbial load and presence of heavy metal in prepared *Sphatika* tablets was under the limits prescribed by The Ayurvedic Pharmacopoeia of India.

Keywords: *Mutravaha srotas*; Urinary disorders; Urinary system; *Sphatika*; *Fitikari*; *Mutrakrichra*; Urolithiasis.

Introduction

Water is an essential component liable for digestion, circulation, elimination, body temperature regulation (Brunton, Chabner, & Knollmann, 2011; Nilore, 1984). The urinary system pivotal function is to maintain the normal composition and volume of body fluid that can be executed by glomerular filtration, tubular reabsorption, tubular secretion of soluble and filterable components present in plasma (Satoskar, Rege, & Bhandarkar, 2015). The urinary system, the bowels, the skin and the lungs are four excretory custom of the human body (Brunton *et al.* 2011).

(Chunekar & Pandey, 2004; Sharma, 2004; Sivananda, 2006; Tripathi, 1994; Vagbhata, 1961). It is a colourless, white transparent, odourless crystalline masses or a granular powder with a sweetish astringent taste contains Potassium, Aluminium, Hydrogen, Sulphur and Oxygen (K₂SO₄Al₂(SO₄)₃.24H₂O) (Roqaiya & Begum, 2015). When heated it melts and at about 200°C and loses its water of crystallisation with the formation of the anhydrous salt. It is soluble as 1 part in 7.5 parts of water, 1 in 0.3 of boiling water, and 1 in 3 of glycerol (ALtaei & AI-Jubouri, 2005). Two types of *Sphatika* has been explained in the classics i.e.



A SYNOPTIC OVERVIEW ON *BOERHAVIA DIFFUSA* FOR ITS MEDICINAL IMPORTANCE Jaswinder Kaur¹, Saurabh Singh¹, Amit Mittal¹, Anand Kumar Chaudhary² and Dileep Singh Baghel^{1*}

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Abstract

Punarnava (*Boerhavia diffusa*) belongs to family Nyctaginaceae. It is also named as spreading hog weed and used comprehensively in Ayurvedic system of medicine to cure diseases like *Hridya rog* (cardiac disorders), *Pandu* (anaemia), *VayasthapanalRasayana* (rejuvenator), *Sotha* (inflammation with swelling), *Mutravahshorhogai vikar* (urinary tract disorders), *Jwara* (fever), *yonitrog* (vaginal disorders), *suikarog* (female disorder), *kushharog* (skin diseases) *mrida bhakshana janya rog* (disorders originated due to eating of clay), *Basti karma* (enema), *balatrog* (disorders of children's), *madhumeha* (anti-diabetic) etc. Its synonyms, morphology, therapeutic potential is described in *Ayurvediya Samhitas* and *Nighantus*. In this synoptic work attempt has been done to summarize the synonyms, therapeutic potential and phytoconstituents of *Punarnava* (*Boerhavia diffusa*).

Keywords: *Punarnava*, *Boerhavia diffusa*, *Diffusa*, spreading hog weed

Introduction

Herbs play an essential function in our everyday life. They have been the most effective source of drugs in olden days (Ekor, 2014). Even nowadays herbs are similarly essential to modern drugs as they have got fewer side effects whilst in comparison to synthetic medicines (Sen & Chakraborty, 2019). The growing belief on the use of medicinal plants within the industrialized societies were traced to the extraction and improvement of several medicines and chemotherapeutics from these plants in addition to from traditionally used rural herbal remedies (Ekor, 2014; Kim, Lee, Jerng, & Choi, 2019).

emphasis on balanced *ahaar* (diet), *nidra* (sleep) and *brahmcharya* (celibacy) for the maintenance of health (Chandaliya, Chandaliya, Tukaram, & Vinayak, 2015; Sastri, 2013).

Boerhavia diffusa (*Punarnava*) turned into named in the honor of Dutch physician Hermann Boerhaave on the 18th century (Banjare, Prasad, & Naik, 2012). The genus *Boerhavia* L. (Family: Nyctaginaceae) consists of 40 tropical and sub-tropical species (Heywood, Moore, Richardson, & Stearn, 1993) found growing wild in diverse terrestrial habitats, ranging from managed grass-lands, waste-lands, agroecosystems to large forest gaps. *Punarnava*

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RESEARCH ARTICLE

Formulation and Evaluation of *In vitro* Potential of *Punarnava ghan* Tablet against Urolithiasis (*Mutrakrichra*)

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ABSTRACT:

Background: *Punarnava* (*B. diffusa* Linn.) is usually prescribed in India as traditional medicine in the management of renal miseries including CaOx (calcium oxalate) urolithiasis (*Mutrakrichra*). It occurs commonly as a weed throughout India. Out of the forty species of this genus *B. diffusa*, *B. chinensis*, *B. erecta*, *B. rependa*, and *B. rubicunda* have Indian originates. **Objective:** To prepare *Punarnava ghan* tablet and evaluate its potential against Urolithiasis (*Mutrakrichra*) by *in vitro* technique. **Materials and Methods:** *Punarnava kwath churna* were prepared as per the methodology mentioned in *Sharandhar samhita* and *ghan* was prepared by evaporating the watery portion from prepared *kwath*. Prepared *ghan* was compressed into tablet, and for evaluating the quality of tablets standard parameters were determined. **Results:** Physicochemical and stability studies have not shown any remarkable variations with prepared tablet dosage form. *In vitro* studies showed 50 % of crystal inhibition at 650µg/ml. **Conclusion:** The prepared tablets of *Punarnava ghan* did not have remarkable variation during physicochemical and stability studies. The prepared tablets were able to so remarkable *in vitro* activity against Urolithiasis (*Mutrakrichra*).

KEYWORDS: *Mutrakrichra* *Boerhavia diffusa* *Punarnava ghan* Tablet Stability studies Antiuro lithic

RESEARCH ARTICLE

Formulation, Evaluation and Assessment of *In Vitro* Potential of *Gokshur Ghan* Tablet against Urolithiasis (*Mutrakrichra*)

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ABSTRACT:

Background: Tribulus species spread in the warm, temperate regions and prevalent in zone where hot summers and dry soil present. Tribulus species comprises of more than 25 species. Tribulus terrestris L. (Zygophyllaceae) consists of ripe, dried, whole fruit and an annual herb, rarely perennial that grows worldwide, particularly in the temperate regions. It is used as traditional medicines in India, China, South Africa, Bulgaria and many other countries. It is used for strengthening (*balya*), nutritive (*brimhan*), rejuvenator (*rasayan*), diuretic (*mutral*), anti-inflammatory (*shothahara*), renal calculi (*ashmani*) and urolithiasis (*Mutrakrichra*). **Objective:** To prepare *Gokshur* tablet and evaluate its potential against Urolithiasis (*Mutrakrichra*) by *in vitro* technique. **Materials and Methods:** *Gokshur kvatha churna* was prepared as per the methodology mentioned in *Sharandhar samhita* and *ghan* was prepared by evaporating the watery portion from prepared *kvatha*. Prepared *ghan* was compressed into tablet, and quality of tablets were evaluated. **Results:** Physicochemical and stability studies have not shown any remarkable variations with prepared tablet dosage form. *In vitro* studies showed 58.24% of crystal inhibition at 650µg/ml. **Conclusion:** The prepared tablets of *Gokshur ghan* did not have remarkable variation during physicochemical and stability studies. The prepared tablets were able to show remarkable *in vitro* activity against Urolithiasis (*Mutrakrichra*).

KEYWORDS: *Mutrakrichra*, *Gokshur*, *Ghan*, Tablets, Stability studies, Tribulus terrestris, Urolithiasis.

GOKHARU (TRIBULUS TERRESTRIS): AN ABBREVIATED REVIEW

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ABSTRACT

Gokharu, a commonly available weed which possess substantial therapeutic value in the field of traditional systems of medicine, viz. *Ayurveda*, Chinese, *Siddha*, and *Unani*. It is well known medicinal plants in the folk medicine of many countries for a number of diseases i.e. diuretic, aphrodisiac, antiurolithic, immunomodulatory, antihypertensive, antihyperlipidemic, antidiabetic, hepatoprotective, anticancer, anthelmintic, antibacterial, analgesic, anti-inflammatory, antihyperlipidemic, and cardioprotective activity.

Keywords : *Gokharu*, *Tribulus terrestris*

An overview of Sphatika (Alum)

Nitika Anand, Saurabh Singh*, Simranjeet Kaur, Sakshi Sabharwal, Dileep Singh Baghel, Vibhu Khanna, Sajisha V. S
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Abstract

Sphatika is considered as very good and useful drug in Ayurveda which is known as alum in English. This is found in crystal form which resembles to salt. It is a mineral origin drug. Work done by the Greek scholars or physicians is considered as the healthy discussions on the topic of sphatika. Ancient scholar Razi from Arab gives the full details about sphatika. He characterized alum in the types of vitriol, due to the same activity like astringent qualities. Sphatika is a drug with good therapeutic efficacy. It is used as krimighan, jwaraghan, shulprashnam. Sphatika is used as major ingredient in so many herbo-mineral formulations. This drug comes from that category of drugs which are used internally as well as externally. Sphatika is used for many kinds of antimicrobial and antiseptic purposes since ancient time. The present review highlights the important activities and detailed description of sphatika. Which will be beneficial to promote the natural potential antimicrobial drug.

Keywords: Sphatika, Alum, Natural, Antimicrobial, Ayurveda

1. Introduction

Most of the mineral origin drugs are found in crystal form. Sphatika is a mineral origin drug. Commonly it is known as fitkari in India. Alum, name given to it because of its astringent properties^[1]. It is completely soluble in warm water. As per Unani medical literature it is considered as kashya^[2]. It is transparent but some time slightly translucent^[3]. According to historical review of alum, was first prepared in Asian countries. In ayurvedic literature

AN OVERVIEW ON: APAMARGA KSHARA

Sakshi Sabharwal, Nitika Anand, Simranjeet Kaur, Vibhu Khanna, Dileep Singh Baghel, Saurabh Singh*, Amit Mittal
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ABSTRACT:-

Apamarga (*Achyranthes aspera*, Amaranthaceae) is a vital healing dravya originate as a unwanted plant all over India. In conventional medication framework all pieces of this plant are utilized like beej, moola and shoots. In Atharvaveda, it is said to be kshetriya roga nasak and yatudhan Krimi nasak. In Yajurveda it is said to be used for hawan purpose due to its Rakshoghan Property. No other plant has better water removing property than Apamarga. Apamarga kshara is an alkaline Ayurvedic medicine, in powder form which is prepared from Apamarg- Prickly Chaff-Flower. Kshara is a caustic, alkaline in nature obtained from the ashes of Apamarga dravya. It is adaptable, because even in such places that are difficult in approach by ordinary measures can be treated by kshara. This alkaline preparation may be a amalgam of numerous dravyas or it can be a single dravya. It has a vast series of explanation about kshara explained through Acharaya Sushruta. Kshara has lots of therapeutic usages and also replace many surgical procedures. The large number of phytochemical elements have been extracted out from this plant which possess some properties like Arsha, Kusthaghana, paapraga nasak, Duhswapnana, unmade, Apasmara, Ashmari, Hikka-Swaas, Vish Chikitsa etc. The compacted form of herb is utilized in pneumonia and mixture of the root is utilized as gentle astringent in bowel disorders. Traditionally, it is used as anti-diabetic, anti-inflammatory and abortifacient.

KEYWORDS: Apamarga kshara, Rakshoghana, kushthaghana, Kshetriya roga, Yatudhan Krimi, Paapraga .

INTRODUCTION: -

Kshara is the water-soluble ash of drug which is in the form of solutions, powders which is alkaline in nature. It is

8.3.9



8.3.10



8.3.11

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Invention Title: A NOVEL FORMULATION FOR THE MANAGEMENT OF UROLITHIASIS

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Applicant:

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Abstract:

The present invention discloses a novel formulation for the management of urolithiasis. The formulation consists of sphatika, surya kshar, apamarg kshar, gokshur extract, punarnava extract, acacia powder, and talc. The formulation reduces recurrence of stone episodes in urolithiasis and it can be formulated in various dosage forms.

Complete Specification

8.4 PLAGIARISM REPORT

8.4.1

PhD 02-05-2021

ORIGINALITY REPORT



8.4.2

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