Pharmaceutical standardization and Quality Control aspect of marketed sample Ashwagandha Kalpa with respect its Active constituents

A THESIS

SUBMITTED IN PARTIAL FULFILLAMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

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IN

AYURVEDA

BY

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Statement by the candidate

This is to submit that this written submission in my thesis entitled **Pharmaceutical standardization and Quality Control aspect of marketed sample Ashwagandha Kalpa with respect its Active constituents** represents original ideas in my own words and where others' ideas or words have been included, i have adequately cited and referenced the original sources. I also declare that i have stuck to all principles of academic honesty andintegrity and have not miss presented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the school and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required. Patients related to API, process, product, method and equipment, if any, have been examined to ensure non- infringing approach to the existing patents.

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Certificate by Supervisor

The work described in this thesis entitled**"Pharmaceutical standardization and Quality Control aspect of marketed sample Ashwagandha Kalpa with respect its Active constituents"**has been carried out by **Ms. Ambika Thakur_**under my supervision. I certify that this is her bonafied work. The work described is original and has not been submitted for any degree to this or any other university.

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Place:

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This is certified that the work described in this thesis entitled**"Pharmaceutical standardization and Quality Control aspect of marketed sample Ashwagandha Kalpa with respect its Active constituents"** has been carried out by **Ms.Ambika Thakur** at the School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

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DEVOTED TO -

MY PARENTS
MY GUIDE
MY CO- GUIDE

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Date:

Place :

(Ambika)

ABBERIVATION

A.H.Chi.	Ashtanga Hridaya Chikitsathana
A. S. Chi.	Ashtanga Samgraha Chikitsasthana
Ch. Chi.	Charaka Samhita Chikitsasthana
Ch. Su.	Charaka Samhita Sutrasthana
Ch. Vi.	Charaka Vimanasthana
Ka. Sam. Khi.	Kashyapa Samhita Khilsthana
Ma. Ni.	Madhava Nidana
Sha. M. Kh.	Sharangadhara Samhita Madhyama Khanda
Su. Chi.	Sushruta Samhita Chikitsasthana
Su. Su.	Sushruta Samhita Sutrasthana
B. P.	Bhavaprakasha
C. D.	Chakradatta
D. G. V.	Dravyaguna Vigyana
G. N.	Gadanigraha
Y.R	Yogaratnakara
Percentage	%
Millimeter	ml
Meter	m
Centimeter	cm
Milligram	mg
Minute	min
Gram	g
Hours	hrs
Degree celisus	^{o}C
Hydrochloric Acid	HCL
Nitric acid	HNO_3
Number	No.
Kilogram	kg
Hydrogen peroxide	H_2O_2
DAK	Dhataki
MAK	Madhuka

YAK	Yeast
L.O.D	Loss on drying
A.I.A	Acid insoluble ash
HPTLC	High Performance Thin Layer Chromatography
W/W	weight/weight
V/V	volume/volume
NMT	Not more then
NLT	Not less than
Fig.	Figure
Std.	Standard
No.	Number
DAK	Dhataki ashwagandha kalpa
MAK	Madhuca ashwagandha kalpa
YAK	Yeast ashwagandha kalpa

CONTENT

CONTENT OF TABLE

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Chapter-1
INTRODUCTION

Ayurveda is the oldest medical science, which originated in India around 5,000 years ago.[1]The term *Ayurveda* is derived from *'Ayur'* means life and '*Veda*' means science. The objective of *Ayurveda* is: To maintain and promote health by preventing physical, mental, and spiritual condition of human being. To cure diseases through natural medicine, diet, and regulated lifestyle.[2]

Medicine manufacturing part of *Ayurveda* is deal in *Rasashastra* and *Bhaishajya kalpana*. It deals with drug identification, selection, manufacturing, collection, preservation, processing, storage, product analysis, efficacy, toxicity safety, dose determination and finally clinical application. In *Ayurveda*, the pharmaceutics is deal under of *Bhaishajya kalpana*. *Bhaishajya kalpana* deal with wide range of *panchavidhkashayakalpana*, *swarasa* (expressed juice), *Kalka* (paste), *kashaya*(decoction), *hima* (cold infusion), *phanta* (hot infusion). When some liquid *kashaya,swarasa* and some drugs or food drugs liked jaggery or honey or sugar are mixed and put together for some time to achieve fermentation, are known as *sandhan*.[3]

Sandhan Kalpana

Sandhan Kalpana is derived from two different words i.e. *Sandhan* + *Kalpana.*

Sandhan kalpana are one of the dosages forms of *Ayurveda* in practice since thousand of year. *Sandhan Kalpana* contains alcoholic and acidic contents in smaller or larger percentage produced by fermentation. They contain special properties which make it more beneficial than other preparations. The *Madhya sandhan* contains both water soluble and alcohol soluble active principles of the drug. It can be stored for longer time without losing their therapeutic activity. The basic pharmaceutical principle in *sandhan kalpana* is to extract active constituents of raw drug through a bio- chemical process of fermentation in a mildly self-generated alcoholic medium. This ensure better extraction of both water and alcohol soluble constituents with most favorable growth of micro-organism. There are about 150 *asava* and *arishta* formulations mentioned in ancient text out of which about 45 in popular practice.[4]

Asava

When soft drugs mentioned are added with required quantity of water, to which jaggery or sugar as prescribed in the formula is added. This is poured into the fermentation vessel. The container is covered with a lid and the edges are sealed with cotton cloth in seven continuous layers.[5]

Arishta

When from coarsely powdered raw drugs *kashaya* is prepared and kept in the fermentationpot with sugar or honey or jaggery according to the formula is dissolved, boiled and added. At the end, fermentingagentsshould be properly cleaned and added. The mouth of the pot, vessel is covered with a lid and the edges sealed with cloth would in seven consecutive layers and kept either in a special room, or in a heap of paddy, for specific duration of fermentation.[5]

Ashwagandha Kalpa is polyherbal formulation which is used for internally for treating high blood pressure, paralysis, debility, loss of concentration, loss of sleep or insomnia,mental disorders. The review study is an attempt to develop some new approaches for the quality control, standardization and check the effect of batch and time variation on the concentration of active principle.

Standardization

Standardization of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observations.[6]

Qualitative evaluation of herbal drugs:

1. **Organoleptic**
   a. Colour
   b. Odour
   c. Taste etc.
2. **Botanical**
   a. Macroscopy
   b. Microscopy

3. Physical

a. Foreign matter

b. Loss on drying

c. Extractive value

i. Alcohol soluble extractive value

ii. Water soluble extractive value

d. Ash value

i. Total ash

ii. Acid insoluble ash value

4. Biological

a. Microbial contamination

b. Toxicological

5. Chemical

a. Quantitative

b. Qualitative

Chapter-2
TERMINOLOGY

2.1 *Sandhan Kalpana*

Sandhan Kalpana is derived from two different words i.e. *Sandhan* + *Kalpana*.

2.2 *Aasuta* (Begetting a new form)

2.3 *Abhisuta* (Extraction may indicate extraction from fermented smash)

2.4 *Sura*

The fermented liquid prepared by using cooked, rice, barley, etc. is known as *sura.*

2.5 *Sidhu*

The rasa of *Ikshu* boiled some period of time and then that dravya were closed into vessel the formulation is called *Sidhu*.

2.6 *Madaya*

Those preparations which are prepared by fermentation method and developing alcohol.

2.7 *Lepana*

To prevent the leakage of liquid,and reduce the porosity of port.

2.8 *Sandhana Dravya*

To initiate or potentiate the fermentation process in desired direction.

2.9 *Prakshepa Dravya*

Prakshepa dravya are the additives which are added to preparation to give good taste, smell and therapeutic action.

2.10 Filtration

Filtration is the procedures of separation of particles passing it through a permeable membrane which hold the solid but allows the fluid to pass.

2.11 Fermentation

Fermentation is the process of conversion of larger complex molecules into simple molecules through reduction by means of enzymes in an anaerobic condition.

2.12 *ShuktaVarga*

The products which attains *Amlata* (sourness), after fermentation process is known as *Shukta.*

2.13 *Asava*

Asava as *Madya* which is prepared without boiling the drug in water.

2.14 *Arishta*

The formulation which can stay for long period without spoiling is called *Arishta.*

2.15 *SandhanaKala*

For each and every formulations, specific time period is given for fermentation period, it may be given for the particular time duration.

2.16 Labeling

The finished products i.e. should be properly labeled.

2.17 Standardization

Standardization of drug means confirmation of its identity and determination of its quality and purity and detection of raw material.

2.18 Liquid material

Liquid material is play an important role for fermentation, provides favourable growth of micro organism.

2.19 *Sandhan*

To complete absorption of ferment with fermenting material is called as *Sandhan.*

2.20 *Varuni*

The liquid prepared with juice of *Tala* and *Kharjura* is called as *Varuni.*

2.21 *Sura*

The fermented liquid prepared by using cooked, rice, barley, etc. is known as *sura.*

2.23 *Dhupana*

Is used to prevent the antimicrobial activities and provide the long stability of drugs.

2.24*Madhur Dravya*

Madhur dravya is very important role for fermentation .It helps growth of bacteria, yeast, and microorganism essentially requires nutrients.

Chapter-3
REVIEW OF LITERATURE

3.1 Historical Review:

The knowledge of *Sandhan kalpana* starts from *Vedic period*. The process of fermentation (*Sandhan)* was probably discovered by observing the changes in the juices of several fruits and other substances that had been kept for a day or more. The different terms associated with fermentation in various texts are as follows –

Aasuta (Begetting a new form)

Abhisuta (Extraction may indicate extraction from fermented mash)

Parishruta (Foaming, fermenting, that is the state of fermenting)

S*andhana* (Complete absorption of ferment with fermenting material)

All these terms are part of the process fermentation. Except the last terminology all other terms are found in *Vedic* literature.[7]

3.2 Evidence of fermentation in the *Rigveda*

Admixture of a thick juice of *Soma* with barley powder the statement is clear indication of fermentation with barley "15 day old highly intoxicatedSoma",15th day probably refers to the fermentation process. *Soma,* after treatment becomes red all these statements given an indication of fermentation technology involved n the preparation of *Soma*.[7]

3.3 Evidence of fermentation in the *Yajurveda*

The *Shukla Yajurveda* describes the formation of two stimulating drinks – *Sura* and *Parishruta*. *Sura* is supposed to be prepared from germinated paddy, germinated barley and parched rice with the help of fermentation. The Katyayana *SrautaSutra*10 also gives a complete description of the preparation of *Sura*. According to this method either boiled rice or boiled barley is mixed with *Mamsa Rasa* and the entire mixture is kept in a jar. The jar is then kept in a pit for three nights.[7]

3.4 Evidence of fermentation in the *AtharvaVeda*

Madya which is used for the treatment purpose is known as *Aristha*11, and preparation process is mentioned as *AbhishavaPrakriya* the reference indicates the process of fermentation. *Dadhi* (curd) is also used in the Vedic period, shows another example of

fermentation. Therapeutically uses of *Sandhan Kalpana* are started from the *Samhita Kala.* The basic principles to prepare *Sandhan* i.e. the different types of constituents required for their preparation along with their proportions, the method of preparation the time require to complete process, the fermentation pots, the fermenting materials, the place and time (season) of fermentation etc. are found in the *Samhitas* but in a scattered way.[7]

3.5 Evidence of fermentation in the *Charaka Samhita*

In *Charaka Samhita* nine *yoni* are described. *Dhanya* (Cereals),*Phala*(Fruit),*Mula* (Roots),*Sara*(Exudates),*Khanda*(Stem),*Patra*(Leaves),*Twak*(Bark),*Pushpa*(Flower),*Shark ara*(Sugar). In the same chapter 84 fermentative products are described 24 different *Asava* and *Arishta* are described in *Chikitsaathana*, for *Shaman* therapy of various diseases and 4 *Asava-Arishta* in *Kalpasthana* for *Virechana*.[9]

3.6 Evidence of fermentation in the *Sushruta Samhita*

Acharya Sushruta mentioned the use of *Madya Sandhana* during surgery. *Sandhan Kalpana* less described as compared to *Acharya Sushruta*. In *Sushruta Samhita* total 11*Asava Arishta* described rest of 46 *Madya* Varga.[10]*MadyaVarga* of *Sutrasthana* in 45th chapter deals with the 27 types of fermented preparations. Commentator Dalhana has classified the fermented preparations in '*Madya'* and '*Shukta'* groups on the basis of their alcoholic and acidic contents respectively. He has also defined the *Asava Arishta* on the basis of consistency. In this text, 21 *Asava Arishtayoga* are mentioned, but the detail description regarding the contents and the method of preparation is found only for 7 Yoga. *Guda(*jaggery*)* is used in the *Phanita* form (*Shodhana* process of *GudaPhanita*) 19, to prevent unwanted growth of microorganism.[11]

3.7 Evidence of fermentation in the *Ashtanga Hridaya and Ashtanga-Sangraha*

The use of *Dhataki Pushpa* as a fermentation initiator is first time reported in *Ashtanga Hridaya*. In *Ashtanga Hridaya* total 8 *Asava-Arishta* are mentioned.[12]In *Astanga – SangrahaSutrasthana* Herbal medicine was fully developed in this period, and reflected various formulation of different *Sandhan Kalpana* Such as *Asava Arishta, Madya, Sukta,* and *Varuni*. A total 17 *asava- arishta* are quoted in *AshtangaSangraha*.[12]

3.8 Evidence of fermentation in the *Gada Nigraha*

A classical text by *Acharya Shodhala* of 12th century is very innovative in its approach for *Kalpana* wise distribution which shows the importance of pharmaceutics. *Acharya Shodhala*, a pioneer of the experiments in the pharmaceutics, has mentioned 60 *Asava Arishta.* Different preparation methods are described in many of the formulations. The text fulfills all the concepts, which are established today. Some of the examples are quoted here.[13]

Different liquid media : Other than water, *Narikela Jala* (coconut water), *Mutra* (urine), *Mastu* (liquid part of curd) are used e.g.*Narikelasava.*[13]

3.9 Evidence of fermentation in the *SharangadharaSamhita*

In medieval period, *Sandhan Kalpana* is elaborately described in this text. In *madhyam Khand* (10th Chapter) explains acidic and alcoholic fermentation, various formulations prepared from the barley, rice, sugarcane juice, grapes juice etc.[14]

3.10 Evidence of fermentation in the *Bhiasihajya Ratnavali*

In *Bhiasihajya Ratnavali* the preparation of *Sandhan Kalpana,* ingredients and the specific duration to keep the container- for 15 days or 1 month is mentioned. A*charya Govind Das* has mentioned in these books 50 *Sandhan Kalpana* 15 are *Asava* and 29 are *Arishta* and remaining 2 *Chukra*, 2*Sura*, and 1 *kanji Kalpana.*[15]

3.11 Evidence of fermentation in the *Rasa Darpan*

In this text book history of *Sandhan Kalpana* and definition method of preparation are described.[16]

3.12 Evidence of fermentation in the *Yogaratnakra*

Compilation of 12 *Asava Arishta* are mentioned in this text.[17]

3.13 Evidence of fermentation in the TheAyurvedic Formulary of India

Part I and part II of Ayurvedic Formulary of India total 40 *Asava Arishta* are described. These publications by department of AYUSH, Government of India, and every formula are described by sequence.[18]

3.14 Evidence of fermentation in the *Bharat Bhesjya- Ratanakara*

In this book 8 *Asava Arishta* are described their method and preparation.

3.15 Evidence of fermentation in the *Shahstrayogam*

In this book 15 *Arishta* and 10 *Asava* are described.[20]

3.16 Evidence of fermentation in the *Arogya Prakashan*

In this book *Arishta* are described their different formulation are described. In these book described use of *Abhyarishta* Constipation, Urinary disorder, Swollen around the anus *(bawasir).*[21]

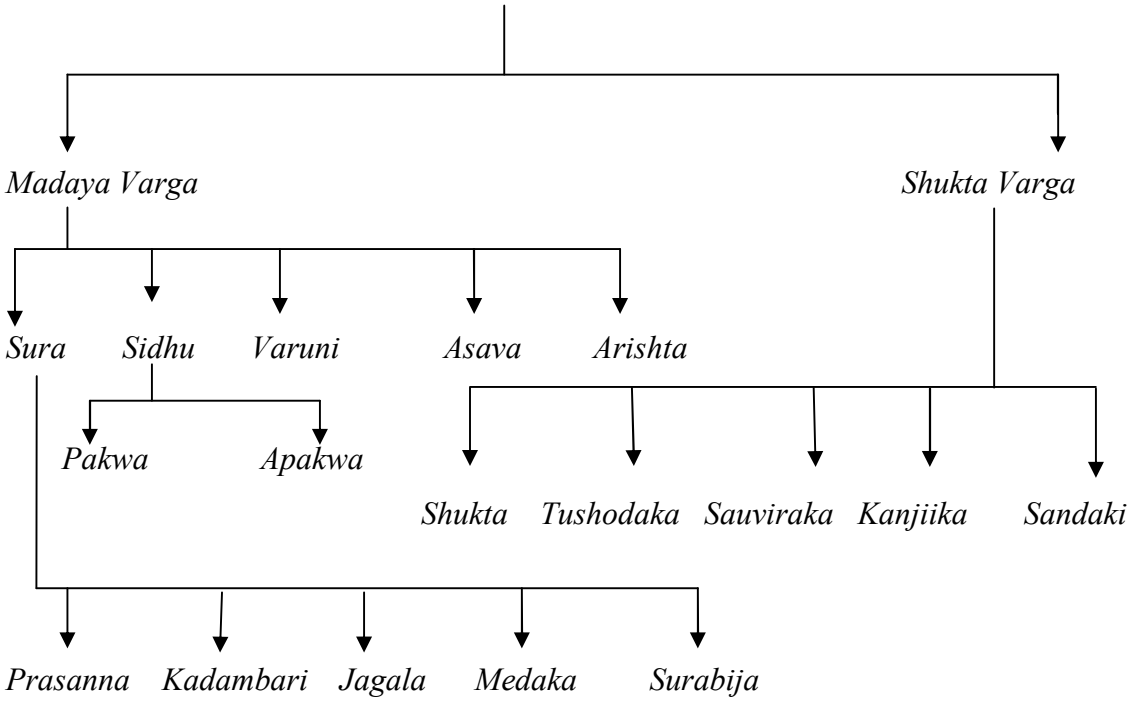
3.17 Evidence of fermentation in the *Bhela Samhita*

In this *Samhita* alcohols are used as a treatment of alcohol are used of various diseases and define the merits and demerits of *Madaya*.[22]

3.18 Evidence of fermentation in the *Kashyapa Samhita*

Sandhana Kalpna is explains separate in *Brihatrayi* but *Kashyapa Samhita* includes it in 7 basic *Kalpna* of *Bhaishajya Kalpana*.[23]

Classification of *Sandhana Kalpna*[24]



Systematic diagram of classification of *Sandhana Kalpana*

3.19 *Sukta Varga*

Name	Definition	Properties & uses
Shukta	Fermentation are used for purpose dietary nutritive supplement, then long period of time it can stored and suddenly its taste are change are converted into alma taste**.**	*Laghu, Usna,Tiksna,Katuvipaka* *Anemia, Urinary, Kaphara,* Expectorant.
Tushodaka	A fermented uncooked *yava* is pounded along with *Tusha* and Kept for *Sandhan.*	*Digestive, Haridya***,** *Anemia, Purgative , Grahai*.
Sauviraka	A fermented *yava* is prepared by boiled but without husk.	*Krimirga, Haridya,* Anemia
Sandaki	It is prepared by fermenting Radish& mustard.	*Rochan ,Laghu*
Kanjiika	A fermented product is prepared by half boiled of dhanya with *manda*.[13,16]	*Dahanashak,Mukhavairasyahara,* *Dourgandhyahara* (Ashtang Haridya)[24,25]

3.20 *Madaya Sandhan kalpna*

Those preparations which are prepared by fermentation method and developing alcohol. Then called *Madaya kalpna.* Those alcoholic preparations which have depressant activity.

Synonyms

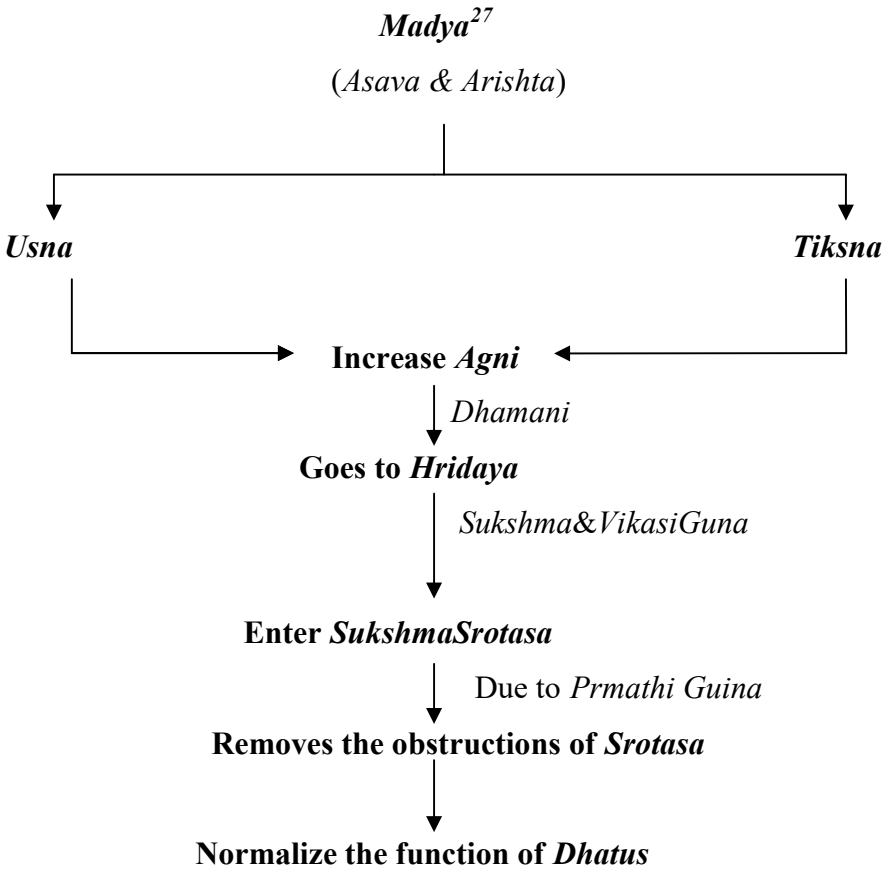
Madya, hala, sura, shundi, Madira, varunatmaja, kalya, Devasprishta, Varuni-Madanpala nighantu.

According to Acharya Sushruta Eight properties of *Madya*

Usna, Tiksna, Visndh, Ruksh, Ashukari, Vikashi, Vayyahi

Madya Dosha

Viscid**,** Produces a burning sensation, Disagreeable taste**,** Unpleasant**,** Immature**,** Slimy[23,24,25,26]



3.20(A) Different types of *Madya*

Name	Process	Properties & uses
Sura	The fermented liquid prepared by using cooked, rice, barley, etc. is known as *sura*.[26] ***Sura* further typed it** i) *Prasana*- The clear supernatant fluid of sura is known as *Prasana.* ii) *Kadambari*-Slightly thicker than *prasana.* ii) *Jagala*- jagala is thicker and present lower than *kadambri*. iii) *Medaka*- It is thicker to *jagala.* iv) *Surabija* filtration it's called *vakkasa, Surabija, or kinwa*.[26]	*Guru,Balya,Meda,Kaphavrdhaka.*[26] *Glum, arsh, Grahai Mutrakricha*[26]
Sidhu	The *rasa* of *Ikshu* boiled some period of time and then that *dravya* were closed into vessel. The formulation is called *Sidhu.* Sugar cane *Ikshu* is important in *Madhur dravya*. It can be	*Agni – Bala – Varnakrita, Hridya, Rochana, Snehana, Vata-pittahara*.

	elaborated by *Acharya Sushruta* preparation of sindu with help of guda and sacra jamuna rasa.[26] *Sidhu* is of two types: *Apakwa rasa sidhu&Pakwa rasa sidhu.*	Useful in *Vibandha, Shopha, Arsha, Udara*.[27]
Varuni	The liquid prepared with juice of *Tala* and *Kharjura.*[28]	*Laghu,Tiksna, Haridya, Vibandha, Swasa, Vaman, Shula.*[28]

3.20 (B) *Asava*

In these drugs and *Madhur dravya Dhataki pushpa* added into water (*jala*). After addition they are mixed without decoction and filled into closely tightly container for a specific period of time to make the alcohol is called *Asava*. There is no such a big difference between *Asava* and *Arishta.* In *Arishta* it is prepared by the help of heat and where as in case of *Asava* is prepared without Heating[23,24]

Use of *Asava*

Digestive fire, Sleeplessness sorrow, Beneficial to *Hridaya* including dieresis.

Properties- Pleasant in taste, appearance and odor.[20,23]

3.20 (C) *Arishta*

In which drugs are coarsely powdered and *Kashaya* is prepared. Sugar, jaggery or honey, is added and heated, filtered out. *Praksepa dravya* are fine powdered and added. At the end *Dhataki Puspa,* added should be properly cleaned and added. The reason behind the preparation of *Arishta* is because all the phytoconstituent present in *dravya* finally extracted out.[20,23]

Uses of *Arishta*

G.I.T disturbance, Anemia, Fever, Appetizer, Diseases of spleen[20,23]

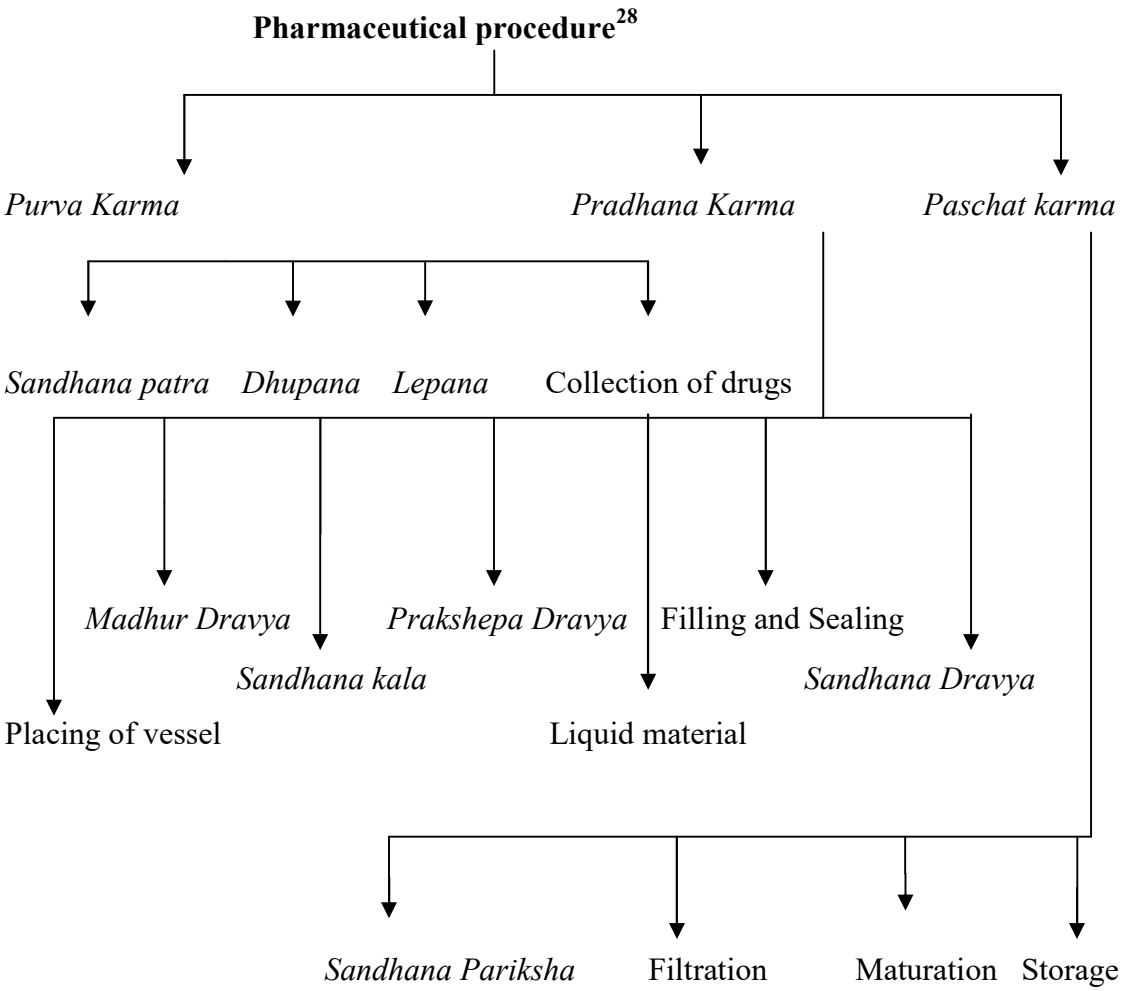
3.21 General pharmaceutical procedure underlying the preparation of *Sandhan Kalpana*

During formulation it may be divided into:

3.21(A) *Purva karma*

3.21(B)*Pradhana karma*

3.21 (C) *Paschat karma*



(A) *Purva Karma*

Selection of container

In ancient time mud port were used for preparation of *Asava* and *Arishta Madaya, Sukta Kalpana.* But the preparation of fermentation process is very difficult and limited limitations. These pots are easily available, cheap and temperature can be maintained but major problem it can easily breakage and water oozes where the inner side of product come out. Sometime these ports are broken during the preparation of *Sandhan Kalpana*. These vessel porosity are present gases which are liberated during the process of fermentation are expelled out in some quantity hence less chance of expulsion.[23,24] Wooden containers are used preparations of Fermentation. Main reason of new wooden container is not easily available and not easy for cleaness. We cannot use the same container for different *Asava Arishta.* Because wooden are organic material chance for

growth of fungal or bacterial is more. In modern time Stainless steel container Plastic &Glass, China-clay container are used.[26,28,29]

Advantages of Stainless Steel	**Disadvantages of Stainless Steel**
It is chemically inert.	They are very costly.
Temperature maintenance is easily.	It change taste and smell due to chemical reaction with this container.[28]
Sterilization and Handling is easy.	
Available in various sizes.	In plastic there may be chances of leaching.
Commercially less costly	Easily breakable & needs good handling.[28,30]

Dhupana

According to *Acharya Caraka* and Acharya Sushruta inside the vessel use for *fumigation* with different drug such as *Guggulu, Jatamansi, Vaca, Candana Karpoor* is used to prevent the antimicrobial activities and provide the long stability of drugs. So after the liquid added into container it is most important.[26, 27]

Table No.:1- Reference regarding the *Dhupana Dravya*

Dhupana Dravya	***Asava/Arishta***	**Reference**
Sharkara, Agaru	*Kankarishta*	Ch. Chi. 16/168
Nambi, Maricha	*Ushirasava*	Sha. Sa. Ma. Kh. 10/13-17
Chandana, Agaru	*Drakshasava*	Ga. Ni. 6/160

Lepana

Lepana are used coating of inner surface of vessel to prevent leakage of liquid and reduce the porosity of port. *Lepana* are used to provide the strength of container. Layer of *Lepana* are fill the pores due to smooth surface of container it help into fermentation. It is used for only *Mritika Patra*. Example of *Lepana* drugs *Ghee, Madhu* which besides their slight disinfectant property, act as base material.[26,28]

Table No.:2- References regarding *Lepana Dravya* in the preparation of *AsavaArishta*

Lepana dravya	***Asava/Arishta***	**References**
Pippali, Chavya, Madhu Priyangu, Ghrita	2nd *Phalarista*	Ch. Chi. 15/153-157
Ela, Mrnal, Agaru, Chandan	*Madhukasava*	Ch. Chi. 15/146-147
Pippali, Madhu	*Loharistha*	Su. Chi. 12/12-19
Lodhra, Dhatki	*Dantyarista*	Chakradatta Arsha/23-26
Maricha, Madhu	*Gandirasava*	Ga. Ni.

Collection of drugs

According to ancient time *Acharya* says collection of drug with respect to season, soil part, day to be collected. Collected the drug in *shard ritu* (October, November) is the best time for collection of herbs. Freshly collected able to produce actions according to properties, but now a time guidelines are used for collection of drugs such as GMP and GAP guidelines are essential to be followed collection of drug.[31]

3.21 (B) *Pradhana karma*

Liquid material

In *Sandhan Kalpana* Liquid material/Aqueous solution is play important role for fermentation, provides favourable growth of micro organism. It helps to grow any microorganism *Asava ArishtaSukta* are prepared by *Swarasa, Kwatha, Takra,* Cold or hot infusion.[32]

Table No.:3-Amount of water required in *Sandhan Kalpana*

Drugs	**Water / Required**
Mridu dravya	4 time water
Madhyama dravya	8 time water
Kathin dravya	16time water [32]

Kwatha

Kwatha is a well known *Ayurveda* dosages form that is used for the therapeutic purpose. In modern terminology it is known as decoction. *Kwatha* is the filtered obtained by boiling coarse powder of drugs in proportion of *Mridu dravya4*, *Madhyam dravya 8*, *Kathina dravya16* quantity of water.[32]

Table No.:4-Different *Acharya* have different opinion regarding to *Kwatha*[33,34,35]

Reference	**Nature of drug**	**Quantity of water**	**Reduction upto water**
Sushruta	-	8 or 16 times	1/4th
Vagbhata	-	8 times	1/4th
Indu	-	16 times	1/4th
Ksharapani	*Mridu, Kathina*	4 or 8 or 16	1/4th
Sharangadhara	-	16 times	1/8th
Sharangadhara	*Madhyama*	8 times	1/4th
Varaha Mishra	1 *masha*-1 *pala*	16 times	1/4th
	1 *pala* – 1 *Kudava*		
	1 *Kudava*- 1 *prastha*		
	1 *prastha*- 1 *Khari*		
General rule	Unspecified	8 times	1/4th[34]

Table No.:5-Different method of *Swarasa* used in the *Asava Arishta*

***Swarasa* Method**	***Asava*/*Arishta***	**Reference**
Powdered drug + water	*Pindasava*	Ch. Chi. 15/160 – 162
Squeezing of drugs	*Dhatrayaristha*	Ch. Chi. 16/111 – 113
Bhasma + warm water + *Phanita*	*Palashadi Arista*	Su. Chi. 10/7
Squeezing of *Kumari Juice*	*Kumariasava*	Sh. Sa. Ma. Kh. 10/18 – 27

Madhur Dravya

Madhur dravya is very important role for fermentation. It helps growth of bacteria, yeast, and microorganism essentially requires nutrients. *Guda (*Jaggery*) Sugar, Mishra,* Honey, Sugar cane *munaka* are example of *Madhur* dravya. According to Acharya *Saragdhara Samhita* the general ratio of water and *Guda* is 39.06%. Yeast can developed of high concentration of sugar and grow well in solution containing 40% sugar. Jaggery forms

the primary source for the metabolism of the micro-organism and help to formation of alcohol.[24,25]

Table No.:6- Use of sweetening agents in classics[36]

Acharya	Min. % of Sugar	Max. % of Sugar	% of <40% of sugar*	% of >40% of sugar*
Ch.	20.00	156.22	25.00	54.16
Su.	39.00	200.00	18.18	45.45
A.H	39.00	156.00	25.00	50.00
Ga.Ni.	19.53	312.50	10.71	60.71
Sha.sa.	32.03	156.25	30.76	61.53

At higher sugar concentration exceeding 400g/2% alcohol formation decreases.

Table No.:7- Effect of sugar concentration on alcohol formation[36]

Sugar concentration of wort (%)	Ethanol concentration in % (formed in 2 month)
37	8.6
42	6.3
47	5.9
55	3.4
75	0.0

Sandhan Dravya

Sandhan dravya or fermentation initiator is probably the contribution of *Acharya Charaka* has been used *Dhataki* in the *Sandhan Kalpana* is extensively used. The following drugs that had been used for their role as fermentation initiator such as;

a) *Dhataki pushpa*

b) *Madhuca pushpa*

c) *Surabija*

d) Yeast

Yeast (favourable pH for growth is 3 to 4.5 Alcohol inhibits the growth of yeast in concentration of 5 to 7%). *Dhataki* is also known as *Tamra pushpa*. It is Latin name is

Woodfordia fruticosa. It contains 20% of tannic acid. The presences of tannin produce environmental for yeast growth. It is much more fermentation indicator. *Madhuca Indica* consists of 50 to 60% of sugars, cellulose enzyme yeast.[24]

Prakshepa Dravya

Prakshepa dravya are the additives which are added to *Asava* and *Arishta* preparation to give good taste, smell and therapeutic action. Most of *Prakshepa dravya Clove Ela, Nagakesara, Trikatu* etc. These drugs act as adjuvant to the therapeutic action and give in the formulation aroma color taste which is patients easily accepectable and palatable. Generally *Prakshepa dravya* are very fine powder but in the case of *Asava* and *Arishta* very fine powder are not added.Corasly fine powder are added into *sandhana patra.*[23,26]

Filling and sealing of *Patra*

Filling & sealing of fermentation vessel should be done after speculating the head space i.e. the space to be left vacant within the fermenter vessel .Head space usually occupies more of the volume of the fermenter. Head space must be left at the top of the fermenter above the liquid medium.[24,28]

Role of Temperature

Temperature is an important factor because there are chances that can decompose some of the thermo- liable active constituents. Therefore during the preparation of decoction temperature should be maintained between 85- 90 ºC. Optimum temperatures as well as maximum and minimum temperature which can be tolerated are distinctly different for yeast respiration, fermentation cell growth and alcohol tolerance. Higher the temperature faster the fermentation and faster its cessation. Fermentation intensity diminishes above a certain temperature which is generally above 35ºC. Also at very low temperature the multiplication of cell decreases.[37]

***Sthanavimarsha* (placing of *Sandhanapatra*)**

In ancient time *Acharya Charka* and *Acharya Sushruta* both they mentioned placed of vessel according to *Desha* it should be kept in open space and also *Asava Arishta* places into paddy husk where the temperature is maintained. It is developed because fermenting microbes are highly sensitive to high temperature fluctuations and easily get damaged so

it is required higher or lower than required temperature. Temperature to be maintained depends on the micro-organisms usually ranging from 25◦c to 35◦c.[22, 25]

Placing of *Sandhana patra*

- The place must be cleaned and dry.
- Place should be away from direct air.

Table No.:8-*Sandhana kala* (Duration of the process)

Name of Samhita	**Maximum**	**Minimum**
Caraka Samhita	Half month	**7** Days
Sushruta Samhita	4 month	**7** Days
Ashtanga Hridaya	1 month	14 Days
Ashtanga sangrha	6 month	7 Days[3,26]

3.21(C) *Paschat Karma (Sandhan Pariksha)*

Observation at the initial stage	**Observation after the onset of fermentation**	**Observation after fulfillment of fermentation process**
To begin *Pottali* of *Prakshepa dravya* float over the liquid.	Floating Pottali of the *Prakshepa dravya* persistent.	The *Prakshepa Pottali* will sink into the bottom.
Liquid appear viscous and sticky.	The color of the liquid gets darker.	A strong alcoloholic odour noticed.
The temperature of inside and the periphery of the fermenter stand same.	Mild Alcoholic odor appears.	No heared sound or bubbling.
Nature of the foams, flocculation.[3]	Effervescence will be visible.	The temperature of *Asava* or *Arishta* is observed to be decreased.[3]
	A typical hissing sound audible from the vessel.[3]	

Filtration

Filtration is the procedures of separation of particles passing it through a permeable membrane which hold the solid but allows the fluid to pass. At the point when the *Asava* and *Arishta* placed into the container then observed outside the close to the port some sound stable hearing. After complete the *Sandhan* then it stopped these it means *Sandhan Kalpana* is completed then it filtered with the help of sieve. Then filtration with the help of sieve and fill into the container. For larger commercial purpose, various newer techniques like-electric filtration press, hydrolic filters, cold centrifuge method can be followed.[28,38]

Maturation

After complete this fermentation process filtration it can stained for 4 to 6 days. In these ways fine powder of *Asava* and *Arishta* settled down again it can filtered to separate the sediment.[20,23]Acharya Dalhana commentator of Sushruta *Samhita* had opined in the qualities of *Jirna Madya*. On the commentary on *Jirna* he opined one year old is known as *Jirna*, and is consumable with desired organoleptic quality.[27]

Packaging, Labeling and Storage :

Packaging : Prepared Asava Arishta kept in well stoppered bottles (amber colour bottle) leave a gap of at least 2 inches at the top the bottles for gas. Before packing, the container should be properly cleaned and dried. After the completion of packing, they are to be properly sealed. Proper attention should be given to the exact measuring/correct weighing of the medicine.

Labeling : The finished products i.e. Asava Arishta should be properly labeled and contain the following details. Name of the medicine, Reference to the formulation, List of ingredients, Percentage of self-generated alcohol, Date of manufacture, Batch number, Price, Date of expiry, Name and address of the manufacturer.

Storage

After compounding the *Sandhana Kalpana* it is essential that these should be stored properly. The stability of *Asava, Arishta* during its storage depend on the type of container and closure used during dispensing. Amber color glass container is used for

storage because it has the capacity to filter out U.V radiation. Slightly headspace should be maintained in the storing container so as to any of the gas released later.[26,28]

3.22 Test of *Asava Arishta*

Lime water test:

Sandhan Patra to test tube, which is filled with limewater and gas produced in *Sandhan Patra* to test tube. During this test bubbles will be seen in limewater and limewater turns milky, due to release of CO_2 during fermentation. Following chemical reaction occurs during this process. The temperature of fermenting liquid will be found slightly raised.[24,23]

Burning matchstick Test

Burning matchstick or candle will be stifled, if taken inside the container. Burning matchstick or candle will continue to burn, when introduced into the fermenter. No change can be found in limewater test.[20,23]

3.23 Definition according to modern science

Fermentation is the process of conversion of larger complex molecules into simple molecules through reduction by means of enzymes in an anaerobic condition. It results into the release of energy along with carbon dioxide.[39]The term 'fermentation' is derived from the Latin verb *'fevere'* means 'to boil'. Conversion of Sugar to Alcohol using yeast chemical conversion of carbohydrates into alcohols or acids. Any energy-releasing metabolic process that takes places only under anaerobic conditions.[3]

Table No.:9- Master Formula used for *Ashwagandha Kalpa*

Sr.no	Name of Ingredients	Botanical name	Parts used	Qty. of Ingredients
1.	*Ashwagandha*	***Withania somnifera***	Root	4 part
2.	*Sarpgandha*	***Rauwolfia serpentina***	Root	3 part
3.	*Brahmi*	***Bacopa monnieri***	Whole plant	2 part
4.	*Vacha*	***Acorus calamus***	Rhizomes	1 part
5.	*Sharkra*			10 part

DRUG REVIEW

3.24 Pharmacodynamics of *Kwatha Dravya*

Drugs	Rasa	Guna	Virya	Vipaka	Dosha Karma
Ashwagandha	*Tikta, Kasaya*	*Laghu*	*Usna*	*Madhur*	*Kaphvatashamk*[40]
Sarpgandha	*Tikta, Katu*	*Ruksa, Laghu*	*Usna*	*Katu*	*Kaphvatashamk*[41]
Brahmi	*Tikta,Kasaya, Madhura*	*Laghu, Sara*	*Sita*	*Madhura*	*Kaphvatashamk*[42]
Vaca	*Katu, Tikta*	*Laghu, Tiksna*	*Usna*	*Katu*	*Kaphvatashamk, Pittvardhak*[42]

3.25 Pharmacokinetics of *Kwatha dravya*

Drugs	Karma	Rogaghnata	Pharmacology
Ashwagandha	*Vatakapha, Balya, Rasayana, Vajikarana*	*Sothhara, Vednasthapan, Depan, Anuloman, Sulaprashman, Swashar*	Anti- inflammatory, Antistress, Antibiotic, Antioxidant, Anti- aging, Antiparkinsonian[43]
Sarpgandha	*Vatahara, Kaphahara, Mutrala, Dipana, Rucya, Pacana, Nidraprada, Visaghna, Kamavasadaka, Hrdayavasadaka*	*Nindrajan, Pittvardhak, Vishghan, Javarghanraktbharsham k,Hridyavashadk, Ampachan, Jwaraghan*	High blood pressure, Insomina, In insanity, Hysteria Itching.[44]
Brahmi	*Vatahara, Kaphahara, Rasayana, Ayusya, Medhya, Matiprada, Svarya, Prajasthapana, Visahara, Mohahara*	*Sothahar, Vishghan, Medhya, DepanPachan, Rakyshoodhak, Hridya, Kaphghan, mutravirechan*	Wound healing, Cytotoxic and Antitumour, Memory enhancing, Immunomodulating Antiprotozoal, Mental retardation.[45]
Vacha	*Vatahara, Kaphahara, Mala Mutravisodhani, Dipani,Kanthya, Krmihara, Vamaka, Medhya*	*Aruchi, Ashmri, Manashdoshr, Vednasthapn, Apasmar, vathara*	Antibacterial, Antidiabetic, Antifungal, Antinflamatory, Anticancer, Antihepatotoxic, Antispasmodic, and Anti-diarrheal**.** [46]

3.26*Ashwagandha* (Root)[40]

Ashwagandha consists of dried mature roots of ***Withania somnifera*** Dunal. (**Fam. Solanaceae),** a perennial shrub, found in waste land, cultivated field and open grounds throughout India, widely cultivated in certain areas of Madhya Pradesh and Rajasthan, roots collected in winter, washed and cut into short pieces.

Synonyms

Sanskrit: Hayagandha, Vajigandha

Assamese: Ashvagandha

Bengali: Ashvagandha

English: Winter cherry

Gujrati: Asgandha

Hindi: Asgandh

Kannada: Angarberu, Hiremaddina-gida

Kashmiri: Asagandh

Malayalam: Amukkuram

Marathi: Asagandha, Askagandha

Oriya: Aswagandha

Punjabi: Asgandh

Tamil: Amukkaramkizangu

Telugu: Pennerugadda

Urdu: Asgand

Constituents - Alkaloids and withanolides. Other constituents are amino acids, choline, beta- sitosterol, chlorogenic acid, scopoletin, withaferin. The main constituents of Ashwagandha are alkaloids and steroidal lactones. The other alkaloids are Somniferine, somnine, Somniferinine, Withanine, pseudo- Withanine, Tropine, pseudo Tropine, 3-α-gloyloxytropane, choline, Cuscohygrine, isopelletierine, anaferine and anahydrine. Two acyl steryl glucosides Sitoindoside VII and Sitoindoside VIII have been isolated from roots. The leaves contain steroidal lactones, which are commonly called as "Withanolides". The withanolides have C28 steroidal nucleus with C9 side Chain, having six membered ring.[47]

Table No.:10-Classical Reference properties of *Ashwagandha*

Sr. No	Reference	Drug Name	Description
1.	**Priya Nighantu**[48]	*Ashwagandha*	Properties, Therapeutic action are mentioned in page no.95.
2.	**Bhavaprakash Nighantu**[49]	*Ashwagandha*	Different Synonym, Properties, Uses mentioned in page no.363.
3.	**Raj Nighantu**[50]	*Ashwagandha*	Different Synonyms, Part Used, dose mentioned in page no.83.
5.	**Shankar Nighantu**[51]	*Ashwagandha*	Different Synonyms, color, Properties mentioned in page no.13

3.27*Sarpagandha* (Root)[41]

Sarpagandha consists of air dried root of ***Rauwolfia serpentina*** (Linn.) (Fam. **Apocynaceae);** a perennial undershrub widely distributed in India in the sub-Himalayan tracts upto 1,000 m as well as, in the lower ranges of the Eastern and Western Ghats and in the Andamans.

Synonyms

Sanskrit: Nakuli, Candrika, Chandramarah

Bengali: Chaandar

English: Rauvolfia Root, Serpentina Root

Gujrati: Amelpodee

Hindi: Chhotaa Chaand, Dhavalbaruaa

Kannada: Sutranaabhu

Malayalam: Amalpori

Marathi: Adkai, Chandra

Oriya: Dhanbarua, Sanochado

Tamil: Sarppaganti

Constituents - Rauwolfia contains indole alkaloids, such as reserpinine, serpentinine and ajmalicine. Serpentine, Sarpagine, Reserpine, Serpentine, Rauwolfinine, Yohimbine, Ajmalimine.[27]The other alkaloids present in the drug are Ajmaline, ajmalicine, Rauwolfinine, rescinnamine, reserpinine, Yohimbine, serpentine and serpentinine. The

major alkaloids reserpine and rescinnamine are esters derived from methyl reserpate and trimethoxybenzoic acid in reserpine and trimethoxycinnamic acid in case of rescinnamine. Syrosingopine is methyl carbethoxy syringoyl reserpate.[44,47]

Important Formulation-*Sarpagandhadi Curna, Sarpagandhayoga, Sarpagandha Vati, Sarpagandha Ghana Vati.*

Table No.:11-Classical Refernce Properties of *Sarpgandha*

Sr. No.	Reference	Drug Name	Description
1.	**Priya Nighantu[48]**	*Sarpgandha*	Properties and action mentioned in page no.106
2.	**Dravyaguna-Vijnana[52]**	*Sarpgandha*	Different Synonym, Properties, Therapeutic Action mentioned in page no. 36-38.
3.	**Indian Materia Medica[53]**	*Sarpgandha*	Uses, Properties, habitat, Chemical Constituents mentioned in page no.1051-1052.

3.28 *Brahmi* (Whole Plant)[42]

Brahmi consists of dried whole plant of ***Bacopa monnieri*** (Linn.) Syn. *Herpestis monnieria* (Linn.)**(Fam. Scrophulariaceae**; a glabrous, succulent, small, prostrate or creeping annual herb, found throughout India in wet and damp places.

Synonyms

Sanskrit: Sarasvati, Kapotavanka

Assamese: Brahmi

English: Thyme Leaved Gratiola

Gujrati: Neerbrahmi, Bamanevari

Hindi: Manduka Parni

Kannada: Nirubrahmi, Valabrahmi, Ondelaga, Mandukaparni

Malayalam: Bhahmi

Marathi: Jalnam, Brahmi, Birami

Oriya: Brahmi

Punjabi: Brahmibuti

Tamil: Nirabrahmi, Brahmi vazhukkai

Telugu: Sambarenu, Sambrani

Urdu: Brahmi

Constituents – It contains an alkaloid which is known as Brahmine. The plant also yields beulinic acid, gamma Sitosterol, Stigmasterol, Mannitol, Saponinbacoside A. From the whole plant D-mannitol, beta sitostrol, Stigmasterol, Stigmastanol and bacoside A and B are reported. Nicotine, luteolin and luteolin-7-glucoside.Jujubogenin and pseudojujubonin obtained by degradation of bacoside A.[45,47]

Important Formulation-*Sarasvatarishta, Brahmi Ghrta, Ratnagiri Rasa, BrahmiVati, Sarasvata Curna, Smrtisagara Rasa.*

Table No.:12-Classical Reference properties of *Brahmi*

Sr. No	Reference	Drug Name	Description
1.	**Priya Nighantu[48]**	*Brahmi*	Properties and Therapeutic action mentioned in page no.97.
2.	**Bhavaprakash Nighantu[49]**	*Brahmi*	Different Synonym, Properties and Therapeutic action mentioned in page no.461.
3.	**Dravyaguna-Vijnana[52]**	*Brahmi*	Different synonym, Properties, chemical Constituents are mentioned in page no.6-8

3. 29*Vacha* (Rhizome)[43]

Vacha consists of dried rhizome of ***Acarus calamus*** Linn. (**Fam. Araceae**); a semiaquatic herb, wild or cultivated throughout the country ascending upto 1800 m in the Himalayas.

Synonyms

Sanskrit: Ugragandha, Ugra, Sadgrantha

English: The Sweet Flag

Gujrati: Ghoduvaj, Ghodvach

Hindi: Bach, Gora-bach

Kannada: Baje, Narru Berua

Malayalam: Vayambu

Marathi: Vaca, Vekhandas

Punjahbi: Varch, Ghodavaca

Tamil: Vasambu, Pillai maruntho

Telugu: Vasa

Urdu: Waja-e-Turki

Constituents- Volatile Oil (principal constituents of the Volatile oil are Asamyl alcohol, Eugenol and Asarone), also contains a bitter principle Acorin (Glucoside), Starch and Tannin. Calamus contains constituents such as alkaloids, Flavanoids, gums, lectins, mucilage, phenols, quinine, saponins, sugar, tannins, and triterpenes. Calamenone as well as calamendiol and isocalamendiol also occur in the roots. 27 other compounds that are identified in Acorus calamus were 4-Terpineol,2- Allyi-5ethoxy-4-methoxyhenol, Lysidine, Borneol, Octadecatrien-1-ol, Butyl butanoate, Geranylacetate, Acetic acid, Camphor Linolenic acid.[47,46]

Important Formulation - *Vacadi Taila, Vaca Lasunadi Taila, Sarasvata Curna, Sarasvatarishta, Manasamitra Vataka.*

Table No.:13- Classical Reference properties of *Vaca*

Sr. No	Reference	Drug Name	Description
1.	**Priya Nighantu[48]**	*Vacha*	Properties and Therapeutic action mentioned in page no.83.
2.	**Bhavaprakash Nighantu[49]**	*Vacha*	Properties, Uses and Therapeutic action are mentioned in page no.43.
3.	**Indian Materia Medica[53]**	*Vacha*	Botanical description, uses are mentioned in page no.35-37.
4.	**Dravyaguna-Vijnana[31]**	*Vacha*	Different Synonym, Chemical constituent, Properties and uses are mentioned in page no. 28-30.

3. 30 *Gur* (Jaggery)

Gur (Jaggery) is a natural, traditional sweetener made by the concentration of sugarcane. *Gur* is prepared by concentrating the sugarcane juice and it is available in the form of solid form and in semi-solid form. It is prepared by boiling the fresh cane juice at about 100-110 for 3-4 hours in an open iron plate.[50]Jaggery is a dark, course, unrefined sugar, sometimes referred to as "palm sugar:" It can be made either from the sap of various palm trees or from sugar-cane juice. It is primarily used in India, where many categorize

sugar made from sugar-cane as jaggery and that processed from palm trees as *"gur"*. Jaggery has a sweet, wine-like fragrance and flavor that lends distinction to whatever food it embellishes.[54]

Biological Name: *Saccharum officinarum*

Family:**Poaceae**

Part used: Whole plant

Pharmacodynamics :

Rasa : *Madhura***Guna** : *Natisheeta, Snigdha*

Virya: *Ushna***Vipaka** : *Madhura*[54]

Doshaghnata :*Tridoshashamaka* (with different *Anupana*)

Karma :*Deepana, Pachana, Anulomana, Vrishya, Hridya, Mutra-Raktashodhaka, Increase Medodhatu, Kapha and Krimi, Pittaghna.*

Sweetening substances used in the *Ayurvedic* formulations for the purpose to increase it's palatability, for preservation and also to have, tonic effect. They are responsible for the generation of alcohol in *Asava Arishta*. Besides this, sweetening substances, which are important ingredients of *Asava Arishta,* have unavoidable role in the process of fermentation.[55]

Modern Review:

It improves digestion, prevents fatigue, purifies blood and provides strength to the muscles. Jaggery is rich in minerals, vitamins iron and instant glucose. It is not only easily digestible but has various minerals and vitamins in right proportion, which is extremely useful for our body. Jaggery and sugar are not only different in their composition but also in their effect on the human metabolism. Carbohydrate, which is prominently present in sugar, need B vitamins for their proper utilization by the body and the nature has so arranged it that, in their natural states, both cereals and natural sugar items (like, cane-juice, fruits, nuts etc.), and also protein foods, have more than enough of the B-vitamins needed for the assimilation of all the carbohydrate present.[56]

1	**Sucrose**	65–85 grams.
2	**Fructose and glucose**	10–15 grams.
3	**Protein**	0.4 grams.
4	**Fat**	0.1 grams.
5	**Iron**	11 mg, or 61%.
6	**Magnesium**	70-90 mg, or about 20%
7	**Potassium**	1050 mg, or 30%[57]

Pharmacological characters of *Guda* :

A work has been carried out regarding the identification of fermenting organism in *Asava - Arishta.* The organisms are isolated from jaggery. According to this study Bacillus species is present in both the new and old jaggery. Among the Bacillus species B. acetoethylicus and B. Polymyxa are reported to bring about alcohol production (Prescott and Dunn, 1959). Old and new jaggery yield almost equal percentage of alcohol.[58]

Table No.:14- Classical Reference properties of Jaggery

Sr.No.	Reference	Drug Name	Properties of fresh jiggery	Properties of Old Jaggery
1.	**Bhavapraksh Nighantu[49]**	Jaggery	*Swasa, Kasha, Kapha*, Enhance the digestion.	*Madhur, Viryavardhak, Vatanashak, Laghu, Pathya* are mentioned in page no. 796.
2.	**Yogaratnakara[59]**	Jaggery	*Guru, Usna, Kaph-vatnashak, pittanashak*.	*Vayunashak, Tridoshnashak, Jwarnashak, Pandu, Prameha, Laghu* are mentioned in page no.111

Dhanvantari Nighantu[60]

In *Dhanvantri Nighantu* different Synonym of *guda,* properties and therapeutic action are mentioned in page no. 144.

3.31 Sugar

Sugar is the generic name for sweet, soluble carbohydrates, many of which are used in food. There are various types of sugar derived from different sources. Simple sugar are called monosaccharides and include glucose, fructose, and galactose.[61]

Chemistry

Monosaccharides are also called "simple sugar" the most important being glucose. Almost all sugars have the formula $C_n2_nO_{n.}$ Glucose has the molecular formula $C_6H_{12}O_{6.}$

Preparation :

Commercially from the sugar cane, beet root and sorghum. Originally sugar cane was the only source, but at present the root of vulgaris is used largely in Europe and to an increasing degree in this country. The sugar cane is crushed and the juice amounting to about 80% is expressed with roller mills. The juice after "defecation" with lime and removal of excess of lime by carbonic acid gas is run into vacuum pans for concentration and the saccharine juice is evaporated in this until it begins to crystallize. After the crystallization is complete, the warm mixture of crystals and syrup is run into centrifuges, in which the crystals of raw sugar are drained and dried. The syrup resulting as a by product from raw sugar is known as molasses. Raw beet sugar is made by a similar process but is more troublesome to purify than that made from sugar cane.[62]

***Dhataki* (Flower)[40]**

Dhataki consists of flowers of ***Woodfordia fruticosa*** (**Fam. Lythraceae)** much branched, semi deciduous, undershrub or shrub, 1-3 m high, rarely upto 3 m, found throughout India, asascending to 1500 m in Himalayas and also in the Gangetic plains also cultivated in garden.

Synonyms

Sanskrit: Bahupuspi, Tamrapuspi, Vahnijvala

Assamese: Dhaiphool

Bengali: Dhaiphul

English: Fire flame bush

Gujrati: Dhavadi, Dhavani

Hindi: Dhai, Dhava

Kannada: Dhataki, Tamrapushpi

Malayalam: Tattiripuvu, Tatire

Marathi: Dhayati, Dhavati

Oriya: Dhaiphula, Dhatuki

Punjabi: Davi, Phul Dhava

Tamil: Kattati, Kattathi, Kattattipoo

Telugu: Aarl Puruvu

Constituents - Tannin and glucoside, hydrolysable tannins and flavonoids. β-sitosterolgallic acid, Polyphenols-ellagic acid, polytachoside and myricetin-3-galactoside, anthocyanins pelargonidine-3, 5-diglucoside and cyaniding 3, 5-diglucoside; octacosanol, chrysophanol-8-o-beta-d-glucopyranoside, beta-sitosterol, hecogenin, mesoinositol, flavone *i.e*. glycosides-quercetin-3-rhamnoside, naringenin-7-glucoside and kaempferol have been reported from flowers. A high proportion of ellagic acid and polyphenols have been detected in the leaves and flowers.[63]

Pharmacological Action :

Antimicrobial activity, Hepatoprotective activity, Antiulcer activity, immunomodulatoryacivity, Antifertitlity activity, Antihyperglycemic activity, Antibacterial, Wound healing activity. [64]

Properties and Action

Rasa: *Kasaya, Katu*

Guna: *Laghu*

Virya: *Sita*

Vipaka: *Katu*

Karma: *Grahi, Visaghna, Garbhasthapana, Krminut, Sandhaniya*

Table No.:15- Classical Reference properties of *Dhataki*

Sr.no	Reference	Drug Name	Description
1.	**Bhavapraksh Nighantu[49]**	*Dhataki*	Different synonyms, properties, Use, dose mentioned .
2.	**Indian Materia Medica[53]**	*Dhataki*	Two Species of *Dhataki* are mentioned .
3.	**Raj Nighantu[50]**	*Dhataki*	Different Synonyms, Properties, Use are mentioned .
4.	**Nighantu Adarsa[65]**	*Dhataki*	Different synonym of *Dhataki* and properties are mentioned.
5.	**Charka Samhita[11]**	*Dhataki*	Properties of *Dhataki pushpa* are mentioned .

***Madhuca* (Flower)[46]**

Madhuca consists of flower usually without stalk or calyx of ***Madhuca indica*** Syn**. *M. latifolia*** (Roxb.) Macbride, Bassia latifolia Roxb. (***Fam. Sapotaceae***); a medium sized deciduous tree occurs in mixed deciduous forests throughout India, and also cultivated.

Synonyms

Sanskrit: Gudapuspa

Assamese: Mahua, Mahuwa

Bengali: Mahuwa

English: The Indian Butter tree, Mahawash tree

Gujrati: Mahudo, Mahuwa

Hindi: Mahuwa

Kannada: Hippegida, Halippe, Hippe, Hippenara, Madhuka, Ippa, Eppimara

Malayalam: Irippa, Ilippa, Iluppa, Eluppa

Marathi: Mohda

Oriya: Mahula

Punjabi: Maua, Mahua

Tamil: Katiluppai, Kattu Iluppai, Iluppi

Telugu: lppa Puvvu

Urdu: Mahuva

Constituents – Sugars, α-amyrin acetate, α-spinasterol, erythrodiolmonocaprylate, betulinic acid and oleanolic acid caprylates Some of theconstituents of both leaves and bark arementioned in the β-amyrin acetate, 21-Hydroxy-3-oleanylmyricitate, Ursolic acid, β-carotene and xanthophylls; erthrodiol, palmitic acid, myricetin.[66,67]

Pharmacological activity

Antioxidant activity, Wound healing activity, Antimicrobial activity, Antipyretic activity, Anticancer activity.[66]

Properties and Action

Rasa: *Madhura*

Guna:*Guru*

Virya:*Sita*

Vipaka:*Madhura*

Karma:*Vatahara, Pittakara, Sukrala, Sramahara, Balya, Ahrdya*

Table No.:16- Nutrition aspect of *Mahua* flower[68]

Sr.no.	Constituents	Flower
1.	Moisture(%)	19.8
2.	Protein(%)	6.37
3.	Fat(%)	0.5
4.	Reducing Sugar(%)	50.62
5.	Total Inverts(%)	54.24
6.	Cane sugar(%)	3.43
7.	Total Sugar(%)	54.06
8.	Ash(%)	4.36
9.	Calcium(%)	8

Important Formulation - *Madhukasava, Drakasadi Kvatha Curna, Eladi Modaka*

Therapeutic Uses -*Trsna, Daha, Srama, Svasa, Ksata, Ksaya.*

Table No.:17-Classical Reference properties of Madhuca

Sr.no	Reference	Drug Name	Description
1.	**Bhavprakash Nighantu**[49]	*Madhuca*	Properties, Therapeutic are mentioned in page no.579-580.
2.	**Priya Nighantu**[48]	*Madhuca*	Flower are Useful for fermentation are described in the nighantu page no.49
3.	**Nighantu Adarsh**[65]	*Madhuca*	Different synonyms of *madhuca* are described in page no.306-307
4.	**Shankar Nighantu**[51]	*Madhuca*	Different synonyms of *madhuca*, therapeutic action are mentioned in page no.210
5.	**Raja Nighantu**[50]	*Madhuca*	Different synonyms, properties, uses are mentioned in page no.358-359.

Yeast

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom. The yeast lineage originated hundreds of millions of years ago, and 1,500 species are identified. The yeast doesn't itself take part in the fermentation process, but it secretes a complex set of enzymes that act upon the sugars and convert it to alcohol and carbon dioxide gas.. Yeasts can break down simple sugars only (monosaccharide) into CO2 and ethanol. So, enzymes that break down starch and disaccharides (sucrose = glucose + fructose is most common sugar in plants) must be added before adding yeast except for honey and some fruit juices.[70]

Table No.:18-Total Calories present in yeast[70]

Sr. no	**Calories**	**%**
1	Saturated Fat	1g
2	Polyunsaturated Fat	0g
3	Monounsaturated fat	4.3g
4	Total Carbohydrate	41g
5	Dietary fiber	27g
6	Sugar	0 g
7	Sodium	51 mg
8	Potassium	955mg

Chapter-4
RATIONAL AND SCOPE OF THE STUDY

4.1 Rational of Study

High blood pressure, paralysis, loss of concentration, loss of sleepness, mental disorder are very commonly found diseases in our society. For such diseases *Ayurveda* is best called as remedial measure. The formulation like *Asava Arishta*, *Ghrita, Taila*, *Vati* etc. are very well known to be found helpful to cure these diseases. *Ashwagandha Kalpa* is also among one of such formulations. It is a patent product of Baijnath Pharmaceuticals Paprola Himachal Pradesh. This medicine is used in indication mainly high blood pressure, paralysis, loss of concentration, loss of sleepness, mental disorder. Presently the method that the company is using to prepare this formulation is time consuming. They are also finding batch variations due to the fermenting agent used in the formulation. My project will be mainly focused on the improvement in method of preparation of *Ashwagandha Kalpa*. For the desires purpose I will work on two factors –Time and fermenting agent used in the preparation of *Ashwagandha Kalpa.* My efforts will be to provide the company a best product by using minimum tools in adequate time period.

Aim of the study

To study the impact of variables time and fermenting agent on different batches of *Ashwagandha Kalpa* a marketed formulation.

4.2 COMPREHENSIVE PLANE

1 Collection of ingredients of *Ashwagandha Kalpa.*
2 Authentication of ingredients of *Aswagandha Kalpa.*
3 Pharmacognostic study of ingredients.
4 Quality evaluation of ingredients.
5 Preparation of different batches of *Ashwagandha Kalpa.*
6 Finished product analysis-Analytical Study of Prepared batches of *Ashwagandha Kalpa.*

7 Stastical evaluation of results by PCA.

1. COLLECTION OF RAW MATERIAL

The raw material for the preparation of *Ashwagandha Kalpa* will be collected from local areas of Paprola Himachal Pradesh and Baijnath Pharmaceutical Paprola Himachal Pradesh.

2. AUTHENTICATION OF INGREDIENTS

Pharmacognostic Study

a) Morphology
b) Macroscopic study.
c) Microscopic study

3. QUALITY EVALUATION OF INGREDIENTS

a) L.O.D
b) Ash Value
c) Acid insoluble Ash
d) Extractive Values-Water and Alcohol
e) pH
f) Qualitative analysis
g) Quantitative analysis
h) Chromatography fingerprint

4. FINISHED PRODUCT ANALYSIS

a) Physcio-chemical parameters-L.O.D
b) Total dissolves solutes
c) Ph
d) Sugar Content
e) Alcohol content
f) Test for methanol
g) Chromatographic analysis-HPTLC,HPLC.

5. STATSTICAL ANALYSIS

The data results will be analysed by Principal component analysis to find the area and the variables responsible for discrimination among the different batches of *Ashwagandha Kalpa.*

Scope of the study

In the present day, jaggery is widely used instead of sugar, honey and sugarcane. One of the major problem is fungal growth during *Sandhan* period. Considering these view the study was planned to compare the effect of the different fermentening agents, Time period & sweetening agents used in fermented preparations to search out prevention measures for fungal growth during *Sandhan* period. So the study was carried out in such a manner that the problems are to be ruled out.

Chapter-5
OBJECTIVE OF THE STUDY

Objective

1. To prepare different batches of *Ashwagandha Kalpa* the basis of two variables- Time and Fermenting agent.
2. To study out the impact of variables on the different batches of *Ashwagandha Kalpa* by using analytical parameters like physicochemical evaluation, chromatography study etc.
3. Evaluation of batch results by stastical tool principal component analysis(PCA).

Chapter-6
EXPERIMENTAL STUDY

Ashwagandha kalpa- a liquid dosage form is an example of *Sandhan Kalpana* (alcoholic fermentation). Method of preparation of *Ashwagandha Kalpa* is similar to *Arishta* preparation, because it fulfills all aspects of pharmaceutics of *Arishta Kalpana* (Sweetening agent i.e *Sharkara*(sugar) and *Gud* (Jaggery), *Ikshu* (Sugarcane), *Prakshepa Dravya* (adjuvant), use of *Dhataki pushpa, Madhuca pushpa,Yeast* as fermenting initiator make it an ideal example of *Sandhan kalpna*. It includes all fundamental principles of *Ayurvedic* pharmaceutics i.e the *Samskara.*

Aim and Objective

In the present study, three batches of *Ashwagandha kalpa* were prepared by taking following constants as variables

1. Practicle 1:*Ashwagandha Kalpa* prepared by different sweetening agents- -Jaggery,sugar and *Ikshu rasa.*
2. Practicle 2: *Ashwagandha Kalpa* prepared by using different Fermenting agent- *Dhataki Pushpa, Madhuka Pushpa* and yeast.

Material And Methods

Ingredients and their proportions in Ashwagandha kalpa

Dravya	Drug	Part used	Proportion
Kwatha Dravya	*Ashwagandha*	Root	4 part
	Sarpgandha	Root	3 part
	Brahmi	Whole Plant	2 part
	Vacha	Rhizomes	1 part
Sandhan Dravya	*Dhataki*	Flower	5 part
	Madhuca	Flower	5 part
	Yeast	-	2 part
Madhur Dravya	Jaggery	-	40 part
	Ikshu Rasa	-	40 part
	Sugar	-	40 part
Drava Dravya	Water	Reduced up to $1/8^{th}$	1800 Lit.

The raw materials were collected from the host organization Baijnath pharmacy. The ingredients used in the preparation of *Ashwagandha kalpa* were first identified by pharmacognostical study (Chapter no.7)& evaluated for their quality by employing Analytical parameter (Chapter no.8)Tap water was used in mentioned batches. Bhatti Electricity Furnace were used for heating purpose.

Method of Preparation of *Ashwagandha kalpa*

1) Preparation of boiled water
2) Preparation of *Kwath*
3) Preparation of wort
4) After fermentation

1) Preparation of boiled water

Date Start : 23 /12/2016	**End Date:** 23/12/2016
Time Start: 9:50 A.M	**Time (Stop):** 10:50 p.m

Batch Code: 1

Principle: Boiling

Duration:1/5 hours

Equipments: Furnace, Cotton Cloth, Measuring cylinder

Water:163 Lt.

Procedure: Tap water was boiled for 1 hours after coming it at boiling stage,it was made cool up to 60º C.

Observation: Clear layer was seen on upper surface of water..

ph of water before boiling:8.0 by pH paper

ph of water after boiling:7.6 by pH paper

2) **Preparation of *Kwatha***

Date Start : 24 /December/2016	**End Date:** 24/December/2016
Time Start: 9:50 A.M	**Time (Stop):** 5:00 p/m

Preparation of *Kwatha*

Batch Code:1.1

Principle:Boiling

Duration:6.50 hours

Ingredients:*Ashwagandh* : 1200g ***Sarpgandha*** : 900g

Brahmi : 600gm ***Vacha*** : 300g

Water : 160 Lt.

Equipments: Stainless steel vessel

Heating Device:Heating mental

Capacity:200 Lt.

Length:12.50 inch

Diameter:22. 50 inch

Measuring jar plastic: Jar 1 lt.

Capacity:1lt.

Length:7.0 inch

Diameter:4.5 inch

Thermometer: Measuring 360ºC temperature.

Cotton cloth

Ph paper

Procedure :

- *Yavakuta churna*(coarse powder) of *Kwatha dravya* was taken in vessel.
- Boiled tap water was used for overnight soaking of materials.
- Next day, *Kwatha* was prepared by reducing water to 1/8th.
- *Kwatha* was filtrated with double layered cotton cloth and measured.

Observations:

- Change in colour of water was observed immediately after adding water in *Kwatha* Dravyas.
- Materials were allowed for soaking up to 15.20 hours.
- It took 17.30 hrs for *Kwatha* preparation (it was prepared in two days.)
- The maximum temperature was observed as 92 ºC during preparation of *Kwatha.*
- Typical pleasant smell was fell during boiling of *Kwatha.*

Precautions:

- Mild heat was applied during preparation of *Kwatha.*
- Continuously stirring was done after 15- 20 mint.
- Vessel are sterilized by *Guggul, Jatamansi.*
- Cleaned cotton cloth was taken.
- Vessel was covered properly during overnight soaking to prevent from external dust and insect.

Results:

Before Boiling water (in L)	**After boiling *Kwatha* (in L)**	**Loss (in L)**	**% of Loss**
160 Lt.	20 Lt.	140lt	87.5%

Practical 1- Preparation of *Aswagandha Kalpa* using Sugar as sweetening agent

1.1 Preparation of A.K –Fermenting agent: *Dhataki*

Sweetening agent: Sugar

Fermentation vessel: Earthen Pot

Date Start: 25/12/2016	**End Date: 25/12/2016**
Time : 9:00 a.m	**Time Stop: 11:00 a.m**

Batch code:1.2

Principle: (Mixing)

Duration :2.0 hours

Ingredients:***Kwatha*:**2 Lt. **Sugar**: 425 gm ***Dhataki***:25 gm

Equipments :Earthen Pot

Diameter :23 inch

Length :12 inch

Capacity :2 Lt.

Measuring Jar: I L capacity (measurement as above)

Procedure :

- After the straining of *Kwatha* 425 gm sugar was added and dissolved.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- After cooling of *Kwatha dhataki pushpa* were added.

Observation:

- Temperature of *Kwatha* was 36ºC when *dhataki pushpa* added into the *Kwatha.*
- *Dhataki Pushpa* floated on surface when added into the *Kwatha.*

Precautions:

- Fresh *Dhataki Pushpa* were dried and used.
- *Dhataki Pushpa* were used after once dipped into the boiled water.

1.2 Preparation of A.K –Fermenting agent : *Madhuca Pushpa*

Sweetening agent : Sugar

Fermentation vessel : Earthen pot

Date Start: 25/12/2016	**End Date: 25/12/2016**
Time : 9:00 a.m	**End Date: 25/12/2016**

Batch code:1.3

Principle :(Mixing)

Duration: 2.0 hours

Ingredients:*Kwatha:* 2 Lt **sugar:**425gm ***Madhuca:*** 25 gm

Equipments :Earthen pot

Diameter :23 inch

Length :12 inch

Capacity:2Lt.

Measuring Jar : I L capacity (measurement as above)

Procedure :

- After the straining of *Kwatha*425 gm sugar was added and dissolved.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- After cooling of *Kwatha Madhuca pushpa* were added

Observation

- *Madhuca Pushpa*floated on surface when added into the *Kwatha.*
- Temperature of *Kwatha* was 36ºC when *dhataki pushpa* added into the *Kwatha*.

Precautions:

- Fresh *Madhuca Pushpa* were dried and used.

1.3 Preparation of A.K –Fermenting agent: *Yeast*

Sweetening agent : Sugar

Fermentation vessel: Earthen pot

Date Start: 25/12/2016	**End Date: 25/12/2016**
Time : 9:00 a.m	**Time Stop: 11:00 a.m**

Batch code :1.4

Principle:(Mixing)

Duration:2.0 hours

Ingredients:***Kwatha***: 2 Lt **sugar:** 425gm **Yeast :**4 gm

Equipments: Earthen Pot

Diameter:23 inch

Length:12 inch

Capacity :2 Lt.

Measuring Jar: 2 L capacity (measurement as above)

Procedure :

- After the straining of *Kwatha* 425 gm sugar was added and dissolved.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- After cooling of *Kwatha* Yeast were added.

Observation:

- Yeast floated on surface when added into the *Kwatha.*
- Temperature of *Kwatha* was 36ºC when Yeast added into the *Kwatha*.

Precautions:

- Fresh yeast were used.

1.4 After Fermentation place to maturation

Date Start: 26/12/2016	**End Date: 23/1/2017**

Batch code :1.5

Equipments : Earthen pot

Capacity:2liter

Length :14.50 incm

Diameter :09.50 inch

Circumference:30.50 inch

Measuring jar: Plastic – and 1 liter capacity

Glass bottle:2 (5 liter and 3 liter capacity)

Capacity:05.00 liter 03.00 liter

Length:11.50 inch 10.40 inch

Diameter :06.50 inch 05.90 inch

Circumference:21.00 inch 18.00 inch

Cotton cloth

Procedure :

- On 30dayth it was filtered and kept for maturation in Earthen pot.

Precautions :

- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- All the vessels used in the procedure were sterilized by heat treatment.

Practical – 2 : Preparation of *Ashwagandha kalpa* by using Jaggery as a sweetening agent.

Unit Operations:

1) **Preparation of *Kwatha***
2) **Preparation of wort**
3) **After fermentation**

1. Preparation of *Kwatha*

Date Start : 24 /12/2016	**End Date:** 24/12/2016
Time Start: 9:50 A.M	**Time (Stop):** 5:00 p/m

Batch Code:1.1

Principle: Boiling

Duration: Same as per practical no. 1.1

Ingredients : As per practical no. 1.1

Equipments : Same as per practical no.1.1

Procedure : Same as per practical no.1.1

Observation: Same as practical no. 1.1

Result: Same as practical no.1.1

Reason: Evaporation of water.

2.1. Preparation of A.K –Fermenting agent:*DhatakiPushpa*

Sweetening agent: Jaggery

Fermentation vessel: Earthen Pot

Date Start : 25 /December/2016	**End Date:** 25/December/2016
Time Start: 9:00 A.M	**Time (Stop):** 11:00 a.m

Batch code :1.6

Principle :(Mixing)

Duration :2:00 hours

Ingredients :*Kwatha* :2 Lt. **Guda***:* 425gm ***Dhataki***: 25 g

Equipments : Earthen pot

Diameter :23 inch

Length :12 inch

Capacity :2 Lt

Procedure :

- After straining of *Kwatha* whole quantity of *Guda* (jaggery) i.e.was added and dissolved and again it was strained.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- After cooling of *Kwatha*, *Dhataki flowers* were added.

Observations :

- Gradually, wort became viscid, as jaggery was dissolved.
- *Dhataki flowers* were floating on the surface.

Precaution :

- Fresh *Dhatki flowers* were used.
- After straining, *Guda* (jaggery) was added immediately.

2.3 Preparation of A.K – **Fermenting agent:** *Madhuca Pushpa*

Sweetening agent: Jaggery

Fermentation vessel: Earthen pot

Date Start : 25 /December/2016	**End Date:** 25/December/2016
Time Start: 9:00 A.M	**Time (Stop):** 11:00 a.m

Batch code :1.7

Principle :(Mixing)

Duration :2:00 hours

Ingredients:*Kwatha*: 2 Lt**Guda :** 425 g ***Madhuca***: 25 g

Equipments : Earthen port

Diameter : 23 inch

Length :12 inch

Capacity :2 Lt.

Procedure :

- After straining of *Kwatha* whole quantity of Guda (jaggery) i.e.was added and dissolved and again it was strained.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- After cooling of *Kwatha*, *Madhuca flowers* were added.

Observations :

- Gradually, wort became viscid, as jaggery was dissolved.
- *Madhuca flowers* were floating on the surface.

Precaution :

- Fresh *Madhuca flowers* were used.
- After straining, *Guda* (jaggery) was added immediately.

2.4 Preparation of A.K –Fermenting agent: *Yeast*

Sweetening agent: Jaggery

Fermentation vessel: Earthen pot

Date Start : 25 /December/2016	**End Date:** 25/December/2016
Time Start: 9:00 A.M	**Time (Stop):** 11:00 a.m

Batch code : 1.8

Principle : (Mixing)

Duration : 2:00 hours

Ingredients : ***Kwatha :*** 2 Lt. **Guda :** 425 gm **Yeast:** 4 gm

Equipments: Earthen port

Diameter : 23 inch

Length: 12 inch

Capacity: 2 Lt.

Procedure :

- After straining of *Kwatha* whole quantity of *Guda* (jaggery) i.e.was added and dissolved and again it was strained.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- After cooling of *Kwatha,* Yeast were added.

Observations :

- Gradually, wort became viscid, as jaggery was dissolved.
- Yeast were floating on the surface.

Precaution :

- Fresh Yeast were used.
- After straining, *Guda (*jaggery) was added immediately

2.5 After Fermentation place to maturation

Date Start : 25 /December/2016	**End Date:**23/1/2017

Principle :*Sandhana(fermentation)*

Duration :30 days (1 months)

Equipments : Same equipment as above used practical no. 1.4

Procedure : It was strained and kept for maturation for 10 days.

Rest observations were same as practical no. Above 1.4

Practical – 3 : Preparation of Ashwagandha kalpa by using Ikshu rasa (sugar cane)

Preparation of *Kwatha*

Procedure is same as practical no. 1

3.1 Preparation of A.K –Fermenting agent:*DhatakiPushpa*

Sweetening agent:*Ikshu Rasa*

Fermentation vessel: Earthen pot

Date Start : 24 /12/2016	**End Date:** 25/12/2016
Time Start: 9:00 A.M	**Time (Stop):** 11:00 a.m

Batch code :1.9

Principle: Mixing

Duration :2:00 hours

Ingredients: *Kwatha*: 2 Lt ***IkshuRasa* :** 425 Lt.***Dhataki :*** 25 g

Equipments: Earthen pot

Diameter:23 inch

Length :12 inch

Capacity:2 Lt.

Procedure :

- After straining of *Kwatha* whole quantity of *Ikshu rasa* i.e.was added and dissolved and again it was strained.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.

- After cooling of *Kwatha*, *Dhataki flowers* were added.

Observations :

- Gradually, wort became lightly viscid, as *Ikshu* was dissolved.
- *Dhataki flowers* were floating on the surface.

Precaution :

- Fresh *Dhataki flowers* were used.
- After straining, *Ikshu rasa* was added immediately.

3.2 Preparation of A.K –Fermenting agent:*MadhucaPushpa*

Sweetening agent:*Ikshu Rasa*

Fermentation vessel: Earthen pot

Date Start : 24 /12/2016	**End Date:** 25/12/2016
Time Start: 9:00 A.M	**Time (Stop):** 11:00 a.m

Batch code :2.0

Principle: (Mixing)

Duration: 2:00 hours

Ingredients: ***Kwatha*:** 2 Lt. ***Ikshu Rasa:*** 425 Lt.***Madhuca*:**25 g

Equipments: Earthen pot

Diameter :23 inch

Length :12 inch

Capacity :2 Lt.

Procedure :

- After straining of *Kwatha* whole quantity of *Ikshu* rasa i.e.was added and dissolved and again it was strained.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- After cooling of *Kwatha,Madhuca flowers* were added.

Observations :

- Gradually, wort became lightly viscid, as *Ikshu rasa* was dissolved.
- *Madhuca* flowers were floating on the surface.

Precaution :

- Fresh *Madhuca flowers* were used.
- After straining, *Guda* (jaggery) was added immediately.

3.3 Preparation of A.K –Fermenting agent: *Yeast*

Sweetening agent: *Ikshu Rasa*

Fermentation vessel: Earthen pot

Date Start : 24 /12/2016	**End Date:** 25/12/2016
Time Start: 9:00 A.M	**Time (Stop):** 11:00 a.m

Batch code :2.1

Principle:(Mixing)

Duration :2 hours

Ingredients :*Kwatha* :2 Lt. ***Ikshu Rasa* :** 425 Lt.**Yeast :**4 gm

Equipments : Earthen port

Diameter :23 inch

Length :12 inch

Capacity :2 Lt.

Procedure :

- After straining of *Kwatha* whole quantity of *Ikshu rasa* i.e.was added and dissolved and again it was strained.
- Earthen pot was sterilized with hot water and was dried by sun rays. It was fumigated by *Guggulu* for 20 minutes.
- After cooling of *Kwatha,* Yeast were added.

Observations :

- Gradually, wort became viscid, as jaggery was dissolved.
- Yeast were floating on the surface.

Precaution :

- Fresh Yeast were used.
- After straining, *Guda* (jaggery) was added immediately.

After Fermentation place to maturation

Date Start : 25 /December/2016	**End Date:** 23/1/2017

Principle:*Sandhana* (fermentation)

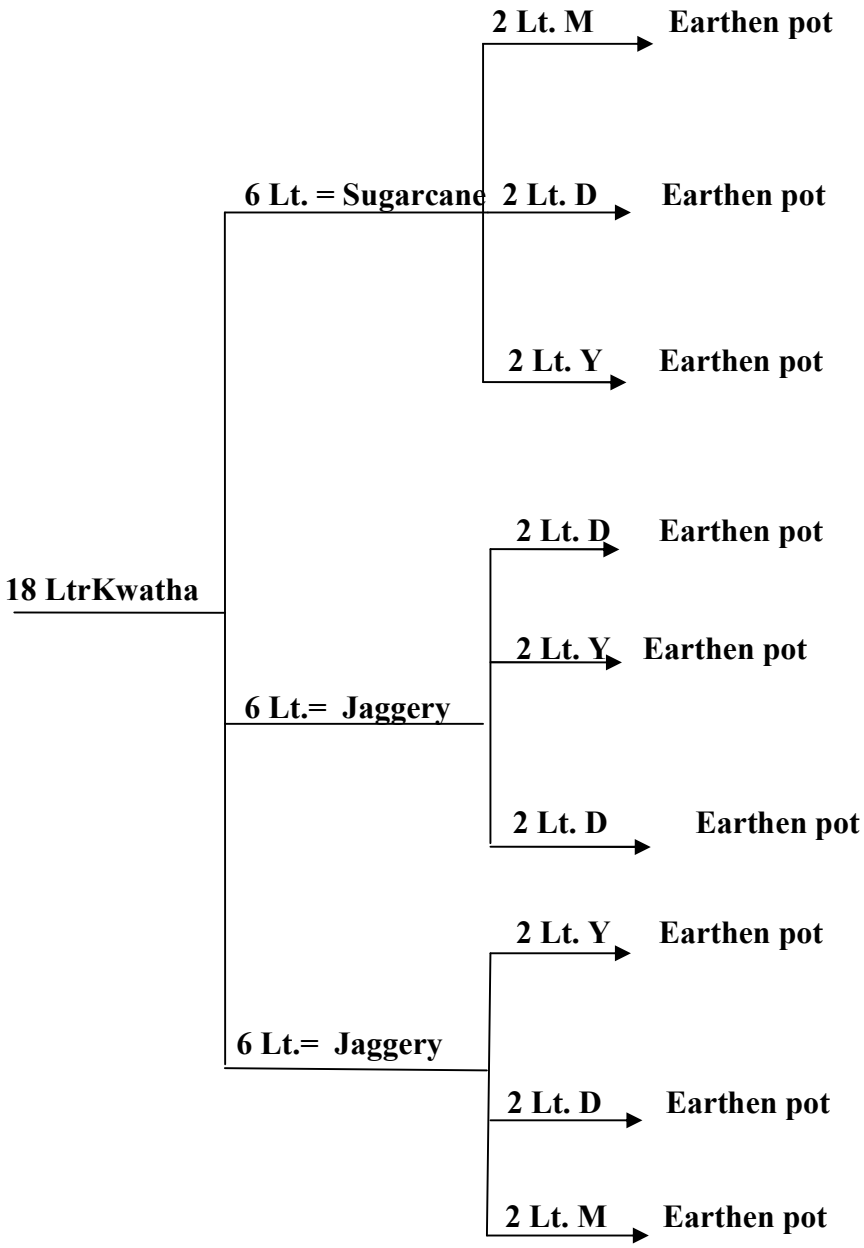
Duration :30 days (1 months)

Equipments : Same equipment as above used practical no. 1.4

Procedure : It was strained and kept for maturation for 10 days.

Rest observations were same as practical no. Above 1.4

Schematic presentation for preparation of wort



[M:MadhukaPushpa, D:Dhataki Pushpa, Y:Yeast]

Table No.: 1- Sandhan Sidhi Lakshan

Sr. NO	Parameter	15 Days			1 Month		
		DAK	MAK	YAK	DAK	MAK	YAK
1	Lime water test	Positive	Positive	Positive	Positive	Positive	Positive
2	Match stick test	Positive	Positive	Positive	Positive	Positive	Positive
3	Sound test	Hising sound	Hising sound	Hising sound	Hising sound	Hising sound	Hising sound

Table NO.:2- Comparison, observation and result of(Dhataki, Madhuca,yeast) Sugar, Jaggery,Ikshu, sample of Ashwagandha Kalpa after fermentation:

Sample	Organoleptic character	DAK	MAK	YAK	After fermentation
Sugar	Color	Dark brown	More dark brownish	Brownish	Dark brown
	Smell	Pleasant, mild alcoholic	Pleasant, mild alcoholic	Pleasant, mild alcoholic	mild alcoholic
	Taste	Bitter, Astringent, Madhur	Bitter, Astringent, Madhur	Bitter, Astringent, Madhur	Astringent, Madhur
Consistency		Thicker, sticky	Thicker, sticky	Thicker, non-sticky	Clear, non sticky
Jaggery	**Color**	Brownish	Dark brownish	Light brown	Brown
	Smell	Pleasant, mild alcoholic	Pleasant, mild alcoholic	Pleasant, mild alcoholic	mild alcoholic
	Taste	Bitter, Madhur	Bitter, Madhur	Bitter, Madhur	Bitter, Madhur
Consistency		Thick, sticky	Thick, sticky	Thick, sticky	Clear, non – sticky
Sugarcane	**Color**	Dark brown	Dark brownish	Light brown	Brownish
	Smell	Pleasant, mild alcoholic	Pleasant, mild alcoholic	Pleasant, mild alcoholic	Mild alcoholic
	Taste	Kashaya, Madhur	Kashaya, Madhur	Kashaya, Madhur	Madhur, Bitter
Consistency		Thick, sticky	Thick, sticky	Thick, sticky	Clear, non – sticky

Table No.:3-Details of Preparation of wort of each batch

Batch	Sugar Wort			Jaggery Wort			Sugar cane		
	DAK	**MAK**	**YAK**	**DAK**	**MAK**	**YAK**	**DAK**	**MAK**	**YAK**
***Kwatha* (L)**	2 L	2 L	2L	2L	2L	2L	2L	2L	2L
Sugar	425 gm	425 gm	425gm	-	-	-	-	-	-
Jaggery	-	-	-	425gm	425gm	425gm	-	-	-
Sugar cane	-	-	-	-	-	-	425 ml	425ml	425 ml
Dhataki	25 gm	25 gm	25 gm	25 gm	25 gm	25 gm	25 gm	25 gm	25 gm
Madhuca	25 gm	25 gm	25 gm	25 gm	25 gm	25 gm	25 gm	25 gm	25 gm
Yeast	4 gm	4 gm	4 gm	4 gm	4 gm	4 gm	4 gm	4 gm	4 gm

Table No.:4-General Observations of Fermentation process of wort

Parameter	'O' day	Fermentation starting day	During fermentation	After fermentation
Colour	Light brown	Light brown	Brown	Dark brownish
Odour	Kwatha	Slightly Kwatha and Pleasant	Pleasant	Mild Alcoholic
Taste	Bitter, Astringent, Sweet	Bitter, Astringent, Sweet	Bitter, Astringent, Sweet	Bitter, Astringent, Sweet
Sound	No	Slightly present	Present	No
Effervescence	No	No	Slightly present	No
Consistency	Thicker sticky	Thicker, Sticky	Thick	Liquid, slightly thick
Fermenting agent	Floating	Floating	Floating	Negligible floating
Temperature	Room temp.	Raised	Raised	Room temp.

Table No.:4-Fermentation chart

Sr. no	Days	DAK	MAK	YAK
1.	Fermenting Starting day	10^{th}	15^{th}	6^{th}
2.	Completion day	30 day	30 day	30 day
3.	Maturation day	10 day	10 day	10 day
4.	Total duration	40	40	40

B: Batch S: sugar J: Jaggery IK: Ikshu rasa

RESEARCH AND METHODLOGY

Chapter-7
Pharmacognostical Study

Pharmacognosy is a branch of pharmaceutical science, which deals with naturally occurring biologic products especially those derived from plants. The term Pharmacognosy is derived from two Greek words '***Pharmacon***' means drugs and '***gignosco'*** or gnosis - to acquire knowledge means knowledge on drugs. Any plant that is used in medicine needs elaborate study prior to its use. This detailed study of plants enables us to differentiate between closely related species of the same genus or related genera of the same family. It may be defined as an applied science which deals with the biological, biochemical and economical features of natural drugs and their constituents. For product standardization, the raw material should be of standard quality. The quality of finished product entirely depends on the quality of the raw materials. Therefore first step of standardization is the quality control aspects of raw material. It can be achieved by macroscopic and microscopic examination of the crude drugs. In the present study an attempt was made to standardize the seed used for the pharmaceutical process.[71]

Pharmacognostic study

Conventional Pharmacognostic methods were used for the study of morphological and microscopic characters of the plant part used in the formulation *Ashwagandhakalpa.*

S.No	Plant	Part used (examined)
1	*Aswagandha*	Root
2	*Sarpagandha*	Root
3	*Vacha*	Rhizome
4	*Bramhi*	Whole plant

Macroscopic study method

Morphological characters of all raw ingredients were studied by observing under the dissecting microscope and noted down. The organoleptic characters of the powdered material were also evaluated.

Microscopic study method

From the preserved or freshly collected samples (as per requirements) free hand section were taken and examined. They were cleared with chloral hydrate and stained with various reagents.

For powder microscopy, slides were prepared by using water, chloral hydrate as a clearing agent, stained with Phlorogucinol and conc.HCl for lignified tissues, iodine for starch grains and glycerin as mountant.[72]

Chapter-8
Analytical Study

Analytical study used to determine the quality of the pharmaceutical products. It also gives the information about the purity and safety of the products. Analytical study is the application or process in order to identify the chemical constituents and also about standards of the preparation.In the present scenario, the attraction towards the *Ayurveda* is increasing day by day due to its less unwanted side effects. Hence, qualitative and quantitative study of a particular formulation should be carried out by the use of various parameters which helps in standardization and authentication of the drug, using the modern techniques and instruments.

This chapter is divided into following divisions.

1. Raw material analysis.

2. Finished product analysis along with in process quality control.

1. Raw material analysis

Before the raw material used for the preparation of the finished product proper identification of all plant material is the extremely necessary for its genunity. This can be done by evaluating their quality by various parameters. So here all the raw materials were analysed by employing various parameters such as pharmacognostical evaluation,Physico-chemical parameter, qualitative screening, quantitative assay for plant metabolite and chromatographic fingerprint.

Parameters employed were as followed:

1.1Foreign matter determination

1.2 Determination of Loss on drying

1.3 Determination of Total Ash

1.4 Determination of Acid Insoluble Ash

1.5 Determination of Water Soluble Extractive

1.6 Determination of Alcohol Soluble Extractive

1.7 Qualitative chemical tests

1.8 Quantitative estimation

1.9 Quantitative estimation of saponin glycoside

1.10Quantitative estimation of total alkaloid content

1.11 Quantitative estimation of total tannin content

1.12 Chromatographic study-TLC, HPTLC

Physciochemical Parameter

Determination of Foreign Matter[73,74]

Weigh 100-500g of the drug sample to be examined or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6 xs). Separate and weigh it and calculate the percentage present.

Calculation:

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

1.2. Determination of Total Ash Value[73,74]

Incinerate about 2 to 3 g accurately weighed ground drug in a tared platinum or silica dish at a temperature not exceeding 450º C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerated the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450 ºC. Calculate the percentage of ash with reference to the air dried drug.

Calculation:

$$\text{Percentage of Total ash} = \frac{\text{Weight of ash}}{\text{Weight of Sample}} \times 100\%\text{w/w}$$

1.3.Determination of Acid Insoluble Ash[73,74,75]

Boil the ash obtained in for 5 minutes with 25ml of dilute hydrochloric acid; Collect the insoluble matter in a Goonch crucible or on an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid- insoluble ash with reference to the dried drug.

Calculation:

Percentage of Acid Insoluble ash = $\frac{\text{Weight of A.I.A}}{\text{Weight of Sample}} \times 100\%\text{w/w}$

1.4.Determination of Sulphated Ash[73,74]

Heat a Silica or Platinum crucible to redness for 10 minutes; allow cooling in a dessicator and weigh it. Put 1 to 2 g of the substance, accurately weighed, into the crucible; ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of Sulphuric acid, heat gently until white fumes are no longer evolved and ignite at 800º± 25 until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of Sulphuric acid and heat. Ignite as before, allow cooling and weighing. Repeat the operation until two successive weighings do not differ by more than 0.5mg.

Calculation:

Percentage Sulphated ash = $\frac{\text{Weight of sulphated ash}}{\text{Weight of Sample}} \times 100\%\text{w/w}$

1.5.Determination of Alcohol Soluble Extractive[73,74,75]

Macerate 5 g of the air dried drug, coarsely powdered, with 100ml of Alcohol of the specified strength in a closed flask for twenty- four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol –soluble extractive with reference to the air-dried drug.

Calculation:

Percentage of A.S.E= $\frac{\text{Weight of residue } \mathbf{x} \text{ vol. made}}{\text{Weight of Sample } \mathbf{x} \text{ vol. taken for evaporation}} \times 100\%$ w/w

1.6.Determination of Water Soluble Extractive[73,74,75]

Proceed as directed for the determination of alcohol- Soluble extractive, using chloroform water instead of ethanol.

Calculation:

Percentage of W.S.E= $\frac{\text{Weight of residue x vol. made}}{\text{Weight of Sample x vol. taken for evaporation}} \times 100$ %w/w

1.7.Determination of Moisture content (Loss on Drying)[73.74,75]

Place about 10 g of drug after accurately weighing it in a tared evaporating dish. After placing the above amount of the drug in the tared evaporating dish dry at 105 for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighings corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01g difference.

Calculation:

Percentage of L.O.D = $\frac{\text{Weight of moisture}}{\text{Weight of Sample}} \times 100$%w/w

1.8.QUALITATIVE CHEMICAL TESTS

The methods employed to isolate active substance are termed as extractive method. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different types of components. Qualitative tests are used to detect the presence of functional groups, which play very important role in the expression of biological activity.Using the methanol, water and chloroform soluble extracts of samples carried out qualitative tests. These tests indicate the types of phyto-constituents present in the sample.

1.8. A. Test for Alkaloids[74]

Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute HCl. Shake well and filter. With filtrate, perform following tests:

- **Dragendorff's test**

  To 2- 3 ml filtrate, add few drops Dragendorff's reagent. Orange brown ppt formed

- **Mayer's test**

  2-3ml filtrate with few drops Mayer's reagent gives white ppt.

- **Hager's test**

  2-3ml filtrate with Hager's reagent gives yellow ppt.

- **Wagner's test**

2-3ml filtrate with few drops Wagner's reagent gives reddish brown ppt.

1.8. B. Test for Tannins[74]

To 2-3 ml of aqueous or alcoholic extract, add few drops of following reagent:

- **5 % $FeCl_3$ Solution**: Deep blue black Color
- **Lead acetate Solution**: White ppt.
- **Acetic acid Solution**: Red color Solution.
- **Potassium dichromate**: Red ppt.

1.9.C. Test for Glycosides[74]

Baljet's test: A thick section shows yellow to orange color with sodium picrate.

1.8.D. Test for Steroid[74]

Salkowski reaction: To 2 ml of extract, add 2 ml. Chloroform and 2 ml conc. H_2SO_4. Shake well Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

1.8.E. Test for reducing sugars

Fehling's test: Mix each 1 ml Fehling's A& B solutions; boil for 1 min., add equal volume of test solution. Heat in boiling water bath for 5-10 min. First a yellow, then brick red precipitate is observed.

1.8.F. Test for Saponins[73,74,75,76,77]

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

1.10. QUANTITATIVE ESTIMATION

1.9.A. Quantitative estimation of total alkaloid content

Requirement

a) Methanol (95%),

b) Dil. HCl (2N)

c) Chloroform

d) Ammonia solution

e) Phenolphthalein

f) Anhydrous sodium sulphate

PROCEDURE

10 gm Plant material+100 ml Methanol

↓ **Soaked overnight**

Extract filtered and evaporated upto residue over water bath

↓

Residue dissolved in 25 ml 6N HCL

↓

Transfer the acidic layer into separating funnel and extract with 25 ml $CHCl_3$

↓

Shake vigourasely and allow standing until two layers separate out

↓

Collect the $CHCl_3$ layer and discard

↓

Basify the acidic layer with ammonia upto pH 8

↓

Extract the basic layer with 25 ml $CHCl_3$,shakevig., allow to separate two layers

↓

Collect the $CHCl_3$ layer and repeat the process repeated 3 times

↓

Combine the $CHCl_3$ layer and wash with water to make it ammonia free. (Checked with Phenophathalein indicator)

↓

Add anhydrous sodium sulphate to $CHCl_3$ extract to remove water

Decant off $CHCl_3$ extract to a previously weighed evaporating dish and evaporate upto residue.

Calculation% Alkaloid content = Weight of residue 100 %w/w

Weight of sample taken

1.9.B. Quantitative estimation of total Saponin content[74,75,76]

Requirement

1) Petroleum ether
2) Methanol
3) Acetone

PROCEDURE

Accurately weighed, 10 gm of powdered plant material was taken in a conical flask

Extract with 25 ml petroleum ether

Decant of petroleum ether and discard, Process repeated 3 times (3X25ml)

Defatted material extracted with 25 ml Methanol

Methanol extract collected, process repeated 3 times (3X25 ml)

Evaporate methanol over water bath upto 30 ml

Add 100 ml Acetone, allow standing overnight

Precipitate filtered, dried up to constant weight

Calculation% Saponin content =Weight of residue- wt. of filter paper x 100 %w/w

Weight of sample taken

1.9.C. Quantitative estimation of total Tannin content[76.77]

Requirement

1) Potassium permanganate
2) Indigo carmine

PROCEDURE

Acc. weighed 1 gm sample dissolved in 100 ml D.W

Add 700 ml D.W

Add 25 ml Indigo caramine

Titrate with N/10 $KMnO_4$ solution

Color changes from blue to golden yellow

Take the blank reading in similar manner omitting the sample

Calculation% tannin content= a-b×0.004157×1000 % v/w

Wt. of sample

2. Finished product analysis was carried out by employing following parameters

2.1.Organoleptic evaluate-Colour, odour, taste

2.2.Physicochemical parameters

2.2.A. L.O.D

2.2.B. Total solid content

2.2.C. Extractive values

2.2.D. pH

2.2.E. Refractive index

2.2.F. Specific gravity

2.2.G. Alcohol content

2.2.H. sugar content

2.3.Test for methanol

2.4.Qualitative screening.

2.5.Quantitative assay-Alkaloid, Tannin, Saponin.

2.6.HPTLC fingerprint

2.7.Microbial profiling

2.7.A. Total bacterial count

2.7.B. Total fungal count

2.7.C. Test for pathogen-E.coli, S. Aureus, P. Arugenosa, S. Typhii.

2.1. Organoleptic evaluation[75]

Color,odour and taste were evaluated by sensory perception.

2.2. Physicochemical Parameters

2.2 A. Determination of Moisture Content (Loss on Drying)[73,74,75]

Method is same as procedure 1.7

2.2 B. Determination of Total Solid Content[73,74,75]

Transfer accurately 50 ml of the clear Asava/Arishta to an evaporable dish, which have been dried to a constant weight and evaporate to dryness on a water bath, then dry at 105 for 3 hours. After cooling the dish containing the residue in a dessicator for 30 min,

weigh it immediately. The weight of residue should comply with the requirement stated under the individual monograph..

Calculation:

Solid Content = Weight of residue ×100%w/w

Weight of Sample

2.2 C. Determination of Alcohol Soluble Extractive[73,74,75]

Method is same as procedure 1.5.

2.2 D. Determination of pH value[73,74,75]

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in g per liter. Although this definition provides a useful practical means for the quantitative indication of the acidity or basicity of a solution, it is less satisfactory from a strictly theoretical point of view. No definition of pH as a measurable quantity can have a simple meaning, which is also fundamental and exact.Theph value of a liquid can be determined potentiometrically by means of the glass electrode, a references electrode and a pH meter either of the digital or analogue type.

2.2 E. Determination of Refractive index[73,74,75]

The refractive index (ŋ) of substances with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light of light passing from air into the substance. It varies with the wavelength of the light used in its measurement. The refractive index is measured at 25º (±0.5) with reference to the wavelength of the D line of sodium (ƛ 589.3 nm)

2.2 F. Determination of specific gravit[73,74,75,76]

Proceed as described under wt.per ml. Obtain the specific gravity of the liquid by dividing the weight of the liquid contained in the pycnometer by the weight of water contained, both determination at 25 unless directed in the individual monograph.

Calculation:

Specific Gravity= Weight of Sample

Weight of Water

2.2. G. Determination of Alcohol content[73,74]

Transfer 25 ml of the preparation being examined, accurately measured at 24.9º to 25.1º to the distillation flask. Dilute with 150 ml of water and add a little pumice powder, Attach the distillation head and condenser. Distil and collect not less than 90 ml of the distillate into a 100ml volumetric flask. Adjust the temperature to 24.9º to 25.1º and dilute to volume with distilled water at 24.9º to 25.1º.Determination the relative density at 24.9º to 25.1º. The values indicated in column 2 of table 1 are multiplied by 4 in order to obtain the percentage of ethanol by volume contained in the preparation, if the specific gravity is found to be between two values, the percentage of ethanol should be obtained by interpolation. After calculation of the ethanol content, report the result to one decimal place.

2.2. H. Estimation of sugars [73,74,75,76,77]

This includes estimation of total, reducing and non-reducing sugars.

Preparation of solution for analysis:10 ml of *Ashwagandha Kalpa* was taken in a glass beaker to which the clarifying agent, 5 ml of 10% lead acetate solution was added and warmed for about 3 – 5 minutes to get the precipitate. Solution was filtered through filter paper. To the filtrate sodium oxalate was added to dissolve excess lead acetate and to get a clear solution. This solution was filtered through the filter bed made up of glass wool, cotton and Whattman no. 1 filter paper to get a clear solution, washing was given by distilled water and the volume was made up to 250 ml.

Determination of reducing sugar:

To 5 ml, of above solution, 25 ml, each of Fehling's A and Fehling's B solutions were added, boiled for three minutes and filtered through filter bed (glass wool, cotton and

Whattman no. 1 filter paper). Repeated washing was given by hot distilled water till clear, colorless filtrate was obtained. Precipitate of cuprous oxide (residue) was then taken with acid ferric solution to dissolve the precipitate completely in it. This solution was titrated against 0.1 N KMnO4 solution using orthophenanthrolin as indicator. At the end point the colour changed to green. From the amount of KMnO4 solution required, the amount of copper was calculated. Then percentage of sugar content was determined from Hammond table.

Determination of Total Sugar:

20 ml of the clarified solution was taken; to it 5 ml of 6N HCl was added and heated on water bath at 69-71°C. This is then treated with diluted NaOH solution by using phenolphthalein as indicator till pink color appeared. Volume was made up to 100 ml. For the determination of total sugar 05 ml of this solution was taken and the remaining procedure was same as that of reducing sugar. Percentage of total sugar was calculated from Hammond table.

Determination of non-reducing sugar:

The non-reducing sugar content was obtained by subtracting reducing sugar from total sugar.

Non reducing sugar = Total sugar – Reducing sugar.

2.3Test for the absence of Methanol

Methanol can be detected by using the iodoform test. Mix sodium hydroxide (NAOH) and iodine (I2) in sample. A yellow precipitate of CHI3 (iodoform) will indicate the presence of methanol. If the yellow precipitate is not present, indicate the absence of methanol.[81]

2.5 Quantitative assay-Alkaloid, Tannin, Saponin.[73,74,75]

Method as same as procedure no. 1.9 A, 1.9 B, 1.9 C

HPTLC

HPTLC profile of different samples of *Ashwagandha Kalpa* was performed for establishing the finger prints and to study out the presence of identical chemical constituents.It is a technique in which a solid undergoes distribution between two phases, a stationary phase acting through absorption and a mobile phase in the form of liquid depending upon the solvent system this helps fairly to distinguish the individual chemical

constituent in the formulation by calculating the Rf value and visual comparison of the size and intensity of the spots.

Preparation of sample:

Raw Material sample preparation

About 2 gm of powdered sample of raw ingredients (each separately) was extracted with 5 ml of methanol. Extract filtered and subjected for HPTLC analysis**.**

Finished product sample preparation

2 ml of sample was dissolved in 2 ml mixture of chloroform and methanol (1:1 v/v) and kept a side for few minutes. Contents were filtered through Whatmann No.1 .Filtrate was collected and centrifuged. The supernatant was transferred to another clean test tube for HPTLC analysis.

Sample coding

Track 1-DAK (*Dhataki* based *Ashwagandha Kalpa*)

Track-MAK (*Madhuca* based *Ashwagandha Kalpa*)

Track 3-YAK(Yeast based *Ashwagandha Kalpa*)

Track 4- *Ashwagandha* methanolic extract

Track 5-Methanolic extract of *Brahmi*

Track 6-Methanolic extract of *Dhataki*

Track 7- Methanolic extract of *Madhuca*

Track 8- Methanolic extract of *Sarpgandha*

Track 9- Methanolic extract of *Vacha*

Chromatographic Conditions[79,80]

Application mode : CamagLinomat V

Development Chamber: Camag twin trough chamber

Stationary Phase : Precoated Silica Gel GF254 Plates

Mobile phase : Chloroform: Methanol(9:1 v/v)

Chamber saturation : 30 min.

Development distance: 7 cm

Scanner : Camag Scanner III

Detection : Deutariumlamp,Tungestun lamp

Data system : Wincats Software

Detection wavelength : 254 nm & 366 nm

Derivatizing agent : Vanillin sulphuric acid

Table no.: 8.1- Organoleptic character of raw material

Sr. no.	DRUGS	COLOR	ODOUR	TASTE	SIZE	SHAPE
1.	***Ashwagandha***	Yellowish	Characteristics	Pungent, bitter	4.4.	Longitudinal
2.	***Sarpgandha***	Creamish	Pleasant	Bitter, acrid	8.2	Longitudinal
3.	***Brahmi***	Light greenish	Characteristics	Sweet, Astringent	7.8	Irregular
4.	***Vacha***	Brownish	Aromatic	Acrid, Bitter	8.1	Longitudinal
5.	***Dhataki***	Reddish yellow	Characteristics	Astringent	1.2cm	Longitudinal
6.	***Madhuca***	Reddish	Characteristics	Astringent	1.2cm	Round

Table No.: 8. 2 (A) -Result of Physicochemical Parameter of *Ashwagandha*

Sr.no	Parameter	Ashwagandha (Root)	
		Standard value	**Result**
1	**LOD**	**-**	7.3%
2	**Foreign matter**	NMT 1%	Nil
3	**Total Ash value**	NMT 7%	5.7%
4	**Acid Sulfated ash**	NMT 1%	4.76%
5	**Water Extractive value**	**-**	13.8%
6	**Alcohol Extractive value**	NLT15%	16.8%

Table No.: 8. 2(B) -Result of Physicochemical Parameter of *Sarpgandha*

Sr.no	Parameter	Sarpgandha (Root)	
		Standard value	**Result**
1	**LOD**	NMT2%	**1.37%**
2	**Foreign matter**	**Nil**	**-**
3	**Total Ash value**	NMT8%	6.59%
4	**Acid Sulfated ash**	NMT1%	1.7%
5	**Water Extractive value**	NLH10%	16.17%
6	**Alcohol Extractive value**	NLT4%	5.6%

Table No.:8.2(C) - Result of Physicochemical parameter of *Brahmi*

Sr.no	Parameter	Brahmi (Whole Plant)	
		Standard value	**Result**
1	**LOD**	**-**	**7 %**
2	**Foreign matter**	NMT2%	Nil
3	**Total Ash value**	NMT18%	12.04%
4	**Acid Sulfated ash**	NMT6%	4%
5	**Water Extractive value**	NLT15%	18.04%
6	**Alcohol Extractive value**	NLT 6%	7.39%

Table No.: 8. 2 (D)- Result of Physicochemical Parameter of *Vacha*

Sr.no	Parameter	Vacha (Rhizomes)	
		Standard value	**Result**
1	**LOD**	**-**	16%
2	**Foreign matter**	NMT 1%	Nil
3	**Total Ash value**	NMT 7%	3.18%
4	**Acid Sulfated ash**	NMT 1%	0.5%
5	**Water Extractive value**	NLT 16%	19.0%
6	**Alcohol Extractive value**	NLT 9%	8.6%

Table No.:8. 2 (E) -Result of Physicochemical Parameter of *Dhataki*

Sr.No	Parameter	Dhataki (Flower)	
		Standard value	**Result**
1	**LOD**	**-**	10.7%
2	**Foreign matter**	NMT 2%	**-**
3	**Total Ash value**	NMT 10%	8.4%
4	**Acid Sulfated ash**	NMT 1%	0.5%
5	**Water Extractive value**	NLT 28%	26.67%
6	**Alcohol Extractive value**	NLT 7%	17.04%

Table No.:8.2 (F)- Result of Physicochemical Parameter of *Madhuca*

Sr.No	Parameter	Madhuca (Fruit)	
		Standard value	**Result**
1	**LOD**	**-**	18%
2	**Foreign matter**	NMT 2%	**-**
3	**Total Ash value**	NMT 5%	5.07%
4	**Acid Sulfated ash**	NMT 0.5%	0.5%
5	**Water Extractive value**	NLT 70%	54.25%
6	**Alcohol Extractive value**	NLH 2.5%	3%

Table No.:8.3 (A) - Result of Phytochemical screening analysis of Raw drug *Ashwagandha* (*Withania Somnifera*)

Sr.no	Name of Drugs	Chemical constituent	Test	Phytochemical Investigation
1.	***Ashwagandha***	Tannin	Lead acetate	+ve
			Potassium dichromate	-ve
			Acetic acid	-ve
			Iodine sol^{n}	+ve
		Carbohydrates	Fehling test	+ve
		Steroid	Salkowski	-ve
		Saponin Glycoside	Foam test	**+ve**
		Alkaloids	Dragendorff reagent	**+ve**
			Mayer reagent	**+ve**

Table No. : 8.3 (B) -Result of qualitative analysis of Raw drug *Sarpgandha (Rauwolfia serpentina)*

Sr.no	Name of drugs	Chemical constituent	Test	Phytochemical investigation
1.	***Sarpgandha***	Tannin	Lead acetate	**+ve**
			Potassium dichromate	**-ve**
			Acetic Acid	**-ve**
			Iodine sol^{n}	**+ve**
		Carbohydrates	Fehling test	**+ve**
		Steroid	Salkowski	**-ve**
		Saponin	Saponin Glycoside	**+ve**
		Alkaloids	Dragendorff reagent	**+ve**
			Mayer reagent	**+ve**

Table No. :8.3 (C) - Result of qualitative analysis of raw drug of *Brahmi* (*Bacopa monnieri)*

Sr.no	Name of drugs	Chemical Constituents	Test	Phytochemical investigation
1.	***Brahmi***	Tannin	Lead acetate	+ve
			Potassium dichromate	**-ve**
			Acetic acid	**-ve**
			Iodine sol^{n}	**+ve**
		Carbohydrates	Fehling test	**+ve**
		Steroid	Salkowskirxn	**-ve**
		Saponin Glycoside	Foam test	**+ve**
		Alkaloids	Dragendorff reagent	**+ve**
			Mayer reagent	**+ve**

Table No. :8.3 (D)- Result of qualitative analysis of Raw drug of *Vacha*

Sr.no	Name of drug	Chemical constituent	Test	Phytochemical investigation
1.	***Vacha***	Tannin	Lead acetate	**+ve**
			Potassium dichromate	**-ve**
			Acetic acid	-ve
			Iodine sol^{n}	**+ve**
		Steroid	Salkowskirxn	**-ve**
		Saponin	Foam test	**+ve**
		Alkaloids	Dragendorff reagent	**+ve**
			Mayer reagent	**+ve**

Table No.: 8.3 (E) - Result of qualitative analysis of Raw drug of *Dhataki*

Sr.no	Name of drug	Chemical constituent	Test	Phytochemical investigation
1.	***Dhataki***	Tannin	Lead acetate	**+ve**
			Potassium dichromate	**-ve**
			Acetic acid	**-ve**
			Iodine sol^{n}	**-ve**
		Carbohydrates	Fehling test	**+ve**
		Steroid	Salkowskirxn	**+ve**
		Saponin glycoside	Foam test	**-ve**
		Alkaloids	Dragendorff reagent	**+ve**
			Mayer reagent	**+ve**

Table No.: 8.3 (F) - Result of qualitative analysis of Raw drug of *Madhuca*

Sr.no	Name of drug	Chemical constituent	Test	Phytochemical investigation
1.	*Madhuca*	**Tannin**	**Lead acetate**	**+ve**
			Potassium dichromate	-ve
			Acetic acid	**-ve**
			Iodine sol^{n}	+ve
		Carbohydrates	Fehling test	**+ve**
		Steroid	Salkowski	**+ve**
		Saponin glycoside	Foam test	-ve
		Alkaloids	Dragendorff reagent	+ve
			Mayer reagent	+ve

Table No.: 8.4-Qualitative estimation of raw material Alkaloids, Saponin

Sr.No	Name of raw material	Alkaloids	Saponin
1.	***Ashwagandha***	0.17%	**-**
2.	***Sarpgandha***	0.98%	**-**
3.	***Brahmi***	**-**	0.0365%

Table No.: 8.5-Organoleptic Character of finished product of *Ashwagandha Kalpa*

Sample	Organolepti c character	15 Days			1 Month		
		DAK	MAK	YAK	DAK	MAK	YAK
Sugar	Color	Light brown	Dark brown	Brownish	Dark brown	More dark brownish	Brownish
	Odour	Pleasant	Pleasant	Pleasant	Pleasant, mild alcoholic	Pleasant, mild alcoholic	Pleasant, mild alcoholic
	Taste	Sweet Bitter, Astringent	Sweet Bitter, Astringent	Sweet Bitter, Astringent	Sweet Bitter, Astringent	Sweet Bitter, Astringent	Sweet Bitter, Astringent
Jaggery	Color	Brown	Dark brown	Light brown	Brownish	Dark brownish	Light brown
	Odour	Pleasant	Pleasant	Pleasant	Pleasant, mild alcoholic	Pleasant, mild alcoholic	Pleasant, mild
	Taste	Sweet Bitter, Astringent	Sweet Bitter, Astringent	Sweet Bitter, Astringent	Bitter, Sweet	Bitter, Sweet	Bitter, Sweet
Ikshu Rasa	Color	Light brown	Light brown	Light brown	Dark brown	Dark brownish	Light brown
	Odor	Pleasant	Pleasant	Pleasant	Pleasant, mild alcoholic	Pleasant, mild alcoholic	Pleasant, mild alcoholic
	Taste	Sweet, Astringent	Sweet, Astringent	Sweet, Astringent	Sweet, Astringent	Sweet, Astringent	Sweet, Astringent

Table No.: 8.6 (A) - Result of Physicochemical Parameter of *Gud (*Jaggery*)Ashwagandha Kalpa* Sample (GAK)

Parameters	15 Days			1 Month		
	DAK	MAK	YAK	DAK	MAK	YAK
LOD	84.0	69.12	83.0	76.0	53.3	75.12
Ash Value	4.2	6.82	3.3	9.3	11.89	8.79
Acid Insoluble ash	2.01	4.98	1.88	8.5	9.1	7.54
Water Extractive Value	29.56	68.9	31.32	41.60	81.92	43.26
Alcohol extractive value	11.23	23.18	13.26	23.45	32.84	25.76
Ph	4.87	4.92	4.84	4.64	4.48	4.57
Specific gravity	1.01	1.05	1.09	1.18	1.15	1.21
Total solid content (%)	15.9	30.2	16.15	23.81	46.7	24.76
Alcohol (%)	7%	4%	7 %	8%	4%	8%
Total sugar	25.34	32.54	27.04	20.65	26.89	20.19
Reducing sugar	18.36	21.73	19.39	16.09	19.76	14.77
Non reducing sugar	6.98	10.81	7.65	4.56	7.13	5.42

Table No.: 8.6(B) - Result of Physicochemical Parameter of *Shakra (*Sugar*)Ashwagandha Kalpa* Sample

Parameter	15 Days			1 Month		
	DAK	MAK	YAK	DAK	MAK	YAK
LOD	39.4	32.3	41.4	33.67	26.54	35.81
Ash Value	1.23	3.38	1.78	2.3	5.57	2.98
Acid Insoluble ash	0.95	2.01	1.10	1.59	3.28	1.97
Water Extractive Value	33.98	41.91	34.51	38.91	49.20	40.28
Alcohol extractive value	23.10	32.74	25.61	29.80	38.71	31.20
Ph	3.84	3.45	3.68	4.05	3.68	3.11
Specific gravity	1.21	1.24	1.27	1.45	1.23	1.56
Total solid content (%)	60.0	67.26	58.12	66.10	72.40	64.20
Alcohol (%)	4%	4%	4%	8%	4%	8%
Total sugar	29.01	36.20	27.81	24.98	30.19	24.67
Reducing sugar	20.1	23.0	16.11	18.70	19.90	15.70
Non reducing sugar	8.91	13.20	11.70	6.19	10.28	8.90

Table No.: 8.6 (C) - Result of Physicochemical Parameter of *Sugar cane (Ikshu rasa) Ashwagandha Kalpa* Sample

Parameter	15 Days			1 Month		
	DAK	MAK	YAK	DAK	MAK	YAKS
LOD	75.00	69.91	72.87	70.09	60.81	67.96
Ash Value	5.9	6.3	5.8	7.20	9.5	7.01
Acid Insoluble ash	1.01	1.98	0.98	2.31	3.76	2.00
Water Extractive Value	31.6	44.3	34.28	36.87	49.02	39.62
Alcohol Extractive value	21.78	29.39	24.58	26.51	31.29	28.10
Ph	2.88	3.01	2.93	3.15	3.78	3.65
Specific gravity	1.01	1.21	1.22	1.17	1.28	1.19
Total solid content (%)	25.0	30.19	27.61	29.91	39.02	32.0
Alcohol (%)	5%	4%	6%	5%	5 %	8 %
Total sugar	25.74	28.91	27.04	20.65	22.50	23.41
Reducing sugar	18.90	17.64	19.10	15.04	12.38	16.7
Non reducing sugar	6.78	11.29	7.91	5.61	10.12	6.71

Table No.:8.7-Results of Phytochemical analysis of finished product of *Ashwagandha Kalpa*

Sr.no	Phytoconstituent	Test	Dhataki	Madhuca	Yeast
1.	**Alkaloids**	Dragendorff reagent	**+ve**	**+ve**	+ve
		Mayer reagent	**+ve**	**+ve**	+ve
2.	**Tannin**	Lead acetate	**+ve**	**+ve**	+ve
		Potassium dichromate	**-ve**	**-ve**	-ve
		Acetic acid	**-ve**	**-ve**	-ve
		Iodine sol^{n}	**+ve**	**+ve**	+ve
3.	**Carbohydrates**	Fehling test	**+ve**	**+ve**	+ve
4.	**Steroid**	Salkowski	**Ve**	**-ve**	-ve
5.	**Saponin glycoside**	Saponin	**+ve**	**+ve**	+ve

(Similar results were obtained for 1 month sample)

Table No.: 8.8 (A) -Qualitative estimation of finished product Gud (jaggery)

Finished product Gud (Jaggery)							
Sr.no.	**15 Days**				**1Month**		
	Parameter	**DAK**	**MAK**	**MAK**	**DAK**	**MAK**	**YAK**
1	**Alkaloids**	0.22%	1.1%	0.52%	3%	1.1%	0.4%
2	**Tannin**	72.90%	44.8%	62.90%	77.6%	53.0%	69.9%
3	**Saponin**	1.3%	4.29%	1.98%	5.1%	7.63%	5.89%

Table No.:8.8 (B) -Qualitative estimation of finished product *Shakra* (Sugar)

Finished product Shakra (Sugar)							
Sr.no.	**15 Days**				**1 Month**		
	Parameter	**DAK**	**MAK**	**YAK**	**DAK**	**MAK**	**YAK**
1	**Alkaloids**	0.21%	0.22%	0.28%	1.0%	0.89%	0.1%
2	**Tannin**	59.3%	48.3%	54.5%	67.4%	55.2%	65.8%
3	**Saponin**	1.1%	2.2%	3.4%	5.9%	5.6%	8.2%

Table no.:8.8 (C) -Qualitative estimation of finished product *Ikshu rasa* (Alkaloids, Saponin, tannin)

Finished product Ikshu rasa(Sugarcane)							
Sr.no.	**15 DAYS**				**1 MONTH**		
	Parameter	**DAK**	**MAK**	**YAK**	**DAK**	**MAK**	**YAK**
1	**Alkaloids**	1.66	1.0	1.9	2.01	1.98	2.0
2	**Tannin**	56.4	38.9	48.3	61.21	43.2	52.5
3	**Saponin**	0.21	1.1	3.13	2.13	1.49	5.56

Table No.: 8.9 (A) - Results of HPTLC Finger at 254 nm

Track No.	**No. of spots**	**R_f (254 nm)**
1	13	0.18,0.22,0.26,0.33,0.42,0.52,0.62,0.65,0.68,0.72,0.82,0.85,0.92
2	13	0.18,0.22,0.26,0.33,0.42,0.53,0.62,0.66,0.82,0.85,0.92
3	14	0.18,0.22,0.26,0.33,0.42,0.52,0.62,0.65,0.68,0.72,0.82,0.85,0.92
4	2	0.33,0.65
5	5	0.24,0.42,0.46,0.62,0.74
6	3	0.34,0.42,0.73
7	7	0.26,0.33,0.42,0.54,0.65,0.73,0.84
8	5	0.18,0.33,0.42,0.54,0.72
9	4	0.33,0.44,0.72,0.85

Table No.: 8.9 (A) - Results of HPTLC Finger at 366 nm

Track No.	No. of spots	R_f max. (366 nm)
1	7	0.07,0.18,0.25,0.41,0.50,0.65,0.84
2	8	0.07,0.17,0.25,0.42,0.51,0.55,0.64,0.84
3	7	0.07,0.17,0.25,0.41,0.50,0.65,0.84
4	2	9.25, 0.410.25,0.41
5	1	0.65
6	2	0.07,0.50
7	2	0.41,0.85
8	9	0,07,0.017,0.25,0.35,0.41,0.45,0.50,0.60,0.74
9	3	0.25,0.40,0.65

Chapter-9
Microbiology study

Ashwagandha Kalpa – a polyherbal formulation is made of 4 drugs. There is every possibility of Contamination from the raw drugs to the final product by various microorganisms. Medicinal plant materials carry a great number of bacteria and molds, often originating in soil, while a large range of bacteria and fungi are the naturally occurring micro-flora of herbs, among these aerobic spore forming bacteria are frequently predominate. Handling and production may cause additional contamination and microbial growth. So this section of study deals with the study of microorganism of end product. Microorganism plays a vital role in the fermentation technology. The fermentation by the microorganism is created by a wide variety of enzymes. The activites and growth of these microorganisms are influenced directly or indirectly by different environmental factors such as oxygen, pH, temperature, accumulation of inhibitory intermediates etc.

Considering these points, an attempt has been made to analyze microorganism from the raw drugs to final product i.e *Ashwagandha kalpa.*

Aims and Objectives

1.Total viable count

1.1Total bacterial count

1.2. Total fungal count

2. Test for pathogen

2.1.E.coli

2.2. S. Aureus

2.3. P. Arugenosa

2.4.S. Typhii.

Different culture media were used for the inoculation of Microbial profiling-**Total bacterial count**, **Total fungal count**, **Test for pathogen**-*E.coli,S. Aureus,P. Arugenosa,S. Typhii*. but the method of the preparation of culture media and inoculum were same. Only ingredients were changed. The common procedure is mentioned below:[81]

Basic method of preparation of medium :

- The solid ingredients were weighed by using an analytical balance. Sufficient quantity of distilled water was added.
- The selected ingredients for particular organism were dissolved by using a glass rod or by using a magnetic stirrer.
- Heat was applied to the solution to dissolve the ingredients. Solution was cooled to room temperature.
- Required pH was set.
- Agar was added afterwards and the solution was heated again to introduce agar in the solution.
- Indicator was added by cooling the solution to about 40 ºC.
- Final volume is made by using distill water.
- The prepared medium was distributed while hot, in the conical flask, bottles and test tubes
- The medium in the various containers were sterilized by autoclaving at 121ºC for 15 minutes. The containers were stored at 2 - 8 ºC.[81,82]

1. Total viable count

1.1.Total Bacterial count

Medium: Casein Soyabean Digest Agar

Procedure

Preparation of sample:

Normal saline (NaCl) of 0.8 % concentration was prepared.Using this normal saline solution, appropriate diluations of sample was prepared with concentration ranging $10^{-4,}$ $10^{-3,}$ $10^{-2,}$ $10^{-1.}$.

Preparation of the plates

Sterile growth media was poured into Petri dishes paced on a level surface. The depth of the medium in the plate was kept appropriate 4 mm. After the media solidifies, the plates were dried for 30 minutes in an incubator at 37ºc to remove excess moisture from the surface.

Sample application

Transfer 0.1 to 1 ml of sample on media plates by spread plate method.(each dilution was applied triplicate).Plates were incubated at 35 C for 12 to 24 hrs. After completion of growth time, the plates were observed under electronic colony counter and total microbial count was calculated.[81,82]

$$\text{Colony count (CFU/ml)} = \frac{\text{No. of colonies x Dilution factor}}{\text{Vol. of dilution in ml}}$$

- Colony count between 30 and 300 should be considered for enumeration.

1.2.Total Fungal count

Medium: Sabouraud Dextrose Agar

Procedure

The method of plate preparation , sample preparation and sample application were same as that described in bacterial count .The plates were incubated at 25 C for 72 hrs.[81,82,83]

2. Test of Specific Pathogens:

Detection of objectionable microorganism

- To detect specified objectionable microorganisms, prepare the sample to be examined was prepared as described above. Accurately measured 1 ml sample wastransferred to 100ml of enrichment medium and incubated at appropriate temperature.
- The enriched medium was subculturedon the plates of specified medium and incubated.
- The appearance of the colonies on the plate indicates presence of objectionable microorganism.
- To check the effectiveness of culture media, suspension of each microorganism separately is used as the positive control.
- **Positive controls:***Escherichia coli (MTCC 43), Staphylococcus aureus (MTCC 3160), Pseudomonas aeruginosa (MTCC 424) and Salmonella typhi (MTCC 733).*
- To test the sterility of media, dilutions and aspectic performance of the test, the method using sterile normal saline as the test preparation was carried out. There must be no growth of microorganism.[81,82,83]

2.1. Escherichia coli

Media: *Casein Soyabean Digest broth,MacConkey broth*, *MacConkey agar*, *Eosin methylene blue agar.*

1) Acc. measured10ml of the test material was dissolved in casein soyabean digest broth, mixed and incubate at 30 - 35ºC for 18 – 24 h.
2) About 1 ml of casein soyabean digest broth was transferred to 100ml of MacConkey broth and incubated at 42- 44ºC for 24 – 48hrs.
3) Mackonkey broth was subcultured on a plate of MacConkeyagar and incubated at 30 - 35ºC for 18 – 72 hrs.
4) Growth of red, generally non-mucoid colonies of Gram- negative rods, sometimes surrounded by reddish zone of precipitation, indicated the possible presence of Escherichia coli.
5) The presence of E.coliconfirmed by making a subculture from MacConkey agar plate on to eosin methylene blue agar.
6) Apperance of dark colour colonies with metallic sheen confirms the presence of E. coli.[81,82,83]

2.2.Salmonella Spp.

Media:*CaseinSoyabean Digest broth, Rappaport Vassiliadis Salmonella Enrichment broth, Xylose, Lysine, Dexoycholate agar, Triple Sugar Iron agar Casein.*

1)About 10 ml of the test material was dissolved in casein soyabean digest broth, mixed well and incubated at 30 - 35ºC for 18 – 24 hrs.
3) 0.1 ml of casein soyabean digest brothwastransferred to 10 ml of rappaportvassiliadis Salmonella enrichment broth and incubated at 30 - 35ºC for 18 – 24 hrs.
4) It was subcultured on a plate of Xylose, lysine, deoxycholate agar at and incubated at 30- 35ºC for 18- 24 hrs.
5)Growth of well developed, red colonies, with or without black centres indicates the possible presences of *Salmonella.*
6) Confirms the colonies, if any, thus produced on triple sugar iron agar using the deep inoculation technique.[81,82,83]

2.3. Pseudomonas aeruginosa

Media: *Casein Soyabean Digest broth, Cetrimide agar*

1) 10 g of the test material was dissolved in casein soyabean digest broth, mixed and incubated at 30 - 35ºC for 18 – 24 hrs.
2) Subculture on a plate of cetrimide agar and incubate at 30 - 35ºC for 18 – 72 hrs.
3) Growth of colonies with a greenish florescence, occurs then apply oxidase test.
4) Apperance of purple colour within 5 – 10 seconds indicates the presence of Pseudomonas aeruginosa.[81,82,83]

2.3.Staphylococcus aureus:

Media: *Casein Soyabean Digest broth, Mannitol Salt agar. DNase test agar.*

1) Dissolve 10 g of the test material in casein soyabean digest broth, mixed and incubate at 30 - 35ºC for 18 – 24 hrs.
2) Subculture on a plate of Mannitol salt agar and incubate at 30 - 35ºC hrs.
3) Growth of yellow or white colonies surrounded by a yellow zone indicates the possible presences of S. aureus.
4) Confirm the catalase positive colonies by deoxyribonuclease test.[81,82,83,84]

Table No.-1:Results of total microbial and fungal count

S.No.	Sample	Dilution	TBCcfu(Mean)	TFCcfu(Mean)
1	SDAK	10^{-1},10^{-2},10^{-3},10^{-4}	456	Negligible
2	SMAK	10^{-1},10^{-2},10^{-3},10^{-4}	972	Negligible
3	SYAK	10^{-1},10^{-2},10^{-3},10^{-4}	388	Negligible
4	JDAK	10^{-1},10^{-2},10^{-3},10^{-4}	578	Negligible
5	JMAK	10^{-1},10^{-2},10^{-3},10^{-4}	1098	Negligible
6	JYAK	10^{-1},10^{-2},10^{-3},10^{-4}	465	Negligible
7	IDAK	10^{-1},10^{-2},10^{-3},10^{-4}	592	Negligible
8	IMAK	10^{-1},10^{-2},10^{-3},10^{-4}	1195	Negligible
9	IYAK	10^{-1},10^{-2},10^{-3},10^{-4}	345	Negligible

Table No.-2: Results of specific pathogen

S.No.	Sample	E. Coli	S. Typhii.	P. aerugenosa	S. aureus
1	SDAK	Absent	Absent	Absent	Absent
2	SMAK	Absent	Absent	Absent	Absent
3	SYAK	Absent	Absent	Absent	Absent
4	JDAK	Absent	Absent	Absent	Absent
5	JMAK	Absent	Absent	Absent	Absent
6	JYAK	Absent	Absent	Absent	Absent
7	IDAK	Absent	Absent	Absent	Absent
8	IMAK	Absent	Absent	Absent	Absent
9	IYAK	Absent	Absent	Absent	Absent

SDAK: *Sugar Dhataki Ashwagandha kalpa* SMAK: *Sugar Madhuca*

SYAK: *Sugar* yeast *Ashwagandha Kalpa* JDAK: *Jaggery Dhataki*

JMAK: *Jaggery Madhuca Ashwagandha kalpa* JYAK: *Jaggery* Yeast

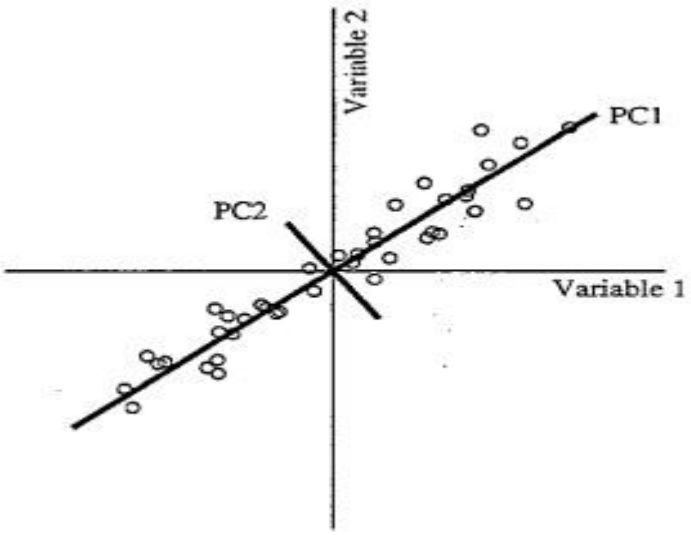
IKDAK :*Ikshu Dhataki Ashwagandha kalpa* IKMAK: *Ikshu Madhuca*

IKYAK: *Ikshu* Yeast *Ashwagandha kalpa*

Chapter-10
Attributes and Principle Component

10.1 Principle Component Analysis (PCA)

PCA is used as a discrimination tool. Principle component analysis is a mathematical manipulation of data matrix where goal is to represent the variation present in many variables using a small number of factors.A new row space is constructed in which to plot the samples by redefining the axes using factors rather than the original measurement variables.These new axes referred to as factors or principal components (PCs),allow the analyst to probe matrices with many variables and view the true multivariate nature of the data in a relatively small number of dimensions[85,86]



PLOT SHOWING VARIABLES AND PCs

For single variable linear model can be used. But where there are N number of components present in sample having multiple variables, it is not possible to define the system by linear model. For such type of data PCA is used for prediction. Validation generates reliable, accurate and precise results. In order to check the reliability of such multicomponent system, PCA like techniques are required because it reduces the

dimensions and so easily we can do prediction. When there are multiple variables, multiattributes are required to describe the results. Stastical tools are very helpfulto describe such data.As per the proposed work different samples of *AswagandhaKalpa* were prepared with variables fermenting agents and time period . Since the sample environment contains multicomponent so it's very difficult to describe the result with single attribute. So PCA has been selected as model for prediction.[85,86,87]

10.2 RESULTS FOR PCA

The PCA results for*Ashwagandhakalpa*with three different fermenting agents mainly *Dhataki, Madhuca* and Yeast shows that sample DAK and YAKare almost similar to that to each other, while sample MAK is different. Sample DAK and YAK falls in the same quadrant of the score plot while sample MAK was found on opposite side.Reason for similarity and difference is the total solid content, alcohol content and pH values.These parameters varies due to different fermenting agents used in the prepartion. The residue plot shows that sample DAK and YAK are present in the same cluster and are very far away from residue while MAK shows maximum residue and seems to be different from other samples. Similar results are obtained in leverage plot where sample YAK shows maximum leverage while leverage for DAK and YAK is almost similar to each other. Variance curve shows that the 100 % data can be explained up to 2 PCs. (Plate no. 10.1 & 10.2)[85,86,87]

When comparison was done between 15 days and 1 month sample, again the similarity was observed for DAK 15 D ,DAK 1M,YAK 15 D and YAK 1M.While samples MAK 15D and MAK 1M were found to be of different nature.

Chapter-11
RESULT AND DISCUSSION

11.1 Result and Discussion for Pharmacognostical study

Ashwagandha

Macroscopic study results

Roots of *Ashwagandha* were straight, unbranched, outer surface buffed, with longitudinal wrinkles, fracture short and uneven; odour characteristic; taste bitter.(Fig no.7.1)

Powder Microscopy

The powder shows presence of features like cork cells, parenchymatous cells, cambium cells etc as identifying characters.[40]

Microscopic study results

T.S of roots shows presence of non lignified cork cells, 2-4 rows of cork cambium, secondary cortex consist of parenchymatous cells, phloem showing sieve tubes, companion cells; medullary rays ; and few xylem.

Sarpagandha

Macroscopic study results

Sarpagandha roots appeared sub cylindrical, thick, outer surface grayish yellow, fracture short, odour and taste was bitter.(fig no. 7.2)

Powder Microscopy

Yellowish brown colored powder of *Ashwagandha* roots shows spherical, simple starch grains; calcium oxalate crystals; vessels; lignified tracheids; lignified xylem and stone cells.

Microscopic study

Transverse section of root shows about 18 layers of stratified cork, underlined by parenchymatous cells coating starch grains and crystals. Inner to this vascular bundle was observed showing phloem fiber, phloem parenchyma and companion cells.

Vacha

Macroscopic study results

The rhizome appears as cylindrical to slightly flattened, rarely straight in shape. Upper surface shows leaf scars and lower surface shows root scars. light brown outside and buff colored inside; taste pungent & bitter, odour aromatic.(fig no. 7.3)

Powder microscopy

Light grey colored powder of *Vacha* shows microscopic features like annular vessels, fibres, simple starch grains as identifying characters.

Microscopic study results

The transverse section of rhizome shows single layered epidermis, spherical shaped cortex cell, fibro vascular bundle and cells containing yellowish color pigment. Endodermis separate,stele consist of parenchymatous cells and vascular bundle.

Bramhi

In present formulation *Bramhi* was used as whole plant so powder microscopy was carried for identification purpose.

Macroscopic study results

Roots of *Bramhi* were observed as thin,branched and creamish yellow in color.Stems were thin, green in color, soft nodes and internodes were present.Leaves were simple, green in color and sessile, ovoid,glabrous and capsule shaped fruits were observed. Tastewas bitter and odour was characteristics.

Powder microscopy

Yellowish brown colour powder of *Bramhi* shows presence of xylem vessels with reticulate thickening,glandular hairs,round and oval starch grains as identifying constants.(fig no. 7.4)

11.2 Result and Discussion for Pharmaceutical study

The ingredients of *Ashwagandha kalpa* mainly *Ashwagandha, Sarpagandha, Vacha, Bramhi. Ashwagandha kalpa* is similar to *Sandhan Kalpana* method.Sweet liquid dosage form leads to the development of new dosage form by all age groups. To increases shelf life, to potentiate the drugs by adding another drugs to get Alcohol soluble active principle from *Swarasa, Kwatha* etc. The process of fermentation was probably

discovered by observing the changes in the juices of several fruits and other substances that had been kept for a day or more. But therapeutic use of *Sandhan* preparation was started from the *Samhita kala.* After proper identification and quality evaluation all the drugs were washed if required and then cleaned. The drugs were made into course powder. First decoction was prepared by boiling the powdered drugs in a specified volume of water for a definite time. After that it was cooled and filtered. Guda, Sugar, *Ikshu rasa* was added and mixed well to get it solubalise. The decoction was divided into three batches. The fermenting agents *dhataki pushpa, Madhuca Pushpa* and yeast were added separately into three batches. *Mruthpatra* (earthen pot) were used as *sandhana patra* and, placed under ground (*sandhanapradesha)* for the process of fermentation. Fermentation process was very slow, small bubbles on surface and match stick was observed as to be positive. The fermentation takes more than 1 month to complete, so in each batch lid was sealed after starting the fermentation and opened after 1 months. The pH of water was 6.5. It become 4.5 acidic in nature, because acid is present in the drugs. After the complete fermentation the final product was filtered and allowed to stand for maturation. The prepared batches of AshwagandhaKalpa were then subjected for analytical study.

Some part of *Ashwagandha, Sarpgandha, Brahmi, Vacha* were observed floating which then had gradually sinked due to absorption of water, so became heavier and sinked. Fermentation was found started 4th day as confirmed by sound and bubble test. After 15 days, half of the samples of each batch were filtered and remaining samples were allowed to undergo fermentation procedure. The 15 days samples and 1 month samples were then subjected for comparative study by employing organoleptic evaluation and analytical parameters. As shown in results for organoleptic characters (Table no.-8.5)The colour reveals that colour of Kwatha was brown which became darker in sugar sample and darkest in the jaggery, and light yellowish brown in Sugarcane. *Madhurata* (sweetness) in sugar and *jaggery, Ikshu* found more in jaggery, and Ikshu then sugar. After fermentation, in *Ashwagandha Kalpa* mild alcoholic odour of was observed. Consistency of *jaggery* was thicker than sugar and *Ikshu* may be due to composition of *jaggery*.

11.3 Result & Discussion for Analytical Study

The composition of *Ashwagandha kalpa* is *Ashwagandha, Sarpagandha, Vacha, Bramhi,* and *guda* were examined for their quality by employing testing parameters like Organoleptic evaluation, physicochemical tests, qualititative screening, quantitative assay for secondary metabolite and HPTLC fingerprint. The results of Organoleptic characters color, odour and taste complies with the standard mentioned in Ayurvedic Pharmacopoeia of India (Table no.-8.1).The results of physicochemical parameters(Table no.-8.2) for *Ashwagandha, Sarpagandha, Vacha, Bramhi and guda* were also observed with in permitted limits as per mentioned in respective volumes of API. The results of organoleptic characters and physicochemical parameters reveals that all the raw ingredients used in the preparation of *Ashwagandha Kalpa* were authentic and are of standard quality.

Analytical study was carried out for in process product and finished product. For in process testing the samples of *Kwatha* and wort containing different fermenting agents mainly *Dhataki, Madhuca* and Yeast were taken as test samples. No marked variations were observed in Kwatha after addition of different fermenting agents. The color of the *Kwatha* remained same as it was initially.

Different batches of *Ashwagandha kalpa* were analysed after 15 days and 1 month. After 15 days, half sample of each batch was filtered and remaining samples were left covered for another 15 days. Samples of *Ashwagandha Kalpa* MAK, DAK and YAK subjected for analytical study by employing organoleptic parameters, physicochemical constants and HPTLC fingerprint.

For Organoleptic evaluation the only difference was observed in color while taste and odour remained same for all three samples of *Ashwagandha kalpa* (Table no.-8.5). The colors of DAK and MAK were dark brown while YAK was light brownish in color.

Significant difference was observed in physicochemical analysis for Three different samples of *Ashwagandha kalpa* (Table no.-8.6). The total solid content was found to be 15.9%w/w, 30.2%w/wand 11.8 %w/w in DAK, MAKand YAK respectively. The results shows high value of total solid content in *Madhuca* based *Ashwagandha Kalpa* as compared to *Dahataki* and yeast. *Madhuca* is more hydrophilic in (greater solubility in water) nature as compared to *Dhataki* and yeast as shown in table no (Table no.-8.2 E &

8.2 F). No marked difference was observed for pH values. For specific gravity, Total solid content and refractive index, similar results were observed for DAK and YAK samples while slightly high values were obtained in MAK (Table no.- 8.6 A & 8.6 B, 8.6 C). These variations are again due to higher water solubility of *Madhuca* as compared to *Dhataki* and Yeast. The results for alcohol content reveal a significant variation where the total alcohol was found to be 7 %, 4% and 7% for DAK, MAK and YAK respectively. Alcohol percentage was found to be less in M*adhuca* based *Ashwagandha Kalpa* as compared to *Dhataki* and yeast. Qualitative evaluation shows similar findings for all the three samples (Table no.- 8.8 A & 8.8 B & 8.8.C). Quantitative assay was carried out for determination of total alkaloid, saponin and tannincontents (Table no.-5). The alkaloid content was found to be 0.22 %, 1.1% and 3 % in DAK, MAKand YAK respectively. Yeast based *Ashwagandha kalpa* shows more percentage of alkaloid as compared to *Dhataki* and *Madhuca*. The content of tannins in DAK, MAK and YAK were found to be 77.7%, 16.2% and 44.8% respectively. The results show more tannin content in dhataki and yeast as compared to *Madhuca.* When samples were analysed for total saponin content again significant variation was observed, where highest percentage was observed in yeast samples as compared to *Dhataki* and *Madhuca.* The results of quantitative assay shows that *Dhataki*and Yeast based *Ashwagandha kalpa* contains more active constituents as compared to *Madhuca* based *Ashwagandha kalpa* which indicates that these two fermenting agents can results in better extraction of Phytoconstituent. The HPTLC method described utilizes silica gel GF 254 HPTLC plates as stationary phase and Chloroform: Methanol (9:1 v/v) as mobile phase which gives good separation of active constituents present in three different samples of *Ashwagandha kalpa*. Extracts of all the ingredients used in preparation were taken as botanical reference. The results reveals that all the three samples of *Ashwagandha Kalpa* DAK, MAK and YAK shows almost similar retention behavior on silica gel (Fig.no 8A & 8B). The separation pattern shows the presence of chemical moieties of the respective ingredients in all the three samples of *Ashwagandha Kalpa* as shown in table no 6(A) and 6 (B).When visualization was done at 254 nm the results Separation of 13 components in DAK,13 in MAK and 14 in YAK. When comparison was done with sample of raw ingredients then all the respective chemical moieties were found to be in formulation samples. The scanning

results at 366 nm show separation of 7 components in DAK, 8 in MAK and 7 in YAK. Greater numbers of components were observed at 254 nm as compared to 366 nm. The reason may be that some of the components are not sensitive at long UV radiation that is 366 nm. Again the active principles were found to be present in *Ashwagandha Kalpa* samples at 366 nm. The 3 dimensional desitogram shows the overlapping peaks of the common components in all the tracks (Fig. no 8.2).The HPTLC results supports the qualitative evaluation.

Significant variations were also observed when comparison was done between 15 days and 1 month samples. For Organoleptic evaluation slight difference was observed in colour while odour and taste were almost same. The colour of 1 month samples was more brownish as compared to 15 days sample (Table No.- 8.5). When different batches of *Ashwagandha kalpa* of 15 days and 1 month were compared for analytical parameters, marked variation were observed. The total solid content was increase in 1 month samples (Table no.-8.6A & 8.6 B & 8.6 C). It might be due to the reason that prolonged time increase extraction efficiency which results in increasing the concentration.

11.4 Result and Discussion forMicrobiology study

All samples of *Ashwagandha Kalpa* were subjected for microbial profiling. Under this section the samples were studied for total microbial count (TMC), total fungal county(TFC), test for specific pathogens. As per available references the permissible limits for TMC is 10^{-5}cfu/gm and for TFC the limit is 10^{-3}cfu/gm. As shown in results bacterial growth and fungal growth was observed in all samples of *Ashwagandha kalpa* but the growth was with in the permissible limits (fig no.9.1, 9.2, 9.3) The objectionable pathogens were found to be absent in all samples of *Ashwagandha kalpa (fig 9.4)* The samples of final product *Ashwagandha kalpa* for the presence of microbial and Fungal load in the local pathological lab (Baijnath research lab).In the report of *Ashwagandha kalpa* there was presence of bacterial and fungal load under the limit given by WHO.

Stastical Analysis

The Stastical results justify the results obtained from experimental study and analytical study. PCA results show that sample DAK and YAK shows similar response while sample MAK was different. For 15 days samples and 1 month (Fig no. 10.1 &10.2).

Chapter-12
SUMMARY & CONCLUSION

- The present study for designed to find out the impact of different fermenting agents in fermentation process of *Ashwagandha kalpa* a marketed formulation. The formulation was comprised of ingredients-*Ashwagandha, Sarpagandha, Bramhi*, Jaggery and Yeast as fermenting agent.
- All the raw herbs were identified by pharmacognostical evaluations and were found to be genuine.
- The ingredients were qualified for their quality according to API protocol.
- *Ashwagandha kalpa* was prepared according to *Sandhan* method mentioned in *Ayurvedic* literature. Three different batches of *Ashwagandha kalpa* were prepared by using different fermenting agents mainly *Dhataki, Madhuca* and Yeast.
- Sample of *Ashwagandha kalpa* were analysed by after 15 days and 1 month by employing physicochemical parameters and HPTLC fingerprint.
- Significant variations were observed when comparison was done taking fermenting agents as variable. The results reveals that *Dhataki* and Yeast based samples were almost similar while *Madhuca* based samples were found to be different.
- When Comparision was done between 15 days and 1 month sample, then it was observed that time period of fermentation also affects the quality of product but upto small extent.
- Alcohol content, total solid content, secondary metabolite was found to be increased in 1 month samples.
- HPTLC method was developed for determining the behavior of fermenting agents on the response of active ingredients of respective herb. The separation was performed over Silica gel G254 plates, and mobile phase Chloroform: Methanol (9:1 v/v).When comparison was done with sample of raw ingredients then all the respective chemical moieties were found to be in formulation samples.

- An attempt was also made to find out the affect of different sweetening agents in fermentation process of *Ashwagandha kalpa.* Three different sweetening agents jaggery, sugar, and *Ikshu rasa* were in preparation of *Ashwagandha kalpa.* Variations were observed when samples were compared for their organoleptic parameters. Sugar and jiggery based samples were darker in color as compared to *Ikshu rasa* samples. Physicochemical parameters were found to be varied upto small extent. Alcohol content was found to be more in sugar and *jiggery* samples as compared to *Ikshu rasa* sample pH, specific gravity and refractive values almost same in all three samples of *Ashwagandha kalpa.*
- Based upon the finding and results it can be concluded that *Dhataki* and Yeast can act as better fermenting agents as compared to *Madhuca.*
- Sugar and jaggery can act as better sweetening agents than *Ikshu rasa*.
- The 15 days fermentation can also give a better product.
- From industrial point of view, to prepare the present product *Ashwagandha kalpa*, yeast as fermenting agent, sugar as sweetening agent and 15 days fermentation time period are the suitable parameters to prepare a better product with suitable economic value.

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Chapter-14

APPENDIX

14.1 Project/Dissertation Topic Approval Performa

14.2 Dissertation topic title Suggested by BRDL.

14.3 Permission for Dissertation Research Work Ms. (Ambika Thakur)

14.5 Authentication letter (raw drugs)

14.6 Plagiarism report

14.7 Poster Presentation Nasyacon

14.8 Poster Presentation ICP (International Conference Pharmaceutical)

TOPIC APPROVAL PERFORMA

LIT (Pharmacy)/Department of Pharmaceutical Sciences

Program : P570-NN7::M.Pharm. (Ayurveda)

COURSE CODE : APH623 **REGULAR/BACKLOG :** Regular **GROUP NUMBER :** PHRRGD0029

Supervisor Name : Dileep Singh Baghel **UID :** 15210 **Designation :** Associate Professor

Qualification : _____ **Research Experience :** _____

SR.NO.	NAME OF STUDENT	REGISTRATION NO	BATCH	SECTION	CONTACT NUMBER
1	Ambika Thakur	11511643	2015	Y1553	9418229810

SPECIALIZATION AREA : Ayurvedic Pharmacy **Supervisor Signature:** _____

PROPOSED TOPIC : Pharmaceutical standardization and quality control aspect of marketed sample of Ashwagandha Kalpa with special reference to its active constituent

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	**Parameter**	**Rating (out of 10)**
1	Project Novelty: Potential of the project to create new knowledge	6.75
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.00
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	7.00
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	7.50
5	Social Applicability: Project work intends to solve a practical problem.	6.75
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	7.25

PAC Committee Members		
PAC Member 1 Name: Dr. Amit Mittal	UID: 13145	Recommended (Y/N): Yes
PAC Member 2 Name: Saurabh Singh	UID: 12208	Recommended (Y/N): Yes
PAC Member 3 Name: Dr. S. Tamilvanan	UID: 16391	Recommended (Y/N): Yes
PAC Member 4 Name: Dr. Navneet Khurana	UID: 18252	Recommended (Y/N): Yes
DAA Nominee Name: Dr. Sazal Patyar	UID: 17050	Recommended (Y/N): NA

Final Topic Approved by PAC: **Pharmaceutical standardization and quality control aspect of marketed sample of Ashwagandha Kalpa with special reference to its active constituent**

Overall Remarks: Approved

PAC CHAIRPERSON Name: 11045::Dr. Monica Gulati **Approval Date:** 25 Apr 2017

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Baijnath Research and Development Laboratory

Paprola, Teh. Baijnath, Distt. Kangra(H.P.)-176115

{A Government Approved Drug Testing Laboratory under the rule 160 A of Drugs and Cosmetics Act 1940 and the Rules thereunder}

CERTIFICATE OF ANALYSIS

{Form-50 (refer 160-D(f)}

Report No. :B.R&DL/16 - 2653

Date : 26/11/16

To Whom It May Concern

It is to certify that **Ms. Ambika Thakur** ,Reg. **No. 11511643** student **of M. Pharm (Lovely Professional University Jalandhar)** is doing her M. Pharm Dissertation work in Baijnath Research and Development Lab and Baijnath Pharmaceuticals Paprola Himachal Pradesh , under the Co-Guidance Of **Dr.RenukaThakur (Incharge Baijnath Research and Development Lab Paprola)**.The Plant specimens brought by the student belongs to following species

1. Ashwagandha-	Withania somnifera-	Soalnaceae
2. sarpagandha-	Rauwolfia serpentine-	Apocynaceae
3. Vacha-	Acorus calamus-	Araceae
4. Bramhi-	Bacopa monnieri-	Scrophulariaceae

Incharge
Baijnath Research & Dev. Laboratory
PAPROLA Teh. Baijnath (Kangra) H.P.

Dr. Renuka Thakur

Incharge (Baijnath Research & Development Lab)

Paper1

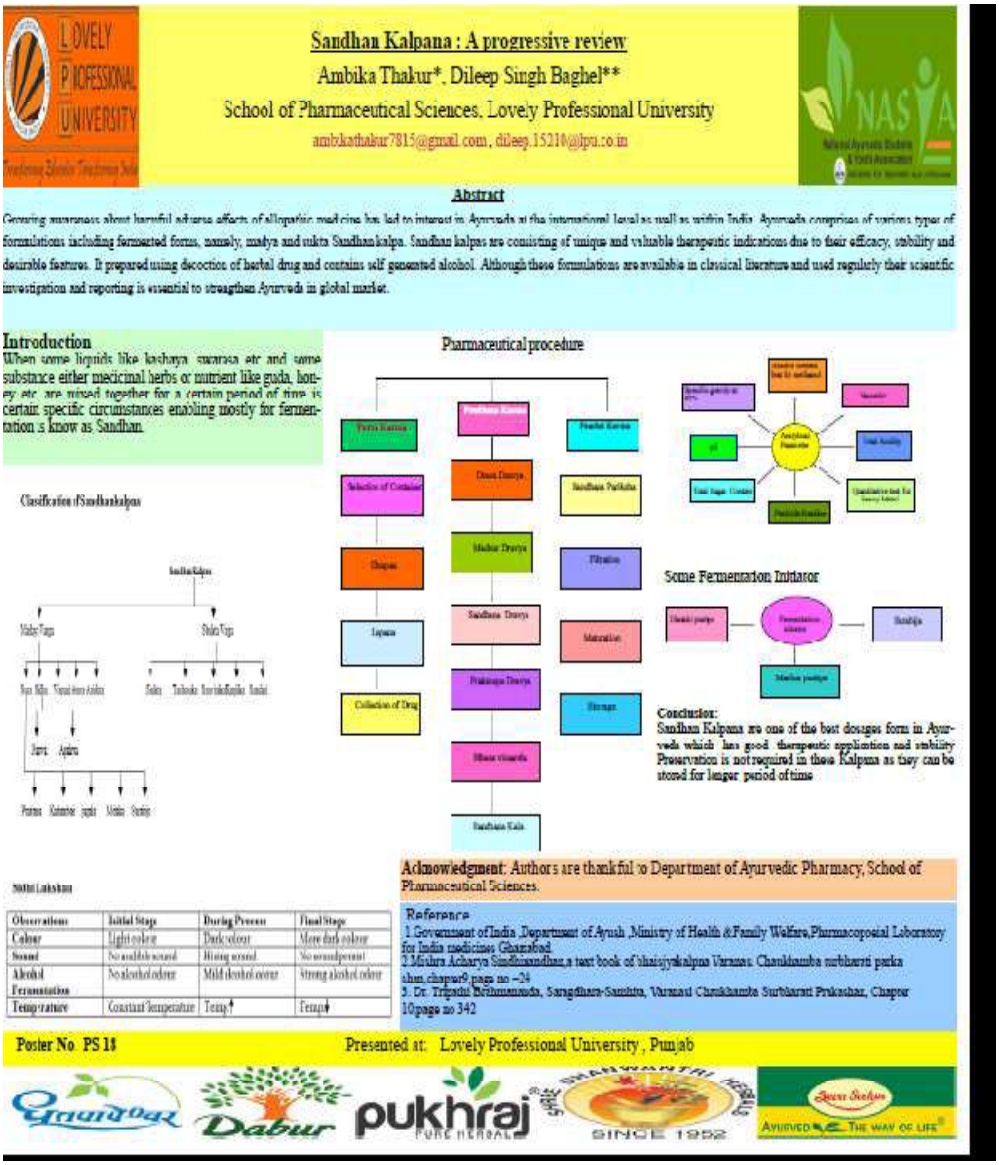
ORIGINALITY REPORT

%17	%15	%9	%8
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

14.7 Poster presentation Nasyacon (2016)

Poster no. PS 18



Sandhan Kalpana : A progressive review

Ambika Thakur*, Dileep Singh Baghel**
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Abstract

Growing awareness about harmful adverse effects of allopathic medicine has led to interest in Ayurveda at the international level as well as within India. Ayurveda comprises of various types of formulations including fermented forms, namely, madya and sukta Sandhan kalpa. Sandhan kalpas are consisting of unique and valuable therapeutic indications due to their efficacy, stability and desirable features. It prepared using decoction of herbal drug and contains self generated alcohol. Although these formulations are available in classical literature and used regularly their scientific investigation and reporting is essential to strengthen Ayurveda in global market.

Introduction

When some liquids like kashaya swarasa etc and some substance either medicinal herbs or nutrient like guda, honey etc. are mixed together for a certain period of time in certain specific circumstances enabling mostly for fermentation is know as Sandhan.

Pharmaceutical procedure

Some Fermentation Initiator

Clasification ofSandhankalpna

Conclusion:

Sandhan Kalpana are one of the best dosages form in Ayurveda which has good therapeutic application and stability Preservation is not required in these Kalpana as they can be stored for longer period of time

Acknowledgment: Authors are thankful to Department of Ayurvedic Pharmacy, School of Pharmaceutical Sciences.

Sidhi Lakshan

Observations	Initial Stage	During Process	Final Stage
Colour	Light colour	Dark colour	More dark colour
Sound	No sound/slow sound	Hissing sound	No sound present
Alcohol Fermentation	No alcohol odour	Mild alcohol odour	Strong alcohol odour
Temperature	Constant Temperature	Temp↑	Temp↓

Reference

1.Government of India ,Department of Ayush ,Ministry of Health &Family Welfare,Pharmacopoeial Laboratory for Indian medicines Ghaziabad.
2.Mishra Acharya Sunilkanandhan,a text book of bhaisjyakalpna Varanas. Chaukhamba surbharti parka shen,chapter9 page no –24
3. Dr. Tripathi Brihmananda, Sarngdhara-Samhita, Varnasi Chaukhamba Surbharat Prakashar, Chapter 10page no 342

Poster No PS 18 Presented at: Lovely Professional University , Punjab

Poster presentation : International Conference pharmaceutical (2017)

Poster no. PCB 113

A DETAILED ANALYTICAL STUDY ON *RHODODENDRON ARBORIUM* (KURVAK/BRAS) FLOWERS

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ID: PCB 113
Code: B

Abstract

Rhododendron arborium Sm. (Fam. Ericaceae) is a medicinal plant known as Kurvak in Ayurveda having deep red or pink flowers. It is native to India, distributed throughout the Himalyas, Himachal Pradesh and also found in Bhutan, China, Nepal. The flowers of this plant has raktastambhak, madhumehar like activities. The flowers are also used to make sharbat, dies etc.

Introduction

Kurvak consists of dried flowers of *Rhododendron arborium* Sm. (Fam. Ericaceae); an evergreen large tree having deep red or pink flowers. It is native to India, distributed throughut the Himalyas, Himachal Pradesh, Uttarakhand, and also found in Bhutan, China, Nepal and Pakistan.

Rhododendron arborium

Method

The present study deals with the detailed analytical study of the flowers. Preliminary physicochemical parameters, phytochemical screening, quantitative estimation , thin layer chromatography (TLC) were carried out in the study. All the tests were done as per mentioned in the API (Ayurvedic Pharmacopoeia of India).

Table 1. Depicting for phytochemical analysis

S. No.	Chemical constituent	Sample A	Sample B	Sample C
1	Alkaloid	Present	Present	Present
2	Tannin	Present	Present	Present
3	Saponin	Present	Present	Present
4	Glycoside	Present	Present	Present
5	Carbohydrate	Present	Present	Present
6	Flavanoid	Present	Present	Present

Table 2. Depicting for physicochemical analysis

S. No.	Parameter	Sample A	Sample B	Sample C
1	Loss on drying	2.58 %w/w	2.14 %w/w	2.35 %w/w
2	Ash value	3.98%w/w	3.20%w/w	3.56%w/w
3	Acid insoluble ash	1.94%w/w	1.86%w/w	1.64%w/w
4	Water soluble extractive	45.92%w/v	31.92%w/v	40.21%w/v
5	Alcohol soluble extractive	46.8%w/v	39.65%w/v	41.23%w/v
6	pH	3.22	3.10	3.21

Thin layer chromatography of *Rhododendron arborium* flower

254 nm

366 nm

Vanillin spray

Mobile Phase
1. Chloroform: Methanol (7:1
2. Toluene:Ethyl acetate: Formic acid (5:4:1)

Detection	Sample A		Sample B	
	No. of spots	Rf value	No. of spots	Rf value
254nm	6	0.15, 0.45, 0.57, 0.72, 0.80, 0.92	6	0.15, 0.45, 0.57, 0.82, 0.92
366nm	4	0.15, 0.45, 0.72, 0.80	4	0.15, 0.46, 0.72, 0.82
Vanillin sulphuric spray	4	0.15, 0.35, 0.60, 0.80	4	0.15, 0.35, 0.60, 0.80

Result

Phytochemical screening reveals the presence of secondary plant metabolites and various physicochemical parametetrs were done. TLC fingerprinting was carried out in two different mobile phase mainly Cholroform:Methanol (7:3) in which maximum 5 spots distinguished and Toluene:Ethyl acetate:Formic acid (5:4:1) 7 spots were distinguished. The components were identified as triterpenoids-Ursolic acid, β-sitosterol and Lupeol.

Conclusion

This study can be used as a tool to identify the *Rhodendron arborium* as more then 1000 species of Rhododendron have been reported.

References

1.Chauhan N.S. Medicinal & aromatic plants of HimachalPradesh. Indus Publishing Company, New Delh: (1999) 353
2.Simultaaeous estimation of threetriterpenoids-ursolic acid, b-sitosterol andlupeol from flowers, leaves and formulations of *Rhododendron arborium Smith.* us-ing validated *HPTLC method Sunita Shilajan, Gouri Swar. International Journal of Green Pharmacy*

International Conference of Pharmacy (ICP-2017) on 7th and 8th April-2017
At: Lovely Professional University