

**DEVELOPMENT, OPTIMIZATION, CHARACTERIZATION
OF NANO FORMULATIONS OF *ALPINIA MUTICA* AND
TRADESCANTIA SPATHACEA FOR ANTI-DIABETIC
ACTIVITY**

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Pharmacognosy

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DECLARATION

I, hereby declared that the presented work in the thesis entitled “**Development, optimization, Characterization of Nano formulations of *Alpinia mutica* and *Tradescantia spathecia* For Antidiabetic activity.**” in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **Dr. Ashish Suttee**, working as Profeseor, in the **School of Pharmaceutical Sciences of Lovely Professional University, Punjab, India**. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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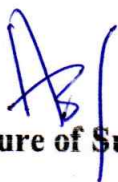
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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled “**Development, optimization, Characterization of Nano formulations of *Alpinia mutica* and *Tradescantia spathocia* For Antidiabetic activity.**” submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Pharmacognosy, is a research work carried out by **Shankaraiah Pulipaka, 41800583**, is Bonafede record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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ABBREVIATIONS

ADP- Adenosine diphosphate

AA- Arachidonic acid

Ag NPs - silver nanoparticles

AMLE Ag- *Alpinia mutica ethyl acetate extract silver*

AM- *Alpinia mutica*

AMEAE: *Alpinia mutica ethyl acetate extract*

AMHAE: *Alpinia mutica hydro alcoholic Extract*

AMME: *Alpinia mutica methanolic extract*

AMPEE: *Alpinia mutica petroleum ether extract*

AMLE- *Alpinia mutica* leaf extract

AMLE AgNO₃- *Alpinia mutica* leaf extract silver nitrate

BHA- Butylated hydroxyanisole

DPPH - 2, 2-diphenyl-1-picrylhydrazyl

DLS- Dynamic light scattering

DMAEMA-Dimethylamine ethyl Methacrylate

FRSA-Free radical scavenging activity

IDF -International Diabetes Federation

MAM- Methyl Methacrylate

SOD- superoxide dismutase

TLC- Thin Layer Chromatography

TTC- Total tannin-content

TFC- Total flavonoidal content

TPC- Total phenolic-content

PDA - Photodiode-Array Detection

Poly-PEGMA- poly (ethylene glycol) methyl ether methacrylate (PEGMA).

PLA -Polylactic acid

PEG-Polyethylene glycol

PLGA- Poly lactic glycolic acid

ROSS - Reactive oxygen species scavenging

SEM- Scanning electron microscopy

SOP- Standard operating procedure

UAE- Ultra sound Assisted Extraction.

USE- Ultra Sound Extraction

WHO- World health organization

Zn O NPs- Zinc oxide nanoparticles

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ANNEXURES

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Annexure 3 : Certificate of analysis of Experimental Work

ABSTRACT: Herbal treatments can heal many ailments. In the modern era, it is necessary to ensure the standardisation of crude drugs in order to guarantee the quality of herbal medicines. Traditional drug usage is on the rise everywhere, but especially in industrialised nations. Nevertheless, one disadvantage is that there isn't enough evidence to support it, which makes it more acceptable. Thus, it is important to focus on the physicochemical and analysis of phytochemical raw medication materials and to keep track of all research done on the nano-drug delivery system of medicines in distribution to create supported evidence. The plant *Alpinia mutica*(A.M.), which is commercially grown in Australia, tropical America, and South India at an altitude of approximately 1000 m, The plant *Tradescantia spathacea* (T.S.), often known as the boat Lilly, is a member of the (*Commelinaceae* family) and is occasionally planted as a decorative plant in gardens in India. A number of qualitative and quantitative evaluation methods, including macroscopy, microscopy, ash value, moisture content, extractive value, and preliminary phytochemical screening, were used in this instance to look at the leaves of (T.S. and A.M.). Leaf stomata, index, palisade ratio, veins islet, and terminations were counted. All of these analyses and results show that both plants adhere to the necessary quality standards. The classic method, which employs Soxhlet's reflux apparatus, has been utilized most often to extract material from plants in recent years. The solvent-intensive method takes a long time. Modern plant extraction methods use innovative extraction methodologies to solve this issue. The Ultra Sound Extraction (USE) method has various advantages over traditional extraction techniques, including reduced solvent usage, faster extraction times, and higher yields and purities of bioactive phytoconstituents. T.S. and A.M. leaves were used to test the effects of extraction on phytoconstituents using ethyl acetate, petroleum ether, methanol, hydroalcoholic, & water. The secondary metabolite content in the USE extract is increased according to the herbs extract and the initial phytochemical screening. To validate these outcomes, the total phenolic and flavonoid content of the extracts was evaluated. The research shows that the USE method significantly increased the phenolic and flavonoid content. Worldwide, diabetes

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affects millions of the population, over 425 million persons in the 20- to 79-year-old age range reported having diabetes in 2017, and it is predicted that number would increase to 629 million by 2045. Hyperglycemia, a metabolic illness associated with the risk of cardiac problems and obesity, characterises type II diabetes. Clinical research has demonstrated that Type II diabetes may be prevented with a better lifestyle that includes maintaining a good body weight and engaging in just moderate physical activity. Moreover, changing one's lifestyle quickly loses its effectiveness in treating diabetes, and maintaining the adjusted lifestyle is challenging. These days, combination therapy using a more oral hypoglycaemic medications shows promise as an effective method of glycaemic control in the treatment of diabetes. However, there are several negative effects associated with the various combo treatments. Combination treatment with phytoconstituents is more effective and has fewer side effects for diabetes control. Phytochemicals are safer than synthetic products. Pharmacological and phytochemical therapies are needed to prevent and cure Type II diabetes without adverse effects. This research screened T.S. and A.M. leaves for quantitative and qualitative assessments to standardize plant materials. The plants leaves extracted and separated, Then prepared and Green synthesized silver and ZnO nanoformulations i.e. AMLE (*Alpinia mutica leaf extracts*), AMLE SNP (*Alpinia mutica leaf extracts silver nanoparticles*), AMLE ZnO NP (*Alpinia mutica leaf extracts zinc oxide nanoparticles*) and TSLE (*Tradescantia spathacea leaf extract*), TSLE SNP (*Tradescantia spathacea leaf extract silver nanoparticles*) and TSLE ZnO NP (*Tradescantia spathacea leaf extract zinc oxide nanoparticles*). The functional groups were analysed, the nanoparticles' shape, size, and average particle size were established, and they were categorised in accordance with the traits that were investigated at and used for T.S. and A.M. Particle size, electrokinetic potential, Nanoparticles in the environment and biology were examined using scanning electron microscopy and X-ray diffraction.

ABSTRACT

Furthermore, their potential for treating diabetes *in-vivo* and *in-vitro* as well as an acute toxicity study were evaluated. Using an alpha amylase inhibition experiment, all of the nanoparticles were studied *in-vitro*. According to the *in-vitro* data, the IC₅₀ values for the AMLE SNP, AMLE ZnO NP, TSLE SNP, and TSLE ZnO NP were found to be significantly higher than those for normal acarbose at 73.72, 73.49, 73.77, 73.93, and 87.26 g/mL, respectively. Also, both plants were assessed for their acute toxicity profile in albino mice in accordance with OECD 425 recommendations in order to determine the safety profile. The toxicity research claims that all nanoparticles have only mild toxicity; however, when measured by biochemical, haematological, and histopathological criteria, SLP, ZnO NPs, AMLE, and TSLE create mild to moderate toxicity. Acute toxicity studies showed that all A.M. and T.S. extracts and natural compounds were safe at 2000 mg / kg, p.o. Plant NPs' *in-vivo* antidiabetic effectiveness was investigated at two dosages., i.e., 100 mg/kg and also 200 mg/kg, p.o., in Albino Wister rats fed an HFD diet and a low dosage of STZ. The results show that the AMLE SNP, AMLE ZnO NP, TSLE SNP, and TSLE ZnO NPs at 200 mg/kg, p.o., significantly lower glucose, triglyceride, and cholesterol levels compared to the experimental group. Both NPs were more potent than metformin, while the other NPs of both plants were less potent. The histology findings of AMLE SNP, AMLE ZnO NP, TSLE SNP, and TSLE ZnO NP reveals that the islets of pancreatic cells maintain their normal shape with relatively mild necrosis, suggesting that these NPs have an anti-diabetic effect.

ABSTRACT

Thus, molecular docking was done for both Nanoformulations (NFs) utilising important targets like alpha-amylase to predict their actions towards these targets. The target HPAA (PDB: 5VA9) was used to study the key components' antidiabetic efficacy in-silico. Among all the plant constituents the Flavokawin B showed the five hydrogen bond interactions to Arg195, Asp197, Glu233, His 299, Asn298 at the binding site of HPAA and showed four stearic interactions to Trp 59, Ile235, Asn 298, His299 at the binding site of HPAA.

In conclusion at the dose of 200 mg/kg, p.o. leads to the inhibition of the alpha amylase enzyme, preserving glycemic control, and lowering the oxidative stress caused by streptozotocin. At 2000 mg/kg, AMLE, AMLE Zn O, T SLE, and TSLE SNPs are safe. Both NPs can treat and study type 2 diabetes. Plant-derived antidiabetic nanoformulations need further investigation to cure diabetes.

Key words: *Alpinia mutica*, *Tradescantia spathacea* Standardization, Characterization of nanoparticles, Acute toxicity, Anti-diabetic activity, Molecular docking

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1 Diabetes

One of the long-term conditions that develop whenever the pancreas's function cannot produce insulin and metabolic abnormalities is diabetes mellitus (DM). There are three types of diabetes, and a lack of insulin may cause any of them. Insulin dependence causes Type I diabetes, but the inability of the body to produce enough insulin causes Type II diabetes. In this situation, the body cannot use the insulin created by our bodies. Gestational diabetes will develop during pregnancy; in very few instances, it will persist after delivery. In general, there are two methods to treat diabetes: pills and injections of insulin. However, most of the time, the drugs have adverse effects, and the insulin injections are painful. We need to employ conventional medication for recovery [1].

Nephropathy, retinopathy, and neuropathy are just a few of the issues that the long-term effects of DM may cause. Obesity, cerebrovascular illness, cataracts, non-alcoholic fatty liver disease, erectile dysfunction, and infectious diseases, including TB, are all severe risks for diabetics. Furthermore, their risk of getting diabetes is increased. A few of the symptoms of DM include polyuria, blurred vision, thirst, and weight loss [2,3]. Hyperglycaemia, a metabolic condition involving lipids, proteins, and carbohydrates, is the hallmark of a variety of illnesses together known as diabetes mellitus. Vascular disease has a significant likelihood as a result. From 1985 to 2000, the number of people with diabetes increased from 30 million to 177 million. Diabetes will affect over 360 million people globally by 2030, according to USFDA data [4].

More people worldwide die from diabetes than from AIDS. Diabetes and AIDS patients in India were 40.8 million and 40 million, respectively [5]. Additionally, it is listed as the sixth fastest-growing condition globally [6]. By 2045, it's predicted that 629 million people will have diabetes, up from the 425 million individuals who reported having the disease in 2017, aged 20 to 79 [7]. According to ethnobotanical research, individuals use almost 800 plants that may have antidiabetic potential out of the numerous therapeutic plants that have antidiabetic activity. Several plants have shown antidiabetic activity when examined using presently available experimental methodologies. Several chemical compounds and a broad spectrum of plant-derived

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active substances may be used to treat type 2 diabetes without insulin. (NIDDS). Alkaloids, Polysaccharides, Hypo-Glycans, Galactomannan Gum, Terpenoids, Peptidoglycans, Glycopeptides, Guanidine, Steroids, Carbohydrates, Amino Acids, and Inorganic Compounds are some examples of these substances. *Galega officinalis* frequent usage is mainly responsible for discovering the extensively used hypoglycemic medication metformin [8]. As a result, medicinal plants might provide antidiabetic medications (and other kinds); nevertheless, the scientific community no longer gives this fact any weight. Many plants and products made from them have been used to treat diabetes, making them possible sources for hypoglycemic medications. Numerous researches on Indian herbs that may be useful in treating different types of diabetes have been published in scholarly journals. According to Ayurveda and other traditional medicinal systems, several plants are used as herbal remedies for treating DM. Herbs that decrease blood sugar levels enhance glucose absorption by muscle or adipose tissue, promote insulin secretion, and lessen the amount of glucose taken from the stomach and generated by the liver [9].

Insulin and oral hypoglycaemic medications such as sulphonyl-ureas and biguanides remain the fundamentals of care, while attempts are being undertaken to develop antidiabetic drugs that are even more effective [10]. Due to unregulated hepatic glucose production and impaired skeletal muscle glucose uptake due to inadequate glycogen synthesis, hyperglycemia occurs. When glucose reabsorption exceeds the renal threshold, glucose leaks into the urine, producing polyuria and osmotic diuresis, which leads to polydipsia (increased drinking), dryness, and dehydration. Insulin deficiency leads to wastage by reducing and breaking down protein synthesis [11,12].

The major causes of non-communicable illnesses in the contemporary world are shifts in food preferences and fashions in lifestyle. Nearly 60% of fatalities worldwide are caused by non-communicable diseases such as heart disease, stroke, diabetes, most malignancies, and lung disorders. Almost 10% of all persons on the earth are afflicted with diabetes mellitus, one of the non-communicable diseases.

The number of people who have the illness on a global scale is rising daily and is predicted to reach 300 million by the end of 2025. According to reports, diabetes affects 2-4% of rural Indians and 4-11% of metropolitan inhabitants. Within a decade, India is predicted to become the global DM hub. Although many medications are

available in many medical systems for treating diabetes, neither synthetic nor natural medicines provide long-term relief from medical conditions, even with frequent usage. Herbal goods are booming due to synthetic drugs primarily damaging the heart, liver, kidney, and other essential organs. In light of this, substantial research is being conducted worldwide on medicinal plants to create brand-new antidiabetic medications with great therapeutic effectiveness and no adverse effects. However, the current problem facing researchers in all medical systems is the creation of innovative antidiabetic medicines [13].

1.2 Classification

Diabetologists generally agree that diabetes may be divided into four main groups.

1.2.1 Diabetes Type I

Type I diabetes is an autoimmune response that selectively damages β -cells, aided by T-lymphocytes, resulting in decreased insulin production. The disease arises due to several viruses that activate the antibodies, which in turn causes the islet cell of the duct gland to be destroyed. Different environmental and genetic variables have an impact on the infection. Pathology and fibrocystic pancreas disease are included in the genetic factors. But the cause is still unidentified. Due to the severe nature of the treatment and its need for insulin, the Type I diabetes patient requires hormone medical attention every day. Changes in diet, regular exercise, and medication can help Type I diabetes symptoms improve [14,15].

1.2.2 Diabetes Type II

Type II diabetes is defined as adult-onset, hypoglycemic-induced diabetes that develops as a result of inadequate internal secretion production or diminished peripheral tissue responsiveness. The lack of a hypoglycemic agent causes a decrease in the amount of glucose available to the cell, which impacts the production of glucose and triglycerides in the veins. Acromegalia, brain doctor syndrome, and glandular disease lead to internal secretion resistance. Along with lifestyle changes, oral hypoglycemic medication will be used to control type II diabetes. Nephropathy, impaired vision, thirst,

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weight loss, and ketosis are symptoms that may be used to diagnose diabetes mellitus. Nephrosis, retinopathy, neuropathy, and disorder are consequences of type II diabetes mellitus [16, 17].

1.2.3 Diabetes During Pregnancy

Pregnant women who do not already have gestational diabetes, a particular kind of diabetes, are susceptible to developing it. Gestational diabetes affects 2 to 10% of pregnancies in the US every year. To promote a healthy pregnancy for you and your unborn child, it is essential to manage your gestational diabetes. Considered to be gestational diabetes, it occurs throughout pregnancy. Hypoglycemia is thought to be caused by increased secretion production during pregnancy, which lowers the hypoglycemic agent's sensitivity. It is a reversible type of diabetes, and treatment throughout pregnancy will consist of food management, except for a brief period when anti-diabetic medication will be required. Because of carelessness and inadequate care during pregnancy, gestational diabetes often transforms into Type-II diabetes after pregnancy [18].

1.2.4 Additional Special Type (Monogenic diabetes)

Monogenetic defects in β -cell function is associated with diabetes in its different manifestations. In these kinds of diabetes, hyperglycaemia often manifests in childhood, according to widespread knowledge. (Generally, before age 25 years). The term "mature onset diabetes of the young" (MODY) characterises these situations, marked by reduced insulin secretion and little to no alterations in insulin action. So far, six particular genes have been found to have mutations on different chromosomes. Hepatocyte Nuclear Factor (HNF)-1, a hepatic transcription factor, is linked to the most frequent kind of chromosome 12 mutations. The β -cell secretes insulin in response to the glucokinase-generated glucose-6-phosphate, which is then metabolised. The "glucose sensor" for the β -cell is hence glucokinase. High plasma glucose levels were required in individuals with glucokinase gene abnormalities to start normal amounts of insulin production. Mutations in additional transcription factors

result in less frequent variants, including HNF-4, HNF-1, and insulin promoter factor (IPF)-1[19].

1.3 Type II Diabetes: Etiology and Pathophysiology

Genetic and environmental factors seem to interact to cause type 2 diabetes, which has a complex aetiology. A susceptible genotype and a diabetogenic lifestyle are likely to combine to cause the illness. (i.e., excessive calorie intake, insufficient calorie expenditure, and obesity). Various body mass indexes (BMIs) apply to different ethnic groups, and being overweight increases the risk of acquiring diabetes for each BMI. Even when they are less heavy, persons with Asian heritage are more likely to achieve diabetes than those with European ancestry [20,21]. White persons are more likely than African Americans to develop prehypertension and hypertension due to hypertension [22]. In addition, type II DM may be predisposed in certain persons by a low birthweight environment during foetal development [23, 24]. Infant weight velocity significantly impacts BMI and waist circumference but has a minor, indirect impact on adult insulin resistance [25]. Overweight or obese type II DM patients comprise around 90% of the population [26]. A major population-based, prospective research found that regardless of weight, a high-energy diet may raise the chance of developing diabetes [27]. The development and progression of type II diabetes may be influenced by environmental pollutants, according to certain studies [28]. A systematic and well-planned platform is necessary to thoroughly study the likelihood that ecological contaminants might lead to diabetes. Glucocorticoids or insulin-deficient circumstances may cause secondary diabetes. (Cushing syndrome, acromegaly, pheochromocytoma).

The pathophysiology of Type II Diabetes comprises several genetic predispositions and environmental risk factors that contribute to developing insulin resistance and irregular insulin production. Insulin resistance could be brought on by changes in the insulin receptor's capacity to bind molecules, the transfer of biochemical signals, or the activation of intracellular effector units [29]. Insulin resistance in the limbs and inadequate insulin production by beta cells in the pancreas are characteristics of diabetes. Insulin sensitivity, linked to greater plasma levels of "free fatty acids" and

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proinflammatory cytokines, is the root cause of decreased glucose transport into muscle cells, enhanced hepatic glucose synthesis, and expedited fat breakdown.

Hyperglucagonemia and hyperglycemia result from the destruction of the reciprocal relationship between the alpha cell that produces glucagon and the beta cell that releases insulin in diabetes mellitus (DM), an islet paracrinopathy. [30]. Beta-cell malfunction plays a crucial role in developing prediabetes and diabetes. Research of obese teenagers by Bacha et al. supports the following, emphasised increasingly in adult debates: Beta-cell malfunction shows up early in the pathogenic process; it isn't always evident after the onset of insulin resistance [31]. When the exclusive focus on insulin resistance as the "be all and end all" progressively evolves, a greater emphasis on addressing beta-cell dysfunction should emerge for early treatment. The rise in postprandial blood sugar levels occurs before transitioning from normal to impaired glucose tolerance. Fasting hyperglycemia eventually manifests when liver gluconeogenesis suppression fails. After introducing insulin resistance, glucose intolerance is accompanied by elevated glucagon levels. (Which might happen due to a high-calorie diet, the injection of steroids, or physical inactivity). However, the reaction to the hormone glucagon-like peptide-1 (GLP-1) is unaffected [32]. Genetic variations connected to beta-cell activity and insulin resistance have been discovered by genomic sequence association analyses employing single-nucleotide polymorphisms [33]. **Figures 1 and 2** illustrate the aetiology and pathophysiology of DM.

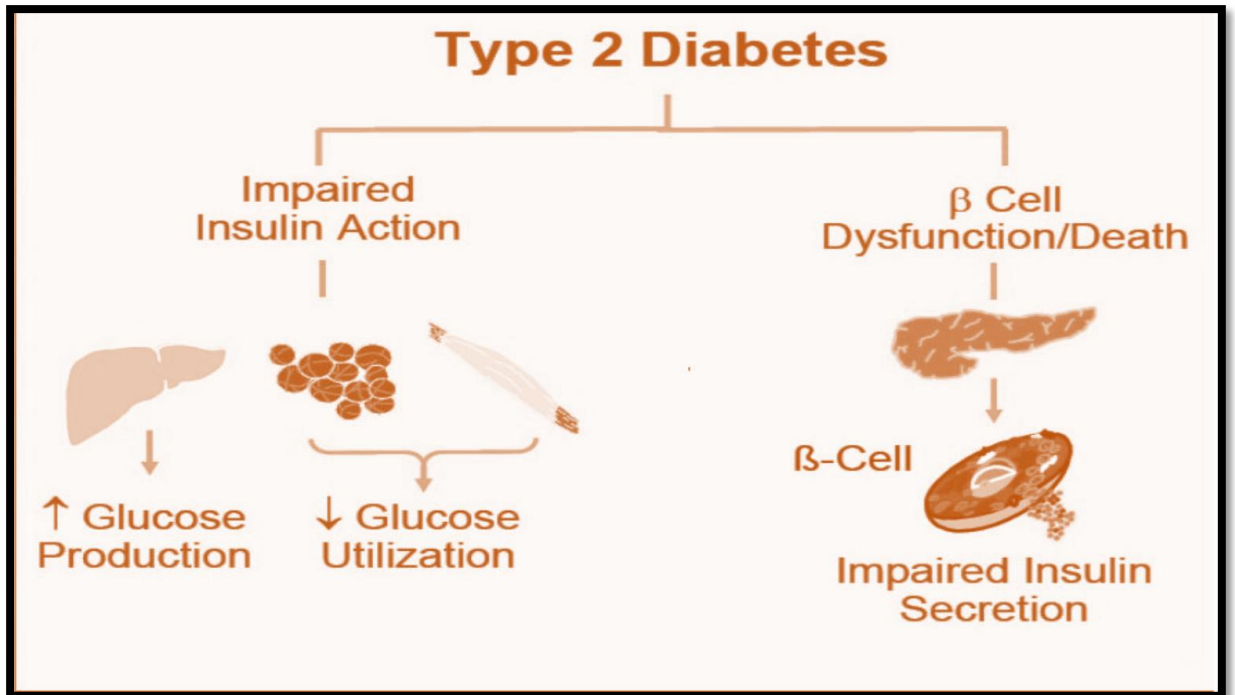


Fig. 1 Etiology of Type II DM

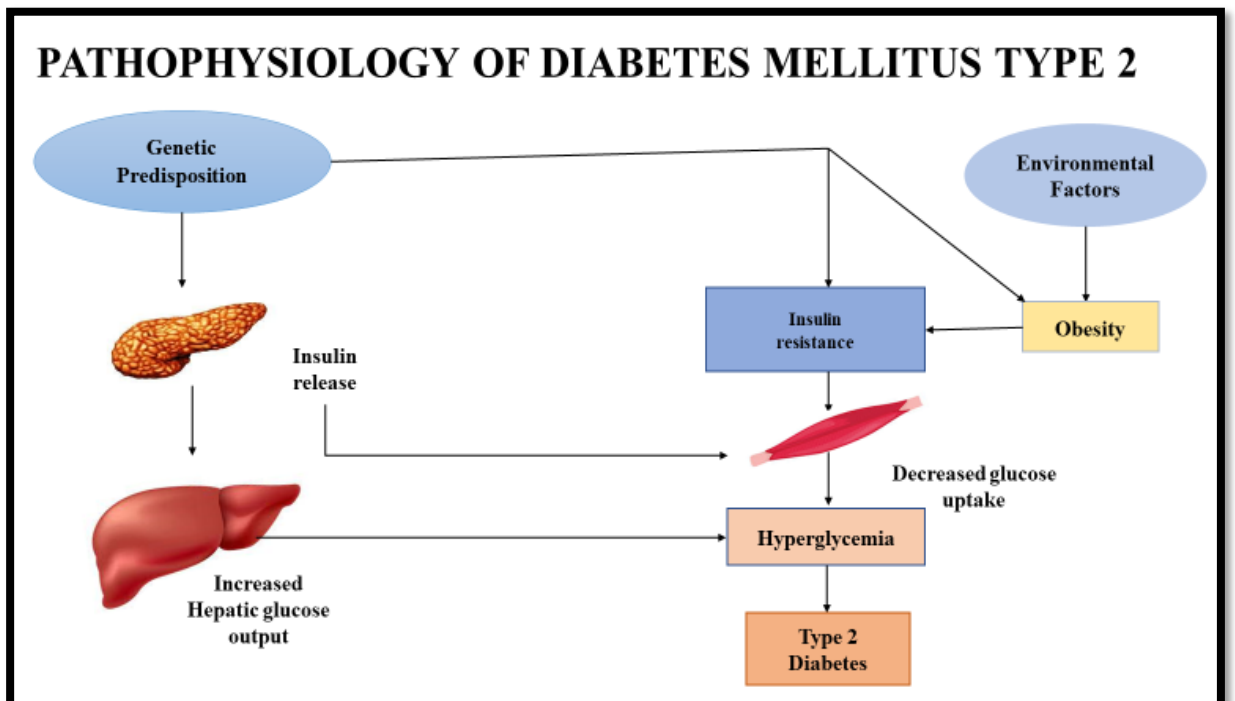


Fig. 2 Patho-physiology of Type II DM

1.4 Diabetic Type II Complications

Most diabetes complications (**Fig. 3**) are caused by tissues exposed to elevated glucose levels for extended periods. Such metabolic problems result in light-headedness due to diabetic complications, often over time. According to the type of diabetes, the disease's pathophysiology varies; nevertheless, the bulk of its symptoms, including microvascular, macrovascular, and neuropathic problems, remain constant. The underlying cause of metabolic and microvascular diseases is hyperglycaemia. Macrovascular disease and hyperglycemia may not be as tightly related. Telomere attrition may indicate diabetes complications and severity. It has to be established if diabetes is the root cause or one of its contributing components [34]. Insulin resistance, elevated LDL cholesterol levels, and other lipid abnormalities increase cardiovascular risk in diabetics. When HDL cholesterol levels are excessively low, high blood pressure and thrombotic deviations, such as higher levels of type-1, usually used to create-activator-inhibitor [PAI-1] and fibrinogen, occur.

Smoking, family history, and increased LDL cholesterol increase cardiovascular risk. Insulin resistance causes liver and muscle smooth lipid accumulation, but not heart lipid buildup [35]. Despite cholesterol-modifying medicines and their advantages, diabetics have persistent lipid abnormalities. The statin dose must be increased, and more lipid-modifying drugs must be added. The effects of insulin resistance are most likely to blame for the increased cardiovascular risk seems to begin before frank hyperglycemia shows. Types of diabetes produced by primary diseases or treatments are classified as secondary diabetes. The most common causes of secondary diabetes are hormonal conditions that promote peripheral insulin resistance, such as acromegaly, Cushing syndrome, and pheochromocytoma, as well as hormonal conditions that interfere with the secretion of insulin (e.g., phenytoin, glucocorticoids, oestrogens) [36]. The most common causes of primary diabetes are acromegaly, Cushing syndrome, and pheochromocytoma.

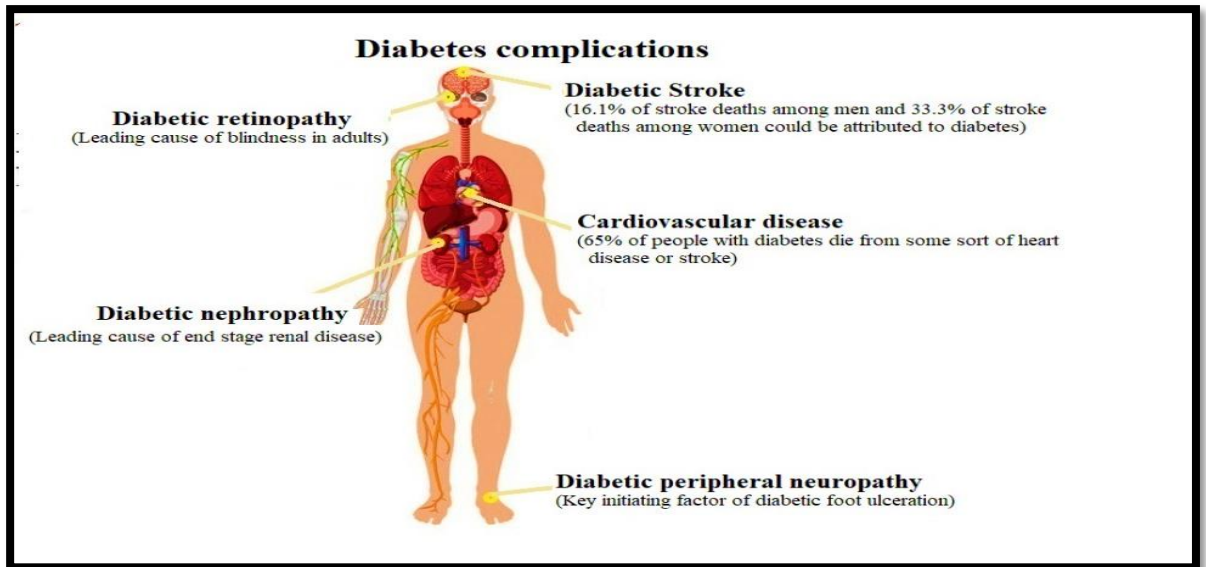


Fig 3. Type II Diabetic Complications

1.5 DM Type II management

Treatment for DM type II involves a progressive strategy, starting with lifestyle modification (therapeutic nutritional treatment and exercise), then progressing to oral anti-diabetic drugs. Determining if diabetes or one of its traditional therapies is to blame for this condition is critical. After taking two or more oral medications simultaneously, insulin is the ultimate consideration [37].

1.5.1 MANAGEMENT OF DIABETES WITHOUT PHARMACOLOGY

1.5.1.1 Lifestyle objectives for people with diabetes include:

i) Promoting health by eating properly; ii) Maintaining healthy weight, growth, and development with energy; iii) Glycaemic control preservation; iv) Obtaining ideal blood lipid levels; v) Customising a diet for each person based on their problems and illnesses; vii) Promoting stress management; and viii) achieving optimal physical activity.

1.5.1.2 Medical Nutrition Therapy (MNT)

Nutritional, behavioural, and physical activity sciences must be used to treat diabetes mellitus. A multifaceted approach is necessary:

- i) A nutritional assessment that takes into account lifestyle, dietary, and metabolic variables
- ii) Setting objectives that are specific to the patient that is realistic, doable, and acceptable to them
- iii) Nutritional intervention includes nutrition instruction and individualised meal planning based on family eating habits.
- iv) Evaluation - To assess progress and make modifications, the diet is based on gender, age, physical activity, body mass index (BMI), and culture. The patient's daily routine should be considered while planning meals, and they should be timed to be personalised, adaptable, and near to the family rhythm. We must eat nutritiously, which is Dietary recommendations include energising foods including carbohydrates, fibre, proteins, fats, and sweeteners. [38] [39] [40].

1.5.1.3 Exercise and physical activity

A crucial part of managing type 2 diabetes is regular physical activity and exercising under controlled conditions. Before prescribing an exercise programme to diabetic patients, a thorough examination should be conducted. The exercise routine must be customised according to a person's skill and capability.

Exercise's advantages:

- Insulin sensitivity.
- Reduced heart disease risk.
- High BP.
- Bone disease.
- Unhealthy weight gain.

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Maintaining flexibility and agility, reducing stress and anxiety, preventing depression; boosting strength and endurance; encouraging sound sleep; enhancing metabolism and digestion; lowering cholesterol; and slowing the ageing process. Doing strength and flexibility exercises at least twice a week and around 150 minutes of aerobic activity per week or equal is advised. Diabetics need a fast-acting carbohydrates snack before and during exercise that lasts longer than usual [41,42].

1.5.1.4 Diabetes and Yoga

An ancient Indian method known as yoga has acquired popularity on a global scale as a way to improve stress-coping abilities and is being advocated more and more as a component of comprehensive diabetes care. Yoga incorporates a variety of elements, including:

- Asanas (postures);
- Pranayama (breathing exercises);
- Dhyana (involving meditation)

Although the specifics of these approaches are beyond these guidelines' purview, there is mounting evidence that people with type 2 diabetes may benefit from yoga. Yogic techniques may positively affect several diabetes care strategies, including lipids, body fat percentage, and glycaemic control. Other advantages include reducing blood pressure, oxidative stress, respiratory and autonomic function, state of mind, sleep, and overall level of life, as well as reducing anti-diabetic drug doses. The potential advantages of yoga and its mechanisms of action in this patient group still need to be confirmed, and several such studies have already been started [43,44,45, and 46].

1.5.2 DIABETIC PHARMACOLOGICAL MANAGEMENT

1. Antihyperglycemic oral drugs
2. Insulin injection
3. non-insulin injection remedy
4. Botanical treatment

1.5.2.1 Anti-Hyperglycaemic-Drugs

Blood sugar levels are affected by intestinal, peripheral (muscle, adipose), hepatic, pancreatic, and stomach hormone release, and kidney glucose processing [47–49]. As seen in **Figure 4**, various anti-hyperglycaemic medications work by altering

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the variables that help manage hyperglycaemia. **Table 1** is a list of the oral hypoglycaemic medications that are presently offered in India.

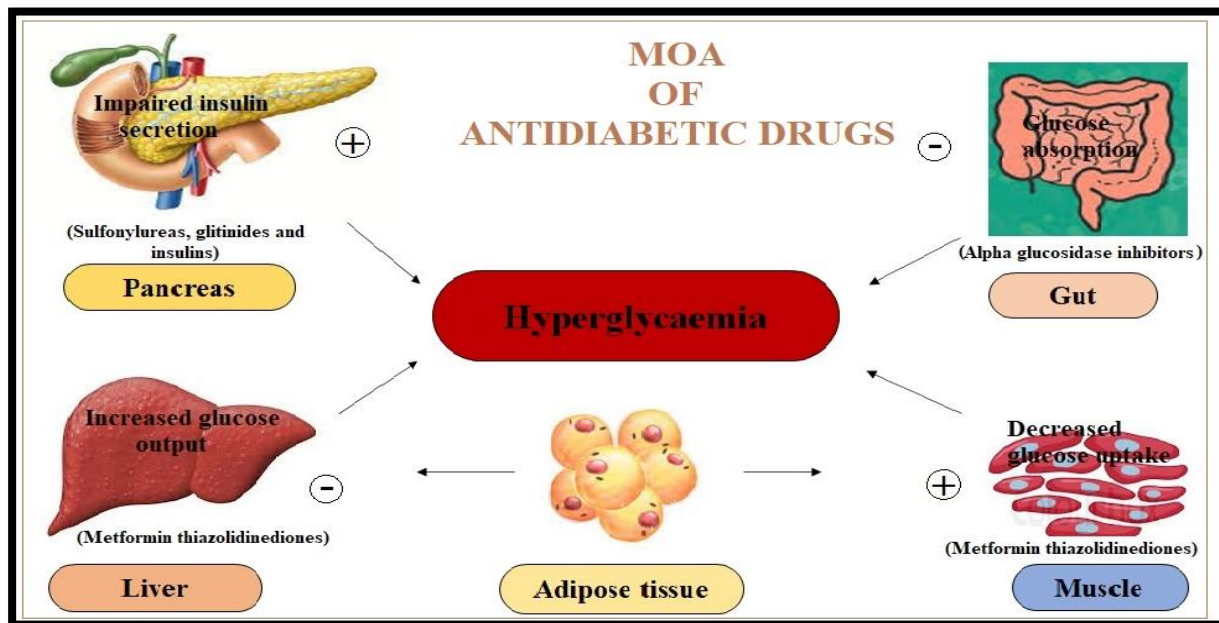


Figure 4: Mechanism of action of anti-hyperglycaemic drugs.

Table 1: India's oral anti-hyperglycaemics

Compounds Biguanides				
Drug name	Daily dose range (min-max) (mg)	Frequency/day	Action duration (hrs)	Excretion mode
Metformine	250-2500	1-3	4-8	Urine.
Metformin SR	500-2500	1-2	18-24	Urine
SULPHONYL-UREAS				
Glibenclamide	2.5-20	1-2	16-24	Urine (50%) Bile (50%)
Glipizides	2.5-20	1-3	8-12	Urine. (80%) Bile (20%)
Glipizides modified release	5-20	1	24	Urine (80%) Bile (20%)
Gliclazides	80-320	1-2	8-12	Urine (80%) Bile (20%)
Gliclazide modified release	30-120	1	24	Urine (80%) Bile (20%)

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Glimepiride	1-8	1	16-24	Urine(60%) Bile(40 %)
DPP - 4 (Dipeptidyl peptidase-4) INHIBITORS				
Sitagliptins	25-100	1	24	Urine(87%) Faeces (13%)
Vildagliptins	25-100	1-2	3-12	Urine(85%) and Faeces(15%)
Saxagliptins	2.5-5	1	2.5	Renal(24-75%) and remaining hepatic excretion
Linagliptin	5	1	24	Entero-hepatic excretion
Tenigliptin	20-40	1	24	Faeces(46.5%) Renal(45%)
Gemigliptin	50	1	18-24	Urine (63.4%) Faeces (27.1%)
THIAZOLIDINEDIONES (GLITAZONES)				
Pioglitazone	Pioglitazone	1	16 - 24	Urine (15 - 30%) and remaining in faeces.
SGLT 2 Inhibitors				
Canagliflozin	100 - 300	1	100 - 300	Urine (33%), Faeces (41.5 %)
Dapagliflozin	5 - 10	1	24	Urine. (75%), Faeces (15%)
Empagliflozin	10 - 25	1	24	Urine (54.4%), Faeces (41.2%)
Alpha-glucosidase inhibitors				
Acarbose	25 - 150	1-3	2	Renal (2%) and rest metabolised.
Voglibos	0.2 - 0.9	1 - 3	1 – 1.5	Urine (5%), Faeces (95%)
Miglitol	25 - 150	1-3	2-3	Renal (95%)
Non-Sulphonyl urea Secretagogues (Glinides)				
Repaglinide	0.5 - 6	3	1	Faeces (90%), Renal (8%)
Nateglinide	60 – 360	3	1.5	Urine (83%), Faeces. (10%)

The Drug Controller General of India (DCGI) disfavours fixed-dose combos (FDCs), especially those with more than two medications.

1.5.2.2. Insulin Therapy

Insulin is essential to type 1 diabetes treatment. To help them reach their glycemic goals, many type 2 diabetes patients may also need insulin injections.

1.5.2.2.1 Insulin should be given to type 2 diabetics upon diagnosis if:

Someone with diabetes who has significant, symptomatic hyperglycemia, weight loss, polyuria, polydipsia, and polyphagia. HbA1c > 9% or Ketosis with severe infections with fasting blood sugar levels > 270 mg/dl.

1.5.2.2.2. Other circumstances in which insulin is advised

Acute hyperglycemia, ketoacidosis in people with diabetes, hyperosmolar hyperglycemic, lactic acidosis stress, hospitalization, lactation, and post-operative pregnancy scenario OHA prejudice or other restrictions renal disease Diabetics on steroids may use oral medications for kidney transplantation.

1.5.2.2.3 Types of insulin preparations

There are several varieties of insulin. They have various pharmacokinetic characteristics. Insulin activity depends on composition, injection method, insulin antibodies, location, and patient response. Only insulin mimics and rDNA-produced human insulin are available. Animal insulin is unavailable. Regular short-acting human soluble insulin; NPH; premixed combinations of these insulins in 25/75, 30/70, and 50/50 proportions; (**Figure 5**). Soluble formulations of the innovative basal analogue's insulin degludec (70%) and insulin aspart (IAsp: 30%) include lispro/lispro protamine, aspart/aspart protamine, and co-formulations of degludec and aspart insulin (IDeg Asp). Rapid acting medications include Lispro, Aspart, Glu [50-52].

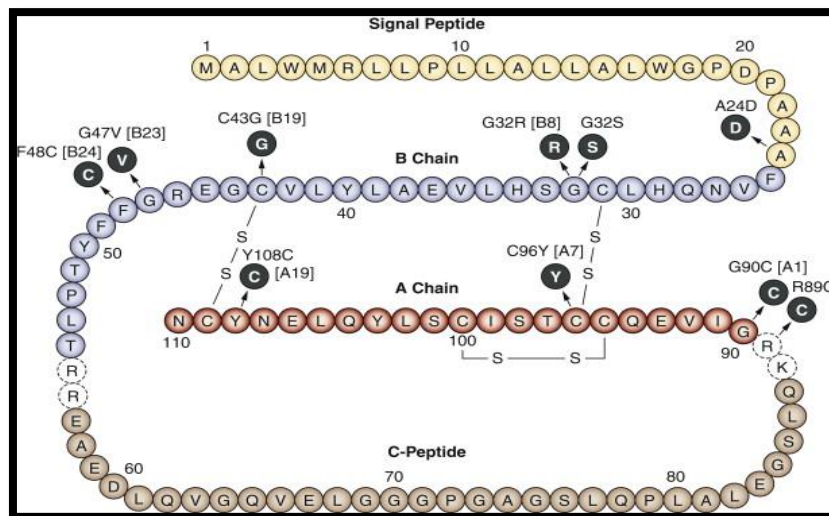


Figure 5: Human Insulin

1.5.3 PHYTOTHERAPY

The application of plant-based treatments (phytotherapy) or therapeutic methods predates recorded history; several places created their ancient medicine archives from plants typically present in their various locations or nations. Modern medicine is built on phytotherapy, and many of the medications still in use today still exist as parts of plants. Most people in low-income nations, where primary healthcare is neither unavailable nor too expensive, still rely on herbal remedies as their primary method of sickness treatment. However, research shows that people living in high-income countries like the Federal Republics of Germany and France have been using herbal medicine at an increasing rate over the last few years to decades. The comprehensive approach to diagnosis and the relatively low side effects compared with traditional medications are some causes. Traditional knowledge and extensive study using experimental models of diabetes mellitus have shown that a wide variety of plants have hypoglycaemia or anti-diabetic potential. The bioactive chemicals found in many plants, which come from various families, are diverse. (**Table 1**).

Even though many medical systems provide different medications for the treatment of diabetes, neither synthetic nor natural medications, no matter how often they are taken, ever provide long-lasting respite from the problems. Herbal goods are expanding rapidly since synthetic medications primarily harm the body's essential organs, including the heart, liver, and kidneys. To create innovative anti-diabetic

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medications with excellent therapeutic effectiveness and minimal side effects, substantial research is being done on medicinal plants all over the globe. However, the development of innovative anti-diabetic medications continues to be a problem for researchers across all medical systems [54].

Table -2: Some of the antidiabetic crude drugs with families

S.NO.	PLANT	FAMILY
1	<i>Allium sativum</i>	Liliaceae
2	<i>Capparis spinosa</i>	Capparaceae
3	<i>Cinnamon zeylanicum</i>	Lauraceae
4	<i>Citrullus colocynthis</i>	Cucurbitaceae
5	<i>Gymnema sylvestre</i>	Apocynaceae
6	<i>Juglans</i>	Juglandaceae
7	<i>Momordica charantia</i>	Cucurbitaceae
8	<i>Ocimum grattisimum</i>	Lamiaceae

1.6 Nanotechnology

Due to his ability to accurately create substances at the nanoscale, the late Norio Taniguchi (The University of Tokyo) was credited for creating the word "nanotechnology" in 1974. The phrase "engineer materials" is often understood to encompass the design, characterization, manufacture, and use of materials. More recently, the scope has been expanded to include systems, devices, and materials. Consequently, nanotechnology is the carefully planned development of nanoscale components, devices, and systems. Thus, nanotechnology is about size and control. Even though all of these technologies can exert control at the nanoscale, some people use the plural word "nanotechnologies" due to the wide variety of applications. Nanotechnology has continued to be used in all sectors where minuscule size plays a crucial role in defining essential qualities, thanks to advancements in materials science, chemistry, and engineering during the last several decades. From biology and medicine to physics, engineering, and chemistry, they are utilized to mark biological molecules precisely, and cadmium telluride nanoparticles are used [55].

According to the definition of nanotechnology, it is the deliberate creation of components, systems, and ecosystems by manipulating their size and form within the

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nanoscale range of 1 to 100 nm. Nanotechnology has the potential to be beneficial for medical applications since nanoparticles may be created to perform a variety of tasks while being comparable in size to biological molecules or systems. The goal of nanomedicine is to cure illnesses at the level of molecules by using nanomaterials' capabilities and physical qualities [56]. The study of health science now includes several expanding research fields, including nanotechnology. For example, nanoparticles display entirely new or amplified features in terms of size, dispersion, and particle form. New uses for nanoparticles and nanomaterials are quickly developing in various scientific fields, including medicine and pharmaceuticals [57].

The name "nanotechnology" comes from the Greek phrase "nanos," which means "short." It is a novel approach to drug growth and also can target itself. The 21st century's most advanced scientific technology is the nano technique. The particle ranges in size from 1 nm to 100 nm. For many years, herbal medicines have been utilised extensively around the globe.

Since they do not have any adverse side effects compared to current medications, they are valued by doctors and patients for their higher therapeutic activity qualities. More knowledge than ever before on using plants for medicinal purposes, especially in pharmaceuticals, has been made available by human investigation in medicine. By reducing toxicity and doing away with pharmacological side effects at the same time, herbal therapy aids in improving the curative value. Additionally, it increases bioavailability [58].

1.6.1 Nanomaterials in medicine

They interact molecularly with living cells and tissues. These nanomaterials and devices are essential by-products of biomedical engineering and are used in physiology and medicine with reasonable accuracy. They provide some degree of technological and biological system integration as a result. Modifying them at the nanoscale scale makes it possible to control and alter the bioactivity of medications, active ingredients, and devices. Thanks to them, solubility, controlled release, and targeted medicine distribution can all be managed [59].

Among the many applications for nanomaterials and nanomedicine are fluorescent biological labels, amino acid, lipid, and protein detection, drug delivery,

other macromolecule detection, pathogen detection, DNA structure probing, tumour identification and detection, tissue engineering, MRI contrast enhancement, and biological molecule purification. Nanomachinery is needed for the design of nanomedicine. Careful control and manipulation of the nanomachinery in the cellular environment leads to a more thorough grasp of the cellular mechanistic study in living cells. In addition, it helps bring about new tools for the prevention, diagnosis, and treatment of illness. Nanoscale imaging, which explains the molecular mechanisms inside living cells, is made possible by advances in biomedical engineering and nanomedicine [60].

1.6.2 Types of Nanoparticles

1.6.2.1 Carbon-based NPs

NPs formed from carbon include nanotubes made from carbon (CNTs) and fullerenes, which are nanomaterials with spherical hollow cages like allotropic carbon. Nanocomposites are used as fillers, environmental gas adsorbents, and catalyst support medium [61-64].

1.6.2.2 Metal NPs

Pure metals are used in the production of nanoparticles because of their renowned LSPR (localised surface plasmon resonance) qualities. Nanoparticles (NPs) have unique optoelectric properties. Nanoparticles (NPs) made of alkali and noble metals, including copper, silver, and gold, absorb a wide range of the visible spectrum. Synthesising metal NPs with a high degree of control over their size, shape, and number of facets is crucial for modern high-tech products. Due to their exceptional optical properties, metal NPs have applications in various scientific disciplines. For superior SEM photos, they focus on enhancing the electronic stream [65].

1.6.2.3 Ceramics NPs

Heated and cooled inorganic non-metallic ceramic nanoparticles (NPs). Several types include amorphous crystalline dense, porous, and hollow. Researchers are interested in these NPs because of their potential uses in various activities, including catalysis, photocatalysis, dye photodegradation, and imaging [66-67].

1.6.2.4 Polymeric NPs

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Researchers have recently turned their attention to nanotechnological procedures utilising medicinal plants, creating numerous novel nanoparticle delivery systems. These polymer-based materials, which are biodegradable and biocompatible, offer a choice for regulated medication administration. Targeted research will focus on polymeric nanoparticles because they are a promising drug delivery system formulation [68-69].

Mixing systems known as polymeric nanoparticles may be used to target the release of drugs. When compared to standard formulations, polymeric nanoparticles provide superior solubility of contents, reduced therapeutic dosage, and increased absorption of active substances. The advantages of employing nanoparticles in the blood include their long shelf life, lack of toxicity, lack of thrombogenicity, lack of immunogenicity, lack of inflammation, lack of neutrophil activation, and lack of entry into the reticuloendothelial system. Polymeric nanoparticles may occasionally be employed as cell surfaces or to access particular tissues. Depending on their intended use and payload, numerous methods can be used to create polymeric nanoparticles. These particles are biodegradable polymers.

Natural materials are preferred because they distribute numerous active compounds using a constant carrier, lengthen body residence time, provide a sustained-release strategy, and minimise negative effects. The diameters of nanoscale systems, called sub-micrometre systems, are one micrometre. Researchers like them because they provide several delivery pathways, site-specificity, and increased therapeutic efficacy. Traditional bonded formulations taken orally may have side effects, and the stomach's acidic pH encourages the breakdown of active ingredients. These problems can be lessened using polymeric nanoparticles. Nanoparticles improve ocular bioavailability and reduce side effects by regulating active component release during ophthalmic administration. Pharmaceutical protection may be provided by polymeric nanoparticles from 10 to 1,000 nm. They will manifest as nanospheres (NSs) and Nano capsules (NCs) with different compositions and structural layouts.

Nano capsules' polymeric membrane may absorb and disseminate the active component in the oily core. Only polymeric structures with retained or absorbable

active ingredients can create nanospheres. Many polymers have been widely employed for compound nanoparticles, including Poly Lactic Acid (PLA) and copolymers incorporating glycolic acid (PLGA) [70-72].

1.6.2.5 Liquid Crystalline Systems

A significant portion of condensed structures are liquid crystals, which exist in a condition that is midway between an isotropic liquid and a crystalline solid; their easy outflow reveals whether they are ordered or disordered. In LCs, mesophases may be cubical or polygonal [73-74]. LCs may be divided into the subcategories of Thermotropic LCs and Lyotropic LCs. When heated to a certain temperature, TLCs move from one phase to another, and this transition is isothermal. The mesophase molecule makes up its main component. LLCs are formed of amphiphilic molecular aggregates called functional unit micelles. Amphiphiles have three types of polar tails: small, large, and oversized (hydrophobic) polar tails. Concentration, solvent, and temperature all affect the development of mesophases; under some circumstances, micelles will self-organize and produce structures with a high level of complexity [75-77]. Researchers may work towards developing a safe, effective, and trustworthy pharmaceutical delivery system to manage illnesses. Drug delivery systems should also include pharmaceuticals with distribution instructions to maximise drug-receptor interaction and minimise adverse effects. Thus, a solution that fulfils all of these needs would be useful. Possible medication delivery systems use LC [78-79].

1.6.2.6 Nanostructured and solid lipid nanoparticles

Solid lipid nanoparticles (SLNs), which first appeared in the 1990s, served as colloidal carriers. They transport pharmaceuticals using liposomes, emulsions, and polymeric nanoparticles, but without their drawbacks. Additionally, to better safeguard labile pharmaceuticals, SLNs have superior physicochemical stability and are easier to make in large quantities [80-82]. SLNs are colloidal particles primarily made up of solid lipids at room temperature and contain excessively pure triglycerides. These structures are made of solid lipids or lipid-based mixes and are stabilised by surfactants [83].

Drug molecules will be chemically protected by the solid lipid particle matrix. As the system evolves, crystallisation takes place, negatively impacting drug release and encapsulation effectiveness. Adding oil to an O/W emulsion which already

includes a solid lipid or mixture of solid lipids, helps produce SLNs. Due to their biocompatibility and tiny size (50–1,000 nm), SLNs may be used in the pharmaceutical business for oral, parenteral, and transdermal delivery. Nanostructured lipid carriers (NLCs) improve encapsulation and minimise active particle outflow. Second-generation NLCs are becoming attractive nanoparticle drug delivery platforms. These methods establish a disorganised liquid lipid matrix for active substances by mixing lipid and solid phases [84-85].

1.6.2.7 Liposomes

Liposomes are small lipid bilayer vesicles in a liquid media. Adsorbed lipophiles are introduced into the membrane, whereas hydrophilic compounds are contained within the liquid compartment. Instead, each type of material will be encapsulated. Phosphor lipids (natural or synthesised), sterols, related compounds, and antioxidants comprise most of these vesicles. Liposomes are classed by size, lamellae type, and surface charge. Liposomes are neutral, cationic, or anionic based on their surface charge. Liposomes may be oligo-, uni-, or multilamellar, microscopic, huge, or colossal. Small unilamellar liposomes (SUVs) have 25–100 nm dimensions, massive ones are a hundred nm to one, and giant ones are more than one and can reach sizes in the tens of microns. Multilamellar liposomes (MLVs) are onion-shaped coaxial lamellae. MLVs exist in more focused systems, whereas ULs are in dilute surfactant solutions [86-87].

1.6.2.8 Microemulsions

In 1943, Hoar and Schulman coined the name "microemulsion" (ME) to describe a system of fluids that could be obtained via volumetric testing. They were made of a direct emulsion containing medium-chain alcohol like hexanol or pentanol. It was initially partially transparent and was titrated until transparent. MEs are transparent emulsions that disperse oil in a fluid medium with a surfactant and cosurfactant. A thermodynamically stable system with nanoscale internal component droplets (nm) results from these conditions. Active substances may be in microemulsions if solubilized in oil or water [88-89]. After the drug is extracted from the dissolving media via a membrane or interface, MEs act as reservoir systems. These systems provide a dimensionally controlled environment with unique properties and may join or connect compounds from other drug teams to improve solubility, standards

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stability, or bioavailability. Micro-emulsified systems can target specific organs or tissues of the body and deliver active substances with different hydrophilic natures/lipophilicity within an identical formulation (**Fig-6 incorporated nanoparticles**) [90-91].

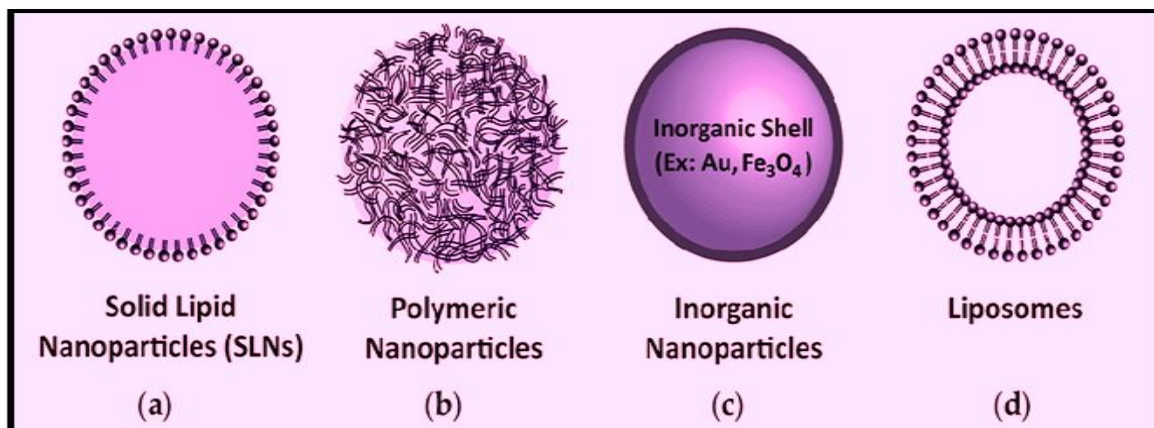


Fig-6 Types of Nanoparticles

Table-3 Nano system features and applications [92].

Types of Nano systems	Size (nm)	Characteristics	Applications
Metallic nanoparticles	<100	Stable, small, and functionalization-friendly colloidal Au and Ag particles have a large surface area.	Gene, medication, radiation augmentation, and thermal ablation.
Carbon Nanotubes.	0.5–3 diameter and 20–1000 Length.	Nanotubes with one or more walls make up the third kind of allotropic carbon sheet. These crystals are strong conductors, semiconductors, or insulators.	Functionalization increases the ability to dissolve, penetrability to cell cytoplasm and nucleus, and gene and peptide transport.
Liposome	50–100	Biocompatible, adaptive, efficient, and simple phospholipid vesicles.	Long-circulating, active and passive genes, amino acids, peptides, and other types of delivery systems.
Nanocrystals Quantum dots	2–9.5	Size 10-100; brilliant fluorescence, wide emission, broad Ultraviolet excitation, and strong photostability; II-VI/III-V column-derived semiconductive material.	HeR 2 labelling, immunoassay, receptor-mediated endocytosis, DNA hybridization, and long-term liver cell imaging.
Polymeric Micelles.	10–100 nm	Biostability, high payload, drug entrapment, block amphiphilic copolymer micelles.	Long-circulation, drug administration with a defined target, and diagnostic value
Polymeric nanoparticles.	10–1000	biocompatible, drug-protective, and degradable.	Transport for drugs. Nanoparticles with changed surfaces and stealth properties may actively and passively disperse bioactive.
Dendrimer	<10	With a core, branch, and surface, the controlled polymer system is highly branched and almost monodisperse.	Macrophage targeting, controlled bioactive delivery, long-circulatory, and liver targeting.

1.7 Herbal Nanotechnology

In this approach, the use of nanotechnology provides a significant role in the herbal formulation of medicines, particularly in drug delivery for rapid dissemination. Nano-natural drug delivery systems can potentially improve activity performance and overcome medicinal plant issues, helping treat dangerous diseases like diabetes, cancer, hypertension, tuberculosis, and others. Nanotechnology can transport water-insoluble herbal medications to cells or tissue, pass strong epithelial and endothelial barriers, release huge herbal molecules, co-deliver two or more medications, and image drug delivery sites using herbal pharmaceuticals [92-94].

Due to scientific reasoning and processing issues, herbal medicines are not used to generate innovative herbal formulations. Modern phytopharmaceutical research can solve the scientific issues and needs of herbal remedies in the NDDS, such as nanoparticles, micro-emulsion solid dispersions, liposomes, solid lipid nanoparticles, etc. Plant herbal medicines can be utilized improvised with better efficiency by integrating them into the modern dose structure. It can be accomplished by designing NDDS in herbal drugs [95]. Since ancient times, natural products such as plant herbal medicines have been used for curing various diseases.

In contrast, the widely used allopathic system and herbal drugs have thousands of ingredients that all function concurrently against the diseases like chronic metabolic disorders. Phytotherapeutics must be scientifically delivered to promote patient compliance and minimise repeated execution. NDDS for herbs may do this. Not only does NDDS decrease the continuous administration for overcoming noncompliance, but it also helps increase the curative values by decreasing toxicity, enhancing bioavailability, and so on [96-98].

1.8 Nano formulations in Diabetes Treatment

The therapeutic management of diabetes mellitus is enhanced by nanotechnology-based methods, and the risk of acute and long-term consequences is reduced [99]. Numerous Nano formulations with various architectural styles have been created to treat diabetes mellitus. Nanocarrier formulations deliver drugs to their targets with the right release pattern. Additionally, Nano formulations enable medication delivery via various pathways [100-101]. By adding appropriate ligands to nanocarriers, medicines' systemically availability and stability may be increased. Drug

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dosage and administration frequency can be decreased through the fabrication of nanocarriers. Manufactured Nano formulations can also decrease the likelihood of hazardous symptoms. As a result, appropriately created nano-formulations of hypoglycaemic medicines may provide enhanced treatment of diabetes. Some of the natural drug Nano formulations show antidiabetic activities (**Table 3**). The progress and efficacy of plant-based nano-based formulations of antidiabetic drugs were highlighted in the following section of this research [102].

Table-4 Some of the natural drug Nano formulations are showing the antidiabetic activity.

Crude Drugs	Curcumin	
Curcuma species	Nano formulation type	References
	Curcumin-loaded self-Nano phospholipid	103,104
	Curcumin-ZnO.	105
	Curcumin with poly-(-benzyl l-glutamate), poly-(ethylene glycol), and poly-(-benzyl l-glutamate).	106
	Curcumin-encapsulated PLGA	107
	Curcumin Nano emulsion	108
	Curcumin-entrapped PLGA-PVA (polyvinyl alcohol)	109
	Curcumin-loaded Pluronic Nano micelles	110
	Curcumin-loaded PLGA	111
	Curcumin-loaded PLA-PEG polymers	112
	Curcumin-loaded chitosan	113
	Curcumin Nano hydrogel	114
	Encapsulated curcumin nanoparticles within gelatine microspheres	115
	Thermosensitive hydrogel in curcumin-assembled gelatine microspheres.	115
Collagen-alginate-encapsulated-curcumin-chitosan nanoparticles.	116	
Grapes, Blueberries	Resveratrol	References
	Resveratrol-loaded layers Polyallylamine hydrochloride with dextran sulphate make 5.5 bilayers in nano formulation.	117
	Multi-layered resveratrol nanoliposome produced by dry film hydration and PEG-amalgamated. (PEGylated).	118
	Resveratrol-loaded nano cochleates	119
	Resveratrol nano emulsion	120
	Resveratrol-loaded solid lipid.	121
	Resveratrol-loaded PLGA.	122
Resveratrol-assembled gold.	123	

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Onions, Grapes	Quercetin	References
	Quercetin-loaded on PLGA.	124
	Quercetin nanorods	125
	Quercetin-loaded solid lipid	126
	Quercetin-succinylated chitosan-alginate NP.	127
	Quercetin-loaded Soluplus micelles	128
	Quercetin-conjugated superparamagnetic iron oxide NP.	129
Chamomile, Celery	Apigenin	References
	Soluplus-pluronic F127 polymer apigenin-loaded nanoscale mixed micelles system.	130
	Apigenin is a solid dispersion in carbon nanopowder.	131
	Apigenin-loaded nanoliposomes	132
Citrus fruits	Naringenin	References
	Self-Nano emulsified naringenin	133
	Naringenin-loaded soluthin-maltodextrin	134
	Naringenin-loaded liposomal NP	135
	Naringenin-loaded chitosan core-shell nanoparticles and naringenin-loaded core-shell polymeric NP.	136
Fruits, Tea	Myricetin	References
	Myricetin-loaded solid lipid NP.	137
Scutellaria, Oroxylum, Thyme.	Baicalin	References
	Baicalin-entrapped nanoliposome.	138
	Plumronic P123 copolymer with sodium taurocholate nanomicelle with baicalin.	139
Carrots, Peppers, Apple skins,	Luteolin	References
	Poly(ϵ -caprolactone)-PLGA-nature oil-luteolin	140
	Luteolin-loaded solid lipid NP	141
Mango tree (Mangifera indica)	Mangiferin	
	Mangiferin-loaded nanomicelles	142
	Vitamin E (co-loaded phospholipidic nanomixed micelles)	143
	Mangiferin-encapsulated -lactoglobulin.	144
Gymnema sylvestre	Gymnemic Acid	References
	Lyophilized Nanoparticles Gymnemic acid	145
	Gymnemic acid-reduced gold NP.	146
	Gymnemic-acid-chitosan	147
Alovera	Emodin	References
	Emodin-loaded nanoemulsion.	148
	Poly-PEGMA-DMAEMA-MAM emodin-loaded	149
Mint, Salvia	Rosmarinic Acid	References
	Polyacrylamide-cardiolipin-PLGA	150
	Polyacrylamide-chitosan-PLGA	151
	Rosmarinic acid-loaded solid lipid.	152
	Rosmarinic acid-chitosan-sodium tripolyphosphate	153
Berberis Sp	Berberine	References
	Berberine-loaded, soy phosphatidylcholine-emulsified anhydrous reverse micelle.	154

	Berberine loaded solid lipid.	155
	O-hexadecyl-dextran entrapped berberine.	156
	Berberine nanosuspension	157
	Berberine-selenium-modified nanostructures.	158
<i>Stevia rebaudiana</i>	Stevia Glycosides	References
	Stevioside-assembled PEG-PLA	159
	Pluronic-F-68 copolymer-based stevioside- PLA	160
	Rebaudioside A-PLA	161
Glycyrrhiza	Glycyrrhizin	References
	Glycyrrhizin insulin-loaded poly (ethyl-cyanoacrylate) nanospheres.	162
	Glycyrrhizin-loaded nanoparticles in nicotinamide.	163
	Glycyrrhizin -polymeric NP	164
<i>Ferula foetida</i>	Ferulic acid	References
	Both PLGA nanoparticles and carbopol 980 hydrogels with ferulic acid nanoparticles are available.	165

1.8.1 Green-Synthesized Phyto Therapeutic Nano Formulations as Antidiabetic Agents

The use of natural remedies, particularly to treat chronic ailments, is becoming more and more popular on a global scale. Products that come from nature with little processing are included in natural remedies. Natural therapeutics have long been considered safe, effective, and economical treatment options. The majority of naturally produced medicines are herbal. The treatment of diabetes and its complications with herbal remedies has a long and influential history, and some plants and these reported Nano formulations are mentioned (**Table 1 and Table 4**) [166-169]. Polymeric or metallic nanoparticles that have been manufactured using herbal items have been discovered to produce more effective therapeutic results than native crude products in the management of diabetes. The clinical equivalent of many commercially available antidiabetic medications has been found in phyto-nanotherapy, which has more excellent biopharmaceutical properties.

Moreover, a synergistic effect can be used to give plant-metal nanoparticles their distinct medicinal capabilities. Green synthesis of silver, gold, and copper oxide nanoformulations for diabetes treatment improves phytochemical stability, pharmacokinetics, and biopharmaceuticals. It has been suggested that many phyto-

nanoformulations created recently are beneficial in reducing diabetes. To create innovative antidiabetic phyto-nanoformulations that are therapeutically effective against diabetes, however, a significant portion of the study is necessary [170-173]. According to various studies (**Figure -7**), Current and Future Health Care Challenges are more.

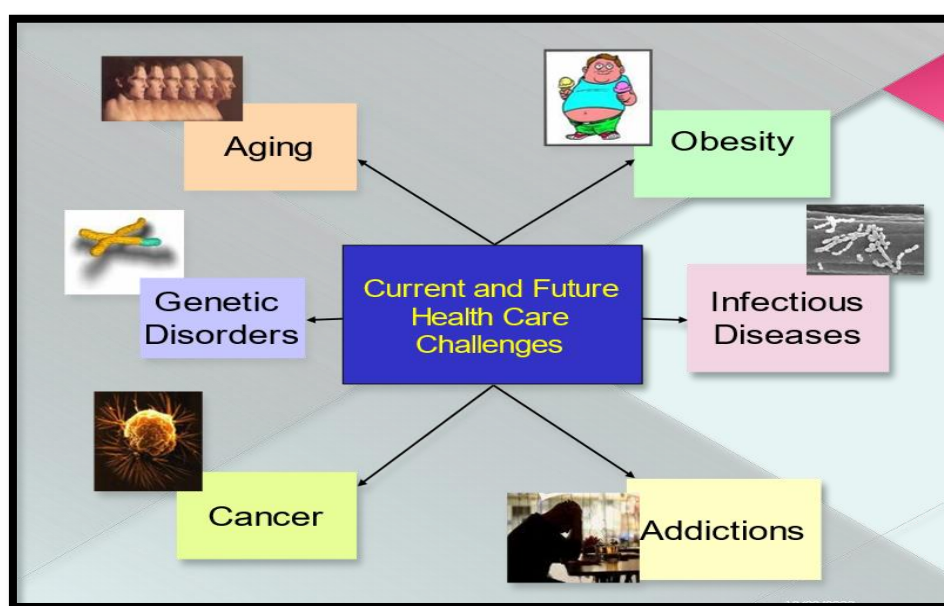


Figure -7 Current and Future Health Care Challenges

1.8.2 Future for Herbal nanotechnology

The use of natural goods and herbal remedies has been studied globally. Many institutions are creating herbal remedies for use in medication delivery systems at the fundamental and clinical trial levels. The primary goal is to develop improved techniques for administering these pharmaceuticals in dosages that will not interfere with the pharmacological treatment to the appropriate locations and throughout the body. It would be wonderful to have a medication that increases a patient's internal fortitude while reducing adverse effects, including toxicity and hypersensitive responses. Future research teams may get intrigued by the prospect of employing herbal nanoparticles to provide cancer medications and potentially produce impressive outcomes. Therefore, adding "herbal remedy" to nanocarriers would increase their

INTRODUCTION

ability to cure many chronic illnesses and benefit health. In the subject of nanotechnology, there are several instances of accomplishment and empirical evidence. Herbal remedies also include a lot of beneficial substances that contain antioxidants and other ingredients that may be employed in functional meals. This kind of collaborative research, including traditional "herbal medications" and more advanced drug delivery methods, such as "Nanotechnology," has developed desirable pharmaceutical medications in a few years that will enhance people's health [174–177].

They had been nano-formulated to increase the pharmacokinetic and clinical performance of numerous naturally occurring antidiabetic drugs. However, the absence of long-term experimental statistics and data has limited most of these analyses. They mainly consider the prolonged protective profile, long-term therapeutic performance, and toxicology of known hypoglycaemic nanoformulations made from plant compounds. Therefore, the bulk of the results is constrained by the size of the laboratory. It will take much work to solve this issue. However, new nano-formulations of plant-derived compounds with antidiabetic properties have been produced and are allegedly beneficial against numerous disorders with aetiologies comparable to or distinct from diabetes. Thus, adjusting these nanoformulations doses may improve diabetic treatment. These approved by the FDA nano-formulations were created to increase the medicinal compounds' physicochemical, pharmacological, and physiological effects [178-181]. More FDA-approved Nano formulations are currently being utilised clinically as pharmaceuticals. Nano-formulations of spontaneously formed hypoglycaemic drugs will enhance diabetes treatment compliance, cost, and toxicity.

CHAPTER 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 *Alpinia mutica* profile

2.1.1 Description- *Alpinia*, the most famous Zingiberaceae genus, contains approximately 230 species spanning Asia's unique and sub-peculiar locations. Southern India's plant has 9 species [182-183]. A perennial plant called *Alpinia mutica* (A.M.) may be found in Thailand and Malaya. It creates horizontal, fragrant plants with subterranean stems. Despite some changes in cultivation, the classified kinds may be found across northern Malaysia. Although various agricultural sources give AM alternatives, many species are spread over the northern Malayan foreland. Locals utilize the fruit of these trees to alleviate edema and ease gastrointestinal problems [184].

2.1.1.1 Biological source- It is obtained from the leaves and Rhizomes of *Alpinia mutica* belonging to family *Zingiberaceae* [185].

2.1.1.2 *Alpinia mutica* - The plant is called Chengkenam, Tiny Cardamom, Fake Cardamom, Narrow-leaved *Alpinia*, and Orchid Gingerpuibai (Indonesia) (Malaysia). *Alpinia mutica* belongs to the Tribe *Alpinieae* of the *Alpinioideae* subfamily, which is part of the Zingiberaceae group of the *Zingiberales* order. It is most common in Borneo, Penang, Malaysia, Singapore, northeast India, and Southern India (Western Ghats of Kerala's Palakkad area and Karnataka). (The district of Coorg). It is a beautiful and therapeutic plant that is native to the Southeast Asian region and India. It is mainly planted for its ornamental value [186]. It grows near Thailand's rivers and is a blooming plant [187]. It grows in high-altitude wetlands close to springs or rivers. It is widely farmed throughout Southeast Asia and South America and thrives in open, sunny areas, woodlands, and brushwood. *Alpinia mutica* has a white corolla with shorter tubes than the calyx, 2.5 to 3 cm long lobes, an oval dorsal that is concave and 1.5 cm wide, and an oblong lateral is 0.60 cm broad. A trilobed ovate labellum needs cross-pollination to produce fruit flavor since flowers are self-sterile [188]. The fruits are ovoid, orange-red capsules 2 cm in diameter and approximately 2.2 cm long. They remain on a plant for months and contain numerous seeds, some of which germinate. It multiplies via rhizome divisions or source (previously soaked in hot water for two days) at 22-24 °C. **(Figure 8).**



a) Harvesting the *Plant*



b) *Alpinia mutica* Leaves



c) *Alpinia mutica* Flower



d) *Alpinia mutica* Fruit



e) *Alpinia mutica* Rhizome



f) *Alpinia mutica* Dried Leaves

Fig -8 Various plant parts of the *Alpinia mutica*.

2.1.1.3 Various names [188].

Narrow-Leaved Alpinia, Dwarf Cardamom, and False Cardamom, Orchid Gingerpuibai (Indonesia); Chengkenam (Malaysia).

2.1.1.4 Taxonomic Classification [188].

Alpinia mutica has a place with the

Kingdom: Plantae

Subkingdom: Green plants –Viridiplantae

Super division: Embryo-phyta

Division: Tracheo-phyta

Subdivision: Spermato-phytina

Class: Magnoliopsida

Superorder: Liliales

Order: *Zingiberales*

Family: *Zingiberaceae*

Sub family: *Zingiberoideae*

Tribe: *Alpinieae*

Genus: *Alpinia* L.

Species: *Alpinia mutica* Roxb

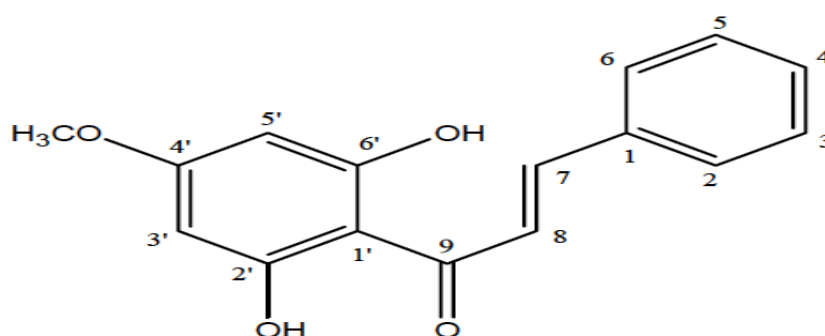
Other species of *Alpinia* are Several *Alpinia* species such as *A. calcarata*, *A. galanga*, *A. malaccensis*, *A. officinarum*, *A. oxyphylla*, *A. purpurata*, *A. conchigera* Griff., *A. javanica* Blume, *Alpinia aquatica*, *Alpinia capitellata*, *Alpinia* cf. *assimilis*, *Alpinia javanica* var. *colorata*, *Alpinia latilabris*, *Alpinia macrostephana*, *Alpinia malaccensis* var. *nobilis*, *Alpinia Murdoch*, *Alpinia oxymitra*, *Alpinia pangenesis*, *Alpinia petiolata*, *Alpinia rafflesia*, *Alpinia scabra*, *Alpinia suriana*, *Alpinia vitellina*, *Alpinia vitellina* var. *centifolia*, and *Alpinia zerumbet* are some examples of alpine plants.

2.1.2 Phytochemistry

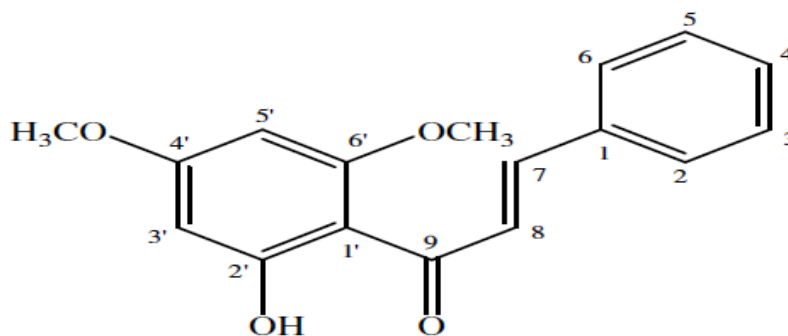
With various components found in rhizome oil, *A.M.* leaf oil has a high concentration of sesquiterpenes, with -Sesqui-Phellandrene as the main component. Even though leaf oil did not contain any of the (E, E)-farnesol that was previously claimed to be a substantial component of rhizome oil's sesquiterpene content, it was.

For the initial time in alpine plants in the Ginger family, leaf and fruit oil extract was extracted and identified to reveal two phenolic chemicals, Aniba A 5,6-dehydrokawain, and an amide, Auranamide [189]. Phenolic compounds like 1,7-diphenyl-3-hydroxy-6-heptene-5-one, flavokawin B, 5,6-dehydrokawain, and flavokawin A have been found in dried rhizomes [190–192] (**Figure-9**) The molecular structures of a few of the chemical components are listed below.

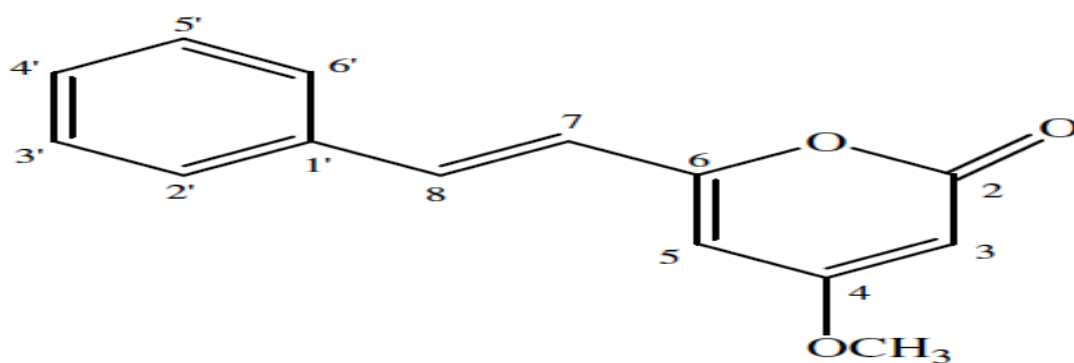
Phytochemistry of the plant *Alpinia mutica*



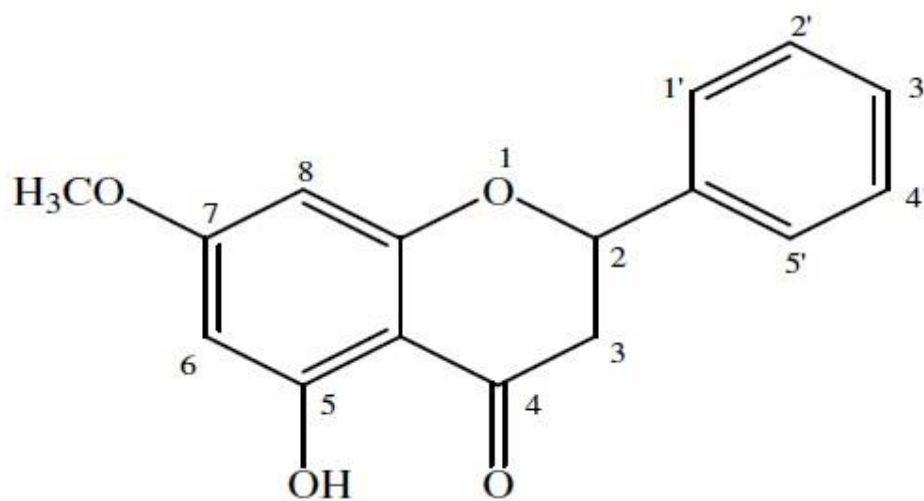
Pinostrobin Chalcone



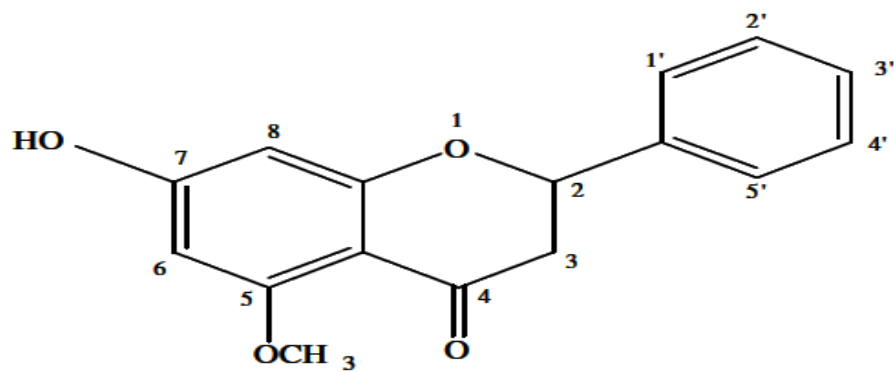
Flavokawin B



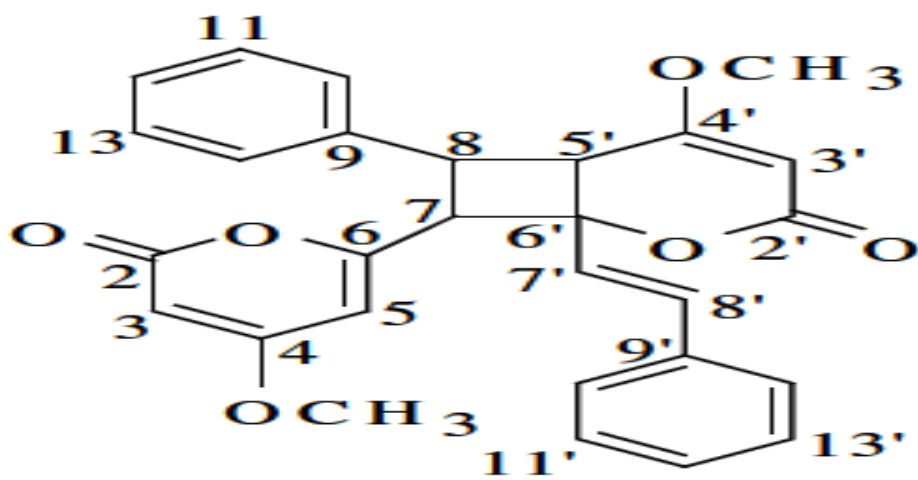
5,6 Dihydrokawin



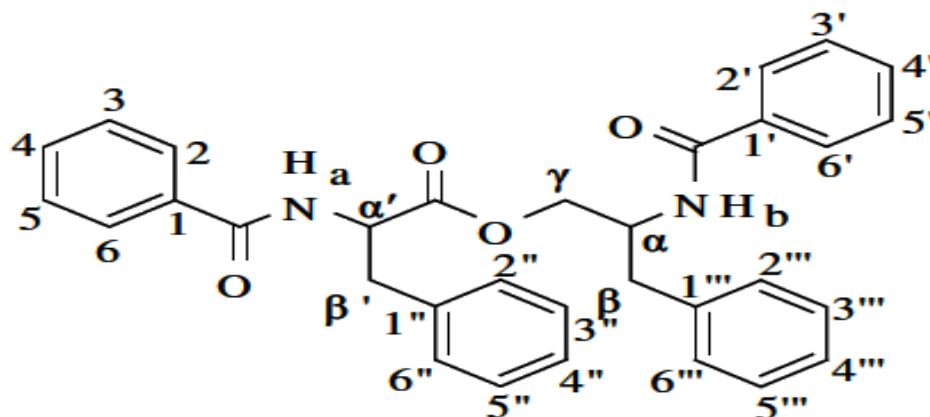
Pinostrobin



Alpinetin



Aniba dimer



Auranamide

Fig-9 Chemical Structures of Various Phytoconstituents of the Plant *Alpinia mutica*

2.1.3 Traditional applications

Fruits have long been used to remedy flatulence and decrease swelling [193]. The rhizomes are also used as a stomach-related remedy [194]. The plants have also been grown for their decorative qualities. Intense platelet aggregation activity was observed for methanol extract and some chemicals obtained from fruits of A.M [195–196]. The rhizome containing ethyl acetate extract also had potent antioxidant and anticancer properties on numerous cancer cells [184, 193].

2.1.4 Pharmacological activity

2.1.4.1 Antimicrobial/antibacterial action:

The oils from A.M.'s ripe and unripe fruits were tested with four types of fungus, two types of Gram-positive bacteria (*S. aureus* and *B. subtilis*), and two types of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas*). (*Candida glabrata*, *Microsporium canis*, and *Trichophyton mentagrophytes*). A.M.'s immature and mature fruit oils did not kill *B. subtilis* (2.50mg/mL) or *S. aureus* (2.50 mg / mL). Although oxacillin is nine times more effective, the anti-*B. subtilis* effects was greatest in ripe fruit oil. The oils had no impact on *M. canis*, *T. mentagrophytes*, and *T. rubrum* at concentrations ranging from 2.50-5.0 mg/mL. Both oils performed as well as

cycloheximide against *T. mentagrophytes*. A.M. mature fruit oil was more effective than unripe fruit (lower MIC versus *Bacillus subtilis* and *M. canis*). Both oils had no effect (MIC >5.0mg/mL) on *E. coli*, *P. aeruginosa*, and *C. glabrata*. [184].

2.1.4.2 Cytotoxic activity of *Alpinia mutica*

A.M. of the Rhizome or Fruits Skin Oils were cytotoxically exercised using the Trypan Violet Rejection Method. Phosphate-supported saline (PBS) was used to suction Dalton's lymphoid ascites (DLA) cells, and a cell suspension containing 1×10^6 cells per millilitre of PBS was prepared. DLA cells (1×10^6 cell/mL) are cultured in PBS containing 0.1% DMSO (vehicle control), and A.M. rhizome skin oil has been modified in 0.1% Solvent DMSO (0.1, 1, 5, 10, and 20 g/ml) for three hours at 37 °C to evaluate malign. After hatching the control and experiment cells, GC-FID (Flame Ionisation Analyzer) and GC-MS expelled and changed dried crude medication and organic skin essential oils. Trypan blue was added to the cells and seen under a microscope. The 47 components of rhizome oil have been identified, of which 40 (92.8%) have been modified. The fruit skin's two horizontal stem sections and its organic oils (camphine, pinene, 1,8-cineole, and camphor) were analyzed by superficial standardization into four essential components. Fruit skin oils are outstanding at strengthening cells, acting as cytotoxic and mildly antibacterial agents, and have extraordinary uses in aromatherapy because of their dried organic manufacture. Unstable oils, precise curves, and evident gravity are also fixed [197].

2.1.4.3 Anti-oxidant activity

The total phenolic substance in leaves from *Alpinia mutica* was calculated using the Folin-Ciocalteu technique. The Antio-oxidant tests included 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging, super-oxide dismutase (SOD), reducing power, and beta-carotene bleaching. All trials showed that ethyl acetate has the most significant antioxidant capabilities. In the butylated hydroxy anisole (BHA) reference material, ascorbic acid, and then the butylated hydroxy anisole (BHA) reference material, ascorbic acid, and the b-carotene linoleate modelling system, the ethyl acetate fraction had more excellent antioxidant activity [185]. β -carotene linoleate modelling system, the ethyl acetate fraction had more significant antioxidant activity [185].

2.1.4.4 Antiplatelet activity

The antiplatelet activity of the *A. mutica* compounds was tested in vitro using an electrical impedance approach and a whole blood aggregometer. Curcumin from *Curcuma aromatica* and 5,6-dehydrokawain from *A. mutica* had IC₅₀ values under 84 μM. Curcumin was the most potent antiplatelet drug, with IC₅₀ values for AA-, collagen-, and ADP-induced aggregation of platelets of 37.5, 60.9, and 45.7 μM [196].

2.1.4.5 Invitro anticancer activity

We have described the potential for natural phytochemicals from *Alpinia mutica* rhizome to inhibit UCK2, a colorectal cancer treatment tool. Here, we used in vitro to test whether or not natural UCK2 inhibitors could effectively kill HT-29 cells. The research used flavokawain B and an alpinetin constituent from the rhizome of *A. mutica* as extracts. According to the research, treatment of HT-29 cells dramatically reduced the level of expression of UCK2 mRNA [198].

2.1.4.6 Antitrypanosomal and cytotoxic activities

Four carbazoles (girinimbine, mahanimbine, murrayafoline, and Murray Anne) and one kavalactone (5,6-dehydrokawain) were tested on in vitro-cultivated *Trypanosoma evansi* cell lines. One *Murraya koenigii* flavonoid, pinostrobin, was tested for antitrypanosomal action. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-cell proliferation test was used to examine the cytotoxic effects of these substances on mammalian Vero cells. Mahanimbine, murrayafoline, and girinimbine are three carbazole compounds with vigorous antitrypanosomal activity, with median inhibitory concentrations (IC₅₀) of drug 3.13, 6.35, and 10.16 μg/ml, respectively. Girinimbine, the least lethal to Vero cells, with a mean cytotoxic concentration (CC₅₀) and selectivity index (SI) of 745.58, 42.38 μg/ml, and 73.38, respectively. The potential antitrypanosomal activity of girinimbine and the other carbazole drugs is comparable to their low toxicity to mammal cells. Mainly, girinimbine is a strong candidate for additional research using in vivo models to examine its potential as an antitrypanosomal drug [199].

2.1.4.7 α -Glucosidase inhibitory activity

The ability of these isolated compounds to suppress the activity of α -glucosidase was examined. Compound 5 demonstrated the most inhibitory action, with an IC₅₀ value of $8.77 \pm 1.04 \mu\text{M}$, whereas compound 3 also exhibited substantial activity, with an IC₅₀ value of $62.77 \pm 2.18 \mu\text{M}$. Moreover, styrylpyrone and flavonoids from *A. mutica* seed arise from cinnamoyl CoA or p-coumaroyl-CoA with malonyl-CoA chain extension, according to a study on the biosynthetic origin of isolated chemicals. Furthermore, compound 6 was the precursor for the synthesis of flavonoids in *A. mutica* seeds, and this is the first report on the isolation of compound 6, an important precursor, from *A. mutica* [200-201]

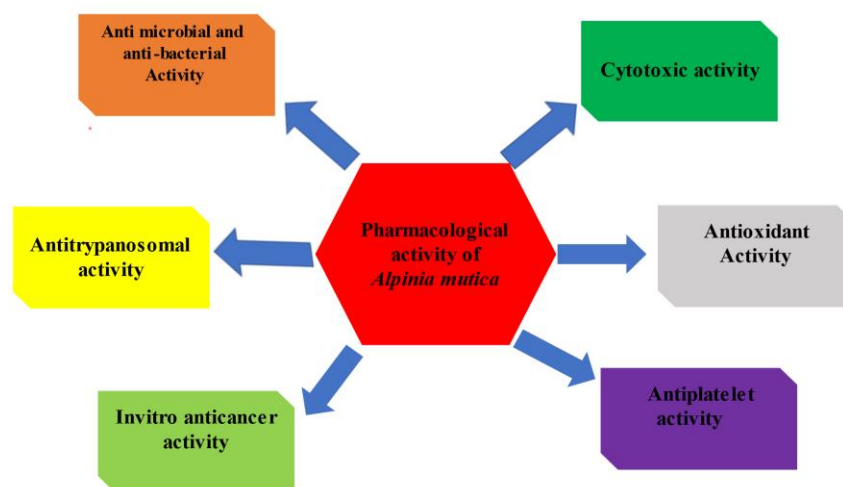


Fig-10 Pharmacological importance of the entire plant *Alpinia mutica*

2.2 *Tradescantia spathacea* Profile

2.2.1 Description

This herb is the boat lily or Moses-in-the-cradle, is a family belonging to *Commelinaceae*, it was first mentioned in early 1788 and had native roots in numerous countries, including the nation of Belize, Guatemala, which was southern Mexico (Mexico, Chiapas, Tabasco, and Peninsula). It's a popular ornamental plant. It lives wild source in Florida, Texas, Hawaii, India, and numerous sea region countries [202]. It is a rosette-shaped herb with a mushy texture, an acaulescent stem, and densely imbricated leaves. The underside of the leaves may be dark purple or green on both surfaces. Two purple bracts have white blooms on them. The plant prefers moist environments and rocks that are just above sea level. As an ornamental, the plant is frequently grown [203].

2.2.1.1 Biological source- It is obtained by whole plant of *Tradescantia spathacea* (Sw.) Stearn, family belonging to *Commelinaceae*.

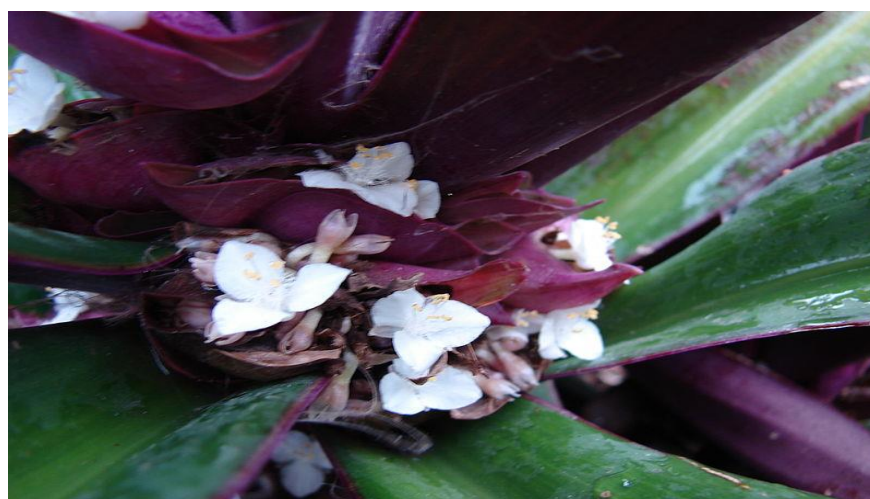
2.2.1.2 *Tradescantia spathacea*.

Its little stems and numerous growing leaves taper at the top make it an excellent bunch-forming plant. Green on top and rose purple on the underside, the leaves are 30–40 cm long and 4–6 cm wide. Flowers are this plant's axillary lymph hubs, which are somewhat ship-moulded brackets; The blossoms have a silvery hue; There are three leaflets, six stamens, three cells per ovary, and a natural product with three valves. (**Figure 11**). An everyday use for T.S is as a cemetery decoration because of its hardiness and ability to survive on rocks. It also thrives in soil with a distinct natural perspective and will grow in sand or coral stone. It is also safe from rain and favours dry conditions. Some people may find the plant fluid to infiltrate and irritate their skin quickly. When consumed, it may cause actual eating scorching pain in the areas of the throat and mouth—generally accepting of the allopathic synthetic medicines (intensifiers that prevent several plants from growing) produced by Australian pine. *T. spathacea* may reproduce by producing seeds, cutting off the plants, and discarding them. Broken portions will readily regrow. The oval-shaped blooms may be easily pollinated by hand or by insects. The size increases for bedding, herbaceous borders, and tropical and subtropical regions. Additionally, cheek colouring has been done using

upsetting juice's blushing effect. Blossom is used medicinally to treat loose stools, enterorrhagia, and hemoptysis [204].



A. *Tradescantia spathacea* Plant



B. *Tradescantia spathacea* flower

Fig-11 Various Parts of the plant *Tradescantia spathacea*

2.2.1.3 Common names [204]

A boating Moses (English).

plant Royster (English)

a boat lily (English)

2.2.1.4 Classification of the crude drug [204]

Kingdom: Plantae

Subkingdom: Viridiplantae – Green plants

Super division: Embryo-phyta

Division: Tracheo-phyta

Subdivision: Spermatophytina

Class: Magnoliopsida

Superorder: Liliales

Order: Commelinales

Family: Commelinaceae

Genus: *Tradescantia* L.

Species: *spathacea*

2.2.2 Phytochemistry

The leaf contains alkaloids, flavonoids, tannins, aromatic mixes, glycosides, and terpenoids. Flavonoids, Anthocyanins, carotenoids, waxes, Coumarin, and Steroidal Constituents are synthetic chemicals that are differentiated or screened [205-206].

2.2.3 Traditional uses:

Western nations employ stems, flowers, and leaves to make tea. Treating diarrhea with leaf infusions is common, as expectorants, and as hypoglycemic agents, while fresh and broken leaves are administered to provide hemostatic to injuries [207–208]. Snake bites may also be treated with this herb [209]. Use dry or fresh leaves to treat colds, haemoptysis, complex hacks, nasal drains, and Mycobacterium TB. The leaves are boiled or soaked in warm water before exposure to cold; it has highly excellent characteristics. It treats fever and asthma in Thailand and the Caribbean Islands. In Cuba, this plant's cataplasms are used to cure wounds. For the therapy of psoriasis, leaves in decoction are used in Puerto Rico. It is used as a traditional medication in Mexico, and the leaves are used to cure "nervios." used for the treatment of superficial mycoses [210-211]. In Myanmar, it is used to treat tuberculosis patients [212].

2.2.4 Pharmacological Studies:

2.2.4.1 Chemoprevention /Anti-tumour

Rosales-Reyes et al. found that different (mostly aqueous) solvent unprocessed T.S extracts decrease hepatic malignant foci in rats. Malignancy is treated with it in Mexico. In order to demonstrate their anticarcinogenic effect, this inquiry is necessary.

Precancer sores have been shown to shrink, which authorises the conduct of further tests to determine potential chemotherapy prevention potential [213].

2.2.4.2 Reactive oxygen species scavenging and Antimutagenic Activities

ROSS and anti-mutagenic tests showed antitoxin action in T.S.'s alcohol pure natural concentrate. According to a study, quercetins, alpha-tocopherols, ascorbic acid, and FRSA may be responsible for the antioxidant activity [206]. -Tocopherol and the vitamin C compound also contribute to the antioxidant action.

2.2.4.3 Stimulation of Human Lymphocyte Proliferative Response

It was determined which extracts from eight traditional Thai medicines may in vitro activate human lymphocyte activity by evaluating the human lymphocyte proliferative response. The extracts boost human lymphocyte proliferation at different dosages. Results indicate therapeutic intervention to modify immunological functioning [214].

2.2.4.4 Microbes inhibition activity:

High phenolic extracts' ability to suppress the growth of three crucial human health-related microbes—*Escherichia coli*, *Listeria innocua*, and other microbes—was tested in vitro. The extracts displayed antimicrobial and bactericidal properties on other bacteria but had little effect on *P. aeruginosa* [215].

2.2.4.5 Anti-Malignancy activity

The protective effects of several fluids and natural extracts against mouse liver cancer were investigated using a hepatocyte-unaaffected model to examine the anti-malignancy activity. Precancer lesions numbered and in a zone are reduced by the pure, watery extracts. Lastly, provide a rationale for continuing research on the chemoprevention mode of action as a possible option for treating malignant illness [213].

2.2.4.6 Antioxidant / Leaves

Herbs are it. For cell strengthening and antimicrobial activity (DPPH, FRS, FRP, and FIC tests), antioxidant activity was investigated on watery leaf extracts of *R.*

spathacea. Combinations and decoctions had similar total phenolic substances and antioxidant benefits when coupled with herbal teas [216].

2.2.4.7 Antiviral Activity

Twenty Malayan herbal treatments were examined for their antiviral properties in order to combat the Chikungunya virus; The strongest toxic inhibitory impacts on Vero cells were obtained by alcohol and chloroform extractions of *T. S* leaves, with cell ability of 92.6%, 91.5%, and 88.8%, respectively. CC50 and EC50 values for this plant's chloroform extract were 285.5 ± 3.1 $\mu\text{g/ml}$ and 69.2 ± 0.6 $\mu\text{g/ml}$ [217].

2.2.4.8 Antimycobacterial action

Some selected Indonesian domestic natural herbs were evaluated for their antibacterial properties to treat multi-drug resistant (MDR) *Mycobacterium TB*. *R. Spathacea* had 100 inhibitions against the MDR strain and 100% inhibition against the *Mycobacterium tuberculosis H37Rv* strain. In addition to potentially being used as conventional supplemental therapies to treat newly emerging MDR variants of *Mycobacterium TB*, *R. spathacea* have strong anti-MDR strains [218].

2.2.4.9 In-Vivo Hepatoprotective Activity

Hepatoprotective activity was reported the, three succulent plants from the family Commelinaceae, *Tradescantia species* have been shown to have hepatoprotective properties. HPLC-PDA-MS/MS was used to characterize *Tradescantia* leaf ethanol extract phenolic phytoconstituents. Phenolic acids, flavonoids, anthocyanins, and glycosides contained 33 polyphenolic compounds in the three plants. Colorimetric analysis of *Tradescantia* yielded the following results for both the overall phenolic and total flavonoid contents (85.11 ± 0.61 , 62.17 ± 1.62 μg), and (35.35 ± 0.14 μg) gallic-acid equivalent/mg of each dry extract and (29.52 ± 0.12 , 11.54 ± 0.01 , and 26.87 ± 0.04 μg quercetin), when compared to silymarin, the ethanolic leaf extracts of the '3' plants showed considerable hepatoprotective effect when given orally to rats who had received carbon tetrachloride injections [219].

2.2.4.10 Treatment of AIDS

Some researchers focused on *T. spathacea*, a pathogen that has to be treated with pharmacological formulations to cure AIDS and the means of transmission. To prevent immune depression and limit viral replication, the treatment needed a combination of various antiretroviral medications. Antiretroviral therapy is complex and expensive because it calls for the simultaneous administration of three or more medications (triple therapy). Excessive doses that fail to provide the intended results can conflict with other medications that should be taken with or without food. Nevertheless, it is known that all *Tradescantia* species are highly toxic, and their detailed introduction to humans would almost certainly result in a toxic reaction. Numerous studies on this plant have been conducted since the early 1990s. It is now known to include specific compounds with the anti-inflammatory effects of flavonoids and coumaric acid [220].

2.2.4.11 Hypoglycaemic Effects

It was utilised in traditional medicine as a haemostatic for wounds using freshly crushed leaves. The leaves are infused and used as a hypoglycaemic agent, expectorant, and treatment for diarrhoea [207].

2.2.4.12 Antidiabetic activity, alpha-amylase, and alpha-glucosidase inhibitory Activity

Tradescantia spatheacea Swartz methanolic extract has excellent antidiabetic action in vivo and inhibits alpha-amylase and alpha-glucosidase, making it beneficial for diabetic patients with hyperglycemic conditions [221].

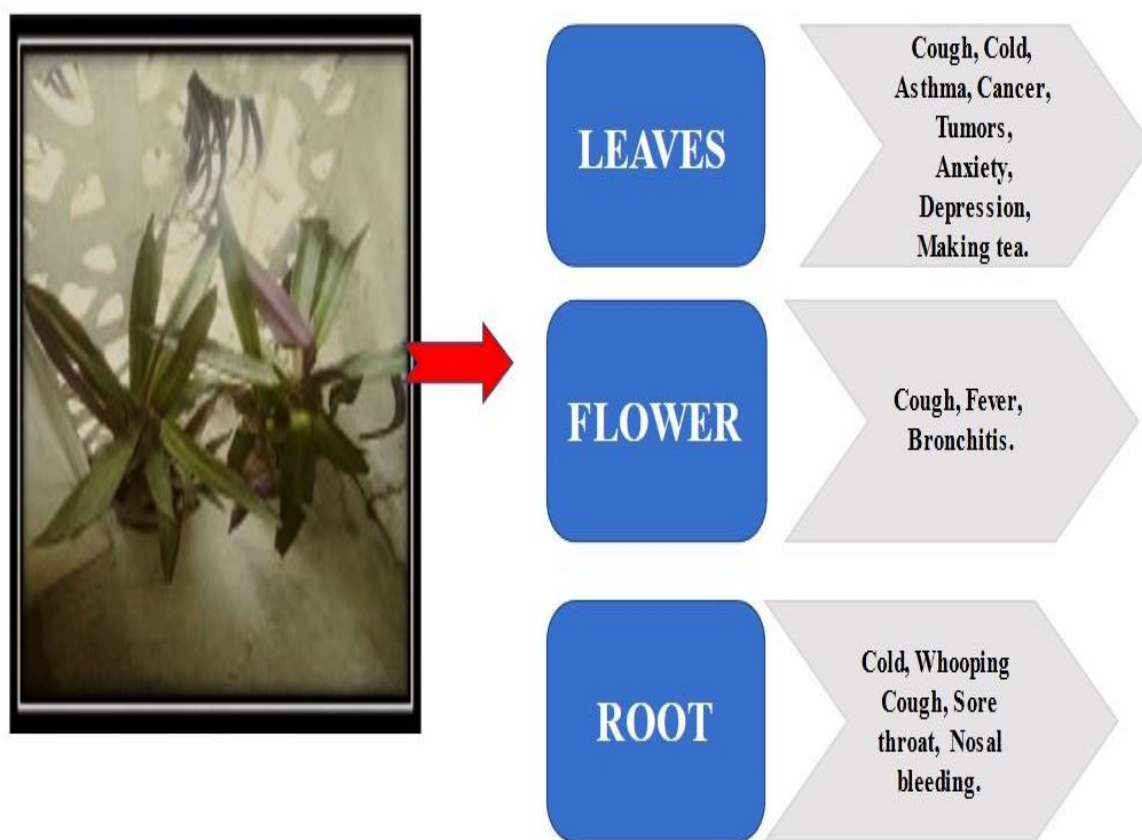


Fig-12 Pharmacological importance of the entire plant *Tradescantia spathacea*

According to a review of the literature, nano-formulation preparations of these crude drugs and antidiabetic activity were not reported above drugs, so I was proceeding to study the development, and characterization of nano-formulations and antidiabetic activity of drugs.

CHAPTER 3
RESEARCH ENVISAGED

3. RESEARCH ENVISAGED

Diabetes mellitus may be a non-infectious endocrine condition that is associated with hypoglycemia and characterised by abnormal glucose metabolism. It's connected with developing several serious diseases like microvascular (neuropathy, retinopathy and nephropathy) and also macro-vascular (peripheral-vascular illness and coronary heart-diseases). Diabetes mellitus, generally known as diabetes, is a group of conditions that have been linked to "sweet urine" and muscular atrophy. Insulin, a hormone secreted by the pancreas, regulates blood glucose levels. When these levels rise, the pancreas releases insulin to maintain the glucose level. Diabetes patients reduced or non-existent insulin production results in hyperglycemia.

Diabetes is the fastest-growing chronic disease worldwide, according to a 2016 WHO study. Between 1980 and 2014, diabetics rose from 4.7 to 8.5%. Diabetes-related elevated blood glucose levels cause approximately fifty percent of deaths before 70. So, there is a need to explore a better therapy for the management of diabetes with fewer side effects. According to the IDF, 415 million people have diabetes in 2016, but 642 million will have it by 2040. According to Aroma World, there are 61.3 million diabetics in INDIA, most of whom are in the 20 to 79 age group. It should double by 2030. India is the world's diabetes capital. Metropolitan India's diabetes rate is growing. Approximately six times as many people in metropolitan areas have diabetes than those in rural areas. The leading causes of diabetes mellitus in the past 20 years have been decreased activity, rising weight and stress, dietary changes, malnutrition, alcohol intake, and viral infections. Because hormones and inflammations behave differently in women, female diabetic patients are more severely affected than male diabetic patients. Less educated individuals are more likely to suffer from diabetes than more educated individuals. The highest percentages of people with diabetes reside in developing nations.

RESEARCH ENVISAGED

Therefore, research is needed to identify a better diabetic medication with fewer side effects. Medicinal herbs are useful for treating type 2 diabetes. However, a safe and effective diabetic medication has not yet been developed. In order to control type 2 DM, it is necessary to investigate phytochemicals, which are thought to be less hazardous than synthetic drugs. The *Tradescantia spathacea* and *Alpinia mutica* plants have both been used to cure diabetes, according to the literature that is currently accessible on them. However, the study was only done on a small scale, and there was no data on the specific plant metabolites with different nanoparticles (metal nanoparticles silver nitrate and Zinc oxide with some specific plants) in charge of the study's therapeutic activity. To examine the plant's safety record, in-vivo antidiabetic capabilities, and fractional anti-inflammatory properties of nano herbal particles, identify the responsible phytoconstituents with nano herbal particles, and predict its possible mechanism of action, *Tradescantia spathacea* and *Alpinia mutica* leaves were chosen based on the literature.

CHAPTER 4
AIMS & OBJECTIVES

4. AIMS & OBJECTIVES

Aim of the Study

Development, Optimization, Characterization of Nano Formulations and Anti-diabetic Activity of *Alpinia mutica* and *Tradescantia spathacea*,

Objectives of the study

The numerous nanoherbal particles derived from *Tradescantia spathacea* and *Alpinia mutica* leaves were assessed for their safety profile in accordance with OECD Guidelines 425 and their ability to treat diabetes using in-vitro and in-vivo models. Additionally, the nanoherbal particles were examined using analytical techniques for the presence of phytoconstituents in order to forecast the likely mode of action of the nanoherbal particles in order to address the issue currently facing the pharmaceutical industry in developing a more effective treatment for diabetes and the objectives are as follows:

- ❖ To extract and standardize plants
- ❖ To observe in vitro antioxidant activity of *Alpinia mutica* and *Tradescantia spathacea* extracts.
- ❖ Green synthesis of Nanoparticles of *Alpinia mutica* and *Tradescantia spathacea* extracts.
- ❖ To observe *in vitro* antidiabetic activity of *Alpinia mutica* and *Tradescantia spathacea* extracts incorporated nanoparticles.
- ❖ To perform *in vivo* antidiabetic activity of *Alpinia mutica* and *Tradescantia spathacea* extracts incorporated nanoparticles.

CHAPTER 5
PLAN OF WORK

5. PLAN OF WORK

The research work was deliberated to carry out as follows:

- a. Collection, identification, authentication, and drying of proposed indigenous medicinal plants (1. *Alpinia mutica* (Leaves) 2. *Tradescantia spathacea* (Leaves))
- b. The extraction process of active fraction from withered plant parts of *Alpinia mutica* – Leaves, *Tradescantia spathacea* – Leaves,
- c. Primary screening of phytochemicals to conclude the phytoconstituents of extracts.
- d. Quantitative assessment of phytoconstituents in extracts, including TTC, TFC, and TPC in *Alpinia mutica* and *Tradescantia spathacea* leaves.
- e. Development, optimization, characterization and evaluation of Nano herbal formulation of *Alpinia mutica*, *Tradescantia spathacea* for antidiabetic activity. Planned to prepare two plants extracts and formulate two different Nano-herbal formulations individually.
- f. Evaluation of *Alpinia mutica* and *Tradescantia spathacea* leaf extracts' in vitro antioxidant activities using reducing power, DPPH, iron chelation, and total phenolic substances.
- g. Evaluation of in vitro antidiabetic activity of different extracts of *Alpinia mutica* – Leaf and *Tradescantia spathacea* – Leaf by Inhibition of Alpha-Amylase enzyme assay (In vitro antidiabetic activity of various plant extracts using alpha-amylase method) and Inhibition of Alpha-Glucosidase enzyme assay (In vitro alpha-glucosidase antidiabetic efficacy of nano-formulation plant extracts)
- h. Determination of antidiabetic activity of different extracts of *Alpinia mutica* – Leaves, *Tradescantia spathacea* – Leaf of contrary to streptozotocin induced damage via pancreatic beta cell lines.
- i. Estimation of antidiabetic activity of different extracts of *Alpinia mutica* (Leaf), *Tradescantia spathacea* (Leaf) contrary to streptozotocin induced damage via Pancreatic beta cell lines.
- j. To assess the acute toxicity of nano-formulations by using mice by following method of OECD Guidelines 425.

PLAN OF WORK

- J. To enrich fractions of different extracts for characterization of the bioactive compounds done by column chromatography and spectroscopic techniques like UV, IR, Mass Spectroscopy, X-Ray Diffraction (X-RD) and scanning electron microscopy.
- k. To analyze the experimental values by using different methods like ANOVA, statistical data analysis, and compilation & data interpretation.
- m. Finally the overview and conclusion.

CHAPTER 6
MATERIALS & METHODS

6. MATERIALS & METHODS

6.1 Plant Material Collection and Authentication

Aerial Parts and Rhizome of *Alpinia mutica* were collected from native areas of Thiruvananthapuram (Kerala) and Local area of Hyderabad, has a voucher specimen number Ref. No. BSI/DRC/ 2019-20/ Tech./305, Roots and Leaves of *Tradescantia spathacea* were collected from Local area of Hyderabad (Telangana), has a voucher specimen number Ref. No. BSI/ DRC/ 2020-21/ Identification / Tech./66, verified by P.V. Prasanna, Scientist G & HoO, Botanical Survey of India, Deccan Regional Centre, Kendriya Sadan, 2nd Floor, Room No. 228-238, CGO Complex, Koti, Hyderabad, Telangana -500095.

6.2. Macroscopy

The macroscopy of the leaves of *Alpinia mutica* and *Tradescantia spathacea* were examined, and Colour, smell, taste, texture, form, and size were observed [222].

6.3. Microscopy

6.3.1 Transverse section

Transverse sections of leaves and rhizomes from *Alpinia mutica* and *Tradescantia spathacea* were cleaned with chloral hydrate, wet with phloroglucinol solution, and added 1-2 drops of Non diluted hydrochloric acid. They were then allowed to sit for 5 minutes and mounted in 50% glycerine. The different enlarged photos were taken with a Motic microscope [223].

6.3.2 Powder microscopy

The aerial portions of *Tradescantia spathacea* and the powdered leaves and rhizomes of *Alpinia mutica* were placed on a slide with 1-2 drops of phloroglucinol concentrated hydrochloric acid and examined under a microscope. Additionally, the slides were made using the weak iodine solution and mounted in 50% glycerine for the identification of calcium oxalate crystals in order to determine the starch granules. The different enlarged photos were taken with a Motic microscope [224].

6.4 Quantitative Microscopy

The plant *Alpinia mutica* and *Tradescantia spathacea* leaves were evaluated for the quantitative microscopy [225-226]. The various parameters analyzed are listed below.

6.4.1 Stomatal number- The average epidermal stomata per square millimetre. The leaves were cleaned with the chloral hydrate solution, after that the epidermal surface, i.e. (upper and lower) was peeled off using forceps and placed in the glycerine onto the slide. The 1 square millimeter was drawn, and the number of the stomata was drawn in the specified area by using stage micrometer and camera Lucida to determine the average stomatal number and calculate the average, the experiment was conducted ten times.

6.4.2 Stomatal index – It represents the percentage of stomata present in the epidermal cells. Every stoma is considered equivalent to one cell. The epidermal cell and stomata number was calculated with the square of 1 square millimeter by stage micrometer and camera Lucida and calculate the average, the experiment was conducted ten times. The index was measured using the formula. **(Eq-1)**

$$\text{Stomatal index} = (S/ E+S) \times 100$$

Where,

S = Stomata number per sq. mm

E = Epidermal cells number in per sq. mm

6.4.3 Palisade ratio- It is defined as the cells available under the surface of an epidermal cell. The 1mm square leaf was cleaned by heating it with a solution of chloral hydrate. Thereafter the epidermal cells were identified, and palisade cells were determined using camera Lucida. The cells that are covered more than half the area of an epidermal cell were considered for calculation. This experiment was conducted 25 times for the estimation of the palisade cell.

6.4.4 Vein-Islet Number: The average vein-islets number available on the surface of the leaf per square mm area. The leaves were cleaned by heating with dilute potassium hydroxide and placed in the glycerine onto the slide. The vein- islets number was calculated on an area of 1 mm square.

6.4.5 Vein-let Termination number: It is defined as the termination available on the leaf surface (per sq. mm) that is lying between the margin and midrib. The same procedure mentioned for the determination of vein-islet number was followed in an estimation of the number of veins-let termination. The average of vein-let terminations was calculated.

6.5 Ash value

6.5.1 Total Ash: A silicon crucible containing 2 grammes of powdered crude drug was placed into a muffle furnace at 450°C for 5 minutes before being cooled. The proportion of ash was calculated for the raw drug [227–228].

6.5.2 Acid Insoluble Ash: Powdered crude drug (2 g.) was placed into silica crucible and add hydrochloric acid (25 ml), heated for 5 min and filtered using filter paper (ashless), rinsed with warm water to get the desired acid insoluble matter that was further ignited and then cooled for 30 min. in the desiccator. The acid-insoluble matter was weighed to determine the acid insoluble ash value [227-228].

6.5.3 Sulfated Ash: The powdered plant material (2 g.) was transferred into the crucible, sulfuric acid (3 ml) was added and gradually incinerated until it becomes carbon-free. The residue was cooled and placed for 30 min. in the desiccator. The sulfated ash percentage value was calculated [227-228].

6.5.4 Water soluble Ash: Powdered plant material (2 g.) was transferred into crucible, add water (25 ml), heated for 5 min. and then filter the solution using filter paper (ashless). The residue was ignited for 15 min in the crucible, but not more than 450°C and then cooled. Subtract the residue mass of the total ash, the resulting mass is water soluble ash value [227-228].

6.6 Identification of Moisture Content: The powdered crude drug (2 g.) was placed in the silica crucible, oven-dried at a temperature of 100°C or 105°C and after that kept in desiccators. The experiment was executed in triplicate to determine the loss of moisture content [229].

6.7 Extractive value

The extractive value is helpful in establishing the quality standards for the plant material and also an indicator of existence for the several secondary metabolites and identification of adulteration. The WHO calculates ethanol-soluble and water-soluble extractive values [230-231].

6.7.1 Ethanol soluble extractive value

The powdered plant material (4 g.) was transferred into the stoppered conical flask, macerated for 6 hrs. using 100 ml of 90% ethanol with frequent shaking, and after that, kept aside for 18 hrs. The plant material was filtered, and the filtrate (25 ml) was placed in a china dish. The evaporation of the solvent was performed on a water bath and placed in a desiccator to achieve the constant weight. The ethanol 90% soluble extractive percentage value was calculated [230-231]

6.7.2 Water-soluble extractive value

A similar protocol was carried out like that for ethanol-soluble extractive value; however, the water was taken in place of 90% ethanol. The water-soluble percentage value was determined [230-231].

6.8 Extraction of the Plant material

6.8.1 Conventional Method

Plant material was air-dried and roughly pulverized at room temperature using a pulverizer. A Soxhlet apparatus extracted the crude medicament with ethyl acetate, petroleum ether, methanol, hydroalcoholic, and Aq-solvents. Distillation and rotational evaporation under vacuum concentrated the solvent. All extracts were kept in desiccators until use [232].

6.8.2 Ultrasound-assisted extraction (UAE)

Ultrasound affects the cell membrane, allowing chemicals to flow out and significant quantities of material to be moved between cells. Between 20 and 2000 kHz of ultrasound frequency are employed. The mechanical effect of ultrasound-induced sound cavitation improves solvent and sample surface contact and cell wall permeability. The process is a simple, affordable technology that can be used for small- or large-scale phytochemical extraction [229-230 and 233]. The extraction process was performed three times to compare the traditional method and UAE's results.

6.9 Preliminary phytochemical testing

Various secondary metabolites were analyzed in all plant extracts, including glycosides, alkaloid compounds, tannins, flavonoids, steroids, triterpenoids, saponins protein, carbohydrates, and amino acids.

6.9.1 Detection of alkaloids

Add diluted HCl drop by drop to the dried extracts, then filter it. The resultant filtrate was checked for alkaloids using preliminary assays—amino acids, carbohydrates, proteins, tannins, triterpenoids, steroids, and saponins. The subsequent tests were run following [234-235].

6.9.2 Identify alkaloids

Add diluted HCl drop by drop to the dried extracts, then filter it. Preliminary tests were performed on the resulting filtrate to test for the presence of alkaloids.

6.9.2.1 Dragendorff's reagent test: Precipitation of oranges and browns, suggestive of alkaloids, was seen after mixing the resulting filtrate and reagents on a watch glass.

6.9.2.2 Mayer's reagent test: On a watch glass, the filtrate mixture and reagent were mixed to look for cream-coloured precipitates, indicating the alkaloids' presence.

6.9.2.3 Wagner's method of test: The filtrate mixture and solution were mixed on a watch glass to search for brownish-red precipitates that indicated alkaloids are present.

6.9.2.4 Hager's reagent test: On a watch glass, reagents were mixed with filtrate liquid to look for yellow precipitates, an indication of the presence of alkaloids.

6.10 Analyse for tannins and phenolic compounds.

Tannins were analysed by mixing dried extracts with ethanol and filtering.

6.10.1 5% FeCl₃ solution: After combining the filtrate with FeCl₃, tannins were checked for a dark blue-black colour.

6.10.2 Lead acetate solution: Tannins formed white precipitates when the filtrate was subjected to lead acetate.

6.10.3 Gelatin solution: After mixing the filtrate and gelatin solution, white precipitates representing tannins were seen.

Iodine test using the diluted solution: The filtrate was mixed with the concentrated iodine solution, and a change of red colour, which indicates the occurrence of tannins, was sought.

6.10.4 Lead acetate solution: The filtrate was added to the solution of lead acetate, and the development of white precipitates, indicating the presence of tannins, was seen.

6.10.5 Gelatin solution: After combining the filtrate with the gelatinous solution, it was discovered that tannins, and white precipitates, were present.

6.10.6 Iodine test using the diluted solution: The filtrate was mixed with the dilute iodine solution, and a change of red colour, which shows the occurrence of tannins, was sought.

6.11 Analyse flavonoids

The various preliminary identifications of flavonoids were carried out by combining ethanol with the drying extracts and filtering.

6.11.1 Shinoda test: The filtrate was then subjected to a 5% ethanol and HCl treatment, followed by the incorporation of magnesium turnings, while also keeping an eye out for the formation of a pink colour, which indicates the existence of flavonoids.

6.11.2 Lead acetate the solution: This mixture was added to the filtrate while being kept an eye out for the emergence of any yellow forms precipitates that would signify the presence of flavonoids.

6.12 Screening for steroids

For preliminary steroid testing, all dry extracts were chloroform-mixed and filtered.

6.12.1 Salkowski test: Filtrate was combined with chloroform (2 ml) and concentrated H₂SO₄, and an appearance of red and a yellowish-green fluorescence, which indicate the presence of steroids and the existence of CHCl₃ and the acid layer, respectively, was seen.

6.12.2 Liebermann-Burchard test: Filtrate was combined with acetic anhydride, chloroform, and conc. H₂SO₄ and then red, blue, and green colouring was seen to determine the presence of steroids.

6.13 Detection of saponins

All dry extracts were mixed with water and filtered to test for saponins.

6.13.1 Foam test: After vigorously shaking the mixture of alcohol and water, persistent foam, which indicates the presence of saponins, was observed.

6.14 Identify proteins and amino acids.

6.14.1 Biuret test: The presence of proteins was determined by adding a reagent to the sample solution and watching for violets or pink colour development.

6.14.2 Test using Million's reagent: The test solution was treated with the reagent, and the presence of white precipitates was monitored. White forms precipitates have been heated and left aside to create a dark crimson protein-revealing solution.

6.14.3 Ninhydrin reagent test: The test sample was mixed with 5% ninhydrin and kept in a water bath for 10 minutes to look for proteins; its blue or purple tint indicated protein presence.

6.15 Carbohydrate test.

All extracts were mixed with water, filtered, and tested for carbs using screening methods.

6.15.1 Molisch's reagent test: The filtrate was combined with the -naphthol solution. Next, the two layers of the test tube's violet ring increased, showing the presence of carbs and glycosides.

6.15.2 Fehling solution: The filtrate was mixed with the two Fehling solutions, boiled for one minute, and tested for carbs and glycosides. In a water bath, this took 5–10 minutes.

6.15.3 Benedict's reagent: Add reagent to the filtrate. Keep for five minutes in a water bath. Check for the presence of a green, yellow, or red colour solution. The colour in the mixture will vary according to how much reducing sugar is present in the sample.

6.15.4 Barfoed test: They combined the filtrate and reagent. Red precipitates indicated the monosaccharide after two minutes in a water bath.

6.16 Triterpenoid screening

Triterpenoids were initially tested by mixing all extracts with ethanol and filtering.

6.16.1 Thionyl chloride test: The filtrate was treated with tin metal beads and thionyl chloride solution to detect triterpenoid.

6.17 Phytochemical quantification

6.17.1 Phenolic content estimation

Ethyl acetate, methanol, and hydroalcoholic extracts were made from 1 g leaves of (A.M.) and (T.S.). Filtering increased the volumetric flask to 50 ml. A test tube contained 1 ml of sample and 10 ml of filtered water. After that, add the Folin Ciocalteu reagents, and let the mixture remain at room temperature for 5 minutes. Add 20% (w/v) sodium carbonate. (4ml). The solution was increased by 25 ml by adding distilled water, and the mixture was vigorously stirred before being kept at room temperature for 30 minutes. The solution's absorbance at 765 nm was measured using an ultraviolet (UV)

spectrophotometer [236]. Three outcomes per outcome. Gallic acid's standard curve quantified the sample.

Calculation (Eq-2)

$$C = c \times V / M$$

C=Total Phenolic Content.,

c=Gallic-acid Conc. attained by Standardization Curve (mg/ml),

with V=Extract-volume (ml),

Mass-Extract =M (g).

6.17.2 Calculation of the Total Flavonoid Content:

Ethyl acetate, methanol, and hydroalcoholic were used to extract *Alpinia mutica* (A.M.) and *Tradescantia spathacea* (T.S.) leaves, each weighing 1 gm. The volume within the volumetric flask was raised to 50 ml after filtering the solution. The 0.6 mL sample contains 2% aluminium chloride. (0.6mL). The combination was then kept at a comfortable temperature for an hour more. The resulting mixture's absorbance at 420 nm was then measured using a U.V. spectrophotometer [237]. The substance was evaluated using the standard flavonoid curve, and each result was replicated three times.

Calculation (Eq-3)

Calculation

$$C = c \times V / M,$$

C - Total Flavonoid content,

c-Quercetin conc. as determined by the standard curve (mg/ml),

V-Extract-volume (ml),

M-Extract Mass (g).

6.17.3 Estimation of total alkaloid content

We used ethyl acetate, methanol, and hydroalcoholic solvent to extract 1 g of leaves from (A.M.) and (T.S.) separately. After filtering, we adjusted the volume to 50 ml in a volumetric flask. A BCG solution and a phosphate buffer (pH 4.7) were added to 5 millilitres of the sample solution. The mixture was then vigorously shaken. After that, 4 millilitres of chloroform were employed to extract the solution. Once the chloroform level was attained, it was transferred to a 10 ml volumetric flask. Absorbance was determined at 470 nm [238-239]. The results were performed in triplicate. Quantification of the sample was accomplished by the atropine standard curve.

Calculation (Eq-4)

$$C=c \times V / M$$

C= Total Alkaloid content.,

c= Atropine conc. achieved by the standard curve (mg/ml),

V=Extract-volume (ml),

M=Mass of Extract (g).

6.18 Study of Thin Layer Chromatography (TLC).

Rf values may assist in identifying compounds under particular conditions. Crude medicinal extracts are compared chromatographically to standard references. Natural medicine extracts generated with a certain solvent system's TLC profile and other characteristics may be used to perform qualitative evaluations of medicinal plants. This herbal evaluation method is becoming popular because to its ease and reproducibility. It examines substances such as isoprenoids, alkaloids, glycosides, steroids, and sugars. Chromatography uses metal plates precoated. The chemical type determined the solvent system [228].

6.18.1 Saturation of Chamber: A sheet of paper filters covered three sides of the chamber and was immersed in a solvent solution for 45 minutes.

6.18.2 Apply test spots as follows: *A.M* and *T.S* leaf solution. A small capillary tube put identical-volume dots 2 cm from the plate's bottom. For the chromatogram, the specified plates were laid out horizontally in a spot where the bubbling liquid reached a depth of about an inch. After 8 cm, a solvent front appeared. Different spots on the chromatogram may show component resolution throughout the *A.M.* and *T.S.* leaves. Visually and in a 365 nm U.V. chamber, the spots were located. The iodine chamber created plates to detect stains since various compounds shine differently under UV light [228].

6.18.3 Steroid detection: Spraying antimony trichloride or vanillin-sulfuric acid reagents over TLC plates revealed the presence of steroids.

6.18.3.1 Antimony trichloride reagent: A solution of 20% tin trichloride in CHCl_3 was spritzed over the TLC plate, and then it was heated for five to six minutes at 100°C . Depending on the light source, steroidal patches might seem brown or violet. Toluene: Ethyl acetate (4:1) is the solvent.

6.18.3.2 Spraying with two solutions: 1% ethanol-based vanillin and 5% ethanol-based sulfuric acid were in solution. I and II were sprayed on the plates in 10 and 5-10 ml, respectively. After 5–10 min at 100°C , the plates were examined under visible light. Steroid dots were violet-blue [228]. Rf was determined using a specific equation

(Eq-3), Return function (Rf) = spot-to-origin distance divided by solvent-front-to-origin distance.

6.18.3.3 Flavonoids:

Up to one centimetre from the upper end, the solvent front moves. After removing the TLC plates, the solvent face was marked with a soft pencil. Air-dried, they were sprayed with 1% ethanolic chloride to dissolve the aluminum solution, dried, and analyzed under 365 nm UV light. Five to six minutes at 100°C heats the TLC plate with methanol solvent system with violet or brown steroidal dots: Hexane: Chloroform (7:2:1v/v/v). The development tank received plates with dried samples. The solvent front was approaching the middle of the "plates" when the growth chamber opened. A soft pencil was used to instantly mark the solvent front's location [228]. The retention factor (Rf) values for each band were then computed.

6.18.3.4 Phenolic compounds:

Before it reached the upper end, the solvent front had some room to manoeuvre. They marked the solvent shown with a soft pencil, followed by TLC plate removal. They were air-dried, coated with a thin coating of green FeCl₃ reagent, and then observed under 365 nm UV light. TLC plates were heated at 100°C for 5-8 minutes. Steroid patches usually appear violet or brown. The different solvent system was (ethyl-acetate, acetic-acid, formic-cid, and water (100: 11: 11: 26 v/v/v/v)). The plates holding dried samples were sealed and put in the development tank. The experiment chamber was opened after the solvent front reached 3/4 of the "plates.". A soft pencil was used to instantly mark the solvent front's location [228]. The values corresponding to the retention coefficient (Rf) were then calculated for each of the colours. The following equation was used to calculate the bands' retention coefficients (Rf): (Eq-5) $R_f \text{ Value} = \text{Distance of spot from origin} / \text{Distance of solvent front from origin}$.

6.19 Metabolites quantification

6.19.1 Antioxidant properties

Plants are a major source of natural antioxidants, according to various research. Antioxidants are phytochemicals, minerals, and vitamins that protect against reactive oxygen species (ROS), ROS destroy various biological structures, causing ageing, cancer, and atherosclerosis.

As a result of their ability to donate electrons, flavonoids' primary mechanism of antioxidant activity is the scavenging of free radicals. In addition to their potential to neutralize free radicals, several flavonoids and other phenolic chemicals are also thought to be antioxidants because they chelate metals. Different ecological, physical, and chemical stresses on cells might result in an imbalance of antioxidant enzymes or ROS. In addition to lipid peroxidation, ROS depletes proteins, inactivates enzymes, and changes DNA. In the scientific literature, several in vitro and in vivo test methods exist for figuring out how well certain chemicals can get rid of free radicals. Strong, slight, and weak antioxidants are the three types of antioxidants that may neutralize free radicals [240–241]. To assess if a material is an antioxidant or not, in vitro approaches are performed. Trolox equivalents, which represent the scavenging of free radicals in terms of Trolox, or IC50 values, which represent concentrations that generate 50% radical scavenging, may be used to quantify the activity.

6.20 FeCl₃ Reducing power

6.20.1 Standard solution preparation

Ten milligrams of ascorbic acid were diluted five times in ten milliliters of deionized water to provide 20, 40, 60, 80, and 100 µg/ml concentrations.

6.20.2 Test preparation

A 10 ml solution was prepared by dissolving the test items in a few amounts of methanol and adding a phosphate buffer. Materials were diluted to 20, 40, 60, 80, and 100 µg/ml in a 10-ml volumetric flask containing phosphate buffer. Utilise fresh reagents.

6.20.3 Phosphate buffer: I.P. made a pH-6.6 0.2M phosphate buffer prepared.

6.20.4 1% solution of Potassium ferricyanide:

1% potassium ferricyanide was made by dissolving 2 g in 200 mL of distilled water. Trichloroacetic acid, 10%: 40 g was dissolved in 400 mL of purified water. One hundred milliliters of pure water and 0.1 grams of iron chloride are combined to produce a 0.1 percent solution.

6.20.5 Reducing Power Protocol

In each 2 ml of the sample and reference solutions, 2.5 ml from a 1% ferricyanide of potassium solution was added. A water bath at 500 °C should hold the mixture for 20 minutes. After cooling, 2.5–10% trichloroacetic acid was placed in a centrifuge for ten minutes at 3000 rpm. 1 ml of 0.1% FeCl₂ and 2.5 ml of filtered water were administered for 10 minutes. The control was made without samples using the same processes. The solution has 700 nm absorbance [241-243].

6.21 The activity of DPPH in scavenging free radicals

6.21.1 standard solution: Ascorbic acid dissolves methanol to generate 20, 40, 60, 80, and 100 µg/ml.

6.21.2 Test sample preparation: To make sample solutions, 10 mg of dried methanolic root extract and leaf extracts and 1 mg/ml methanol were added to 10 ml. In 3.3 ml of Methanol, 4.3 mg of DPPH solution was dissolved, and aluminium foil was used to cover the test tubes to block light.

6.21.3 The estimation procedure for DPPH (1,1-Diphenyl-2-picryl-hydroxyl assay)

Diluting with the sample using methanol yielded 100 liters for each dose level (20, 40, 60, 80, and 100 µg/ml). 150 mL DPPH is diluted numerous times in methanol in every tube. 150 mL of DPPH solution was immersed in three millilitres of methanol and measured at 516 nm instantaneously in the control experiment. A methanol blank was used to measure absorption at 516 nm after 15 minutes using a UV-visible- spectrophotometer (Shimadzu, UV-1800, Japan). Each experiment was carried out thrice to calculate the IC₅₀ and the decrease % [241-243].

6.22 Activity of Iron chelation

Iron chelation activity indicates antioxidant action. The extract, ascorbic acid, ferric chloride, and O-finantrilin solutions were incubated at room temperature for 10 minutes. Solvent absorbance was measured at 510 nm after incubation. Testing occurred three times.

6.22.1 Principle Fe²⁺ chelating ability

Divalent transitional metal ions are the crucial catalysts in the oxidation events that result in the generation of radicals containing hydroxyl and hydrogen hydroxide breakdown reactions in the fentanyl chemical structure. Human cardiovascular disease may be influenced by iron, a metal of transition that may produce free radicals from peroxides. Lowering Fe²⁺ concentrations prevent oxidative damage because Fe²⁺ generates oxyradicals and encourages lipid peroxidation. Additional chelating chemicals prevent ferrioxalate complexes from forming correctly, which causes the complexes to become red. The Fe²⁺-ferrozine mixture's absorbance reduced dose-dependently, while activity increased with concentration. [241-243].

6.23 Green synthesis of nanoparticles of *Alpinia mutica* and *Tradescantia Spathesia*

6.23.1 Green synthesis of Silver Nanoparticles (Ag NPs)

Green Ag NP nanoparticles were synthesized by adding 10 ml of the filtered A.M. and T.S. drug mixture to 45 ml of one mM AgNO₃ in cylindrical flasks with an electric mixer. Ag NPs were produced by forcefully shaking cylindrical flasks for 0, 12, and 24 hours. At ambient temperature, the pale-yellow solution became dark brown in the conical flasks after 12–24 h. For 20 minutes, the colored fluid was spun at 5000 rpm. After the liquid was taken away, only waste was left. The leftover residue was cleaned and dried with distilled sterile water [244].

6.23.2 Biological synthesis of zinc oxide nanoparticles

Forty-two hours of intense mixing were done with 25 ml of plant extraction and 0.1 M zinc nitrate hexahydrate. The filthy precipitate cooled for 24 hours after the process. Precipitate and reaction solution separated after 15 minutes of 6000 rpm centrifugation. The impurities were removed from the precipitate by washing it with deionized water and then drying it at 80°C. A muffle furnace heated the sample in

powder form to 3500C for three hours. Then, 5 μ L of Zn O NPs solutions were poured onto a carbon-dusted copper metal and then cooled before being analysed under a scanning electron microscope [245].

6.24 Green synthetic nanoparticles' characteristics

The shape, average size of particles, and functional group analysis of Zn O and Ag NPs for A.M. and T.S. were genetically engineered. SEM, zeta potential, particle size analyser, and X-ray diffraction were among the tools used to identify the nanoparticles for the green synthesized nanoparticles. A.M. Ag N.P., A.M. Zn O N.P., T.S. Ag NP, and T.S. Zn O N.P.s' crystalline sizes were determined using X-ray diffraction measurements across a 2θ range of 20-80°. (X-ray Diffractometer). To evaluate particle size distribution and zeta potential, a particle size analyzer was used. In order to investigate the existence of the biomolecules necessary for the synthesis of A.M. Ag NP, A.M. Zn O N.P., T.S. Ag NP, and T.S. Zn O N.P.s, a technique called Fourier to transform infrared spectroscopy in a wavelength range of 400–4000 cm⁻¹ was utilized. SEM was used to analyze A.M. Ag NP, A.M. Zn O N.P., T.S. Ag NP, and T.S. Zn O N.P.s to determine their morphology, particle size, shape, and elemental content [246-247].

6.25 Particle size and Zeta potential analysis

Zeta potential and average particle size were determined using dynamic light scattering (DLS) (Malvern Instruments). Particle size was measured at a constant angle of 90° and an average temperature of 25°C. After dispersing the nanoparticle suspension in distilled water, the sample was created by sonicating it for 6 minutes using an ultrasonic bath. Zeta potential data were gathered using electrophoretic scattering of light at 25 °C and 150 V. The zeta potential depends on a charge conductivity concept to stabilize the formulation and mainly normal particle size ranges of nanoparticles are 1-1000 nm and zetapotential is between -10 and 10 mV. [248-249].

6.26 Fourier transform infrared spectroscopy:

The reaction solution was centrifuged at 6000 rpm for 15 min. for FT-IR. The pellets were cleansed multiple times using 20 ml of distilled water to remove other impurities. The substance was dried, crushed with KBr, and then inspected. The samples were measured using a Shimadzu 8400S having a spectrum of 500–4000 m^{-1} and an accuracy of 4 cm^{-1} . The leaf extract's FTIR spectra were analyzed after NP formation to determine what functional groups might be involved in the synthesis of Ag NPs. The FTIR peak values were rearranged, and readings of the spectrum were taken twice to confirm it [250-251].

6.27 XRD analysis:

Powder X-ray Diffraction methods, essential characterization techniques in solid-state science and material research, are primarily used by mineralogists or solid-state chemists to investigate the physicochemical composition of unknown materials. Any compound's unit cell may be rapidly identified using XRD by size, shape, structural parameter resolves, and phase fraction analysis. The peak locations of the Diffraction pattern reveal the size and shape of the unit cell's translational symmetry. XRD was used to inspect the structural properties of produced nanoparticles. Nanoparticles were put into a PAN analytical X-Ray diffractometer set to 4000V and 20mA. The scanning was done with 2θ angle ranging from 20° to 80° at $0.02^\circ/\text{min}$ and 2θ time constants. Crystal structures of all materials were improved to get exact atom positions [251-252].

6.28 Scanning electron microscopes (SEM) analysis: Scanning electron microscopes operate on identical concepts as light reflection microscopes. An image is created in a scanner with electrons when an electron beam strikes an object and reflection occurs, which is captured by a detector. In this study, plants were extracted to make nanoparticles as a capping agent [253-254].

6.29 In vitro anti-diabetic activity

6.29.1 Activity of α -amylase inhibition

The DNS measured α -amylase inhibition. Using 1 mg/ml phosphate buffer, 20–100 g/ml silver nanoparticles were generated. We incubated a 250 μ L sample (2 units/ml) with a solution of amylase at 27°C for 10 minutes. Incubate for 10 minutes before adding 250 μ L of 1% starch solution. The solution was heated for 10 minutes in a water bath after interrupting the reaction with 0.5 mL dinitro-salicylic acid. It was diluted with 5 mL distilled water after cold. Each test sample concentration received a buffer instead of the enzyme to produce a blank. Constant control lacked a 100% enzyme activity sample. Positive control: Acarbose used. A spectrophotometer evaluated the colored solution's absorbance at 540 nm to calculate the inhibitory percentage [255-257]. $(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}) / (\text{Absorbance}_{\text{Control}}) \times 100$

6.30 Materials & Methods for Pharmacological Activity

6.30.1 Chemicals for animal activity

STZ, Methanol, hexane, distilled water, ethyl acetate, ethanol, saline water, ringer solution, Dimethyl sulfide (*DMS*), metformin, silver nitrate, and Zinc hexahydrate.

6.30.2 Experimental animals

Male and female mice weighing 28 to 30 g each were bought from Mahaveer-Enterprises in Uppal, Hyderabad, Telangana, India. For an inquiry into acute toxicity and an in-vivo diabetes study. The polypropylene cages used to house the test animals were kept at ambient humidity and temperature levels for 12 hours daily. Before diet regulation, food (NPD- Normal pellet diet) and water were freely given to the animals. Geethanjali College of Pharmacy's (Approval No. GCPK/IAEC/2020/01) Institutional Animal Ethics Committee (IAEC) approved the animal procedure, and experiments were carried out following CPSCEA regulations for Govt. of India.

6.31 Assay for acute toxicity

According to OECD Guidelines 425, tests for acute toxicity were conducted. Male and female albino mice, aged 8 to 10 weeks, were utilized in the investigation. Weights of 28 ± 4 g were picked randomly, processed, and included in **Table 4**. The mice fasted for 3 to 4 hours. However, water was available at all times before the dose was given. A single oral dosage of 2000 mg/kg; p.o. was given to each group of mice based on the weight of one mouse in the group [248]. For the first 30 minutes and then the next 4 hours, the animals were closely monitored for any symptoms of toxicity. After taking the drug for a couple of hours. The food was restored. After testing the drug-treated mice's endurance, the same drug dose was administered to the remaining four mice. Every mouse in the vehicle control group received the same treatment according to the procedure.

The different groups were closely watched for any adverse side effects. Behavioural characteristics were also recorded for the first 30 minutes, 4 hours, 24 hours, and then at regular intervals, i.e., 14 days. Several times, the mice's body weight was recorded. After the experiment, mice were removed from the body by dislocating their cervical vertebrae while being anesthetized with diethyl ether. Measurements were then taken of the kidney, liver, and heart weights. In order to collect blood samples for biochemical and haematological analysis, the heart was punctured. The blood samples were then taken out and delivered to the pathology lab. The kidney, liver, and heart were preserved in formalin solution (10%) after the organs were removed for histological analysis [258-260].

Table 5: Acute toxicity (OECD 425 Guidelines)

Experiment			
Groups	Treatment with Drug	Drug concentration and rout of administration.	Animals from each category (Mice)
I	Vehicle for Control	1% w/v of CMC (p.o.)	5
II	SNP	2000 mg/kg (p.o.)	5
III	ZONP	2000 mg/kg (p.o.)	5
IV	TSLE	2000 mg/kg (p.o.)	5
V	TSLESNP	2000 mg/kg (p.o.)	5
VI	TSLE ZONP	2000 mg/kg (p.o.)	5
VII	AMLE	2000 mg/kg (p.o.)	5
VIII	AMLESNP	2000 mg/kg (p.o.)	5
IX	AMLE ZONP	2000 mg/kg (p.o.)	5

NPD--Normal Pellet Diet. CMC: Carboxy Methyl Cellulose; SNP: Silver Nanoparticles; ZONP: Zinc Oxide Nanoparticles; TSLE: *Tradescantia spathacea Leaf Extract*; TSLESNP: *Tradescantia spathacea Leaf Extract Silver Nanoparticles*; TSLE ZONP: *Tradescantia spathacea Leaf Extract Zinc Oxide Nanoparticles*; AMLE: *Alpinia mutica leaf extract*; AMLESNP: *Alpinia mutica leaf extract Silver Nanoparticles*; AMLE ZONP: *Alpinia mutica leaf extract Zinc Oxide Nanoparticles*.

6.31.1 Biochemical evaluation

Blood sugar, cholesterol, triglycerides, High-dense-lipoprotein, LDL cholesterol, VLDL, creatinine levels, urea, bilirubin, and alkaline phosphates, total protein molecules, globulins, and albumin were measured in all samples at Clinova Path Labs Pvt. Ltd. in Hyderabad, Telangana.

6.31.2 Analyses of the blood

The pathology laboratory (Clinova Path Labs Pvt. Ltd, Hyderabad, Telangana) tested blood samples for CBC variables, total RBCs, hemoglobin, MCH, MCV, MCHC, WBCs, platelets, lymphocytes, eosinophils, neutrophils, basophils, and monocytes in EDTA tubes.

6.31.3 Histopathological investigation

After sacrifice, the mouse hearts, livers, and kidneys were stored in formalin solution (10%) and analysed by the Kakatiya Medical College Warangal, Telangana pathology department. There Paraffin wax was used to fix the organs. Eosin and hematoxylin were used to stain 5mm paraffin slices. Under the light microscope, the tissue structure was found, and pictures were taken.

6.31.4 Statistical-analysis

The total results were presented as Mean \pm SD, and Turkey's multiple comparison tests and one-way ANOVA were used to evaluate the statistical importance of the results for the various groups. $P \leq 0.05$ was statistically significant.

6.32 An approach to in-vivo antidiabetic activity

6.32.1 Designing an in vivo experiment to induce diabetes

A Wistar rats 180–200 g; was purchased from Mahaveer Enterprises in Uppal, Hyderabad, Telangana, India. The animal research protocol was authorized by (IAEC), and a reference number was given. (GCPK/IAEC/2020/01). Six animals were housed in an acrylic cage with a controlled environment of 25°C, 45–55% moisture, and 12–12 h of light and darkness. A standard laboratory meal and constant access to water were provided for the rats. An intraperitoneal injection of a freshly produced solution of streptozotocin was given to Wistar rats the evening before the experiment. (40 mg kg⁻¹ in 0.1M citrate buffer, pH 4.5). After 48 hours of STZ, rats developed hyperglycemia. The experiment utilised rats with a minimum of 250 μ g dl⁻¹ blood sugar.

6.32.2 Hypoglycaemic-activity in fasting non-diabetic rats (OGTT)

After fasting, rats were divided into 15 groups of six. Group I got 0.5% w/v saline oral in 0.5 ml kg⁻¹. Group II got metformin suspended within a moving vehicle, whereas Group I received a high-fat diet (HFD). (p.o., 50 mg kg⁻¹). **Table 5** lists the various samples that were given here and separated into the groups: diabetic control (STZ 40g/kg), AMLE (200 mg/kg, 400 mg/kg), AMLESNPs (100, 200 mg kg⁻¹), AMLE Zn O NPs (100, 200 mg kg⁻¹), TSLE (200 mg/kg, 400 mg/kg), and TSLESNP (100, 200 mg kg⁻¹) and TSLE Zn O NPs (100 and 200 mg kg⁻¹) were administered to the animals of groups III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV and XV,

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respectively. An Accu Chek measured glucose levels in retro-orbital sinus blood samples taken during ether inhalation. In order to treat type 2 DM, it is essential to analyze and discover possible new anti-diabetic medicines derived from medicinal plant extracts of nanoparticles. Wister rats of either sex was used in this experiment for the HFD and STZ-induced diabetes models [257]. The Wister rats were categorized in different dietetic treatments, i.e., NPD or HFD (17% carbohydrate, 25% protein, 58% fat), and *ad libitum* was provided initially for 2 weeks. The rats were eventually divided and separated into NPD, HFD + STZ, HFD + STZ + Test material AMLE, AMLE SNP, AMLE Zn O NP, TSLE, TSLESNP, and TSLE Zn O NP. (**Table 4**). The ingredients of HFD reported in **Table 6** are according to [257]. All of the HFD-fed rats received STZ (from 40 mg/kg, i.p.) after two weeks of diet management. On the next day, the seventh, measurements of the body's weight and biochemical parameters were made.

The test substances AMLE, AMLE SNP, AMLE Zn O NP, TSLE, TSLESNP, and TSLE Zn O NP were given orally at multiple concentrations, i.e., 200 mg/kg, 400 mg/kg, 100 mg/kg, 200 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg, 100 mg/kg, and 200 mg/kg continuously for seven days. The levels of triglycerides, plasma glucose, and cholesterol were then determined from the blood samples. Half of the rats were dissected at the end of the treatment, and the pancreas was examined histopathologically [261-262]. Diabetic rats are non-fasting rats with blood glucose levels above 300 mg dl⁻¹. Animal water, feed intake was recorded, and rats were given a diet as per the protocol.

Table 6 Experimental design for in-vivo antidiabetic in Wister rats

Experiment			
Groups	Treatment with Drug	Drug concentration and route of administration.	Rats, n=6; number of rats in each group
I	Control (Saline+ CMC)	Saline 0.5% w/v of CMC (p.o.)	6
II	Standard drug	Metformin, 50 mg/kg; body weight (p.o.)	6
III	Diabetic control	STZ (40g/kg; body weight) (i.p.)	6
IV	Diabetic control+ AMLE	200 mg /kg; body weight (p.o.)	6
V	Diabetic control+ AMLE	400 mg /kg; body weight (p.o.)	6
VI	Diabetic control+ AMLESNP	100 mg /kg; body weight (p.o.)	6
VII	Diabetic control+ AMLESNP	200 mg /kg; body weight (p.o.)	6
VIII	Diabetic control+ AMLE Zno NP	100 mg /kg; body weight (p.o.)	6
IX	Diabetic control+ AMLE Zno NP	200 mg /kg; body weight (p.o.)	6
X	Diabetic control+ TSLE	200 mg /kg; body weight (p.o.)	6
XI	Diabetic control+ TSLE	400 mg /kg; body weight (p.o.)	6
XII	Diabetic control+ TSLESNP	100 mg /kg; body weight (p.o.)	6
XIII	Diabetic control+ TSLESNP	200 mg /kg body weight (p.o.)	6
XIV	Diabetic control+ TSLE Zno NP	100 mg /kg; body weight (p.o.)	6
XV	Diabetic control+ TSLE Zno NP	200 mg /kg; body weight (p.o.)	6

NPD-- Normal pellet diet; CMC- Carboxy methyl cellulose; HFD- High fat diet; STZ- Streptozotocin; AMLE: *Alpinia mutica leaf extract*; AMLESNP: *Alpinia mutica leaf extract Silver Nanoparticles*; AMLE ZONP: *Alpinia mutica leaf extract Zinc Oxide Nanoparticles*. TSLE: *Tradescantia spathacea Leaf Extract*; TSLESNP: *Tradescantia spathacea Leaf Extract Silver Nanoparticles*; TSLE ZONP: *Tradescantia spathacea Leaf Extract Zinc Oxide Nanoparticles*.

6.32.3 Biochemical analysis

After administering a light ether anesthetic, capillary tubes collected blood through the retro-orbital plexus of Wister rats. The pathology lab (Clinova Path Labs Pvt. Ltd, Hyderabad, Telangana) examined the blood samples to determine the levels of triglycerides, plasma glucose, and cholesterol.

6.32.4 Histo-pathological study

Rats' separated pancreas was sacrificed, fixed in formalin (10%), and examined by the pathologists in the department at Kakatiya Medical College Warangal, Telangana. There Paraffin wax was used to fix the organs. Eosin and hematoxylin were used to stain 5mm paraffin wax. Under the light microscope, the tissue structure was found, and pictures were taken.

6.32.5 Statistical Investigation

The mainly results were presented as Mean \pm SD, and one-way ANOVA were used to assess statistical significance across the various groups. It was determined that $P \leq 0.05$ was statistically significant.

6.33 Molecular docking studies

The docking studies mainly used different software they are used for molecular modelling includes Chem Draw Pro 12.0.2, Chem Bio 3D Ultra 12.0.2, and Molegro Virtual Docker 6.0 2013. The target receptors the Human pancreatic alpha amylase three-dimensional crystal structure (PDB ID: PDB: 5VA9) taken from Protein Data Bank (PDB). Flavokawin B, 5,6-Dehydrokawain, and Pyroglutamic acid were ligands for all structures created using Chem Draw Pro 12.0.2, and Chem Bio 3D Ultra 12.0.2 was used for energy minimization. The energy minimization aids in determining the ligand's bioactive conformer form. MVD (Molegro virtual docker) ligands must indicate genuine score and correct binding with a receptor in order for docking to be successful.

The Molegro virtual docker score algorithm to be validated for the crystal structure of protein for Alpha amylase (PDB: 5VA9). Docking used one cavity with a large volume of 154.624 and a grid resolution of 0:30, as well as parameters for maximum interactions of 1500, a maximum population size of 50, and a pose generation energy threshold of 100.00. Docking was performed by default on the non-ligand Molegro Virtual Docker, which has five cavities. It employed simplex evolution with a maximum distance factor of 1.00 and stepped 300. Table and Figures in the findings were provided using the Ligand-Human Pancreas Alpha Amylase Receptor Interaction Model [263-264].

CHAPTER 7
RESULTS & DISCUSSION

7. RESULTS & DISCUSSION

7.1 *Alpinia mutica* Profile

7.1.1 Macroscopy

The macroscopy of *Alpinia mutica* leaf revealed the presence of following characters

- Leaves : Straight and conical
- Shape : Conical
- Size : Length: 28-50 cm, width: 3-6 cm
- Base : Alternate
- Margin : Linear-lanceolate
- Taste : Bitter and aromatic
- Color : Heavy green color
- Odor : Aromatic

7.1.2 Microscopy

The leaf of *Alpinia mutica* exhibits various features such as Starch grains, unicellular trichomes, cluster type of ca-oxalate crystals, red color tissue, fibers, Xylem vessels, and xylem fibers. (**Figure 13 and 14**).

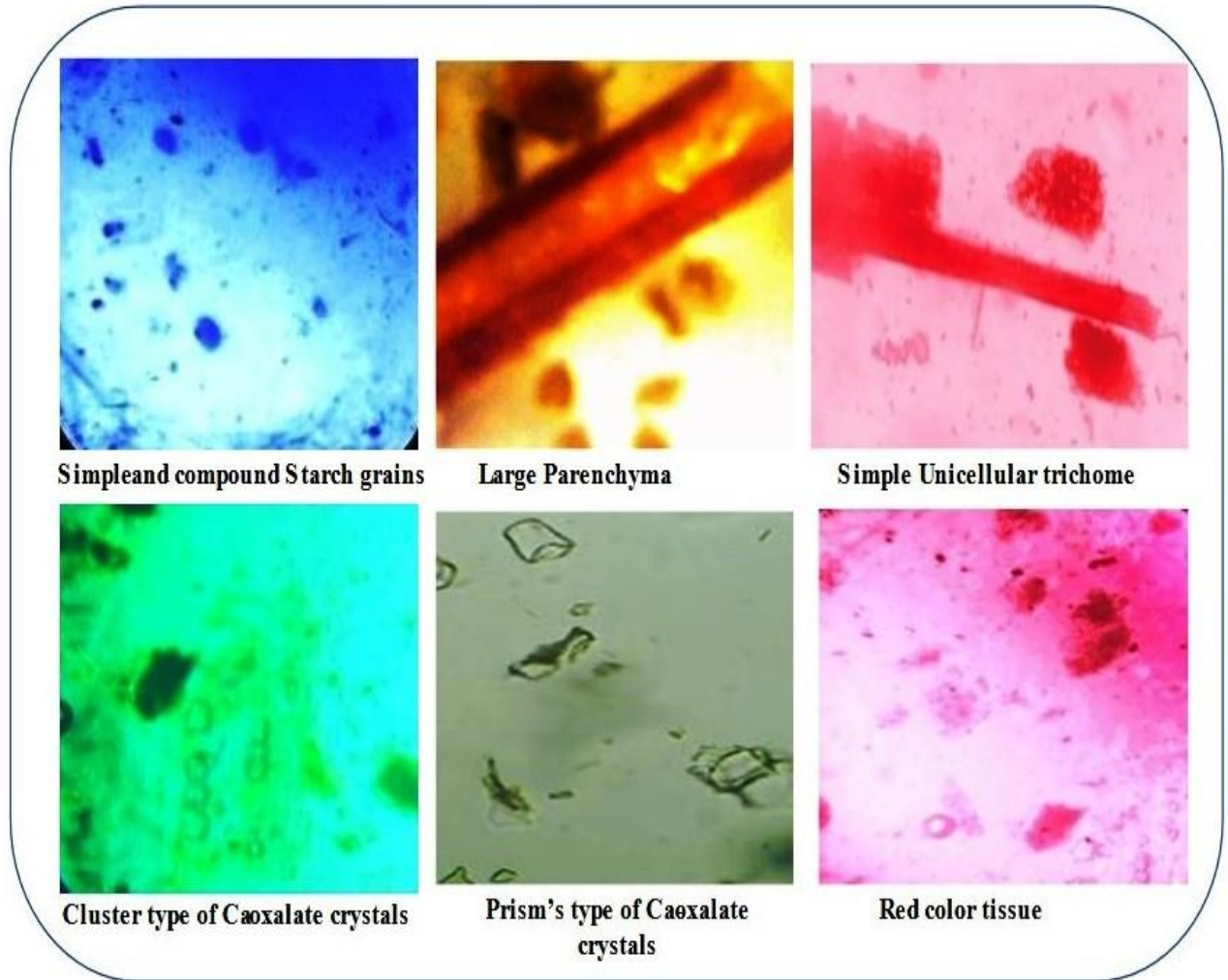


Fig- 13: Powder microscopic characteristics for the Leaves of *Alpinia mutica*

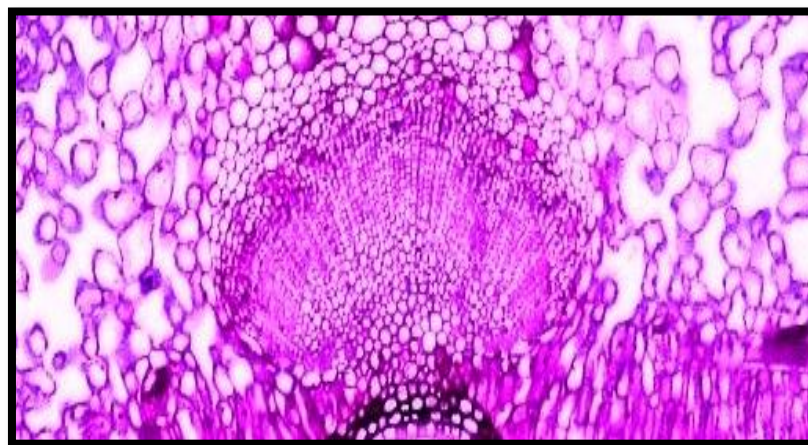


Fig- 14: Transverse section of Leaves of *Alpinia mutica*

7.1.3 Ash Values and Moisture contents

The herb's total, water-soluble, acid-insoluble, and sulfated the ash values of *Alpinia mutica* were found to be (8.45 ± 0.12), (1.07 ± 0.06), (4.27 ± 0.12), and (1.65 ± 0.10), respectively. These numbers demonstrate that the plant satisfies the requirements set forth by the Indian Ayurvedic Pharmacopeia for quality. However, it was discovered that the plant had a moisture content of (2.08 ± 0.10). The all results are shown in **Table 7**

Table 7 Analytical values of the *Alpinia mutica*

S.No.	Ash Type	Ash value (% w/w) Plant material taken (2g)
1	Total amount of ash	8.45 ± 0.12
2	Insoluble ash in acid	1.07 ± 0.06
3	Water soluble ash	4.27 ± 0.12
4	Sulfated ash	1.65 ± 0.10
5	Moisture content	2.08 ± 0.10

7.1.4 Extractive Values

Alpinia mutica had extractive values of 12.54 ± 0.12 and 8.84 ± 0.13 for alcohol and water solubility. These numbers demonstrate that the plant satisfies the requirements set forth by the Indian Ayurvedic Pharmacopeia for quality. The results are shown in **Table 8**.

Table 8 Extractive value of the plant *Alpinia mutica*

S.NO.	Plant material	Extractive values (% w/w), Plant material (4 g)	
		Solubility extractive value in ethanol at 90%	Extraction value in water
1.	Leaf	12.54 ± 0.12	8.84±0.13

7.1.5 Extraction - *Alpinia mutica* plant extract yields increased compared to Ultrasound-assisted extraction (USE). USE extraction is superior to traditional extraction, according to the findings. **Table 9** presents the results.

Table 9: *Alpinia mutica* extracts' colour, consistency, and yield in percentage

Plant Extract	Colour seen in daylight	Consistency	Yield (%w/w) by Conventional Method	Ultrasound-assisted extraction (w/w)
Methanolic-extract	Dark green	Semi-solid	11.56	15.45
Ethyl-acetate extract	Light green	Semi-solid	6.8	12.46
Hydro alcoholic extract	Dark green	Semi-solid	6.91	13.42
Petroleum ether extract	Lightly green	Semi-solid	2.98	6.45
Aqueous extract	Light green	Semi-solid	8.98	13.56

7.1.6 Phytochemical screening

Phytochemical screening was used to identify the phytoconstituents in the extracts. The USE technique increased phytoconstituent content in phytoextracts when compared to conventional extraction. **Table 10** illustrates the outcomes.

Table 10 Phytochemical screening for the various extracts of *Alpinia mutica*

S.N O	Test	Conventional Method of Extraction					Ultrasound assisted Extraction				
		AM ME	AME AE	AMH AE	AMP EE	AM AE	AM ME	AME AE	AMH AE	AMP EE	AM AE
1	Alkaloids	++	++	++	++	++	+++	+++	+++	+++	+++
2	Terpenoid es	+	+	+	+	+	+++	+++	++	++	++
3	Protens	----	----	----	----	----	----	----	----	----	----
4	Tannins	+	+	+	+	+	+++	+++	++	++	++
5	Carbohydr ates	++	++	++	++	++	+++	+++	+++	+++	+++
6	Flavonoid s	+	+	+	+	+	+	+	+	+	+
7	Saponins	++	++	++	++	++	+++	+++	+++	+++	+++
8	Phenolic compound s	++	++	++	++	++	+++	+++	++	++	++

+++ : Strong positive test, ++ : Low positive test, + : weak positive test, - : Negative test. AMME: *Alpinia mutica* methanolic extract; AMEAE: *Alpinia mutica ethyl acetate extract*; AMHAE: *Alpinia mutica hydro alcoholic extract*; AMPEE: *Alpinia mutica petroleum ether extract*

7.1.7 Phenolic content estimation

In mg/g of gallic-acid equivalent, the estimate was provided. The calibration curve for gallic acid was plotted as ($Y = 0.0024x - 0.0073$, $R^2 = 0.9977$) (**Figure 15**), and with the ultrasound-assisted extraction technique, ethyl acetate, methanol, and hydro-alcoholic extracts had a higher phenolic content as compared to extracts made by the conventional method. It was discovered that the methanol extract had the greatest phenolic concentration (4.24 ± 1.83). Results are shown in **Table 11**.

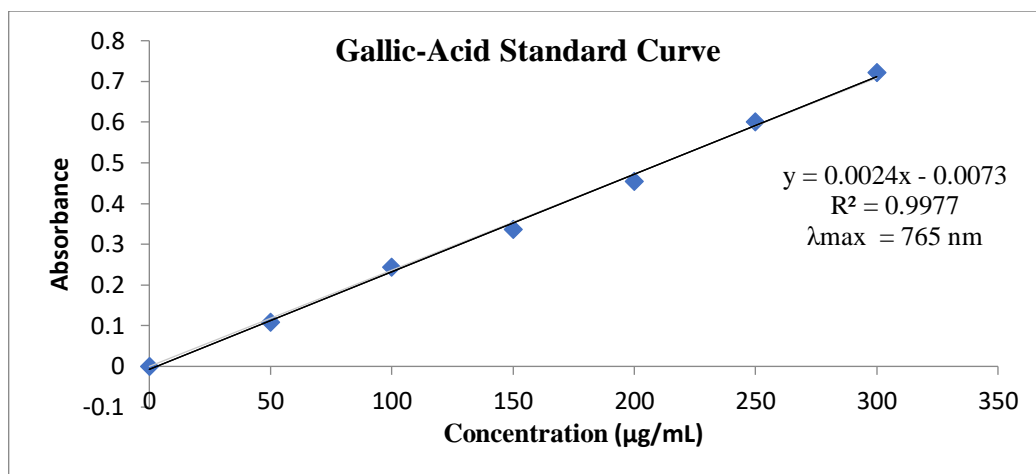


Fig- 15: Gallic acid Standard Curve

Table 11: *Alpinia mutica* total Phenolic Contents

S. No	Test samples (<i>Alpinia mutica</i>)	Gallic acid equivalent in mg/g for conventional extraction's total phenolic content.	USE the total phenolic percentage in milligrams per gram of gallic acid.
1	Methanolic-extract	2.07±1.86	4.24±1.83
2	Ethyl-acetate extract	1.11±0.52	1.45±0.87
3	Hydroalcoholic extract	1.18±0.17	2.01±0.44
4	Petroleum -ether extract	2.35±0.34	2.71±0.26
5	Aqueous extract	2.5±0.098	2.71±0.26

7.1.8 Flavonoid content estimation

The estimate was given in mg/g of quercetin equivalent and calibration curve for quercetin was plotted as ($Y = 0.0038x + 0.001$, $R^2 = 0.9998$) (Figure 16). The flavonoid content was increased when compared to extracts made using the traditional approach in the USE ethyl-acetate, methanolic, and hydro-alcoholic extracts. The methanol extract had the most flavonoid concentration (3.50 ± 1.34). Table 12 displays the outcomes.

According to the study, ultrasonic-assisted extraction techniques significantly increased the extract's phenolic and flavonoid content. The ultrasonic-assisted

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extraction technique also found a quick and efficient solution for plant extraction. Compared to standard extraction techniques, USE approaches enhanced *Alpinia mutica* plant extract yield and phytoconstituent content, mainly phenolic and flavonoid.

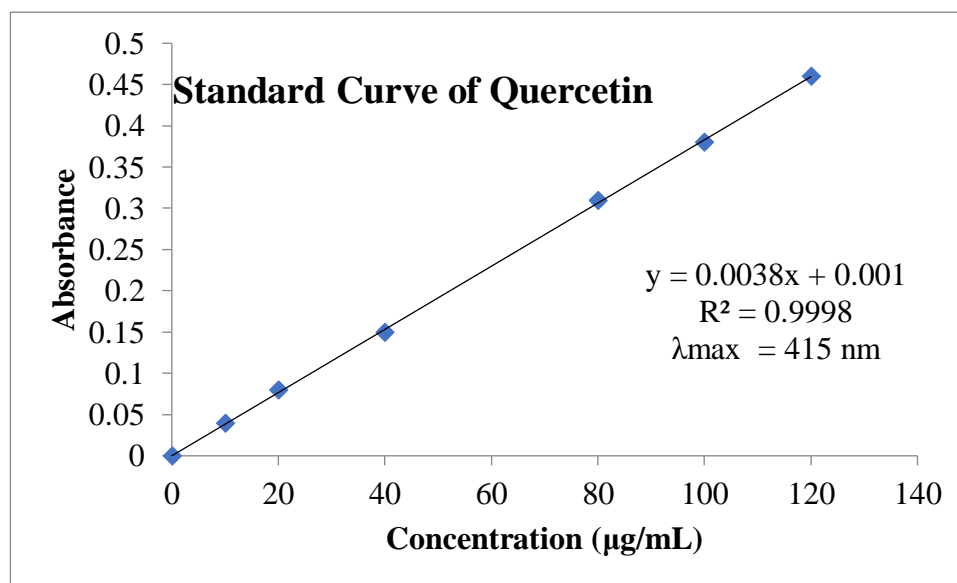


Fig-16: Quercetin Standard Curve

Table 12: Flavonoids total Contents of *Alpinia mutica*

S. No	Test samples (<i>Alpinia mutica</i>)	Gallic acid equivalent in mg/g for conventional extraction's total phenolic content.	USE the total phenolic percentage in mg per gram of gallic-acid.
1	Methanolic extract	2.55±1.00	3.50 ± 1.34
2	Ethyl acetate extract	0.94±0.22	1.05±0.10
3	Hydro-alcoholic extract	0.96±0.16	1.49±0.71
4	Petroleum-ether extract	1.05±0.10	1.31±0.28
5	Aqueous extract	1.09±0.27	1.31±0.38

7.1.9 Alkaloid content estimation

The estimate was given in mg/g of Atropine equivalent and calibration curve for atropine was plotted as ($Y= 0.0003x + 0.0005$, $R^2 = 0.9992$) (**Figure 17**). The alkaloid content was increased when compared to extracts made using the traditional approach in the USE ethyl-acetate, methanolic, and hydro-alcoholic extracts. The methanol extract had the most Alkaloid concentration (35.27 ± 22.47). **Table 13** displays the outcomes.

Accurately measured aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of Atropine standard solution was transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform. The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer (SHIMADZU UV-1800) against the blank prepared as above but without Atropine [238-239].

According to the study, ultrasonic-assisted extraction techniques significantly increased the extract's phenolic flavonoid and Alkaloid content. The ultrasonic-assisted extraction technique also found a quick and efficient solution for plant extraction. Compared to standard extraction techniques, USE approaches enhanced *Alpinia mutica* plant extract yield and phytoconstituent content, mainly phenolic flavonoid and Alkaloid.

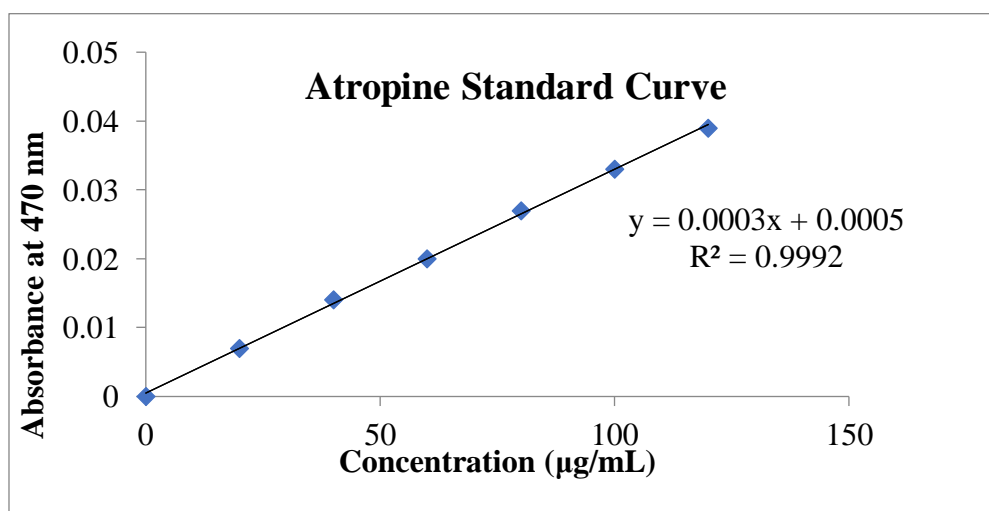


Fig-17: Atropine Standard Curve

Table 13: Total Alkaloid Contents of *Alpinia mutica*

S. No	Test samples (<i>Alpinia mutica</i>)	Atropine equivalent in mg/g for conventional extraction's alkaloid content.	USE the total alkaloid percentage in mg per gram of atropine.
1	Methanolic extract	5.91±2.34	35.27 ± 22.47
2	Ethyl acetate extract	4.69±1.82	27.57±16.2
3	Hydro-alcoholic extract	5.11±2.07	28.55±17.2

7.1.10 Thin Layer Chromatography of A.M extracts:

Different phytoconstituents in a crude extract can be separated using TLC analysis. Solvent systems for herbal extracts were selected through trial and error. After TLC plates were developed, compounds were seen under visible and ultraviolet light prior to and following the administering of the appropriate reagents. The following table contains TLC mobile phases and methods for detection and Rf Values for phytoconstituents in **Table 14, Figure 18**.

Table 14: Phytoconstituent detection solvent system optimisation.

Tests	Total Solvent system	Detection	Rf Values
Steroids	(Toluene): (Ethyl-acetate). (4:1; v/v)	Antimony-trichloride in Chloroform	0.71
Flavonoids	(Methanol): (Chloroform): and (Hexane) (7:2:1; v/v/v)	1 % ethanolic aluminium- chloride solution Detection under U.V (365 nm)	0.81
Phenolic compounds	(Ethyl acetate): (Formic acid): (Acetic-acid): and (water). (100: 11: 11: 26; v/v/v/v)	Detection under U.V (256 nm)	0.80



a) Steroids

b) Flavonoids

c) Phenolic compounds

Figure 18: TLC Analysis of *Alpinia mutica* Leaf Extract Phytoconstituents

7.1.11 Antioxidant activity

7.1.11.1 Reducing power by $FeCl_3$

Both samples and the standard solution contained 2.5 ml 1% potassium ferricyanide. 20 minutes in 50°C water. Using trichloroacetic acid as the solvent at concentrations ranging from 2.5 to 10%, the liquid was spun for ten minutes at 3000 rpm after cooling. One milliliter of 0.1% $FeCl_3$ and twenty-five milliliters of filtered water are administered for 10 minutes. The control was produced without samples. Measured absorbance at 700 nm. The graph shows results (**Table-15,16** and **Figure-19,20**)

Table -15: Scavenging activity power reduction technique:

Conc's (µg/ml)	Ascorbic acid.	Leaf Methanolic extract	Root Methanolic extract
0	0	0	0
20	0.18	0.12	0.1
40	0.34	0.29	0.25
60	0.5	0.39	0.32
80	0.7	0.53	0.44
100	0.85	0.55	0.5

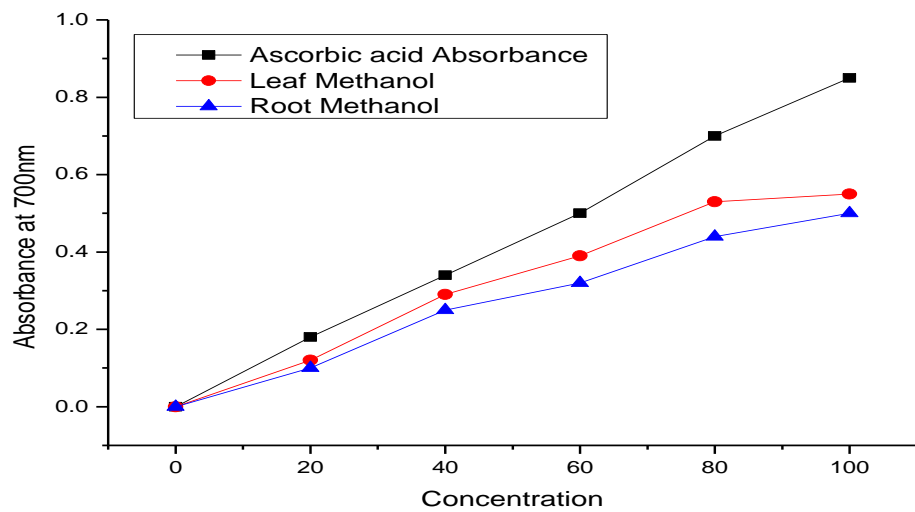


Fig-19: Reduced method Scavenging activity

Table-16: Reduced % Inhibition method: -

Conc. (µg/ml)	Ascorbic acid	Leaf methanol	Root methanol
0	0	0	0
20	83.33	75	70
40	91.17	89.65	88
60	94	92.3	90.63
80	95.71	94.23	93.18
100	96.47	94.54	94

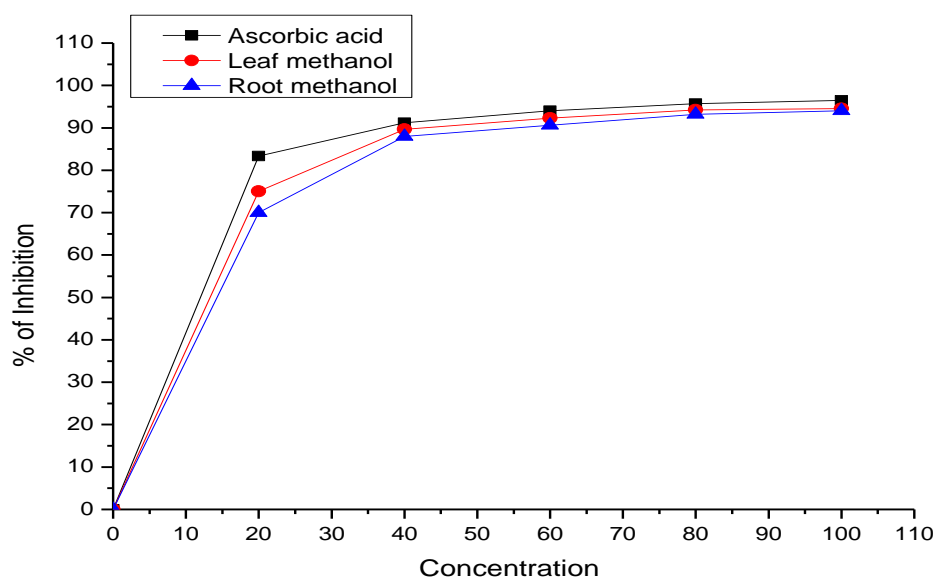


Fig-20: Reduced power method % Inhibition Activity

7.1.11.2 Free radical scavenging action in DPPH

We examined several test volume conc's (20, 40, 60, 80, and 100 g/ml), and for each dose level, 100 liters of the sample were diluted with methanol. A three-fold dilute DPPH solution fills each test tube. Immediately after adding 150 mL containing DPPH solution with 3 mL of methanol for the control measurement, the absorbance at 516 nm was measured. After 15 minutes, a UV and Visible-(Shimadzu, UV-1800, Japan) spectrophotometer with a methanol blank registered 516 nm absorption. It was established that The IC50 and the percentage reduction: There were

three runs of each experiment. The results are shown in (Table-17, 18, and Figures-21, 22).

Table -17: DPPH activity:

Conc'n (µg/ml)	A.A	Leaf Methanol	Root Methanol
0	0	0	0
20	0.1	0.11	0.13
40	0.08	0.12	0.14
60	0.06	0.1	0.12
80	0.05	0.08	0.09
100	0.03	0.05	0.07

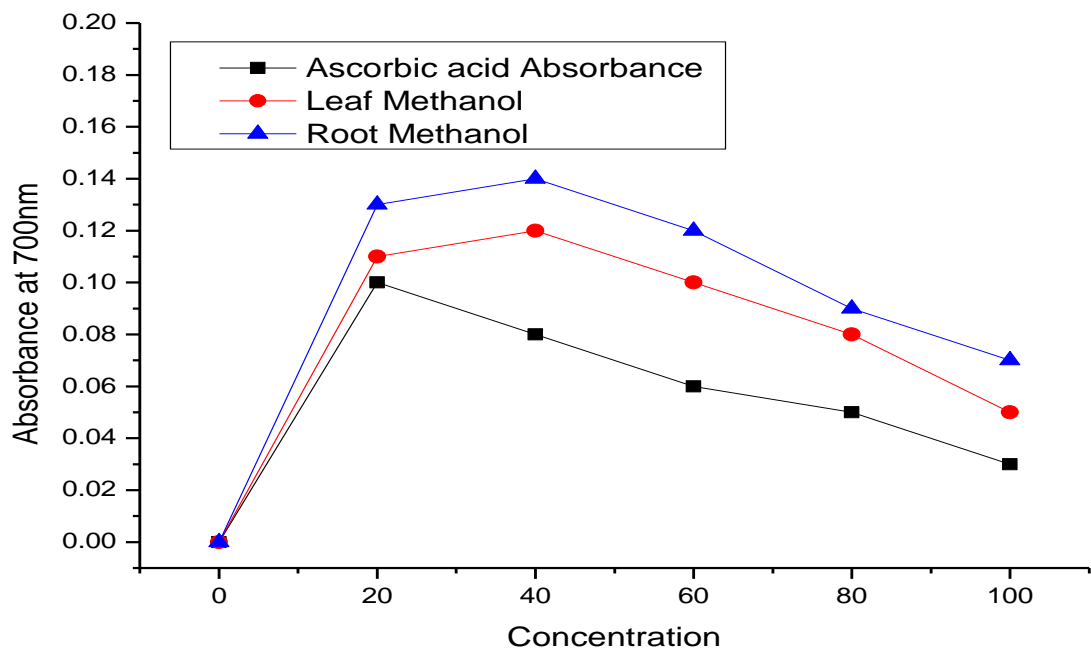


Fig-21: DPPH activity

Table-18: DPPH % Inhibition method:

Conc'n (µg/ml)	Ascorbic acid	Leaf Methanol extract	Root Methanol
0	0	0	0
20	37.5	31.25	18.75
40	50	25	12.5
60	62.5	43.75	25
80	68.75	50	43.75
100	81.25	68.75	56.25

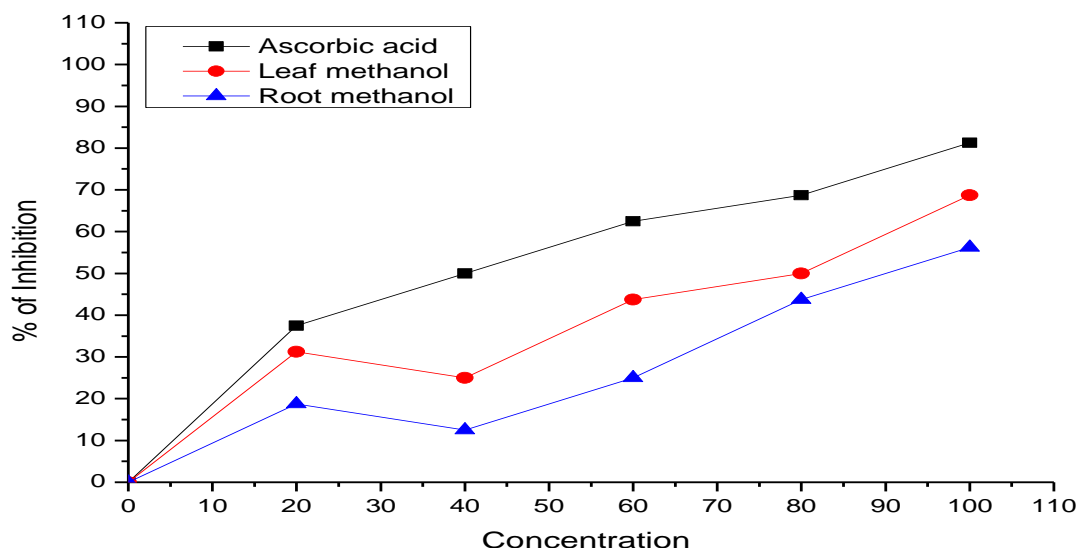


Fig- 22: DPPH Scavenging % Inhibition activity

7.1.11.3 Activity of Iron chelation

Iron chelation measures antioxidant activity. The extract, ascorbic acid (2 mL in 5% v/v methanol), ferric chloride (2 mL, 200 m), and O-Phenophtholin (1 mL, 0.05% w/v) were incubated at the temperature of the room for 10 minutes. Incubated solvent absorbance was 510 nm. Three tests were done. The graph shows results. (Table-19,20 and Figures-23, 24).

Table -19: Chelation activity of Iron.

Conc'n (µg/ml)	Ascorbic acid	Leaf Methanol	Root Methanol extract
0	0	0	0
10	0.07	0.06	0.06
20	0.13	0.12	0.1
30	0.19	0.17	0.14
40	0.25	0.21	0.16
50	0.3	0.24	0.17
100	0.6	0.26	0.18

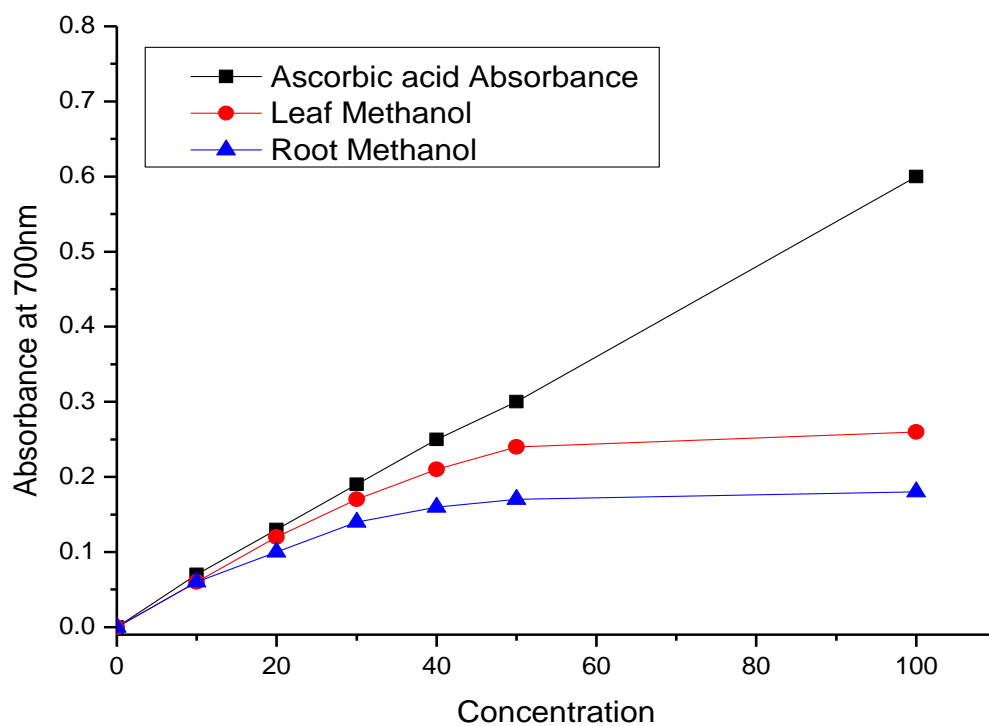


Fig-23: Iron chelation activity

Table-20: Chelation % Inhibition of Iron:

Conc'n (µg/ml)	Ascorbic acid	Leaf Methanol	Root Methanol
0	0	0	0
10	14.28571	0	0
20	53.84615	50	40
30	68.42105	64.70588	57.14286
40	76	70	62.5
50	80	75	64.70588
100	90	76.92308	66.66667

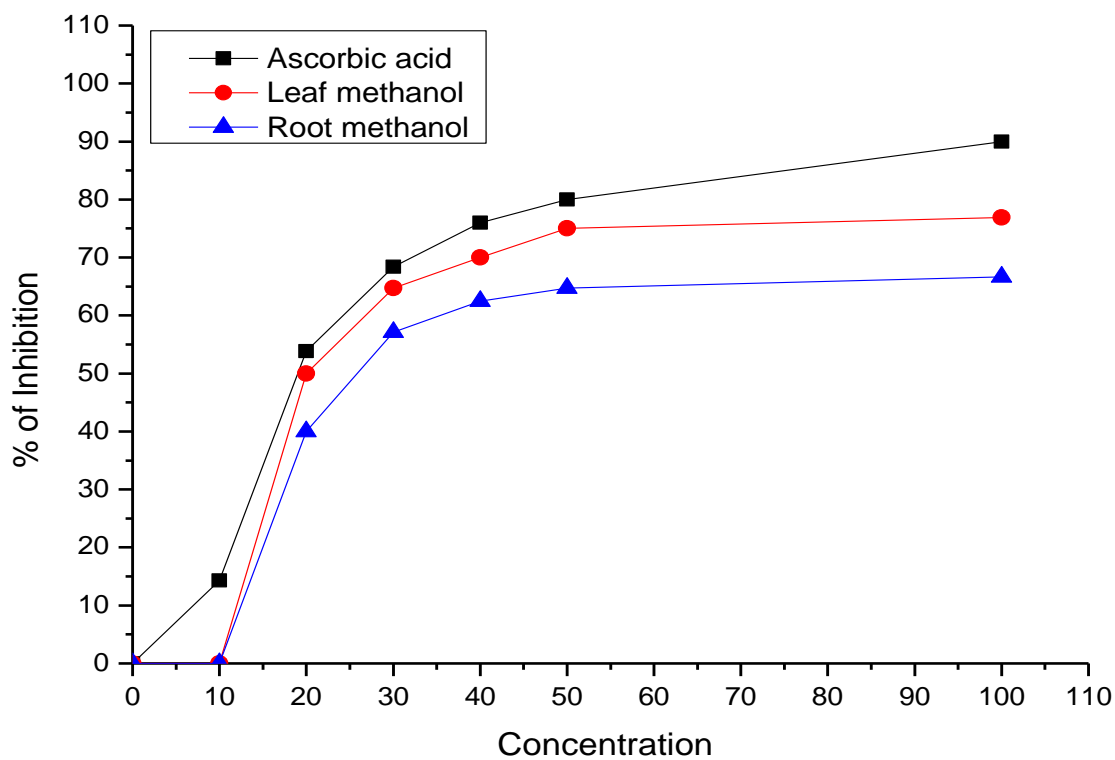


Fig-24: Iron chelation Inhibition activity

7.1.12 Green synthesis of nanoparticles of *Alpinia mutica*

7.1.12.1 Green synthesis of silver nanoparticles (Ag NPs)

Silver nanoparticles were green-synthesized. In magnetic stirrer-equipped conical flasks, 45 ml of 1 mM AgNO₃ solution was introduced dropwise to 10 ml of filter A.M. medication solution. Flasks were forcefully agitated for 0, 12, & 24 hours to produce Ag NPs. After 12–24 hours in 25^{0c} flasks, the solution became pale yellow to dark brown (**Figures 25 and 26**). For 20 minutes, 5000 rpm centrifuged the fully colored solution. Taking out the supernatant left residue. The residue was washed and dried with sterile distilled water.

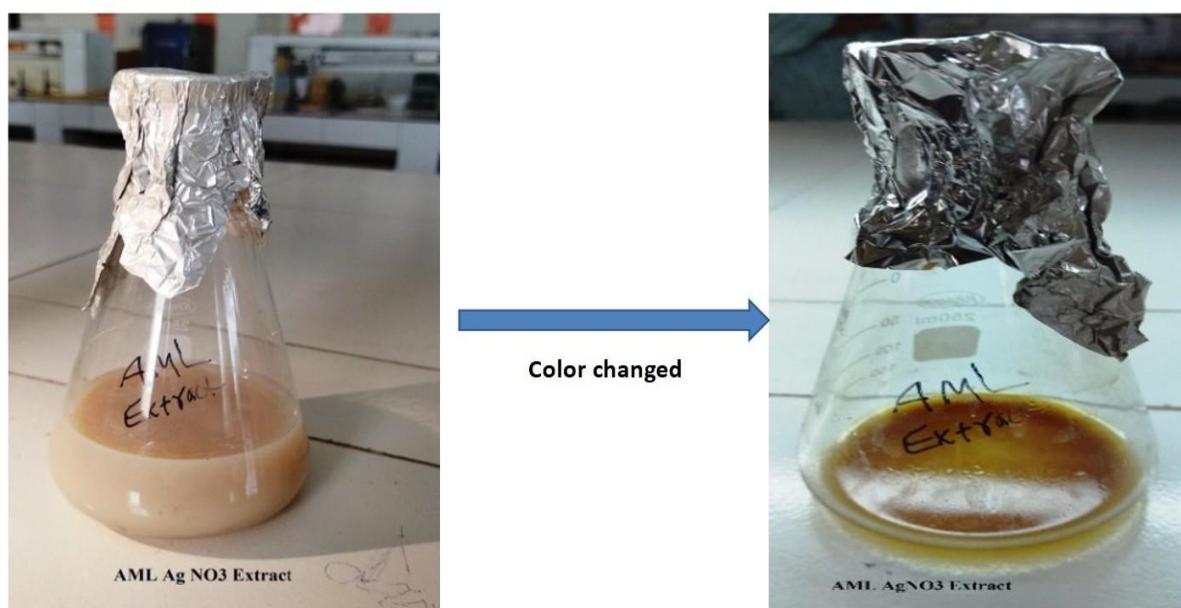


Fig- 25: Green synthesis of Nanoparticles

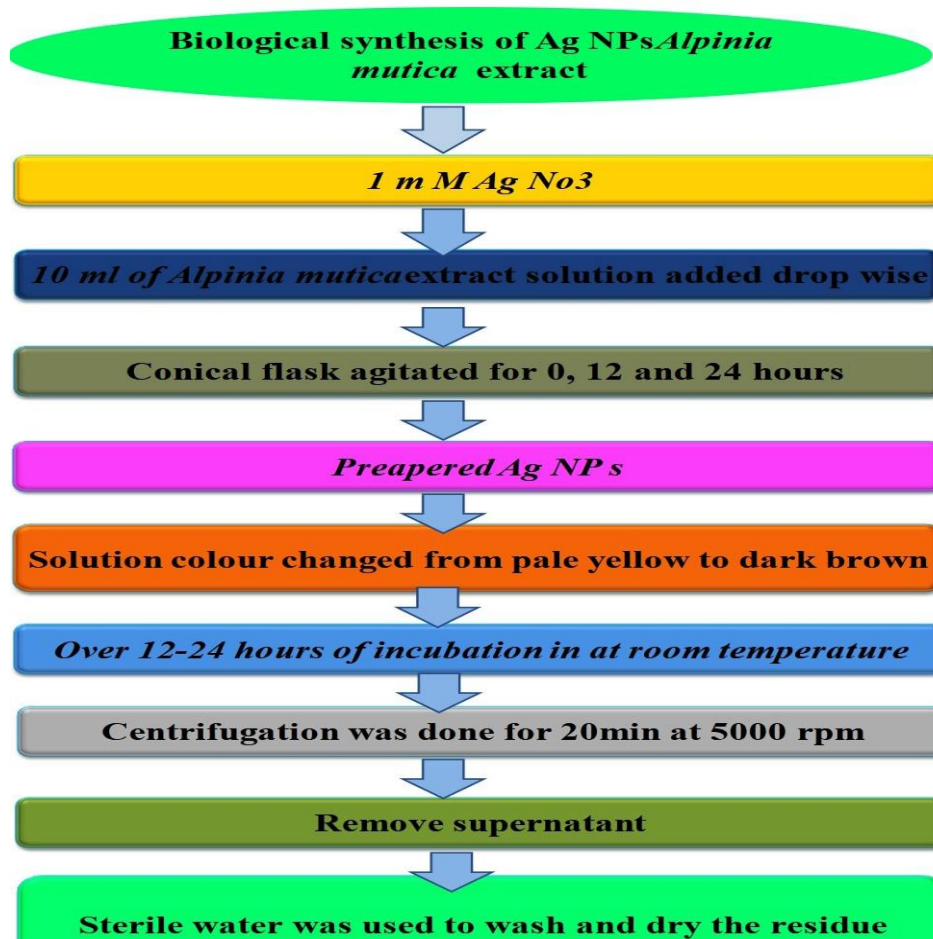


Fig- 26: Green synthesis of Ag NPs from *Alpinia mutica* extract

7.1.12.2 Green synthesis of zinc oxide nanoparticles (Zn O NPs)

Forcefully stirred 25 ml extraction with 0.1 M hexahydrate of zinc nitrate for 2 hours. The dirty colour precipitate was cooled for 24 hours after the process. The reaction solution separated from the precipitate after 15 minutes of 6000 rpm centrifugation. After repeatedly washing with deionized water, the dried product was heated at 80⁰C to remove impurities. After three hours of oxidation at 350⁰C in a muffle furnace, 5 μ L of Zn O Nanoparticle solutions was put on a copper-coated with carbon and chilled before entering the SEM. (Figure-26).

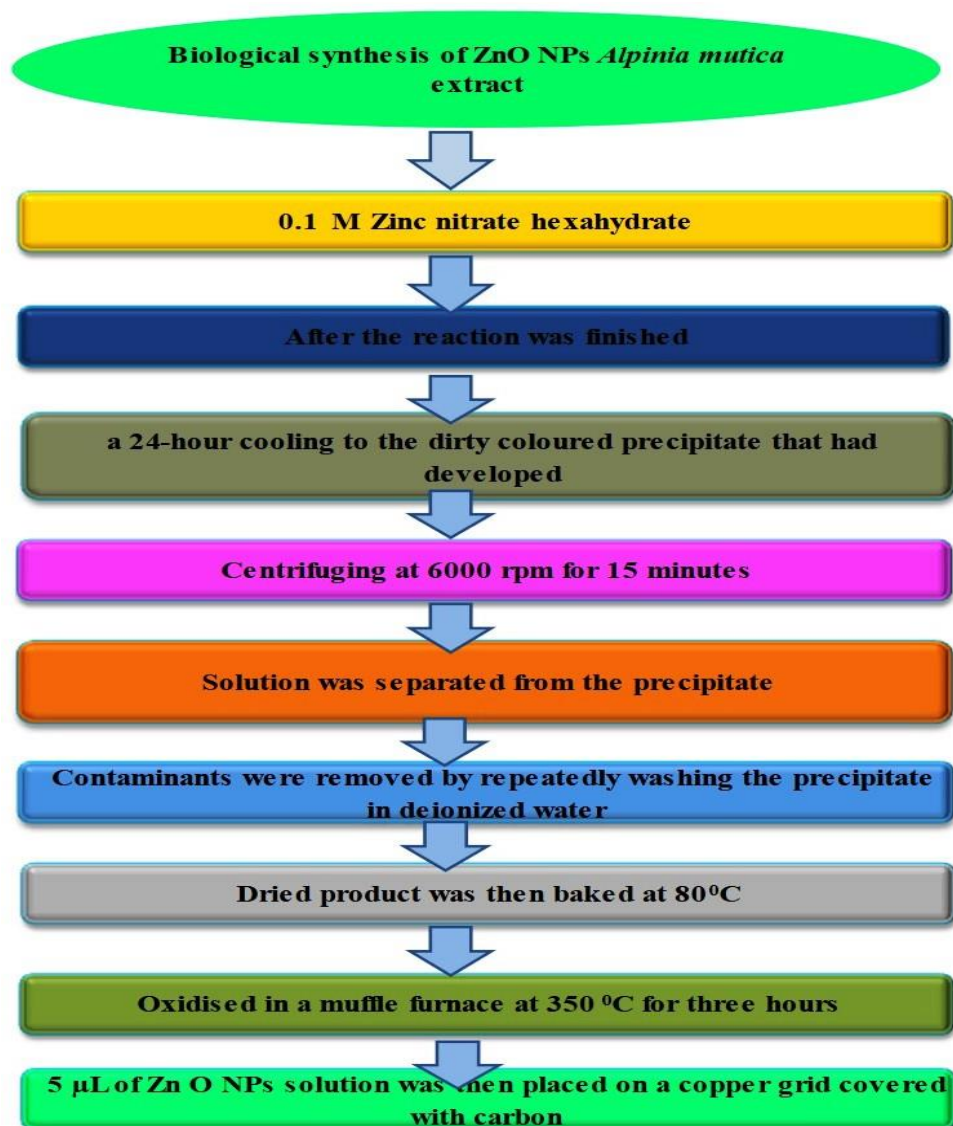


Fig-27: Green synthesis of Zn O NPs from *Alpinia mutica* extract

7.1.13 Characterization of green synthesized nanoparticles

Zn O and Ag nanoparticles' shape, functional group evaluation, the average size of particle dimension, and crystalline nature for A.M., Various tools, Zeta potential, particle size analyser, scanning electron microscopy, and X-ray diffraction, were used to determine the biologically synthesized nanoparticles, respectively.

7.1.13.1 Particle size and zeta potential

Malvern Instruments' DLS determined particle size and Zeta potential. A constant 90° angle and 25°C temperature was used to measure the particle size. The sample was made by dispersing the small particle solution in distilled water and via ultrasound for 6 minutes. At 25 °C and 150 V, zeta potential values were collected

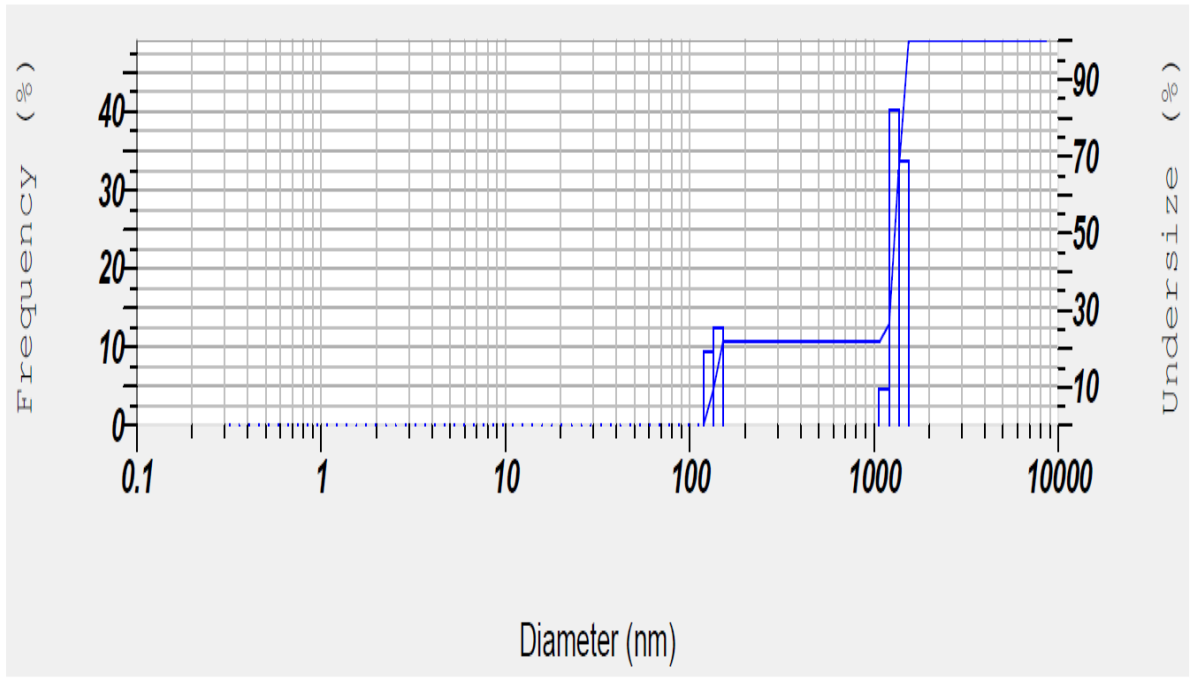
using electrophoretic light scattering. The zeta potential idea depends on the in-control conductivity concept for its formulation to be stable. Zeta potential readings and particle sizes of different nanoparticles are shown (**Figures. 28a, 28b, 28c, and 28d**).

a) AMLE-Ag (Particle size): The synthesized silver nanoparticles using plant extract of *Alpinia mutica* has analysed using particle size analyzer. From the results its clearly indicate that plant extract mediated silver *Alpinia mutica* nanoparticles average mean diameter is between 135 and 136 nm. So, the size of a nanoparticle needed for pharmacological action both in vivo and in vitro.

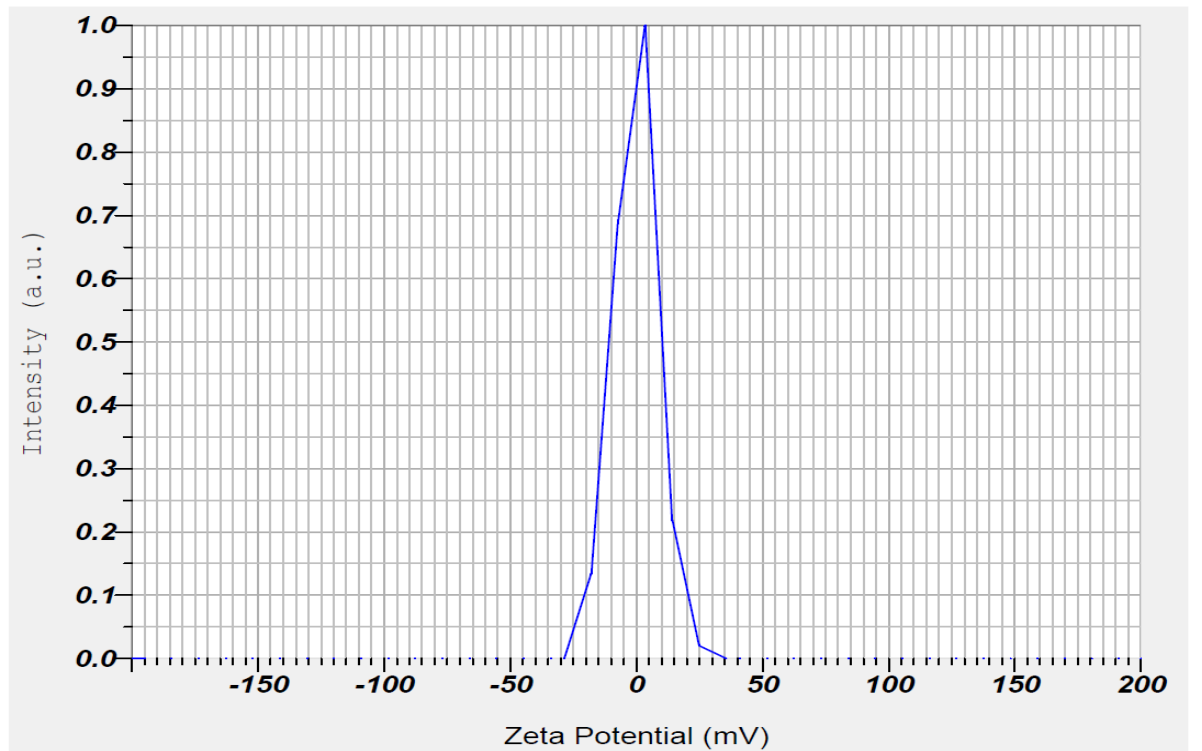
b) AMLE-Ag (Zeta Potential): The synthesized silver nanoparticles using plant extract of *Alpinia mutica* has analyzed using a zeta analyzer. The graphs of zeta potential indicate that *Alpinia mutica* plant extract-mediated silver nanoparticles have a zeta potential value of 2.3 mV. The results of zeta for *Alpinia mutica*-mediated silver nanoparticles depict that synthesized nanoparticles have average stability but are not too bad for performing the activities.

c) AMLE-Zn (Particle Size): The synthesized zinc oxide nanoparticles using plant extract *Alpinia mutica* has analyzed using a particle size analyzer. The results indicate that the average mean diameter of the *Alpinia mutica* plant extract-mediated zinc oxide nanoparticles are 244 nm. So, the size of nanoparticles for in-vitro and in-vivo pharmacological activity.

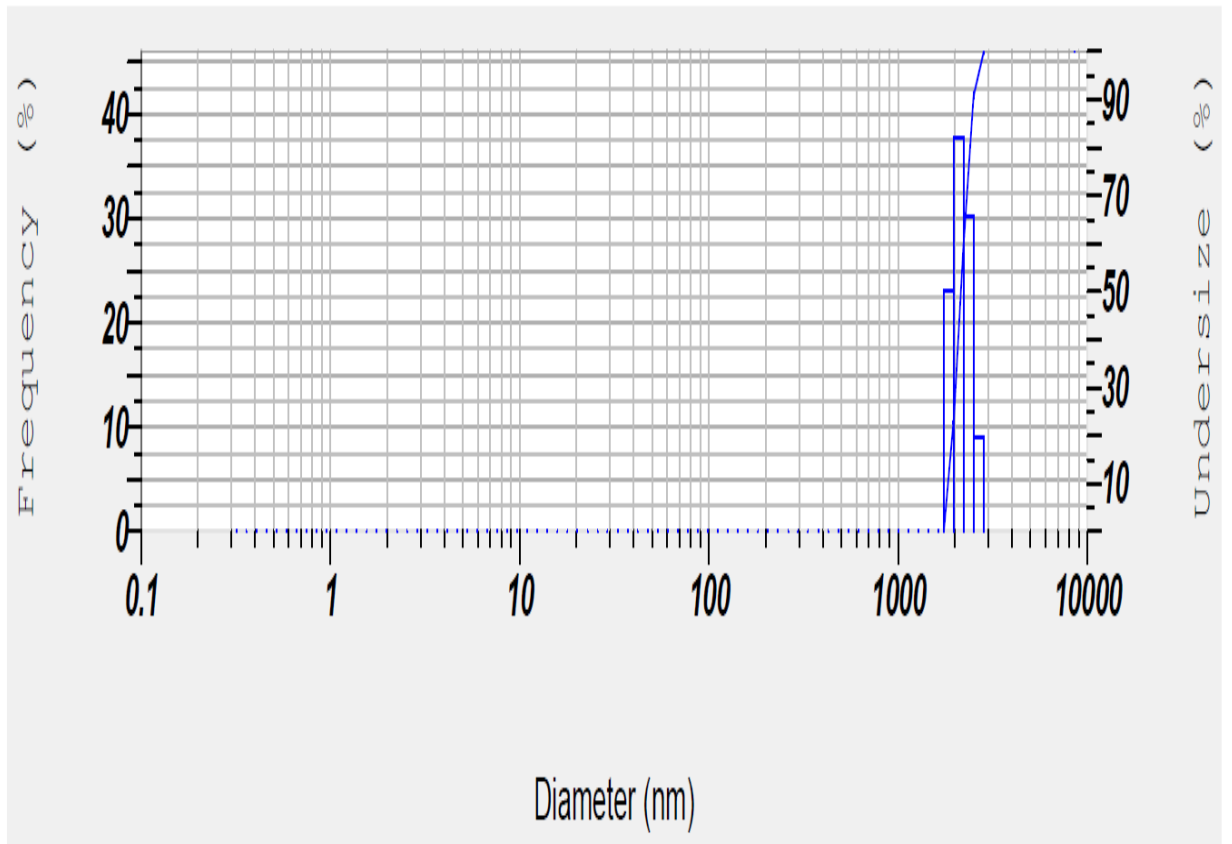
d) AMLE-Zn (Zeta Potential): The synthesized zinc oxide nanoparticles using plant extract *Alpinia mutica* has analyzed using zeta analyzer. The graphs of zeta potential indicate that *Alpinia mutica* plant extract mediated zinc oxide nanoparticles have the zeta potential value of -0.2 mV. From the results of zeta for *Alpinia mutica* mediated zinc oxide nanoparticles depict that synthesized nanoparticles have average stability.



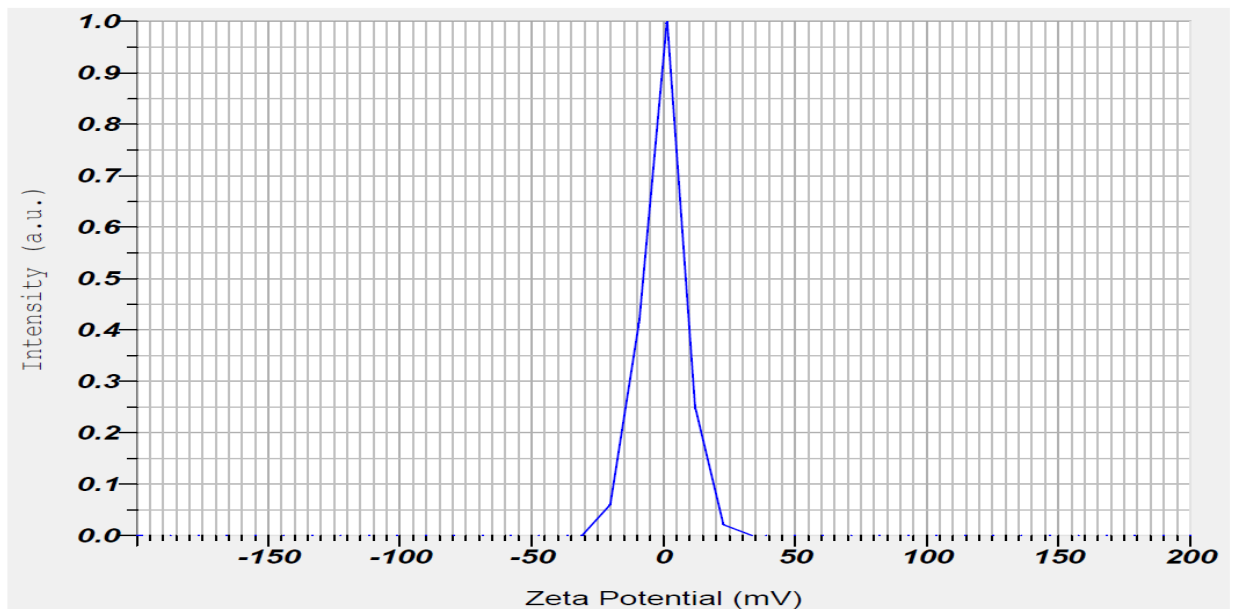
28a) AMLE-AG (Particle size)



28b) AMLE-AG (Zeta Potential)



28c) AMLE-Zn (Particle Size)



28d) AMLE-Zn (Zeta Potential)

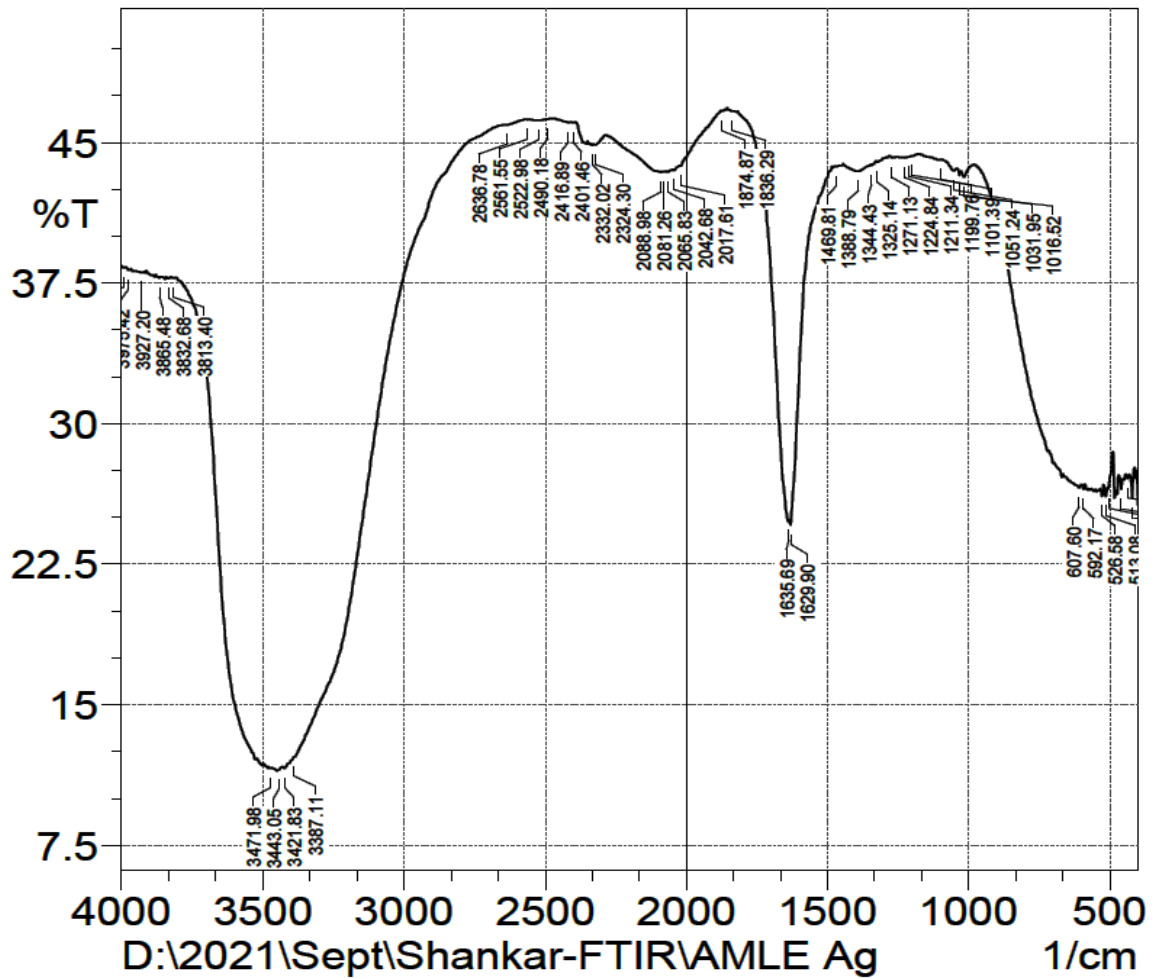
Fig- 28: Zeta potential readings and particle sizes of different nanoparticles

7.1.13.2 Fourier transform infrared spectroscopy: The FTIR spectrum of leaf-extract obtained before and after the formation of Ag NPs with ZnONP was analyzed in order to look into probable functional categories for the production of Ag NPs and ZnONP. FTIR was used to identify leaf extract compounds that reduced ions and sealing agents that maintained nanoparticle solution consistency. (**Figures 29a and 29b**).

a) AMLE Ag NP: To determine which functional category of the extract is crucial in the conversion of nitrates of silver into silver nanoparticles, FTIR tests have been carried out. The Ag, *Alpinia mutica* The FTIR spectrum revealed many peaks at 3927, 3832, 3813, 2636, 1874, and 1629 cm⁻¹. The -COOH bending of phytonutrients and phenols in the leaf extract components may cause peaks at 3927 and 3832.

b) AMLE Zn ONP: It is essential to classify the many groups with functions that produce nanoparticles. For this purpose, we have carried out FTIR experiments. *Alpinia mutica* Zn FTIR spectrum peaks were seen at 3975, 3907, 2636, 2366, 1641, 1525, 1629, and 1288 cm⁻¹. The H-H-bonded leaf extract compound's bending stretching or the stretching of phenolic compounds' -COOH or -OH groups may be responsible for the significant results at 3975, 3907, and 2636.

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Fig-29a) AMLE Ag NP

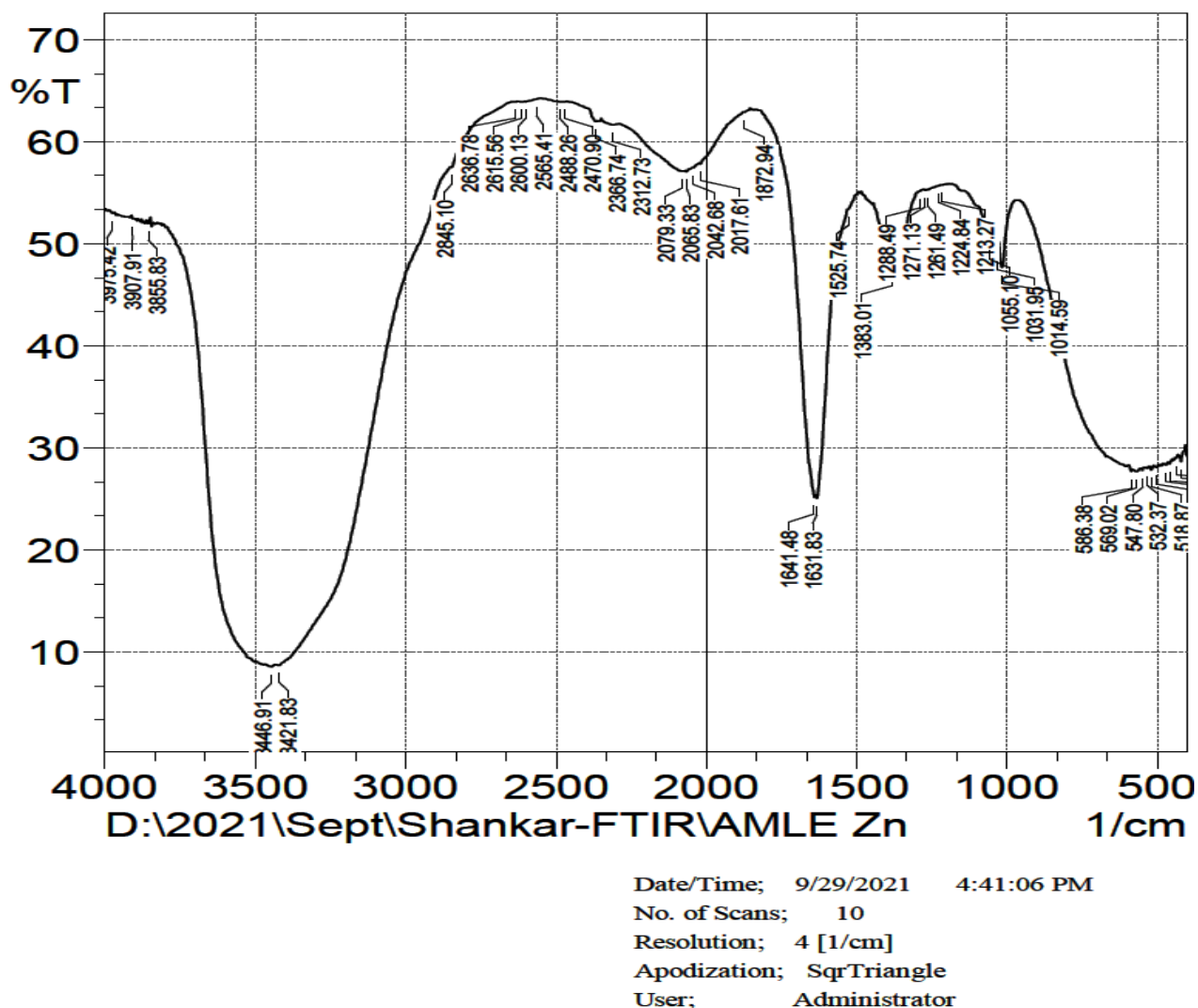


Fig- 29b) AMLE Zn ONP

Fig- 29: Fourier transform infrared spectroscopy

7.1.13.3 XRD analysis: To evaluate the produced nanoparticles' dimensional stability, an XRD examination was conducted. Nanoparticles were put into the PAN analytic X-ray diffractometer, operating at 4000 volts with a current of 20 mA. The scanning was done & using a 2θ range of 20° to 80° at a speed of 0.02° per minute and a 2-time constant. The crystal structures of all materials were improved to provide accurate atom positions (**Figures-30a and 30b**).

a) AMLE Ag NP: XRD analysis has been processed using plant extract of *Alpinia mutica*. The presence of peaks were at 2θ values 41.6° , 64.56° and 84.9° Correspond

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to (111), (200), (220) and (311) planes of Ag, respectively. The synthesis of Ag nanoparticles is seen in the comparatively large and broad XRD patterns.

b) AMLE Zn ONP: *Alpinia mutica* plant extract has been used to process XRD data. Peaks may be seen at 2 values of 44.6° , 64.56° , and 78.9° , which, respectively, correspond to the Zn planes (111), (200), (220), and (311). The existence of peaks at 2 values 27.80° , 32.27° , 46.25° , 57.65° , and 78.12° , which correspond to (111), (200), (220), (311), (400), and (311) is also seen in another spectrum. The comparatively broad and wide XRD patterns show the production of Zn nanoparticles. The kind of extracts employed for the synthesis technique had an impact on the size of the generated Zn crystals, which might be attributed to their therapeutic potential. It can also be reliant on the capping capabilities of the elements contained in the extracts.

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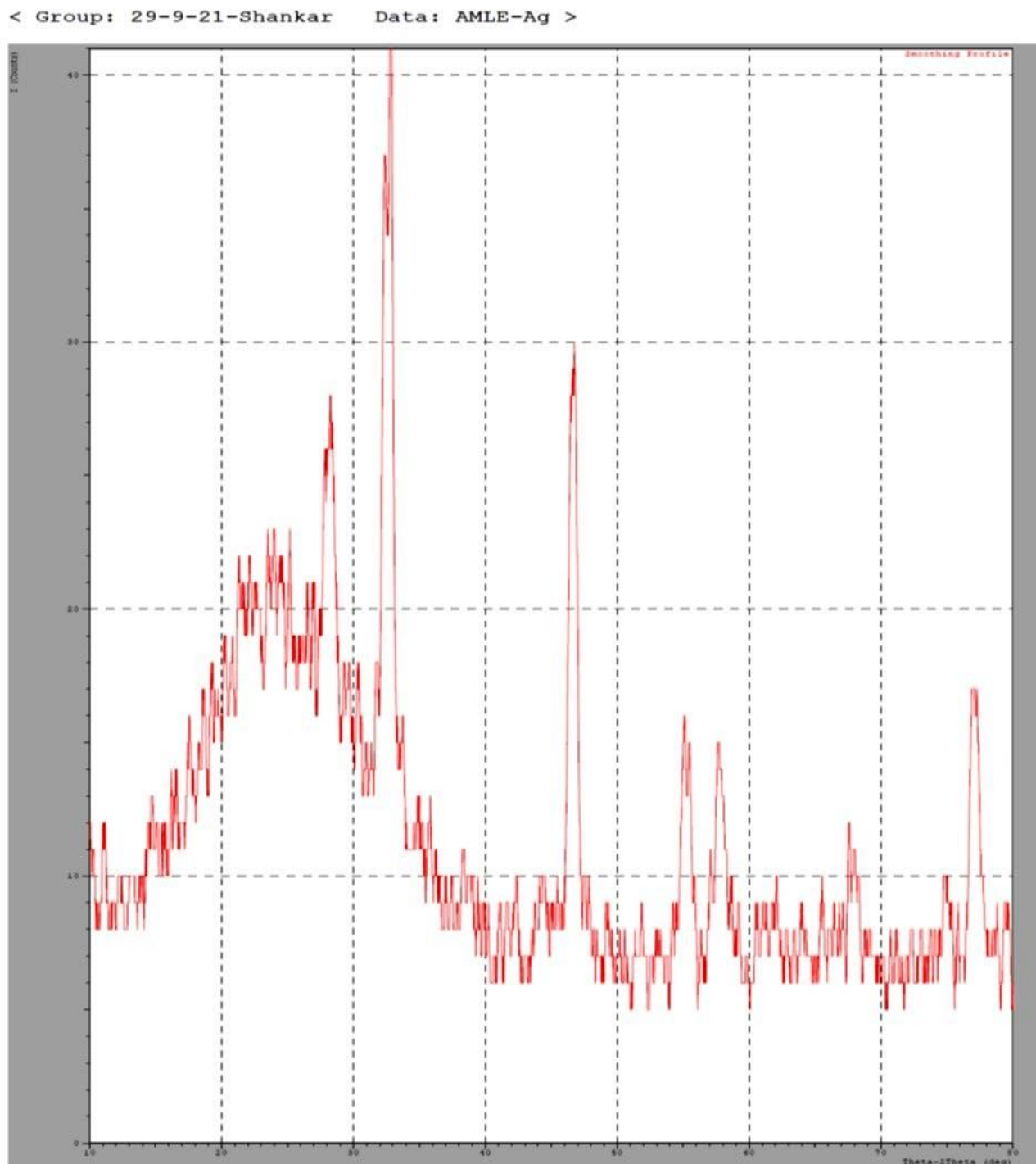


Fig-30a) AMLE-Ag NP

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< Group: 12-11-21-Shankar Data: AML-Zn >

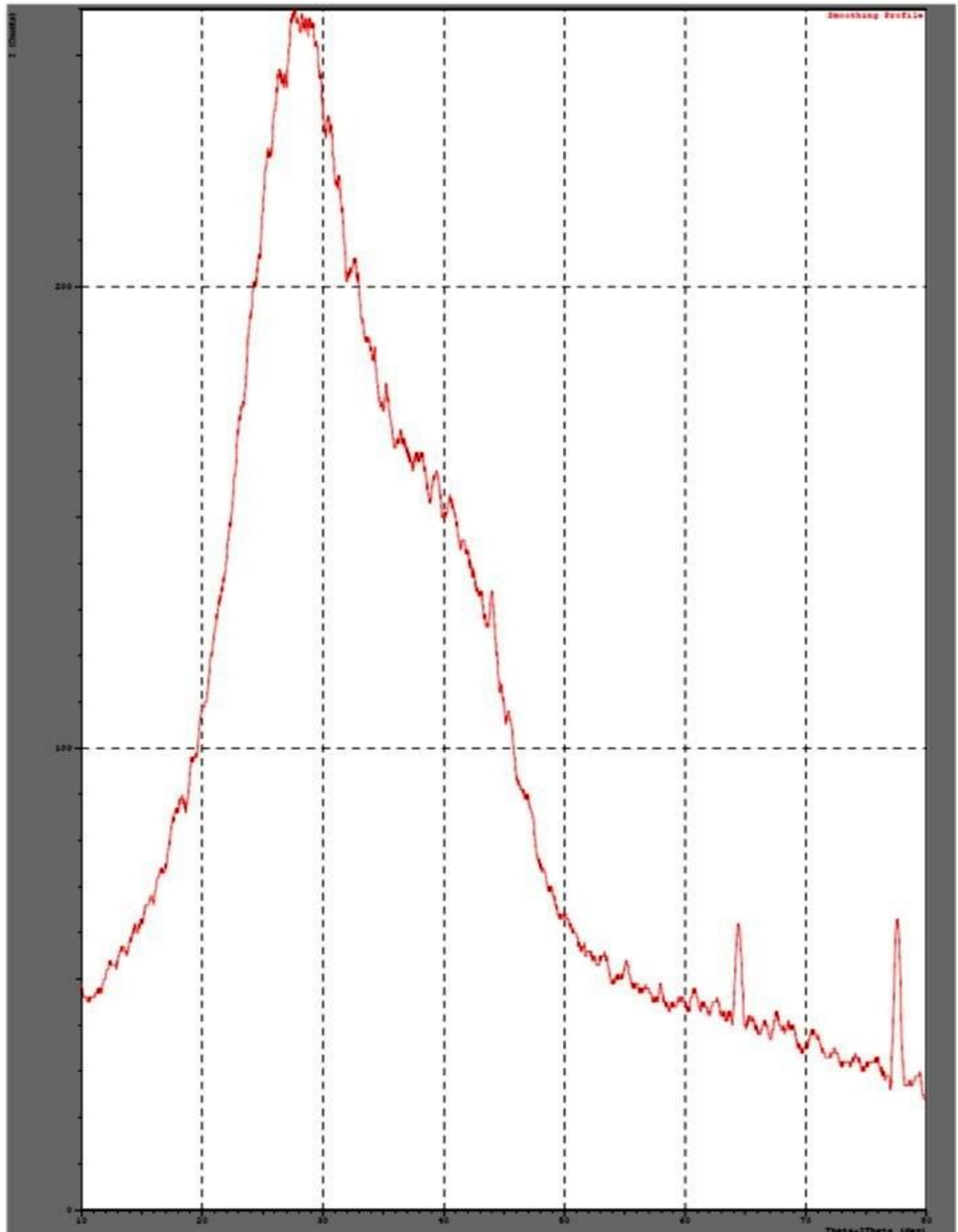


Fig-30b) AMLE Zn ONP

Fig-30: XRD analysis

7.1.13.4 Scanning electron microscopic analysis: Mirror-light microscopes and SEMs both work on the same principles. When an electron beam collides with a target sample's surface in a scanning electron microscope, it is reflected, recorded by the detector, and converted into an image. The plant extract was used for a capping agent in the creation of nanoparticles in this investigation (**Figures 31a and 32b**).

a) AMLE-Ag NP: The silver nanoparticles produced by the *Alpinia mutica* extract of leaves are spherical without aggregation, accessible, and vary in size from 80 to 100 nm.

b) AMLE-Zn O NP: The *Alpinia mutica*-mediated zinc oxide nanoparticles are arranged in an accessible manner and spherical without agglomeration, with the size of nanoparticles ranging from 80-100 nm.

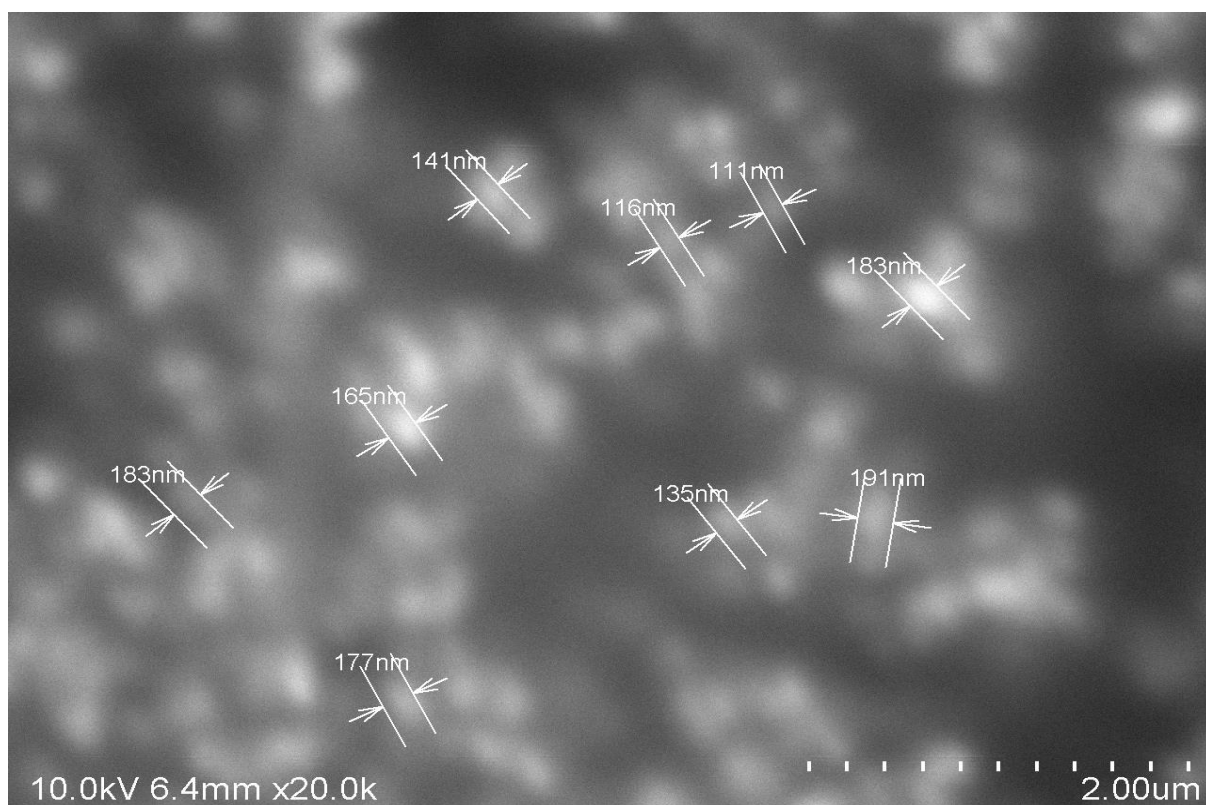


Fig- 31a) AMLE-Ag NP

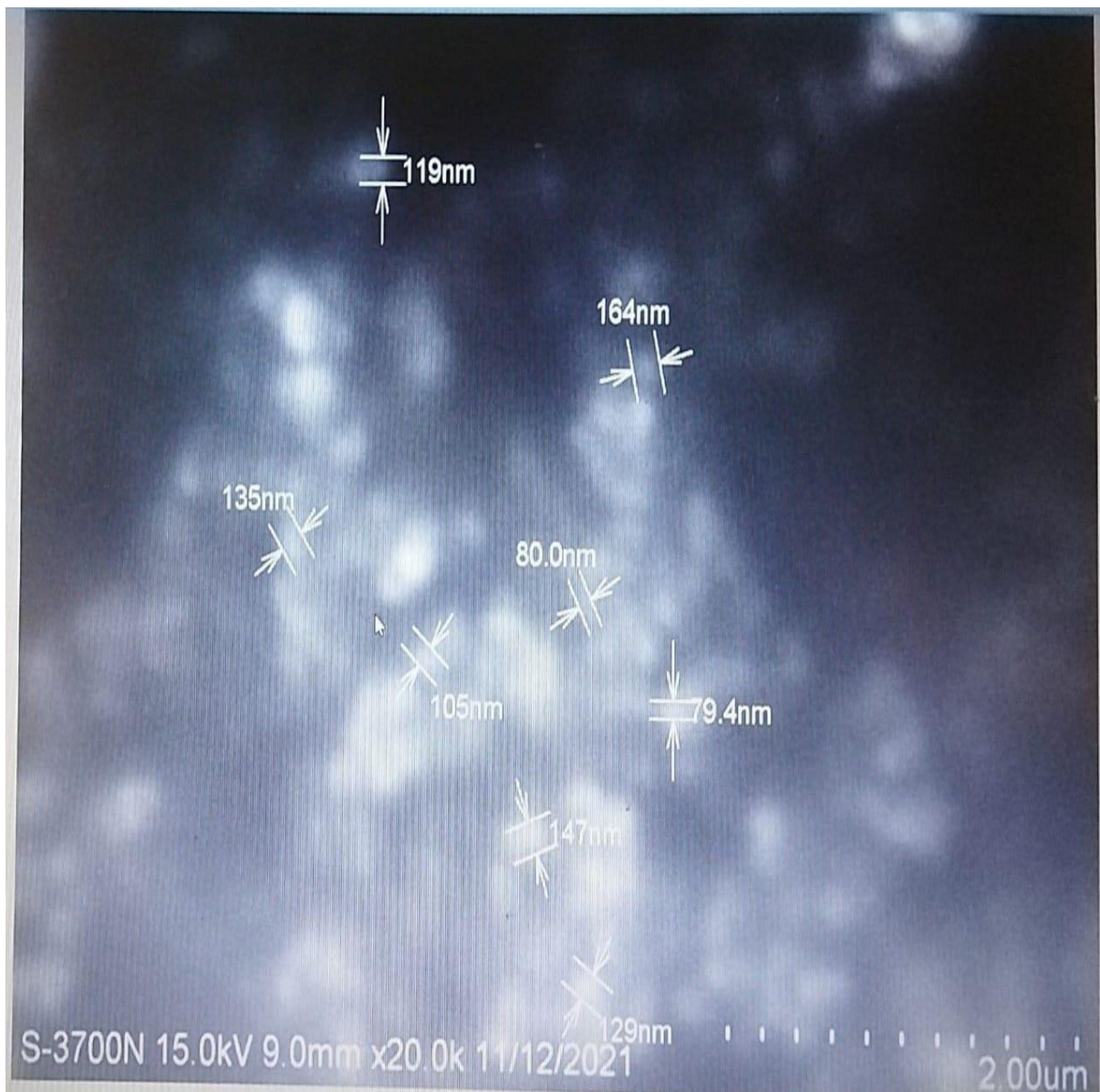


Fig- 31b) AMLE-Zn ONP

Fig- 31: Scanning electron microscopic analysis

7.1.14 In vitro antidiabetic activity

7.1.14.1 α -Amylase-inhibition activity:

Acarbose with alpha-amylase-inhibition AMLE SNP and AMLE Zn ONPs had similar in-vitro antidiabetic effects and inhibition percentages. IC 50 values below 100 $\mu\text{g/ml}$ indicate more decisive action, as shown in AMLESNP (73.72 $\mu\text{g/ml}$), AMLEZnONP (73.49 $\mu\text{g/ml}$), and Acarbose (87.26 $\mu\text{g/ml}$) IC 50 values (Tables 21 and 22, as well as Fig-32 and 33).

Table:21 α -Amylase inhibition-activity of AMLE SNP:

Conc'n ($\mu\text{g/ml}$)	AMLE SNP % Inhibition.	ACARBOSE % Inhibition
0	0	0
20	23.36	17.57
40	30.26	24.14
60	41.85	35.6
80	53.51	47.39
100	64.84	56.44
IC 50 Values	73.72	87.26

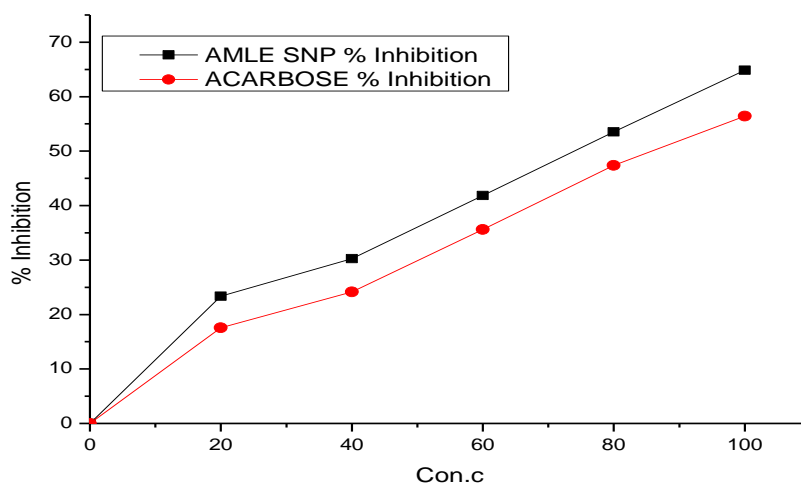


Fig-32: α -Amylase inhibition-activity of AMLE SNP

Table: 22 α -Amylase inhibition activity of AMLE Zn ONP

Conc'n (μg/ml)	AMLE Zn ONP % Inhibition	ACARBOSE % Inhibition
0	0	0
20	23.36	17.57
40	29.94	24.14
60	41.59	35.6
80	53.96	47.39
100	64.97	56.44
IC 50 Values	73.49	87.26

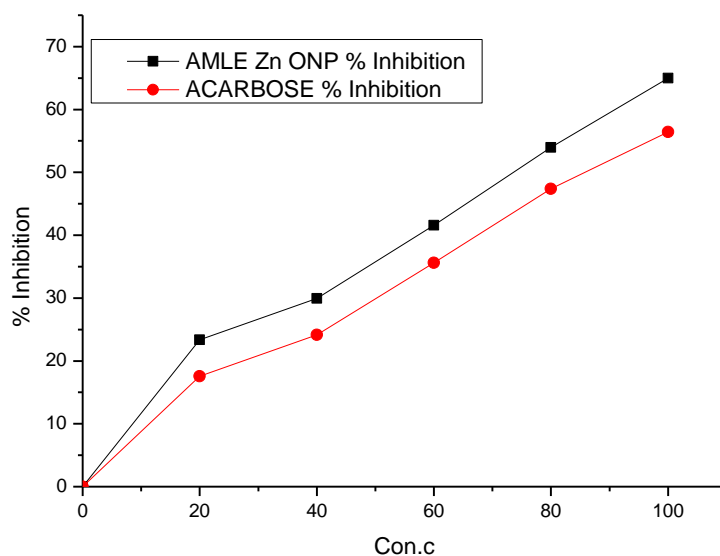


Fig-33: α -Amylase inhibition activity of AMLE Zn ONP

7.1.15 Acute toxicity study

The experiment complied with OECD recommendations 425, SNP, ZnO NP, AMLE, AMLE Ag NP, and AMLE ZnO NP at a 2000 mg/kg dosage. Neither the treatment group nor the vehicle control group had any fatalities. Observations were monitored throughout the 14-day research period, and all the animals were observed regularly.

7.1.15.1 Behavioural pattern and body weight

SNP and Zn ONP experienced tired and drowsy effects throughout the first four hours (**Table 23**). Both the treatment and vehicle control groups saw a slight rise in body weight during the acute toxicity assessment (**Table 24**).

Table 23 Behavioural pattern for the Various Nanoparticles of *Alpinia mutica*

Parameters	30 min						4 hours						24 hours						48 hours						7day						14 day											
	VC	SNP	ZONP	AMLE	AMLE SNP	AMLE ZONP	VC	SNP	ZONP	AMLE	AMLE SNP	AMLE ZONP	VC	SNP	ZONP	AMLE	AMLE SNP	AMLE ZONP	VC	SNP	ZONP	AMLE	AMLE SNP	AMLE ZONP	VC	SNP	ZONP	AMLE	AMLE SNP	AMLE ZONP	VC	SNP	ZONP	AMLE	AMLE SNP	AMLE ZONP						
Skin	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Eyes	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Salvation	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Respiration	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Urination (Color)	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Faces consistency	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Somatomotor activity and behaviour	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Sleep	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Mucous membrane	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Convulsions and tremours	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Itching	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Convulsions and tremours	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Coma	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Mortality	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

U: Unchanged; A: Absent; I: Increased; VC-Vehicle control; SNP- Silver nanoparticles; ZONP- Zinc oxide nanoparticle; AMLE-*Alpinia mutica* leaf extract; AMLE SNP- *Alpinia mutica* leaf extract silver nanoparticles; AMLE ZONP- *Alpinia mutica* leaf extract Zinc oxide nanoparticle.

Table 24 Effect of different *Alpinia mutica* nanoparticles on mice body weight (in grams)

Drug	Group	Day 1st	Day 7th	Day 14th
VC	I	29.14±0.57	28.8±0.47	29.19±0.55
SNP	II	29.45±0.48	28.8±0.37	29.2±0.54
Zn ONP	III	29.05±0.47	29.3±0.42	28.7±0.44
AMLE	VII	29.09±0.51	28.9±0.53	28.9±0.35
AMLE SNP	VIII	29.06±0.69	29.5±0.31	29.0±0.62
AMLEZn ONP	IX	29.2±0.45	28.8±0.70	29.0±0.61

Indicated values are shown as Mean ± SD N= 5;

7.1.15.2 Body-organ ratio index

In either group, organ-to-body weight index did not vary much. At a p.o. of 2000 mg/kg, no damage was found at the organ level in any of the groups (**Table 25**).

Table 25 Organ to body weight index

Drug	Group	Heart	Liver	Kidney
VC	I	0.739±0.102	6.510±0.159	1.540±0.113
SNP	II	0.737±0.101	6.550±0.135	1.530±0.100
Zn ONP	III	0.730±0.103	6.58±0.175	1.526±0.102
AMLE	VII	0.725±0.106	6.56±0.192	1.530±0.102
AMLE SNP	VIII	0.726±0.105	6.62±0.0.205	1.526±0.100
AMLEZn ONP	IX	0.728±0.105	6.61±0.196	1.500±0.109

The organ-to-body weight index is calculated as follows: (organ weight ×100)/body weight; values are shown as Mean SD, N = 5.

7.1.15.3 Biochemical analysis

All nanoparticles (AMLE, AMLESNP, and AMLE Zn ONP) increase total cholesterol, LDL, urea, creatinine, albumin, and AMLE, AMLESNP, and AMLE Zn ONP when compared to the vehicle control. Additionally, globulin and HDL levels dramatically improved in AMLE, AMLESNP, and AMLE Zn ONP and globulin levels. The biochemical results show that all Nanoparticles generate mild toxicity symptoms in albino mice at 2000 mg/kg, but no serious organ damage. (**Table 26**).

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Table 26 Biochemical evaluation of *Alpinia mutica* nanoparticles and extracts

S.NO	Parameters	Unit	VC	SNP	Zn ONP	AMLE	AMLE SNP	AMLE Zn ONP
1	GLUCOSE	mg/dl	93.71±1.71	93.39±2.52	95.33±3.01	95.55±2.71	94.75±2.18	93.97±2.23
2	TOTAL CHOLESTEROL	mg/dl	98.31±3.05	187.02±2.93	106.98±1.95	117.36±1.92	116.36±1.88	117.14±2.04
3	HDL CHOLESTEROL	mg/dl	30.70±1.72	42.75±2.44	32.93±2.23	30.44±1.15	32.48±2.67	31.93±1.94
4	LDL CHOLESTEROL	mg/dl	45.55±1.57	56.19±1.68	64.76±2.36	52.97±2.56	51.30±1.30	51.26±1.61
5	VLDL CHOLESTEROL	mg/dl	22.34±0.77	24.55±1.10	24.18±1.00	23.46±1.33	22.21±1.05	22.64±1.21
6	TRIGLYCERIDES	mg/dl	112.03±3.04	124.14±3.47	124.35±3.29	122.46±4.59	122.3±4.28	122.1±4.66
7	CHOL/HDL Ratio		3.11±0.12	4.38±0.24	3.26±0.22	3.86±0.19	3.60±0.30	3.68±0.23
8	LDL/HDL RATIO		1.48±0.06	1.31±0.075	1.981±0.18	1.743±0.11	1.74±0.11	1.61±0.11
9	UREA	mg/dl	37.12±2.07	47.83±1.90	47.97±1.48	48.23±2.04	47.44±1.06	43.59±1.76
10	CREATININE Level	mg/dl	0.54±0.02	0.54±0.02	0.55±0.02	0.53±0.02	0.54±0.02	0.53±0.01
11	BIT	mg/dl	0.67±0.06	0.75±0.06	0.74±0.06	0.74±0.06	0.74±0.06	0.74±0.06
12	BID	mg/dl	0.26±0.05	0.25±0.05	0.25±0.05	0.25±0.05	0.25±0.05	0.25±0.05
13	BII	mg/dl	0.42±0.02	0.42±0.05	0.42±0.05	0.42±0.05	0.42±0.05	0.42±0.05
14	PROTEIN	mg/dl	6.27±0.27	6.28±0.35	6.35±0.26	6.26±0.21	6.41±0.31	6.27±0.26
15	ALBUMIN	mg/dl	2.27±0.08	2.81±0.22	2.78±0.21	2.74±0.22	2.75±0.21	2.77±0.21
16	GLOBULIN	mg/dl	4.00±0.25	3.57±0.32	3.63±0.27	3.55±0.23	3.49±0.28	3.54±0.14
17	A: G/RATIO		0.653±0.09	0.77±0.136	0.77±0.10	0.77±0.10	0.78±0.10	0.78±0.05
18	SGOT/AST	IU/L	95.92±1.55	131.64±1.63	140.62±7.81	138.47±5.84	138.26±6.11	137.33±6.30
19	SGPT/ALT	IU/L	65.93±5.32	95.53±4.40	95.98±4.60	81.59±8.52	80.08±5.63	80.14±5.39
20	ALP	IU/L	94.88±6.62	125.26±5.36	126.61±5.57	118.68±5.58	108.38±5.89	107.38±3.59

Values are as a Mean ±SD, with a sample size of 5, and statistical analysis was carried out using the one-way ANOVA test. P <0.050, AMLE stands for *Alpinia mutica* leaf extract, SNP for silver nanoparticles, and Zn ONP for zinc oxide nanoparticles.

RESULTS & DISCUSSION

7.1.15.4 Haematological analysis

All the nanoparticles, including AMLESNP and AMLE Zn ONP, induce considerable increases in HGB, HCT, RBC, MCV, MCH, MCHC, MPV, PLT, and P-LCR levels. WBC and PCT levels rise significantly in AMLESNP and AMLE Zn ONPs. In albino mice, all NPs caused either minor poisoning or no symptoms, according to a hematologic investigation. (Table 27)

Table 27 Hematological evaluation of *Alpinia mutica*'s different Nano formulations

S.NO.	Parameters	Units	VC	SNP	Zn ONP	AMLE	AMLE SNP	AMLE Zn ONP
1	HGB	g/dl	13.36±0.07	14.38±0.06	14.38±0.08	13.36±0.05	13.36±0.05	13.37±0.09
2	RBCs	106/ul	8.48±0.07	9.17±0.08	9.21±0.05	9.10±0.03	9.13±0.06	9.10±0.04
3	HCT	%	44.47±0.91	48.52±0.39	49.75±0.08	45.60±0.21	45.88±0.36	45.74±0.21
4	MCV	fL	52.76±0.52	53.9±0.10	53.69±0.14	52.42±0.12	51.47±0.16	51.52±0.17
5	MCH	Pg	15.71±0.11	15.73±0.02*	15.72±0.06*	15.19±0.01	15.19±0.01	15.20±0.01
6	MCHC	g/dl	29.88±0.06	29.52±0.08	29.47±0.01	30.19±0.11	30.20±0.09	30.19±0.11
7	RDW-SD	fL	19.69±0.11	22.17±0.07	22.25±0.11	21.78±0.07	21.77±0.07	21.35±0.12
8	RDW-CV	%	19.10±0.05	19.20±0.07	19.19±0.07	19.80±0.09	19.85±0.05	19.86±0.04
9	WBCs	103/ul	4.94±0.03	4.94±0.02	4.94±0.03	4.80±0.04	4.79±0.02	4.79±0.04
10	NEUT%	%	20.45±0.41	20.79±0.45	20.78±0.38	20.80±0.33	20.80±0.40	20.80±0.41
11	LYMPH%	%	76.39±0.33	75.58±0.33	75.61±0.33	74.93±0.41	75.09±0.54	75.14±0.59
12	MONO%	%	1.00± 0.12	1.44± 0.10	1.42± 0.08	1.51± 0.08	1.50± 0.08	1.51± 0.08
13	EO%	%	2.18± 0.12	2.71± 0.08	2.75± 0.09	2.64± 0.05	2.65± 0.08	2.65± 0.08
14	BASO%	%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	IG%	%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16	NEUT#	103/ul	1.01± 0.02	1.02± 0.03	1.02± 0.03	1.00± 0.02	1.00± 0.04	1.01± 0.04
17	LYMPH#	103/ul	3.78± 0.03	3.79± 0.06	3.79± 0.04	3.61± 0.01	3.60± 0.01	3.60± 0.01
18	MONO#	103/ul	0.05± 0.01	0.07± 0.00	0.07± 0.00	0.07± 0.00	0.07± 0.00	0.07± 0.00
19	EO#	103/ul	0.11± 0.01	0.13± 0.00	0.13± 0.00	0.13± 0.00	0.13± 0.00	0.13± 0.00
20	BASO#	103/ul	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
21	IG#	103/ul	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22	PLT	103/ul	693.48 ± 2.35	785.84 ± 4.00	786.38 ± 4.00	764.30 ± 2.52	764.09 ± 3.90	765.69 ± 2.44
23	PDW	fL	6.75 ± 0.05	7.14 ± 0.04	7.16 ± 0.04	6.75 ± 0.05	6.75 ± 0.05	6.75 ± 0.05
24	MPV	fL	6.25 ± 0.05	7.03 ± 0.03	7.04 ± 0.03	6.67 ± 0.06	6.65 ± 0.06	6.65 ± 0.06
25	P-LCR	%	3.04 ± 0.01	4.70 ± 0.04	4.74 ± 0.04	4.19 ± 0.08	4.18 ± 0.08	4.18 ± 0.08
26	PCT	%	0.51 ± 0.02	0.66 ± 0.03*	0.83 ± 0.03*	0.52 ± 0.01	0.51 ± 0.01	0.52 ± 0.01

Values are presented as Mean ± SD, with a sample size of 5, and statistical analysis was carried out using the one-way ANOVA test. * Denotes a significance level of P <0.050 compared to the vehicle control. AMLE stands for *Alpinia mutica* leaf extract, SNP for silver nanoparticles, and Zn ONP for zinc oxide nanoparticles.

7.1.15.5 Histopathology analysis

At 2000 mg/kg, p.o., AMLESNP, AMLE Zn ONP, and AMLE Extract NPs did not show serious organ markers of toxicity. **Figures 34, 35, and 36** summarise the cardiac, kidney, and liver histology findings. According to the findings, AMLESNP, AMLE Zn ONP, and AMLE NPs only have a mildly toxic effect on the heart, liver, and kidney. However, AMLE Zn ONPs have a mildly toxic to moderately toxic effect on the organ level. Research on biochemical and hematological variables and the organ-to-body weight index supports these results. All plant NPs are thus classified as having acute oral toxicity as category 5 under the GHS (Globally Harmonized System of Classification category).

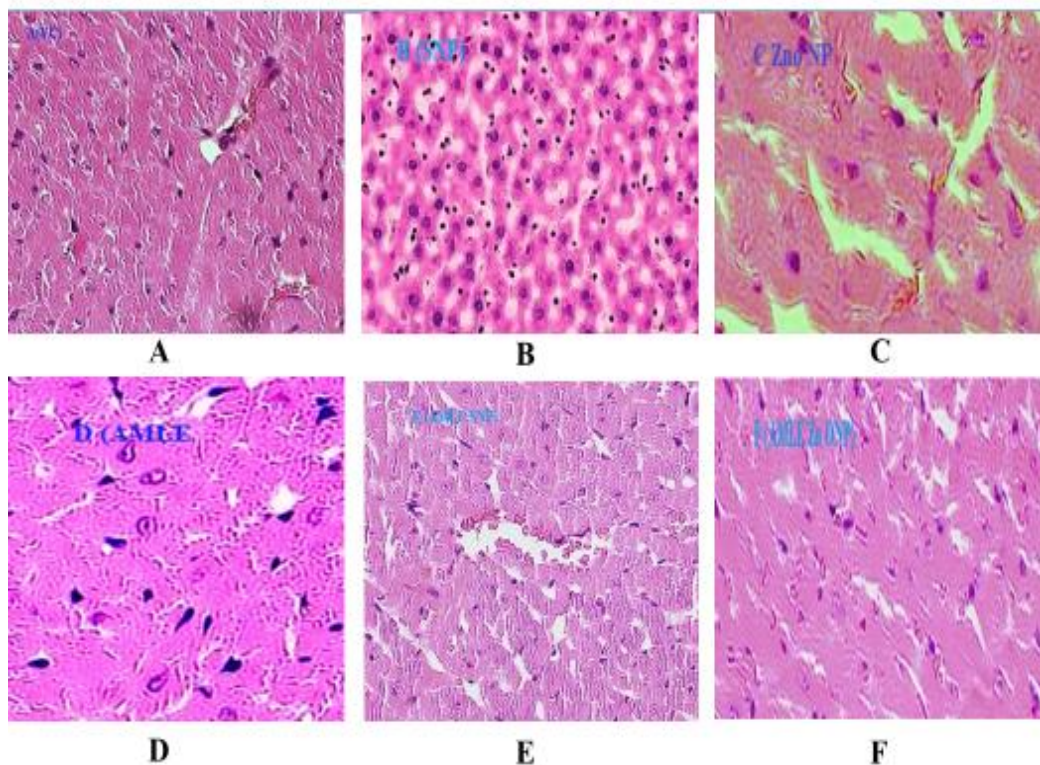


Fig-34: Heart histopathological observations, A (VC): Nothing abnormal detected; B (SNP): Moderate myocardial fatty fiber infiltration; C (ZnONP): Moderate myocardial fatty fiber infiltration; D (AMLE): Moderate myocardial fatty fiber infiltration; E (AMLE SNP): Myocardial fibers have mild granular degeneration; F (AMLE Zn ONP): have mild granular degeneration.

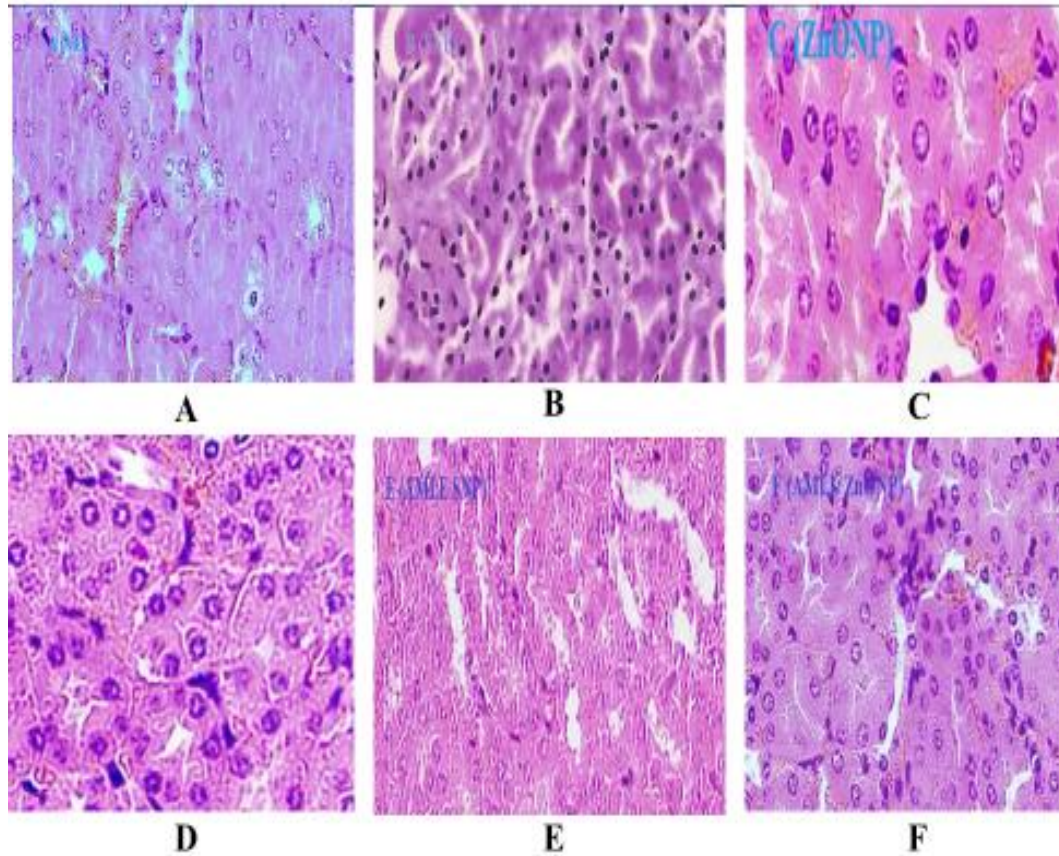


Fig-35: Kidney histopathological observations, A (VC): There is no growth in tissues; B(SNP): Tubular cells of the epithelium have mild degeneration; C (Zn ONP): Moderate tubular epithelial cell degeneration; D (AMLE): Mild tubular like epithelial cell degeneration. E (AMLE SNP): Moderate tubular epithelial cells necrosis and granular degeneration. F (AMLE Zn ONP): Moderate tubular epithelial cell necrosis and granular degeneration

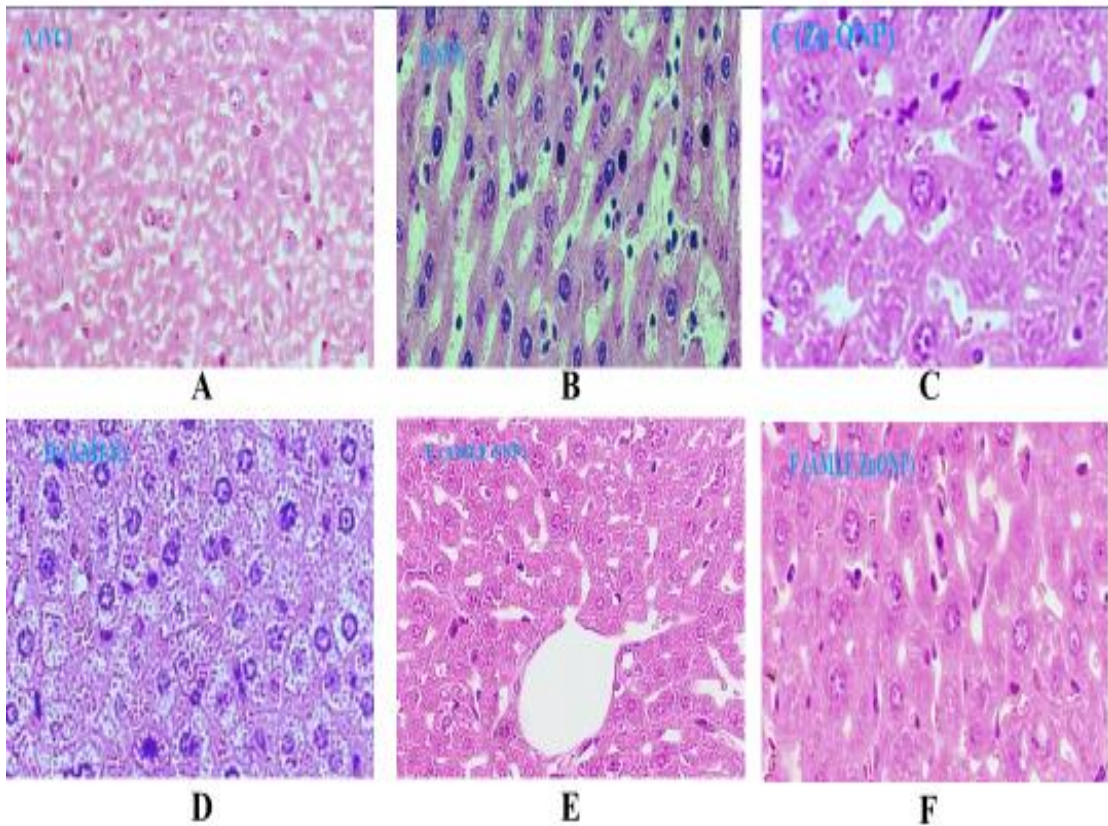


Fig-36: Observations on liver histopathology, A (VC): There is no growth in tissues; B(SNP):Hepatocytes of the liver with minimal granular degeneration; C (ZnONP): Moderate granular degeneration in liver hepatocytes; D (AMLE): Liver hepatocytes show mild granular degeneration.; E (AMLE SNP): Hepatocytes with mild-to-moderate granular degeneration; F (AMLE ZnONP): Mild-to-moderate granular liver hepatocytes..

7.1.16 In-vivo Antidiabetic activity

7.1.16.1 Effect on Body weight

The animals' body weights were recorded on days 1, 15, 22, and 29 (Table 28). On the fifteenth day after a two-week nutritional adjustment, rats given the High-fat diet, as opposed to rats given the Normal pellet diet, showed a substantial difference in body weight. The onset of diabetes is most likely to cause moderate weight loss in all groups given a high-fat diet on the 22nd day after one week of STZ injection. In comparison, the body weight did not significantly decrease from the 22nd to the 29th day.

Table 28 Effect of HFD and STZ model on to the body weight in Albino Wister rats

Group number	Drug treatment	1st Day	15th Day	22nd Day	29th Day
I	Saline 0.5% w/v CMC (p.o.)	254.19 ± 6.03	258.97 ± 6.83	260.90 ± 6.65	260.40 ± 6.12
II	Metformin, 50 mg/kg body weight (p.o.)	249.29 ± 8.75	296.29 ± 7.32	271.48 ± 6.28	258.24 ± 6.09
III	STZ (40g /kg b.w) (i.p.)	249.92 ± 9.60	293.16 ± 8.28	278.86 ± 9.91	256.20 ± 6.09
IV	AMLE 200 mg /kg b. w (p.o.)	252.29 ± 8.97	300.34 ± 6.00	279.29 ± 5.58	269.11 ± 6.18
V	AMLE 400 mg /kg b.w (p.o.)	225.06 ± 5.86	257.25 ± 7.46	253.12 ± 5.05	250.51 ± 4.46
VI	AMLESNP 100 mg /kg b. w (p.o.)	211.97 ± 4.70	230.9 ± 5.07	240.25 ± 9.60	229.54 ± 6.92
VII	AMLESNP 200 mg /kg b. w (p.o.)	210.56 ± 6.97	223.87 ± 5.92	239.33 ± 6.20	230.86 ± 4.41
VIII	AMLE ZnONP 100 mg /kg b.w (p.o.)	210.34 ± 5.56	231.00 ± 5.54	239.97 ± 6.67	229.58 ± 6.65
IX	AMLE ZnONP 200 mg /kg b.w (p.o.)	210.74 ± 4.46	231.55 ± 5.62	239.87 ± 5.43	234.13 ± 6.07

NPD is - Normal pellet-diet, while HFD - is a high-fat diet. STZ stands - for streptozotocin, AMLE stands - for *Alpinia mutica* extract, AMLE SNP stands - for *Alpinia mutica* extract Ag nanoparticles, and AMLE Zn ONP stands - for *Alpinia mutica* extract zinc oxide nanoparticles. The values are mainly given as Mean ± SD, n=6. Statistical analysis was done using a one-way ANOVA test; # denotes p< 0.05 compared—the first day of therapy.

7.1.16.2 Biochemical evaluation

On days 22 and 29, **Table 29** compares plasma glucose, cholesterol, and triglycerides. All HFD+STZ groups had a substantial rise in glucose, cholesterol levels, and triglycerides on day 22 after seven days of STZ injections, indicating type 2 diabetes. On the 29th day of treatment, AMLE SNP & AMLE Zn ONP at 200 mg/kg, p.o. reduced glucose, cholesterol, and triglycerides more than metformin and the experimental group.

The control group, in comparison to the experimental group, did not show a significant difference in AMLE 200 mg/kg, p.o., AMLE 400 mg/kg, AMLE SNP 100 mg/kg, AMLE SNP 200 mg/kg, and AMLE Zn ONP 100 and 200 mg/kg. p.o. The AMLE SNP and AMLE Zn ONP were powerful antidiabetic NPs effective at both dosages but very effective at 200 mg/kg compared to the usual medication (**Figure 36**).

Table 29 Effect of *Alpinia mutica* extract and different nano-particles on plasma glucose levels, total cholesterol and triglyceride levels in albino Wister rats

Group number	Plasma glucose level (mg/dL).		Total Cholesterol levels (mg/dL).		Triglyceride levels (mg/dL)	
	22 nd Day	29 th Day	22 nd Day	29 th Day	22 nd Day	29 th Day
I	104.85 ± 3.15	105.35 ± 3.10	75.03 ± 3.63	77.26 ± 3.70	65.67 ± 3.06	66.46 ± 2.28
II	334.90 ± 4.71#	224.41 ± 3.78##*	173.63 ± 3.38#	116.87 ± 5.89##*	131.83 ± 4.69#	72.37 ± 7.24##*
III	335.91 ± 3.50#	334.12 ± 3.69#	169.21 ± 5.79#	184.15 ± 6.36#	137.18 ± 4.84#	147.98 ± 6.50#
IV	333.72 ± 5.30#	281.49 ± 3.25##*	169.36 ± 6.16#	143.03 ± 6.32##*	138.14 ± 4.85#	119.29 ± 6.22##*
V	334.06 ± 5.52#	281.02 ± 4.44##*	165.76 ± 6.38#	143.27 ± 7.25##*	133.95 ± 6.76#	124.35 ± 4.03##*
VI	332.01 ± 4.10#	244.41 ± 3.27##*	163.95 ± 7.12#	115.78 ± 6.72##*	130.08 ± 4.75#	72.92 ± 7.81##*
VII	334.15 ± 3.38#	250.26 ± 5.02##*	164.97 ± 6.41#	116.46 ± 6.53##*	134.31 ± 7.21#	93.38 ± 7.43##*
VIII	334.27 ± 4.13#	244.04 ± 3.05##*	164.84 ± 6.44#	115.07 ± 4.66##*	132.56 ± 6.25#	74.97 ± 7.24##*
IX	334.95 ± 3.77#	226.5 ± 3.05##*	165.12 ± 6.55#	115.00 ± 6.98##*	134.53 ± 6.95#	77.24 ± 6.16##*

The values are shown as Mean ± SD, where n=6. One-way ANOVA was used for the analysis, followed by Tukey's repeated comparison test, where # signifies a p-value of 0.05 or less compared to the vehicle's control and * denotes a p-value of 0.05 or less compared to the experimental control.

7.1.16.3 Histopathological studies

After receiving a single dosage of STZ, the experimental group had islet-cell necrosis and the onset of type 2 diabetes. After a week with AMLESNP and AMLE Zn NP 200mg/kg p.o., pancreatic islets retained their standard shape and showed moderate necrosis, confirming their potential diabetic benefits. The AMLESNP and AMLE Zn NP100mg/kg p.o. had reduced islet cell recovery. Compared to the experimental control group, the AMLE 200 mg/kg and 400 mg/kg p.o. Treatment groups recover less or not at all (**Figure-37**).

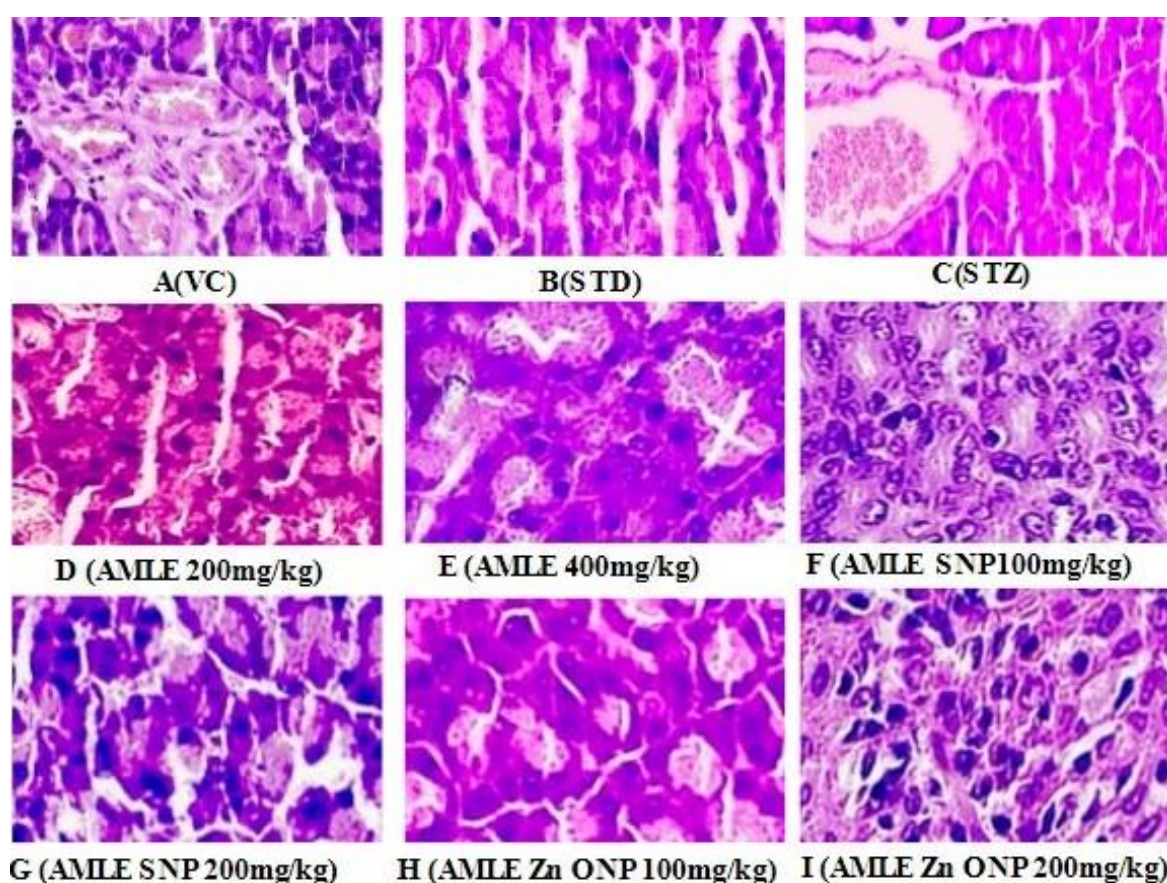


Fig-37: Pancreas Histopathological Observations for the Different Nanoparticles of *Alpinia mutica*, A (VC): There is no growth in tissues; B (Standard): Langerhans Islets have mild necrosis; C (STZ): Necrosis (NC) of Islets of Langerhans; D (AMLE 200 mg/kg): Langerhans Islets have mild-necrosis; E (AMLE 400 mg/kg): Mild-necrosis of Islets of Langerhans; F (AMLE SNP 100 mg/kg): Islets of Langerhans have Mild degeneration and necrosis; G (AMLE SNP 200 mg/kg): Moderate-degeneration and necrosis (MODN) of Islets of Langerhans; H (AMLE Zn ONP 100 mg/kg): Langerhans Islets have mild Necrosis (NC); I (AMLE Zn ONP 200mg/kg): Islets of Langerhans have mild degeneration and Necrosis (NC).

7.2 *Tradescantia spathacea* Profile

7.2.1 Macroscopy

Different macroscopic traits of *Tradescantia spathacea* fresh leaves were noticed, including duration, kind of leaf base, tip occurrence or absence, and lamina features. Some properties that make a lamina unique include composition, incision, structure, venation, border, apex, base, surface, and texture. The root bark's size, form, feeling, fracture, and configuration are examined morphologically. The macroscopy of *the T.S* leaf revealed the presence of the following characters.

- Leaves : Alternative, overcrowded, and expand
- Shape : sword-like, extended, and pointed
- Size : 30-40 cm, 4-6 cm in length and breadth.
- Base : Alternate
- Margin : Entire
- Taste : Bitter and aromatic
- Color : Its bottom side is rose purple and its top surface is dark green.
- Odor : Characteristics
- Surface : Each surface is flat.

7.2.2 Microscopy of T.S Leaf

T.S. leaf has various characteristics, including starch grains, prism-type calcium oxalate crystals, red-colored tissue, fibers, and xylem vessels. In **Figure 38**,

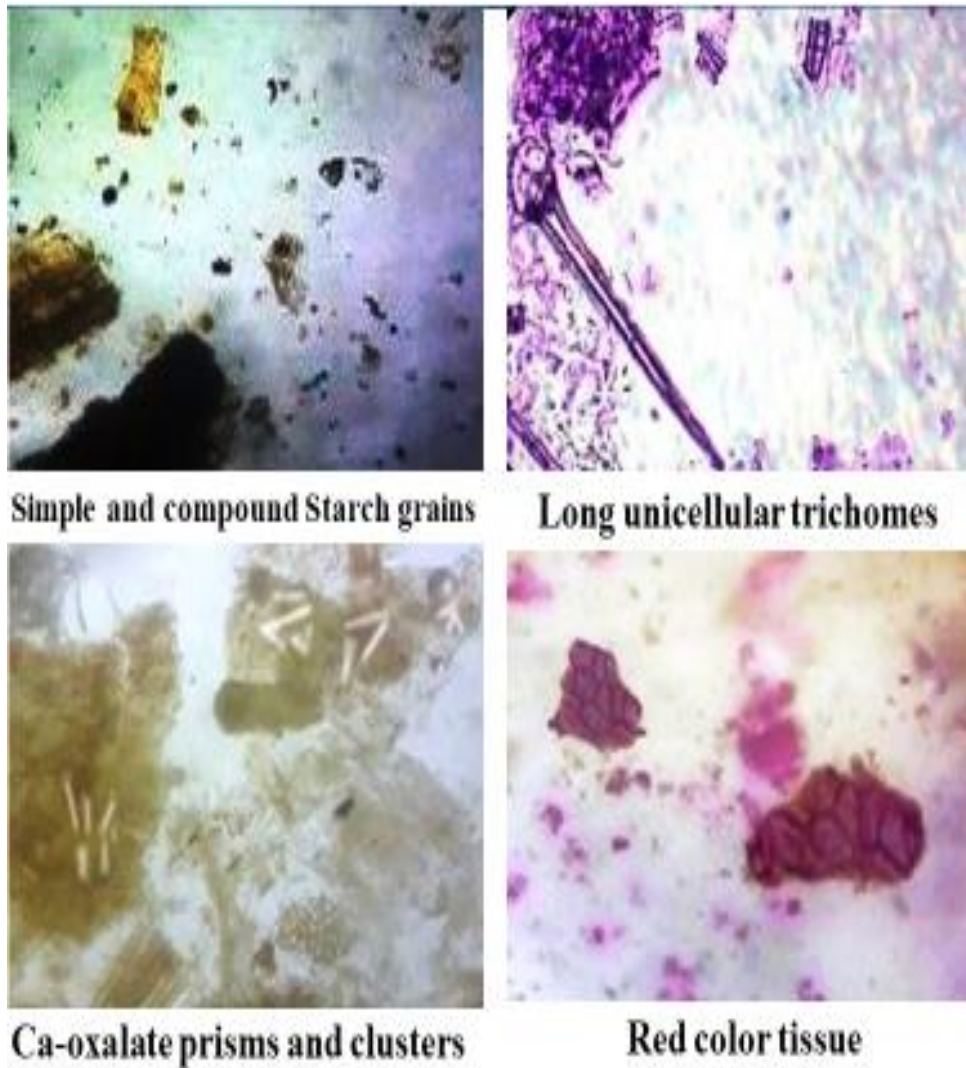


Fig-38: Powder microscopic characteristics for the Leaves of *T. S*

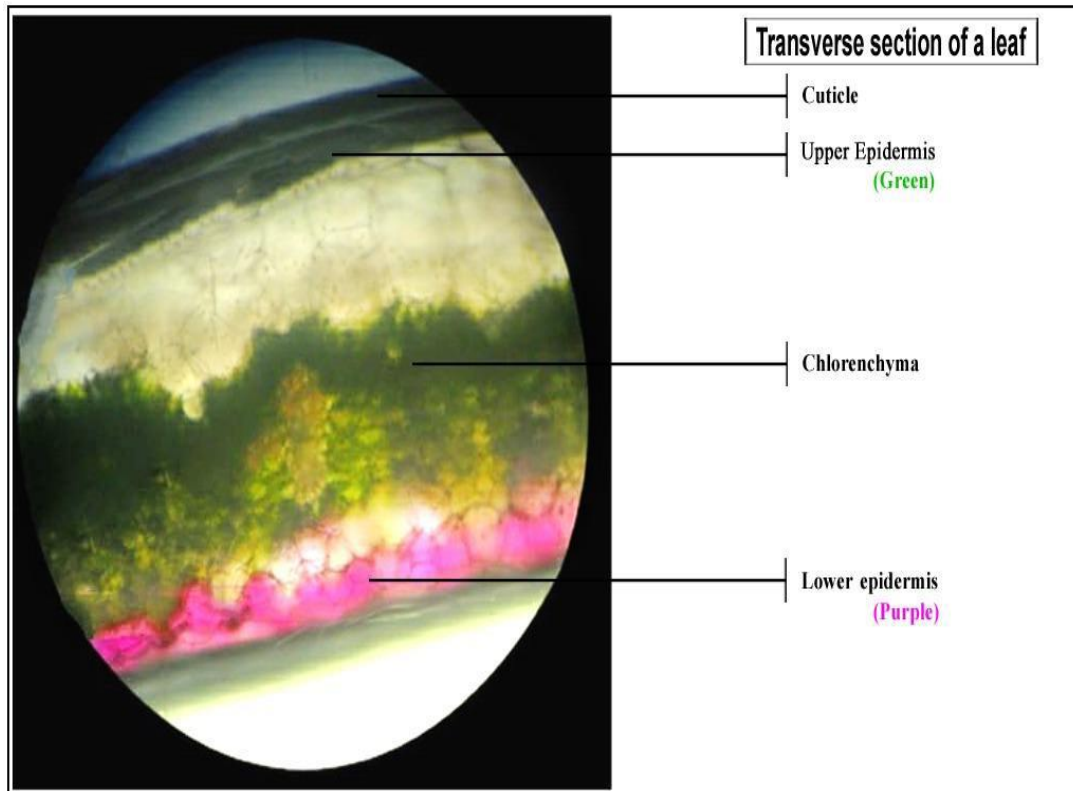


Fig-39: Transverse section of Leaves of *Tradescantia spathacea*

7.2.3 Ash Values and Moisture contents

The values for the plant's total ash (T.S), acid-insoluble ash (A.I.A.), water-soluble ash (W.S.A.), and sulfated ash (S.A.S.) were found to be (11.66±0.10), (7.58±0.15), (7.65±0.17), and (4.64±0.11), respectively. These figures show that the plant meets the standards for quality set out by the Indian Ayurvedic Pharmacopeia. However, the plant's moisture content was found to be (11.23±0.10). **Table 30** displays the outcomes.

Table 30 Analytical values of the T.S

S.No.	Ash Type	Ash value (% w/w) Plant material taken (2g)
1	Total Ash	11.66±0.10
2	Acid Insoluble Ash	7.58±0.15
3	Water Soluble Ash	7.65±0.17
4	Sulfated Ash	4.64±0.11
5	Moisture content	11.23±0.10

7.2.4 Extractive Values

T.S. exhibited extractive values for alcohol and water solubility of 12.8 ± 0.17 and 8.83 ± 0.15 respectively. These figures show that the plant satisfies the quality standards established by the Indian Ayurvedic Pharmacopeia. **Table 31** presents the findings.

Table 31 Extractive values of the plant T.S

S.NO.	Plant material	Extractive values (% w/w), Plant material (4 g)	
		90% alcohol-soluble-extractive value	Water-soluble extractive-value
1.	Leaf	12.8 ±0.17	8.83±0.15

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7.2.5 Extraction - Several plant extracts' yield from T.S increased compared to Ultra sound extraction (USE). The findings imply that the USE is better than the conventional extraction method. In **Table 32**, the results are shown.

Table 32: The colour, consistency, and yield % of T.S. Extracts

Plant Extract	Colour seen in daylight	Consistency	Conventional extraction method Yield (%w/w)	Ultrasound assisted extraction (% w/w)
Methanolic extract	Dark-green	Semi-solid	10.52	16.29
Ethyl acetate extract	Light-green	Semi-solid	6.23	8.56
Hydro-alcoholic extract	Slightly-dark green	Semi-solid	6.56	10.19
Petroleum ether extract	Lightly-green	Semi-solid	2.31	7.85
Aqueous extract	Light-green	Semi-solid	8.25	14.45

7.2.6 Phytochemical screening

Phytochemical screening is used to determine the components of the extracts; the phytoconstituent content in phytoextracts was shown to be higher using the USE methodology than the traditional extraction method. **Table 33** presents the results.

Table 33 Phytochemical screening for the various extracts of T.S

S.NO	Test	Conventional Method of Extraction					Ultrasound assisted Extraction				
		TSME	TSEAE	TSHAE	TSPEE:	AMAE	AMME	AMEAE	AMHAE	AMPEE	AMAE
1	Alkaloids	++	++	++	++	++	+++	+++	+++	+++	+++
2	Terpenoides	+	+	+	+	+	+++	+++	++	++	++
3	Protens	----	----	----	----	----	----	----	----	----	----
4	Tannins	+	+	+	+	+	+++	+++	++	++	++
5	Carbohydrates	++	++	++	++	++	+++	+++	+++	+++	+++
6	Flavonoids	+	+	+	+	+	+	+	+	+	+
7	Saponins	++	++	++	++	++	+++	+++	+++	+++	+++
8	Phenolic compounds	++	++	++	++	++	+++	+++	++	++	++

+++ : Strong positive test, ++: Low positive test, +: weak positive test, -: Negative test. TSME: *Tradescantia spathacea* methanolic extract; TSEAE: *Tradescantia spathacea* ethyl acetate extract; TSHAE: *Tradescantia spathacea* hydro alcoholic extract; TSPEE: *Tradescantia spathacea* petroleum ether extract, TSEAE- *Tradescantia spathacea* aqueous extract.

7.2.7 Estimation of total phenolic content.

The estimate was given in mg/g of gallic acid equivalent. The calibration curve for gallic acid was plotted as ($Y = 0.0024x - 0.0073$, $R^2 = 0.9977$) (**Figure 40**). Compared to traditional extracts, ethyl acetate, a solution of hydro-alcoholic extracts, has increased phenolic compound content. The high phenolic content was found in the methanol extract (4.23 ± 1.70). **Table 34** shows the results

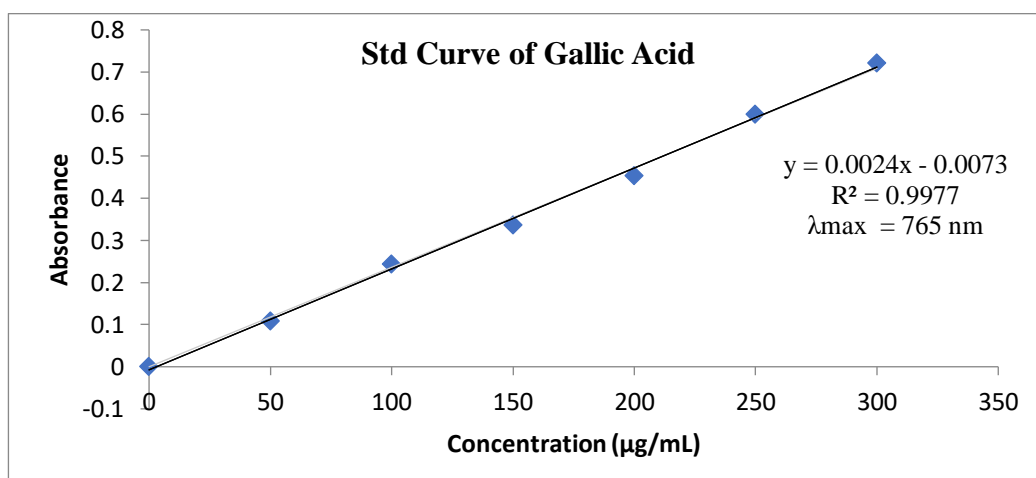


Fig- 40: Standard Curve of Gallic acid.

Table 34: *Tradescantia spathacea*'s phenolic content

S. No	Test Drug (<i>Tradescantia spathacea</i>)	Gallic acid equivalent in mg/g for conventional extraction's total phenolic-content.	USE the total phenolic-content in mg/g of gallic-acid (mg/g).
1	Methanolic extract	1.29 ± 1.05	4.23 ± 1.70
2	Ethyl acetate extract	1.59 ± 0.71	1.80 ± 0.68
3	Hydro alcoholic extract	1.29 ± 1.05	1.53 ± 0.26
4	Petroleum ether extract	2.43 ± 0.17	3.33 ± 0.51
5	Aqueous extract	1.59 ± 0.7	2.22 ± 1.19

7.2.8 Estimation of total Flavonoid content.

In milligrams per gram of quercetin equivalent, the estimate was provided. According to **Figure 41**, the calibration spectrum for quercetin was drawn as ($Y=0.0038x + 0.001$, $R^2 = 0.9998$). Compared to extracts produced using the traditional approach, those made using ultrasound-assisted extraction exhibited increased flavonoid concentration. The maximum flavonoid content was detected in the methanol extract (10.60 ± 6.17). **Table 35** shows the results. Compared to standard extraction techniques, USE methods increased the yield and number of plant-based elements like phenolic and flavonoid content in *Tradescantia spathacea* plant extracts.

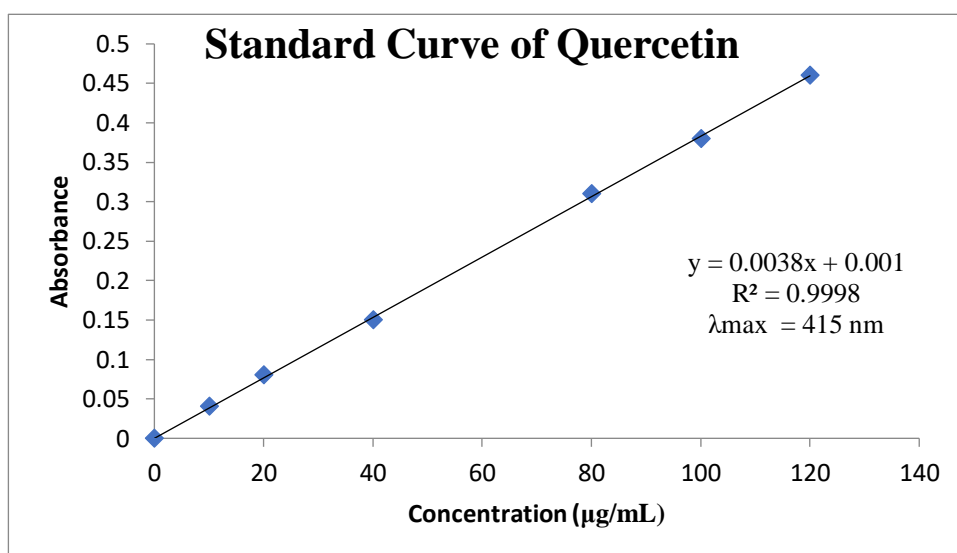


Fig-41: Standard Curve of Quercetin.

Table 35: Flavonoids Contents in *Tradescantia spathacea*

S. No	Test Drug (<i>Tradescantia spathacea</i>)	Gallic acid equivalent in mg/g for conventional extraction's total Flavonoid content.	USE gallic-acid mg/g of total flavonoid content.
1	Methanolic extract	2.27±0.81	10.60 ± 6.17
2	Ethyl acetate extract	2.40±0.91	3.67±0.38
3	Hydro alcoholic extract	1.79±0.61	3.81±0.47
4	Petroleum ether extract	1.66±0.16	3.63±0.52
5	Aqueous extract	2.40±0.87	3.89±0.69

7.2.9 Alkaloid content estimation

The calibration curve for atropine was ($Y = 0.0003x + 0.0005$, $R^2 = 0.9992$) (**Figure 42**). The estimate was in mg/g. Ethyl-acetate, methanolic, and hydro-alcoholic extracts from USE had higher alkaloid concentrations than conventional extracts. The methanol extract had the highest alkaloid content (27.30 ± 15.77). The results are in **Table 36**.

Accurately measured Atropine standard solution aliquots were placed in several separatory funnels. Shake 5 ml of pH 4.7 phosphate buffer with 5 ml of BCG solution at different concentrations. The chloroform-diluted extracts were gathered in a 10 ml volumetric container. In a UV-Spectrophotometer (SHIMADZU UV-1800), the complex's chloroform absorbency was measured at 470 nm against the blank without Atropine [238-239].

Research has demonstrated that ultrasonic-assisted extraction enhances the content of phenolic flavonoid and alkaloid compounds. Ultrasonic-assisted extraction facilitates fast and effective plant extraction. Using these methods increased *Tradescantia spathacea* plant extract yield and phytoconstituent content, primarily phenolic, flavonoid, and alkaloid compounds.

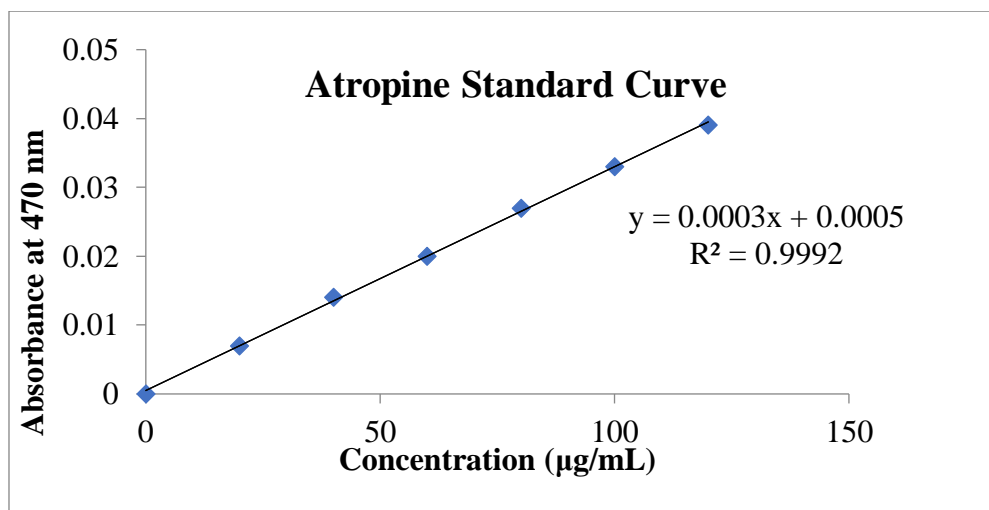


Fig-42: Standard Curve of Atropine.

Table 36: Alkaloid Contents in *Tradescantia spathacea*

S. No	Test Drug (<i>Tradescantia spathacea</i>)	Atropine equivalent in mg/g for conventional extraction's total Alkaloid content.	USE Atropine mg/g of total alkaloid content.
1	Methanolic extract	5.29±2.13	27.30 ± 15.77
2	Ethyl acetate extract	4.84±1.84	22.29±11.91
3	Hydro alcoholic extract	4.77±1.76	23.96±13.00

7.2.10 T. S extract thin layer chromatography:

TLC separates crude extract phytoconstituents. Herbal extracts include chemicals with different physicochemical properties; therefore, solvent systems were selected by trial and error and previous experiments. After TLC plates were created and exposed to visible and ultraviolet light, compounds were applied with suitable reagents and evaluated. The following table contains TLC mobile phases and detection techniques and Rf Values for different phytoconstituents in **Table 37**, **Figure 43**.

Table 37: Solvent System Optimisation for Phytoconstituent Detection.

Test	Solvent system	Detection	Rf Values
Steroids	(Ethyl acetate: Toluene) (1:4, v/v)	Antimony-trichloride in Chloroform	0.71
Flavonoids	(Chloroform: hexane: Methanol) (2:1:7, v/v/v)	1% ethanolic aluminium- chloride solution Detection under U.V (365 nm)	0.81
Phenolic compounds	(Ethyl-acetate: Formic-acid: Acetic acid: water) (100: 11: 11: 26, v/v/v/v)	Detection under U.V (256 nm)	0.80

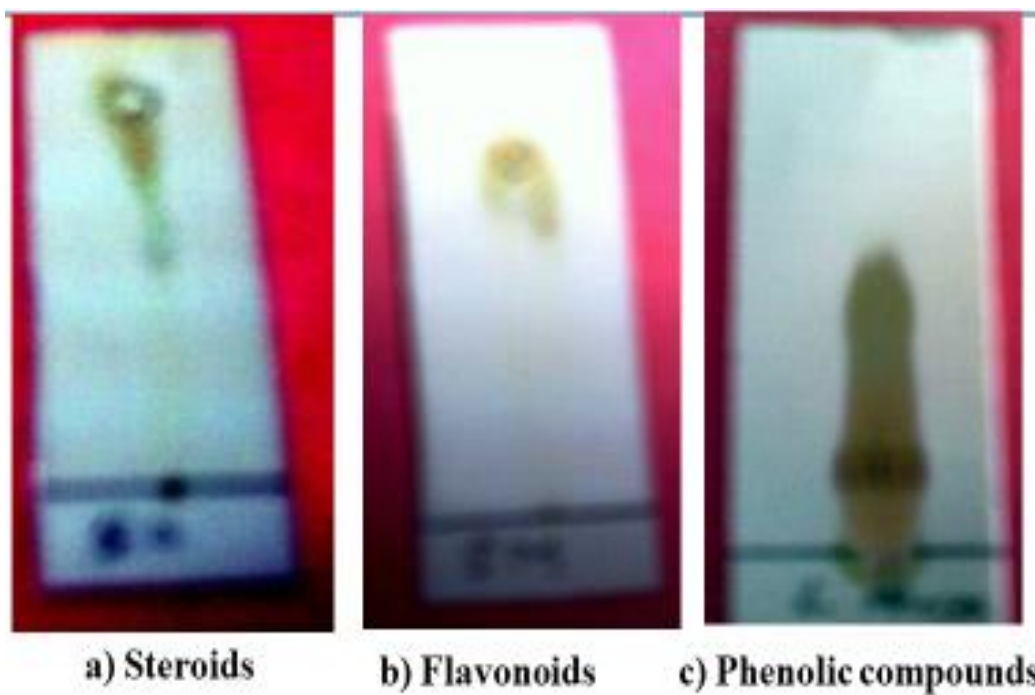


Fig-43: TLC Detection of Phytoconstituents in *Tradescantia spathacea* Leaf Extract

7.2.11 Antioxidant activity

7.2.11.1 Reducing power by $FeCl_3$

In 2ml of each sample and reference solution, 2.5ml of a 1 percent potassium ferricyanide solution was added. For 20 minutes, the mixture should be heated in a water bath to 50⁰C. Trichloroacetic acid was added after cooling, with concentrations ranging from 2.5 to 10%. Ten minutes were spent centrifuging the mixture at 3000 rpm. A 10-minute treatment uses 2.5 ml of sterilized water and 1 ml of iron chloride. Controls were prepared without samples. The solution's absorbance was calculated at 700 nm. (Tables 38, 39, and Figures 44, 45) illustrate the results in the graph below.

Table -38: Reduced method: -

Conc.(μ g/ml)	Ascorbic acid.	Leaf methanol extract	Root methanol extract
0	0	0	0
20	0.18	0.12	0.1
40	0.34	0.29	0.25
60	0.5	0.39	0.32
80	0.7	0.52	0.44
100	0.85	0.55	0.5

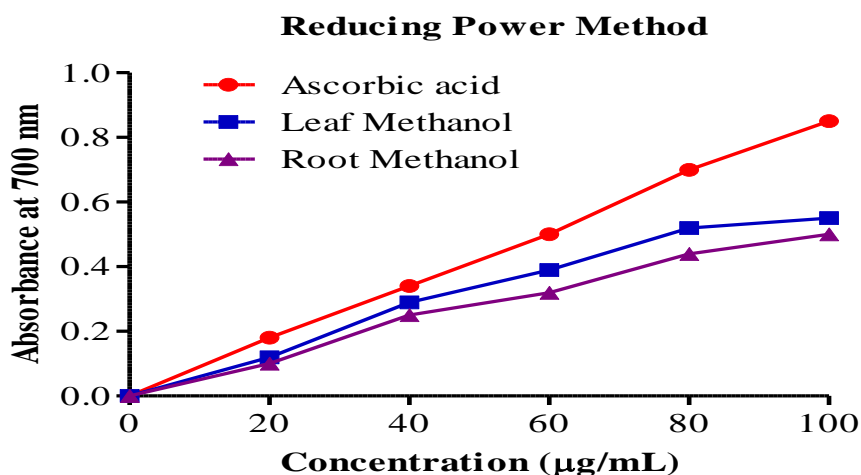


Fig-44: Reduced method Scavenging activity

Table-39: Reducing % Inhibition method:

Conc. ($\mu\text{g/ml}$)	Ascorbic acid	Leaf methanol	Root methanol
0	0	0	0
20	83.33	75	70
40	91.17	89.65	88
60	94	92.30	90.63
80	95.71	94.23	93.18
100	96.47	94.54	94

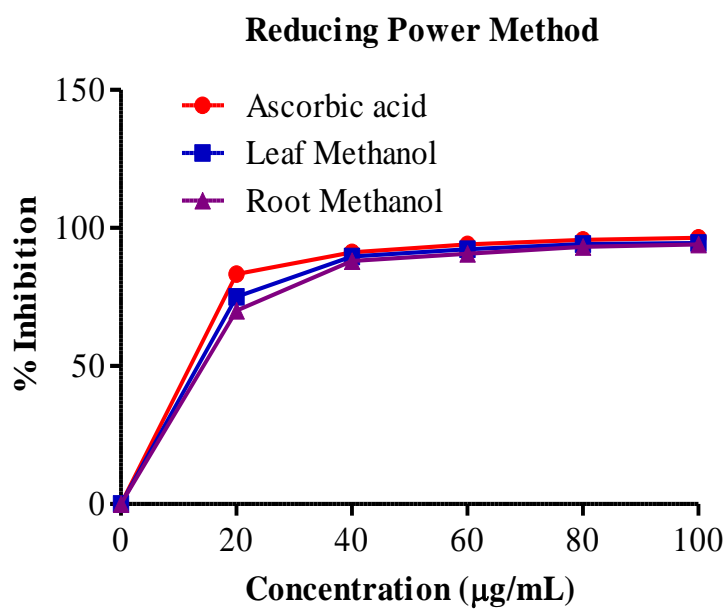


Fig-45: Reduced % Inhibition method

7.2.11.2 DPPH free radical scavenging activity

Diluting the sample with methanol yielded 100 litres for each dosage at 20, 40, 60, 80, and 100 µg/ml. Each test tube contains 150 mL of DPPH diluted twice with methanol. The control reading used three millilitres of methanol and 150 millilitres of DPPH solution to identify the absorption at 517 nm quickly. Using a methanol blank and a Shimadzu UV-1800 ultraviolet-visible spectrophotometer, absorption was measured at 517 nm after 15 minutes. IC50 and % decrease was calculated: 3 times. Results are shown. (Table-40, 41, and Figures 46, 47).

Table -40: DPPH Scavenging activity:

Conc. (µg/ml)	Ascorbic acid	Leaf methanol.	Root methanol
0	0	0	0
20	0.1	0.11	0.13
40	0.08	0.12	0.14
60	0.06	0.09	0.12
80	0.05	0.08	0.09
100	0.03	0.05	0.07

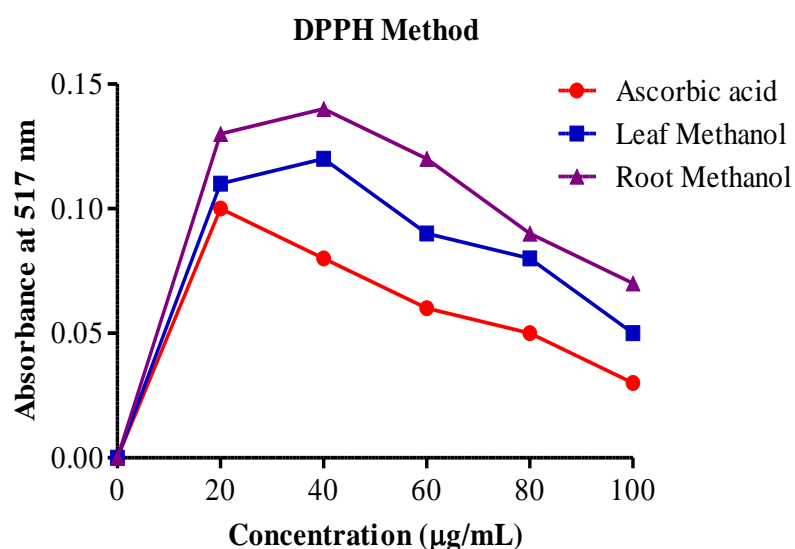


Fig-46: DPPH Scavenging activity.

Table-41: DPPH % Inhibition:

Conc'n (µg/ml)	Ascorbic acid	Leaf Methanol	Root Methanol.
0	0	0	0
20	37.5	31.25	18.75
40	50	25	12.5
60	62.5	43.75	25
80	68.75	50	43.75
100	81.25	68.75	56.25

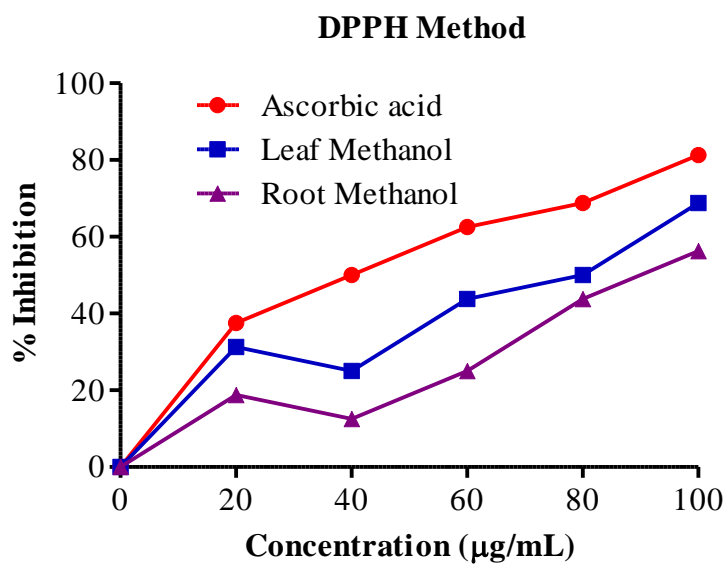


Fig-47: DPPH % Inhibition Method

7.2.11.3 Chelation of iron activity

A technique for assessing antioxidant activity is iron chelation activity. The iron chlorine solution (2 mL, 200 m) and O-Phenanthroline solution of methanol (1 mL, 0.05% w/v) were incubated at room temperature for 10 minutes with the extract with solution (2 mL in 5% v/v methanol).

At 510 nm, the solvents' absorbance was assessed after incubation. Three times were used to conduct the testing. The graph shows results (**Table-42,43 and Figures 48 and 49**).

Table -42: Iron-chelation:

Conc'n (µg/ml)	Ascorbic acid	Leaf Methanol.	Root Methanol
0	0	0	0
10	0.07	0.06	0.06
20	0.13	0.12	0.1
30	0.19	0.17	0.14
40	0.25	0.2	0.16
50	0.3	0.24	0.17
100	0.6	0.26	0.18

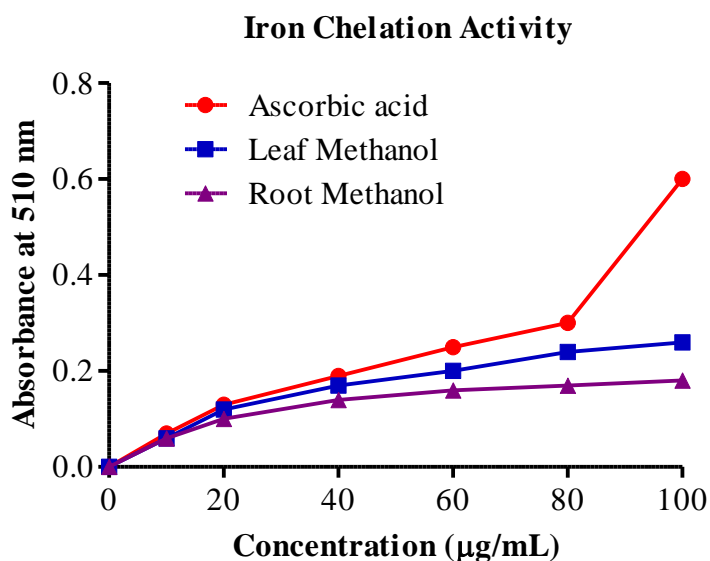


Fig-48: Iron chelation activity

Table-43: Iron-chelation % Inhibition:

Conc'n (µg/ml).	Ascorbic acid	Leaf Methanol	Root Methanol
0	0	0	0
10	14.28	0	0
20	53.84	50	40
30	68.42	64.70	57.14
40	76	70	62.5
50	80	75	64.70
100	90	76.92	66.66

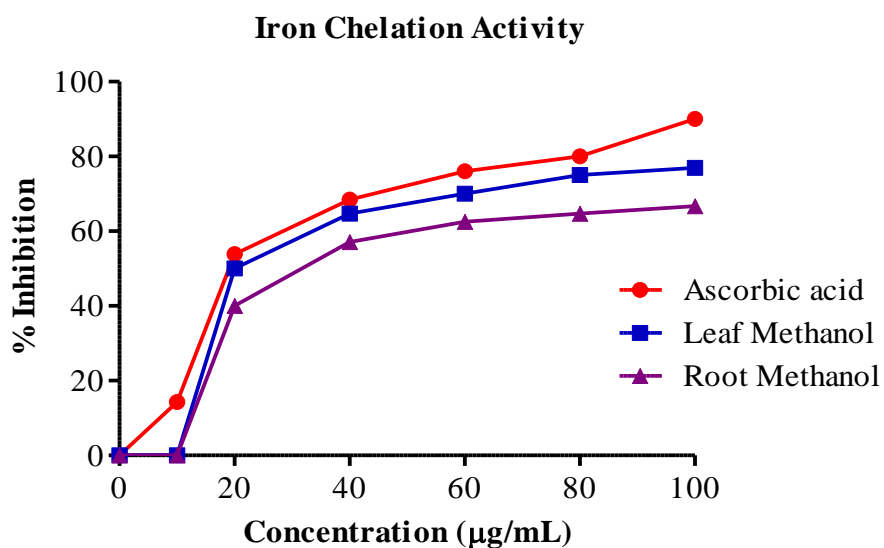


Fig-49: Inhibition activity of Iron chelation

7.2.12 Green synthesis of nanoparticles of *Tradescantia spathacea*

7.2.12.1 Green synthesis of silver nanoparticles (Ag NPs)

The synthesis of AgNP nano-particles was simplified greenly. Add 45 ml of the 1 mM AgNO₃ solution to the conical flasks with a magnetic stirrer, then 10 ml of filtered T.S. drug solution. Ag NPs were made by violently shaking conical flasks for 0, 12, & 24 hours. A 12–24-hour room-temperature conical flask solution got dark brown (Figures 50 and 51). We centrifuged the completely colored solution for 20 minutes at 5000 rpm. Removal of supernatant left just residue. Sterile distilled water was used to wash and dry the residue.

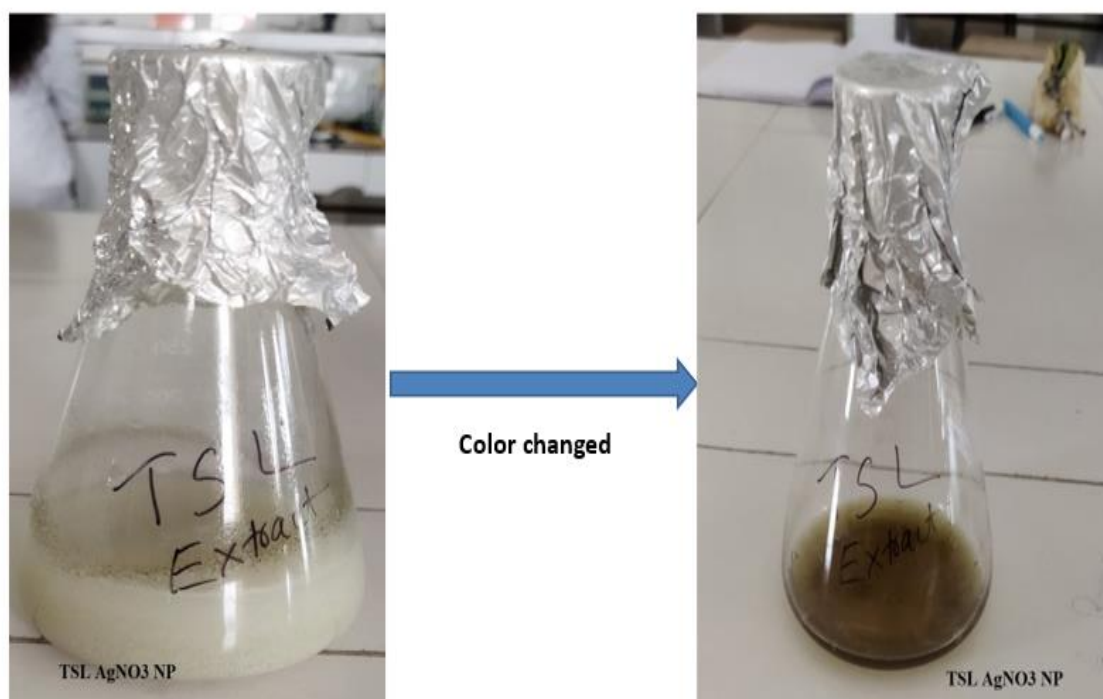


Fig-50: Green synthesis of Nanoparticles

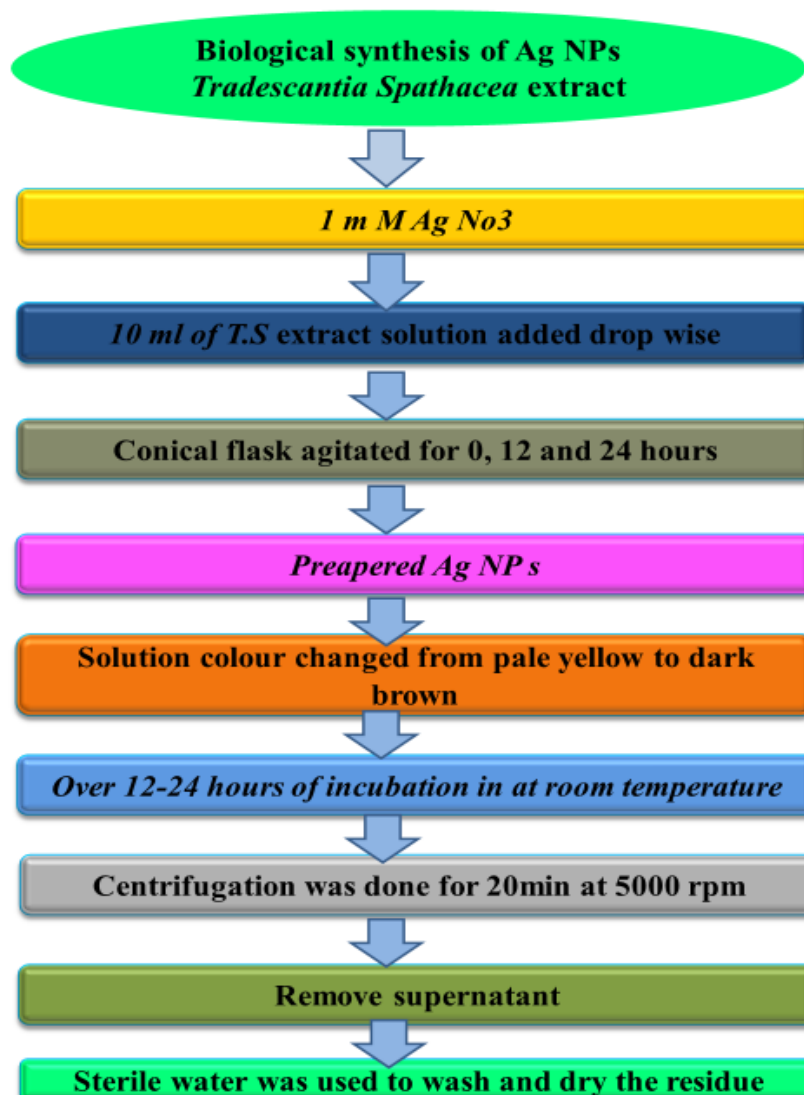


Fig-51: Green synthesis of Ag NPs from *Tradescantia spathacea* extract

7.2.12.2 Green synthesis of zinc oxide nanoparticles (Zn O NPs)

Post-reaction filthy precipitate was cooled for 24 hours. Fifteen minutes of 6000 rpm centrifugation separated the reaction fluid from the precipitate. After repeatedly washing with deionized water, the dried product was burned at 800°C to remove impurities. After oxidizing the powdered sample for three hours at 350°C in the muffle furnace, 5 L of the Zn O NPs solution was placed on a copper-grid covered in carbon, coated, and dried before being carried to a scanning-electron-microscope (Figure 52).

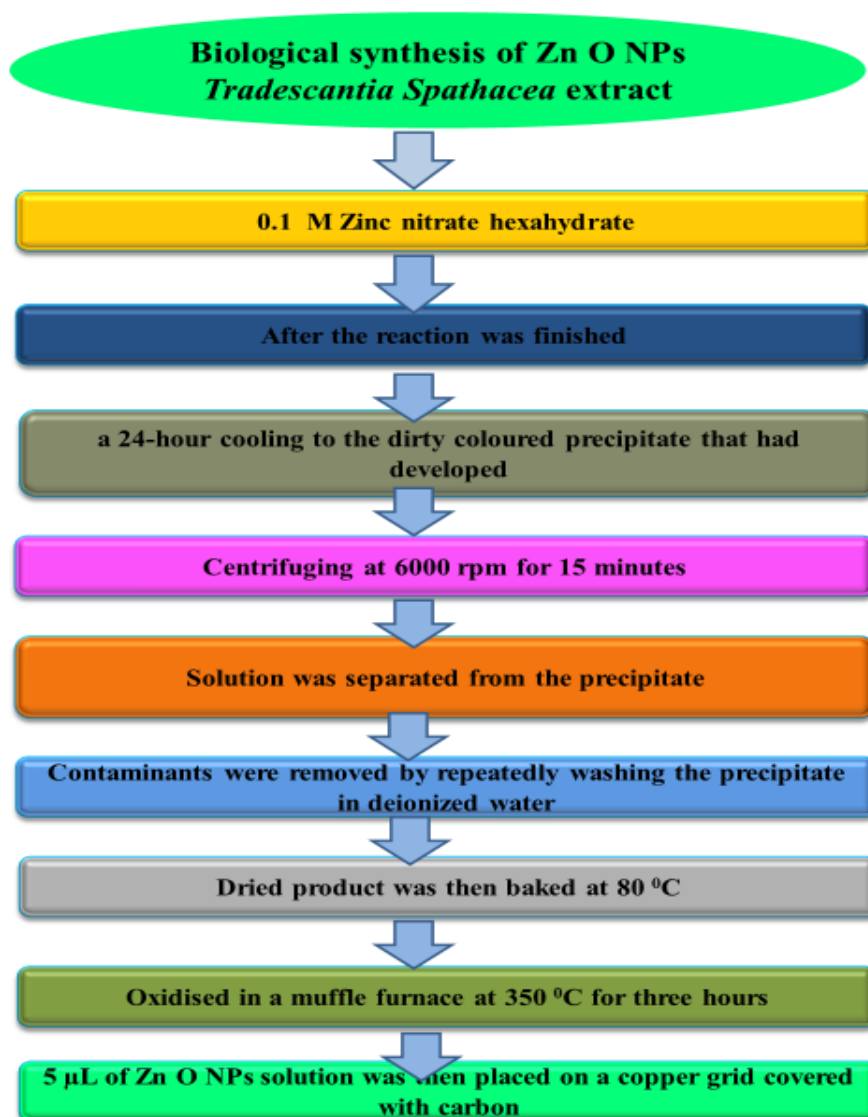


Fig-52: Green synthesis of Zn O NPs from *Tradescantia spathacea* extract

7.2.13 Characterization of green nanoparticles produced synthetically

ZnO and Ag NPs have been studied for (T.S.) to identify their crystalline form, function group evaluation, the typical size of particles, and shape. The properties of physiologically and environmentally produced nanoparticles were studied using a variety of methods, including a particle size analyzer, zeta potential, SEM, and X-ray diffraction.

7.2.13.1 Particle size and zeta potential

DLS (Malvern Instruments) was used to calculate the potential for zeta and average particle size. The particle size was determined at 25°C and a constant angle of 90° and normal ranges of particle size is 1-1000nm. The sample was created by distributing the nanoparticle suspension in distilled water and ultrasonically sonicating it for 6 minutes. Zeta potential measurements at 25 °C and 150 V were gathered via electrophoretic light scattering. The zeta potential is based on the charge conductivity principle to preserve the formulation's stability. The particle size and zeta potential measurements of various nano-particles are included below (**Figures 53a, 53b, 53c, and 53d**).

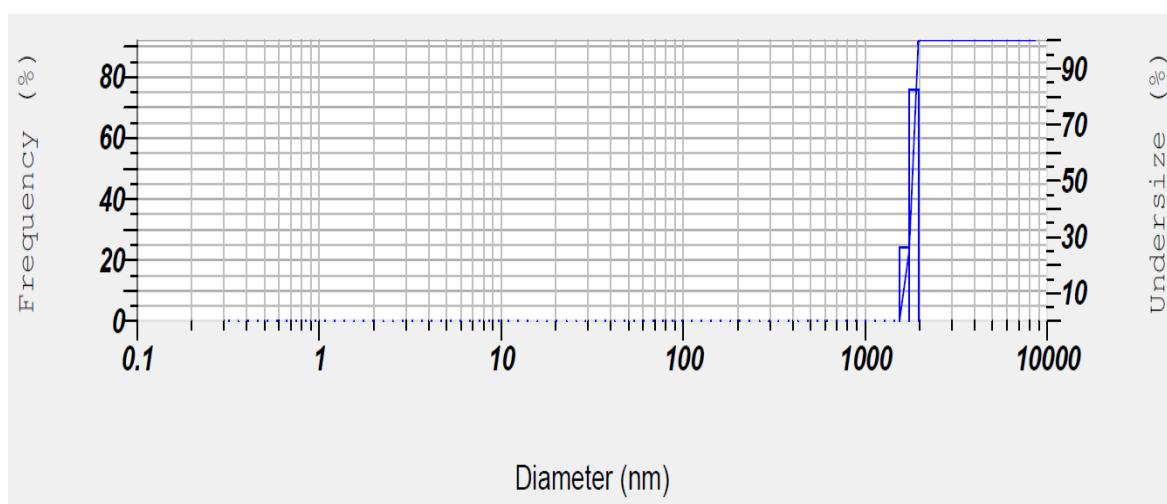
a) TSLE-Ag (Particle size): Green silver nanoparticles made using plant extract T.S. were measured using a particle size analyzer. The graphs of zeta potential indicate that *T.S.* plant extract-mediated silver nanoparticles have an average mean diameter of 1700 nm.

b) TSLE-Ag (Zeta Potential): Green silver nanoparticles made with plant extract T.S. were measured using a zeta sizer. The graphs of zeta potential indicate that *T.S.* plant extract-mediated silver nanoparticles have a zeta potential value of 3.8 mV. The results of zeta for *T.S.* mediated silver nanoparticles depict that synthesized nanoparticles have significant stability.

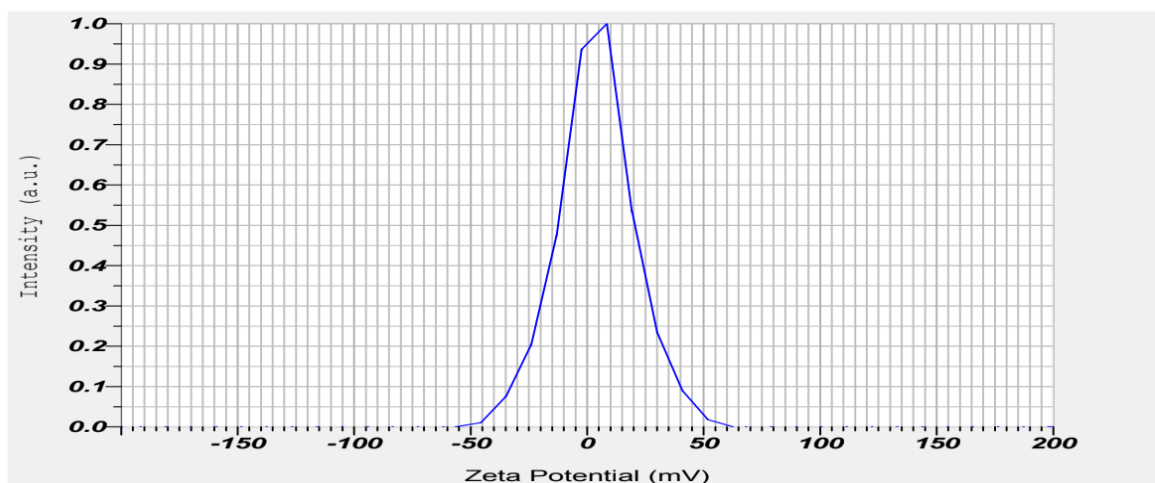
c) TSLE-Zn (Particle Size): Particle size analysis was used to evaluate green Zn O nanoparticles produced using plant extract T.S. Zeta potential graphs show that zinc oxide nanoparticles produced by *T.S.* plant extract have an average mean diameter of 6091 nm.

RESULTS & DISCUSSION

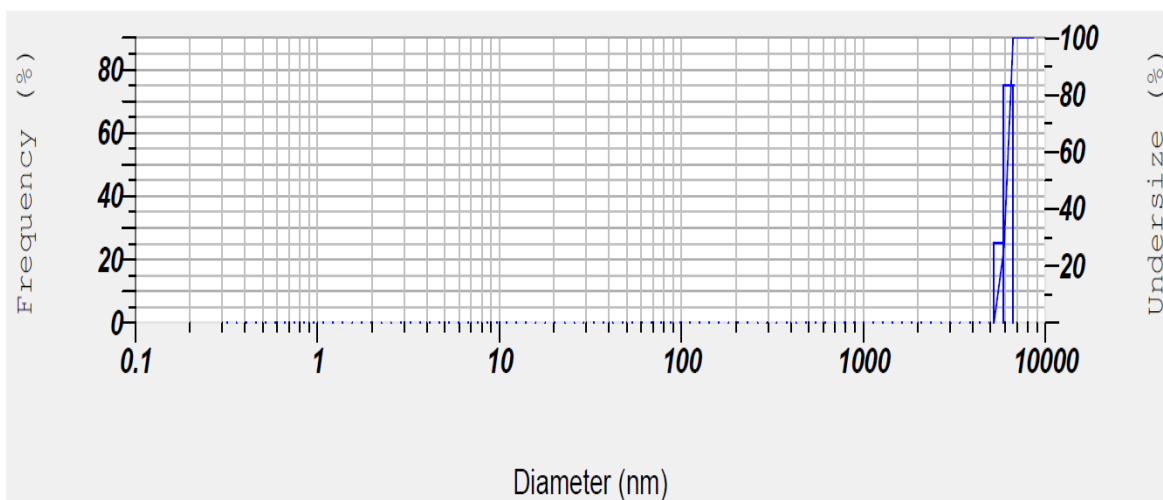
d) TSLE-Zn (Zeta Potential): Zeta sizer was used to determine green Zn O nanoparticles synthesized using plant extract *T.S.* The graphs of zeta potential indicate that *T.S.*, plant extract-mediated silver nanoparticles have a zeta potential value of -0.2 mV. The results of zeta for *T.S.* mediated silver nanoparticles depict that synthesized nanoparticles have low stability.



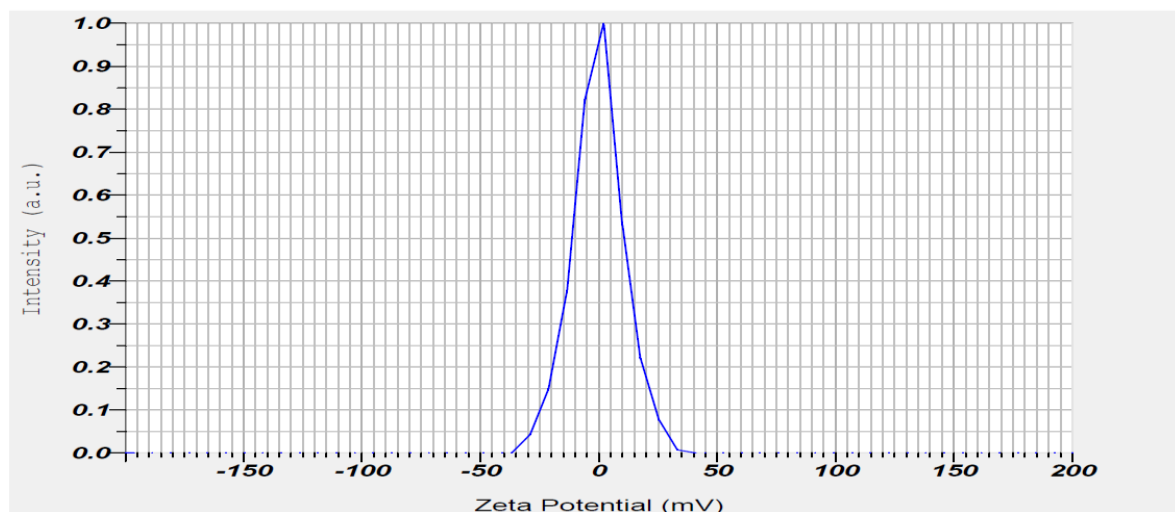
53a) TSLE-AG (Particle size)



53b) TSLE-AG (Zeta Potential)



53c) TSLE-Zn (Particle Size)



53 d) TSLE-Zn (Zeta Potential)

Fig-53: Particle size and zeta potential

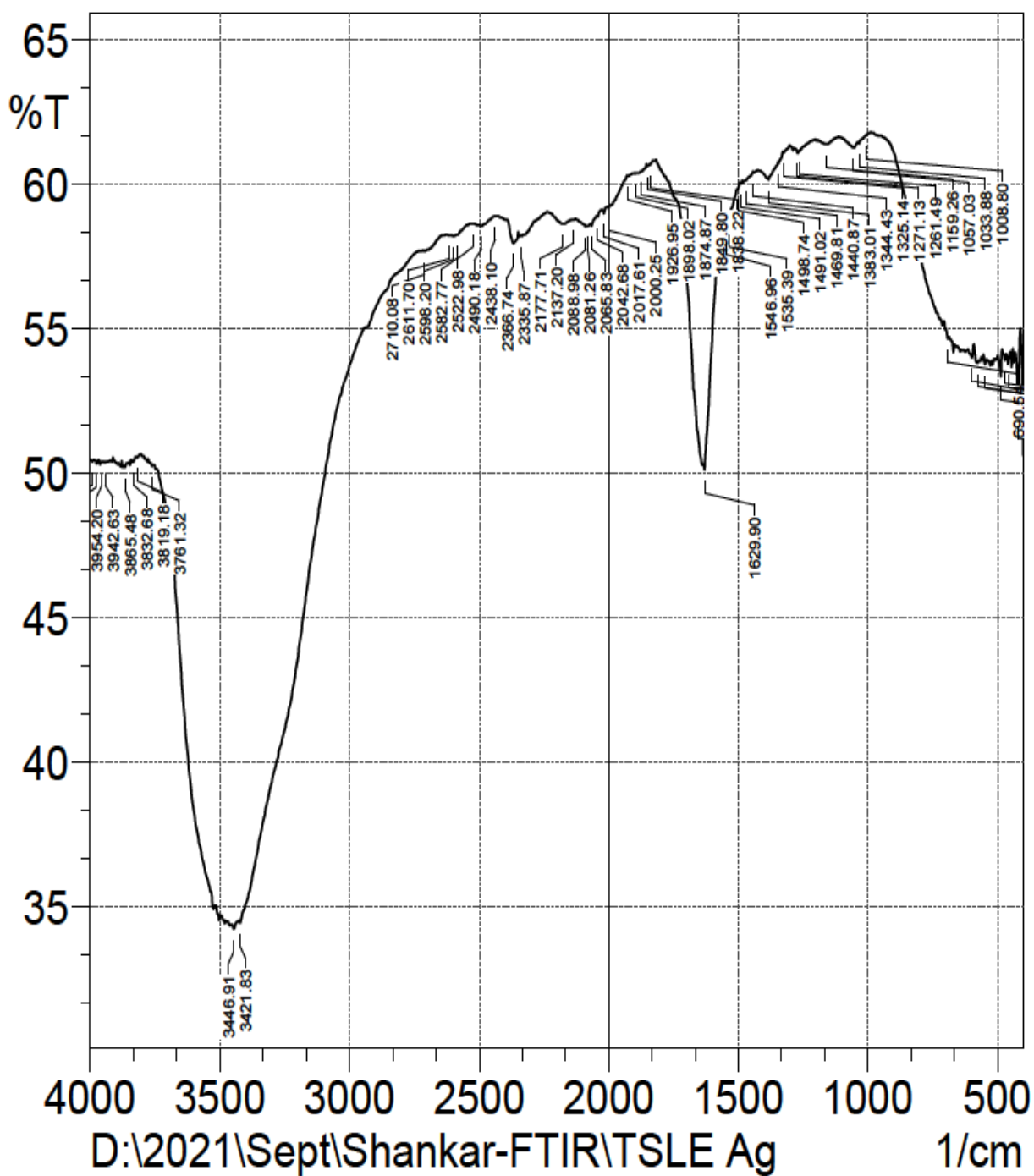
7.2.13.2 Fourier transform infrared spectroscopy: The FTIR spectra of leaf extract of that were analyzed before and after the synthesis of Ag NPs and Zn O NP was studied to discover the probable multifunctional type of compounds that may be involved in producing these materials. The main objective of the FTIR measurement was to pinpoint the leaf extract molecules in charge of ion reductions and the capping agents in charge of the stability of a nanoparticle solution (**Figures 54 a and 54 b**).

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a) TSLE Ag NP: FTIR experiments have been carried out to determine which functional category of the extract is crucial in the reduction of nitrates of silver into silver nanoparticles. *Tradescantia spathacea* Ag FTIR spectra peaks were seen at 3819, 3865, 2933, 2611, 1849, 1546, and 1344 cm⁻¹. Peaks at 2933, 2611, and 1849 may arise due to phenols' [OH] stretching or the leaf extract chemicals' ability to bend and stretch H-H bonds.

b) TSLE ZnONP: It is crucial to identify the many functional groups involved in nanoparticle production. We have conducted FTIR investigations for this reason. The *Tradescantia spathacea*, Zn, FTIR spectrum showed many peaks at 3761, 3817, 2933, 2634, 1635, 1629, and 1383 cm⁻¹. The (OH) stretch of phenols or the bend stretching of hydrogen-bonded leaf extract chemicals may cause peaks at 3761, 3817, and 2933. Proteins, enzymes, or polysaccharides could cause this in the extract, stretching their (OH) bonds.

RESULTS & DISCUSSION



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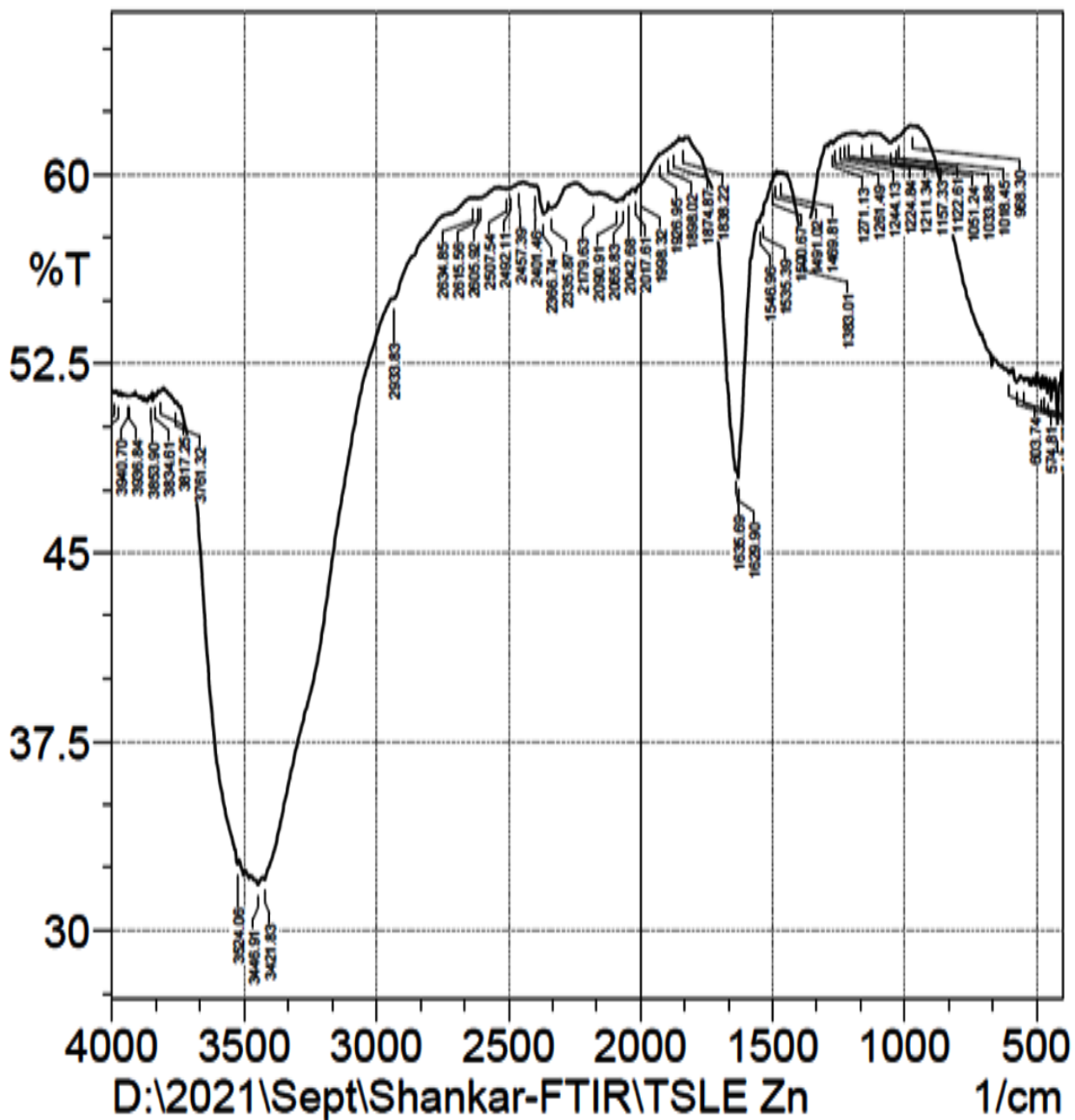
No. of Scans; 10

Resolution; 4 [1/cm]

Apodization; SqrTriangle

User; Administrator

Fig-54a) TSLE Ag NP



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 No. of Scans; 10
 Resolution; 4 [1/cm]
 Apodization; SqrTriangle
 User; Administrator

Fig- 54 b) TSLE Zn ONP

Fig- 54: Fourier transform infrared spectroscopy

7.2.13.3 XRD analysis: An XRD analysis was done to examine the synthesized nanoparticles' structural integrity. A PAN analytical X-Ray diffractometer with a voltage of 4000V and a current of 20mA was loaded with nanoparticles. With a 2θ time constant, the scanning was performed using a 2θ angle between 20° and 80° at a speed of $0.02^\circ/\text{min}$. The crystal structures of all materials were improved to provide accurate atom positions. (Figures 55 a and 55 b)

a) TSLE Ag NP: XRD analysis has been processed using plant extract of *Tradescantia spathacea*. Peaks at 2θ values of 46.6° , 74.56° , and 86.9° are present, and they correspond to the values are (111), (200), (220), and (311) planes of Ag, correspondingly. The peaks at 2θ values, 27.80° , 32.27° , 46.25° , 57.65° , and 78.12° , which correspond to (112), (210), (220), and (311), are also seen in another spectrum (410). The extensive and open XRD patterns indicate the creation of Ag nanoparticles. The kind of extracts employed for the synthesis technique impacted the size of the generated Ag crystals, which might be attributed to the extracts' medicinal potential.

b) TSLE Zn NP: XRD analysis has been processed using plant extract of *Tradescantia spathacea*. Peaks are seen at 2θ values of 44.6° , 64.56° , and 78.9° , respectively, and these values correspond to the values (111), (200), (220), and (311) planes of Zn. Similar to this, another spectrum reveals the existence of peaks at 2θ values of 27.80° , 32.27° , 46.25° , 57.65° , and 78.12° , which correspond to (111), (200), (220), (311), and (400), respectively (311). The comparatively comprehensive XRD patterns show the production of Zn nanoparticles. The kind of extracts employed for the synthesis process impacted the crystalline size of the synthesized zinc, which might be attributed to the extracts' potential for therapeutic application. Additionally, it could depend on the extracts' ability to behave as caps due to the compounds present in them.

RESULTS & DISCUSSION

Group: 29-9-21-Shankar Data: TSLE-Ag >

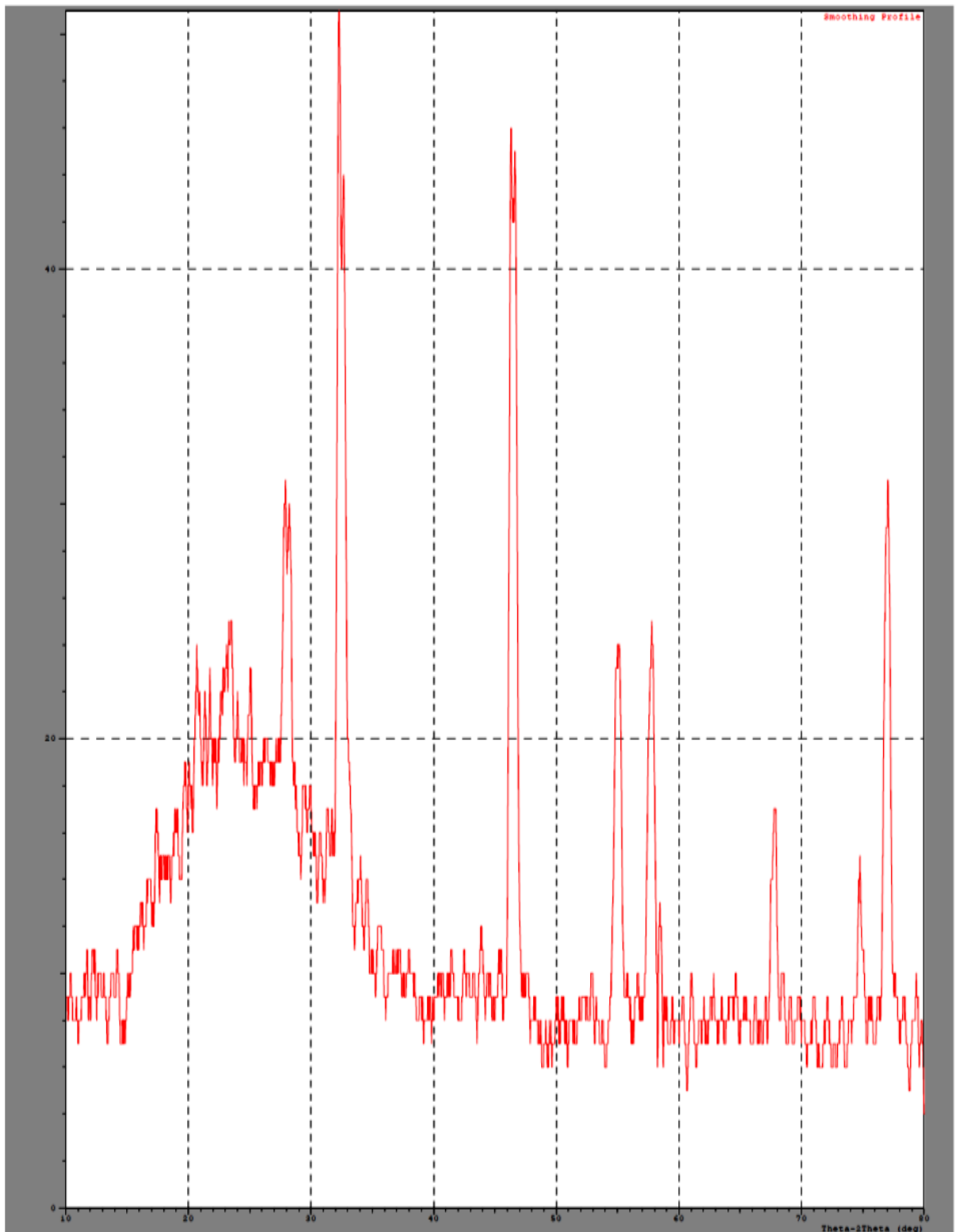


Fig-55a) TSLE-Ag NP

RESULTS & DISCUSSION

< Group: 12-11-21-Shankar Data: TSLE-Zn >

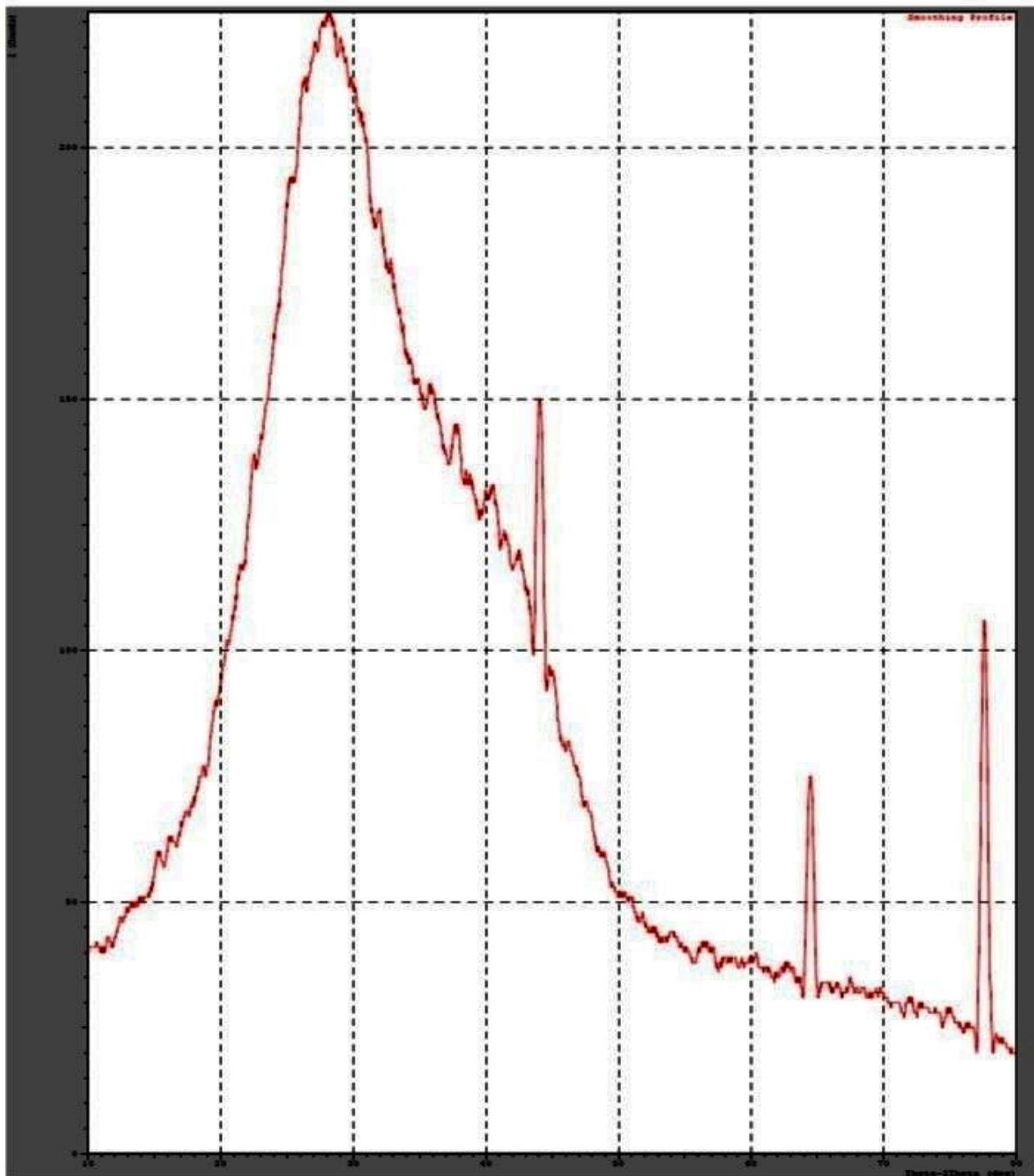


Fig-55b) TSLE Zn O NP

Fig-55: XRD analysis

7.2.13.4 SEM: Similar to reflecting light microscopes, SEMs, work on the same principles. The beam of electrons in a scanning electron microscope is reflected after interacting with the surface of the target material; this reflection is detected by the detector and converted into an image. This work used capping agents made from plant extract to create nanoparticles. (**Figures 56a and 56b**).

a) TSLE-Ag NP: As per the SEM analysis, *Tradescantia spathacea* synthesized silver particles are widely spread and visible to each other. The Particle contains an average size of 113-246 nm on morphology prediction through SEM. No agglomeration appeared, and spherical.

b) TSLE-Zn NP: As per the SEM analysis, *Tradescantia spathacea* zinc oxide nanoparticles are widely spread and visible to each other. The zinc oxide particle contains an average size of 102-159 nm on morphology prediction through scanning electron microscope. No agglomeration appeared, and spherical.

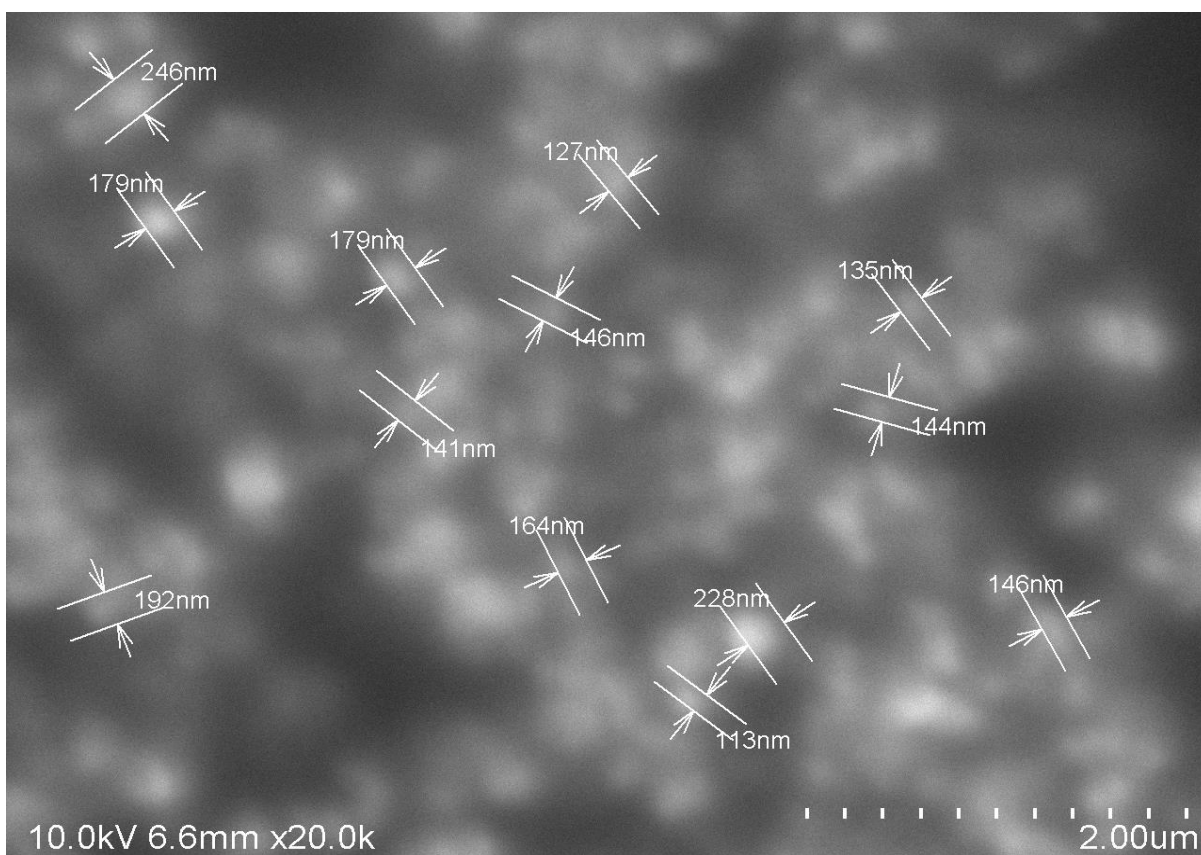


Fig- 56a) TSLE-Ag NP

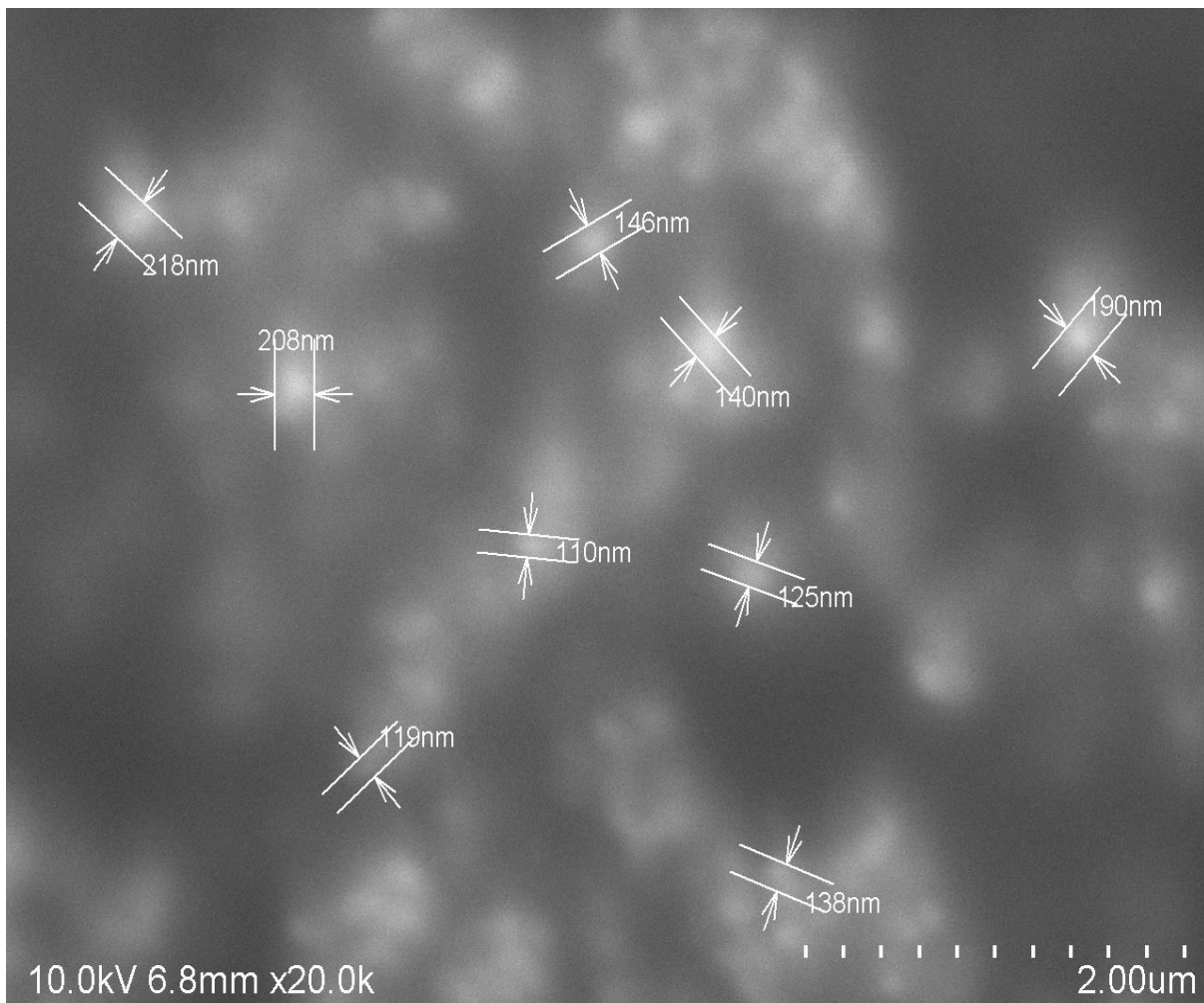


Fig- 56b) TSLE-Zn ONP

Fig- 56: SEM

7.2.14 *In vitro* antidiabetic activity

7.2.14.1 α -Amylase inhibition activity

TSLE SNP and TSLE Zn ONP had equivalent *in-vitro* antidiabetic effectiveness to acarbose, and their percentage of inhibition of alpha-amylase was the same. IC 50 values below 100 $\mu\text{g/ml}$ indicate more decisive action, as shown in TSLE SNP (73.77 $\mu\text{g/ml}$), TSLS Zn ONP (73.93 $\mu\text{g/ml}$), and also Acarbose (87.26 $\mu\text{g/ml}$) estimates in (Tables 44, 45, and Figures 57, 58).

Table:44 α -Amylase inhibition activity of TSLE SNP:

Conc'n ($\mu\text{g/ml}$)	TSLE SNP % Inhibition,	ACARBOSE % Inhibition
0	0	0
20	23	17.57
40	30.33	24.14
60	41.66	35.6
80	53.57	47.39
100	64.64	56.44
IC 50 Values	73.77	87.26

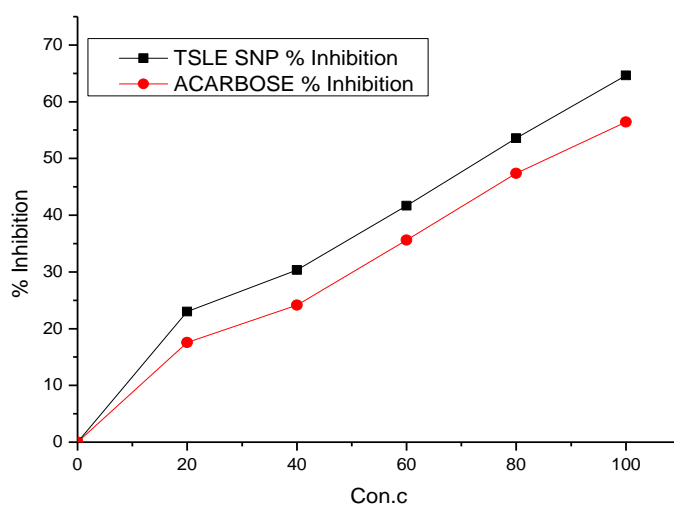


Fig-57: α -Amylase inhibition activity of TSLE SNP

Table: 45 α -Amylase-inhibition activity of TSLE ZnONP:

Conc'n (μg/ml)	TSLE Zn ONP % Inhibition.	ACARBOSE Inhibition %
0	0	0
20	23.26	17.57
40	30.07	24.14
60	41.66	35.6
80	53.25	47.39
100	64.77	56.44
IC 50 Values	73.93	87.26

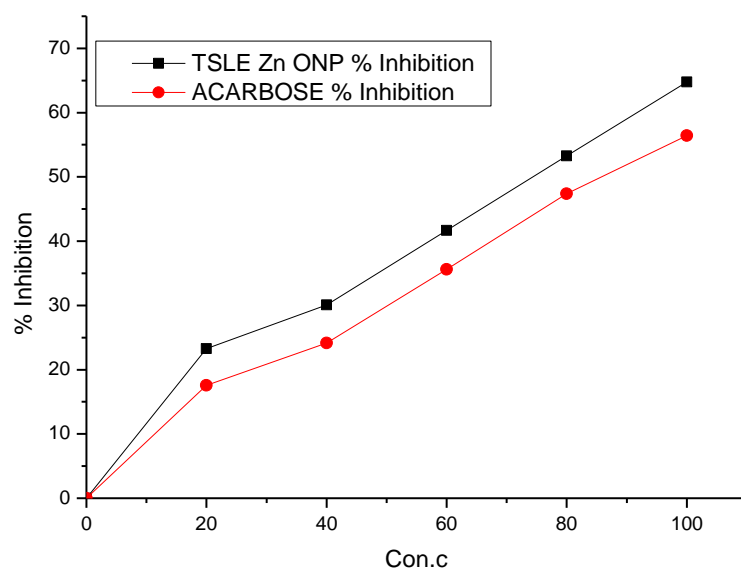


Fig-58: α -Amylase inhibition activity of TSLE Zn ONP

7.2.15 Acute toxicity study

The experiment followed OECD 425 recommendations and used 2000 mg/kg doses for SNP, Zn O NP, TSLE, TSLE Ag NP, and TSLE Zn O NP. Neither the treatment group nor the vehicle control group had any fatalities. All animals were routinely observed throughout the 14-day research period, and observations were noted.

7.2.15.1 Behavioural pattern and also body weight

Within the first four hours, SNP and Zn ONP displayed weary and sleepy symptoms (**Table 46**). During the acute toxicity evaluation, the treatment and solvent control groups' body weights marginally rose (**Table 47**).

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Table 46 Behavioural pattern for the Various Nanoparticles of T.S

Parametrs	30 min						4 hours						24 hours						48 hours						7day						14 day					
	VC	SNP	ZONP	TSLE	TSLE SNP	TSLE ZONP	VC	SNP	ZONP	TSLE	TSLE SNP	TSLE ZONP	VC	SNP	ZONP	TSLE	TSLE SNP	TSLE ZONP	VC	SNP	ZONP	TSLE	TSLE SNP	TSLE ZONP	VC	SNP	ZONP	TSLE	TSLE SNP	TSLE ZONP	VC	SNP	ZONP	TSLE	TSLE SNP	TSLE ZONP
Skin	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Eyes	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Salvation	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Respiration	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Urination (Color)	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Faces consistency	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Somatomotor activity and behaviour	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Sleep	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Mucous membrane	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
Convulsions and tremours	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Itching	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Convulsions and tremours	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Coma	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Mortality	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

U: Unchanged; A: Absent; I: Increased; VC-Vehicle control; SNP- Silver nanoparticles;ZONP- Zinc oxide nanoparticle; TSLE-Tradescantia spathacea leaf extract ;TSLE SNP- Tradescantia spathacea leaf extract Silver nanoparticles; TSLE ZONP- Tradescantia spathacea leaf extract Zinc oxide nanoparticle.

Table 47 Effects of different T.S. nanoparticles on mice body weight (in grams)

Drug	Group	Day 1st	Day 7th	Day 14th
VC	I	29.24±0.62	28.95±0.51	29.20±0.46
SNP	II	29.37±0.56	28.82±0.36	29.30±0.43
Zn ONP	III	28.94±0.60	29.30±0.50	29.02±0.24
TSLE	IV	29.1±0.57	29.22±0.51	29.35±0.43
TSLE SNP	V	28.92±0.33	29.32±0.44	29.24±0.66
TSLE Zn ONP	VI	28.84±0.47	29.50±0.53	29.27±0.66

Mean ± SD, N= 5, is used to represent values.

7.2.15.2 Organ weight index of the body

Organ weight index of the body did not significantly differ between any group. No harm to the organ level was discovered in any of the groups at a p.o. of 2000 mg/kg (Table 48).

Table 48 Organ to body weight index

Drug	Group	Heart	Liver	Kidney
VC	I	0.739±0.10	6.510±0.16	1.540±0.11
SNP	II	0.737±0.10	6.550±0.13	1.530±0.10
Zn ONP	III	0.745±0.09	6.586±0.17	1.526±0.10
TSLE	IV	0.726±0.10	6.564±0.19	1.529±0.10
TSLE SNP	V	0.725±0.10	6.643±0.22	1.523±0.10
TSLE Zn ONP	VI	0.728±0.10	6.610±0.19	1.449±0.10

Values shown as Mean ± SD, N = 5; the organ-to-body weight index is given by the formula (organ weight ×100)/body weight.

7.2.15.3 Biochemical analysis

All nanoparticles (TSLE, TSLESNP, and TSLE Zn ONP) increase LDL cholesterol levels, total cholesterol, urea, creatinine, albumin, TSLE, TSLESNP, and TSLE Zn ONP when compared to the vehicle control. Furthermore, globulin levels considerably rose in TSLE, TSLESNP, and TSLE Zn ONP, as did HDL levels in the

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same three conditions. At 2000 mg/kg in albino mice, all Nanoparticles induce mild toxicity but no organ damage (Table 49).

Table 49 Biochemical Evaluation for the extractions and Nanoparticles of *TS*

S.NO.	Parameters	Units	VC	SNP	Zn ONP	TSLE	TSLESNP	TSLE Zn ONP
1	GLUCOSE	mg/dl	93.70±1.72	93.79±2.82	95.32±3.00	95.46±2.34	92.76±2.01	93.50±2.47
2	TOTAL Cholesterol	mg/dl	98.37±3.04	187.00±2.92	106.97±1.97	117.35±1.92	116.37±1.88	117.16±2.05
3	HDL Cholesterol	mg/dl	30.70±1.72	31.14±1.65	32.47±2.65	30.42±1.15	42.82±2.53	42.88±2.21
4	LDL Cholesterol	mg/dl	45.36±1.21	56.22±1.64	64.88±2.55	54.43±3.34	52.21±1.59	51.42±1.70
5	VLDL Cholesterol levels	mg/dl	22.43±0.79	24.43±1.63	24.22±1.02	23.56±1.37	22.10±1.28	22.72±1.29
6	TRIGLYCERIDES	mg/dl	111.92±2.47	124.70±3.44	124.53±3.32	122.36±4.69	122.22±4.32	122.03±4.54
7	CHOL/HDL RATIO		3.21±0.20	4.38±0.25	3.24±0.24	3.86±0.19	3.60±0.29	3.68±0.23
8	LDL/ HDL RATIO		1.48±0.07	1.24±0.19	1.98±0.18	1.79±0.08	1.59±0.09	1.61±0.19
9	UREA	mg/dl	37.32±1.84	47.94±1.94	48.06±1.52	47.85±1.66	43.83±1.80	43.06±1.42
10	CREATININE levels	mg/dl	0.547±0.02	0.546±0.02	0.555±0.02	0.533±0.02	0.528±0.01	0.527±0.01
11	BIT	mg/dl	0.674±0.06	0.755±0.06	0.741±0.06	0.744±0.06	0.739±0.06	0.733±0.06
12	BID	mg/dl	0.265±0.05	0.259±0.05	0.260±0.05	0.251±0.04	0.252±0.04	0.250±0.04
13	BII	mg/dl	0.430±0.02	0.422±0.05	0.420±0.05	0.424±0.05	0.426±0.05	0.424±0.05
14	PROTEIN levels	mg/dl	6.274±0.27	6.283±0.35	6.354±0.26	6.268±0.22	6.419±0.31	6.40±0.21
15	ALBUMIN	mg/dl	2.270±0.08	2.811±0.27	2.778±0.21	2.745±0.22	2.556±0.29	2.580±0.24
16	GLOBULIN	mg/dl	4.010±0.25	3.548±0.14	3.580±0.32	3.636±0.27	3.551±0.23	3.496±0.28
17	A: G/RATIO		0.568±0.04	0.793±0.10	0.771±0.10	0.779 ±0.1	0.793±0.08	0.784±0.05
18	SGOT / AST	IU/L	95.99±1.62	131.65±1.60	141.51±1.69	138.89±4.08*	138.30±6.11	137.34±6.29
19	SGPT / ALT	IU/L	65.94±5.32*	95.74±4.57	96.00±4.60	80.99±2.78	80.26±5.35	80.15±5.39
20	ALP	IU/L	95.69±6.31	125.28±5.36	126.75±5.45	118.68±4.65	108.83±5.69	107.39±3.58

Statistics: Values are shown as Mean ± SD, N = 5. It was carried out using one-way ANOVA, and * denotes P <0.05 vs. Vehicle Control. TSLE stands for *Tradescantia spathacea* leaf extract. TSLE SNP stands for *Tradescantia spathacea* leaf extract silver nanoparticles. TSLE Zn ONP stands for *Tradescantia spathacea* leaf extract Zn O nanoparticles.

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7.2.15.4 Hematological analysis

All nanoparticles, including TSLESNP and TSLE Zn ONP, significantly enhance HGB, HCT, RBC, MCV, MCH, MCHC, MPV, PLT, and P-LCR levels. In TSLESNP and TSLE Zn ONPs, WBC and PCT levels rise significantly. Hematologic research found that albino mice showed no hazardous or mild poisoning symptoms from any NPs (Table 50).

Table 50 Haematological study of *Tradescantia spathacea* nanoformulations.

S.NO.	Parameters	Units	VC	SNP	ZnONP	TSLE	TSLESNP	TSLE Zn ONP
1	HGB	g/dl	13.35±0.07	14.37±0.05	14.37±0.09	13.36±0.05	13.37±0.05	13.37±0.09
2	RBCs	10 ⁶ /ul	8.48±0.07	9.18±0.08	9.21±0.05	9.11±0.03	9.13±0.05	9.10±0.04
3	HCT	%	45.20±0.76	48.52±0.40	49.76±0.08	45.62±0.20	45.85±0.38	45.74±0.21
4	MCV	fL	52.77±1.08	53.96±0.10	53.69±0.14	52.42±0.10	51.46±0.16	51.52±0.18
5	MCH	Pg	15.69±0.11	15.75±0.03	15.73±0.05	15.19±0.02	15.19±0.01	15.20±0.02
6	MCHC	g/dl	29.88±0.05	25.54±0.09	29.47±0.01	30.20±0.10	30.19±0.09	30.20±0.11
7	RDW-SD	fL	19.69±0.10	22.16±0.06	22.05±0.43	21.47±1.20	21.78±0.08	21.35±0.10
8	RDW-CV	%	19.11±0.05	19.20±0.07	19.19±0.06	19.77±0.09	19.86±0.05	19.88±0.03
9	WBCs	10 ³ /ul	4.956±0.04	4.958±0.02	4.944±0.02	4.796±0.02	4.788±0.21	4.822±0.11
10	NEUT%	%	20.45±0.41	20.79±0.46	20.84±0.28	20.80±0.34	20.81±0.40	20.81±0.41
11	LYMPH%	%	76.39±0.33	75.59±0.33	75.62±0.35	74.92±0.42	74.92±0.42	75.11±0.53
12	MONO%	%	1.01±0.12	1.44±0.10	1.43±0.09	1.51±0.07	1.50±0.07	1.51±0.07
13	EO%	%	2.19±0.12	2.72±0.08	2.75±0.09	2.64±0.05	2.65±0.09	2.65±0.08
14	BASO%	%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	IG%	%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16	NEUT#	10 ³ /ul	1.01±0.02	1.02±0.04	1.02±0.04	1.00±0.03	1.01±0.04	1.01±0.04
17	LYMPH#	10 ³ /ul	3.78±0.04	3.80±0.08	3.79±0.04	3.61±0.01	3.60±0.04	3.60±0.01
18	MONO#	10 ³ /ul	0.056±0.01	0.077±0.01	0.076±0.01	0.077±0.01	0.077±0.01	0.077±0.01
19	EO#	10 ³ /ul	0.116±0.01	0.138±0.00	0.138±0.00	.138±0.00	.138±0.00	.138±0.00

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20	BASO#	10 ³ /ul	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
21	IG#	10 ³ /ul	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22	PLT	10 ³ /ul	693.49±2.33	785.44±3.97	786.40±4.02	765.56±2.02	764.21±3.83	765.69±2.37
23	PDW	fL	6.75±0.05	7.14±0.05	7.16±0.05	6.76±0.05	6.75±0.05	6.75±0.06
24	MPV	fL	6.25±0.05	7.04±0.03	7.05±0.02	6.57±0.19	6.65±0.06	6.64±0.06
25	P-LCR	%	3.04±0.01	4.70±0.05	4.73±0.04	4.20±0.08	4.18±0.09	4.19±0.08
26	PCT	%	0.51±0.02	0.66±0.02	0.83±0.03	0.52±0.01	0.51±0.02	0.51±0.01

Values are stated as Mean ± SD, N= 5, Statistical-analysis was accomplished using one way ANOVA, * represents P<0.050 vs. Vehicle Control; VC-Vehicle control.; TSLE- *Tradescantia spathacea leaf extract*; TSLE SNP- *Tradescantia spathacea leaf extract Silver Nanoparticles*; TSLE Zn ONP- *Tradescantia spathacea leaf extract Zinc oxide Nanoparticles*.

7.2.15.5 Histopathology analysis

All of the NPs from TSLESNP, TSLE Zn ONP, and TSLE Plant Extract did not exhibit any serious organ-level toxicity, suggesting that every one of the NPs is safe at a dosage of 2000 mg/kg, p.o. The results of the histology investigations for the liver (**Figure 59**), kidney (**Figure 60**), and heart (**Figure 61**) are summarised. The findings demonstrate that whereas TSLE Zn ONPs induce mild to moderate organ-level toxicity, TSLESNP, TSLE Zn ONP, and TSLE NPs only cause minor organ-level toxicity in the heart, liver, and kidney. Biochemical, haematological, and organ-to-body weight index investigations support these findings. Therefore, all plant NPs come under the Globally Harmonised System of Classification category 5 for acute oral toxicity

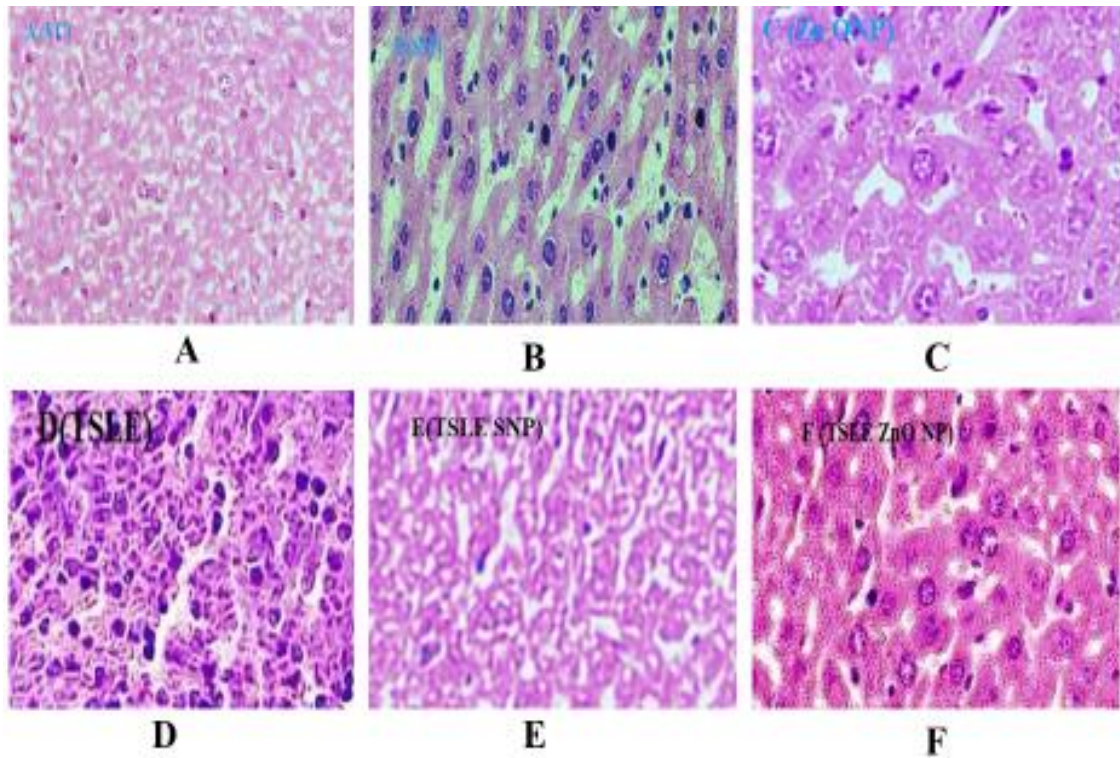


Figure 59: Liver histopathology, A (VC): Nothing abnormal growth detected; B(SNP): Hepatocytes of the liver with mild granular-degeneration; C (ZnONP): Hepatocytes of the liver with moderate-granular-degeneration; D (TSLE): Hepatocytes of the liver with mild-granular degeneration; E (TSLE SNP): Hepatocytes of the liver with mild granular degeneration; F (TSLE ZnONP): Hepatocytes of the liver with mild to moderatgranular degeneration.

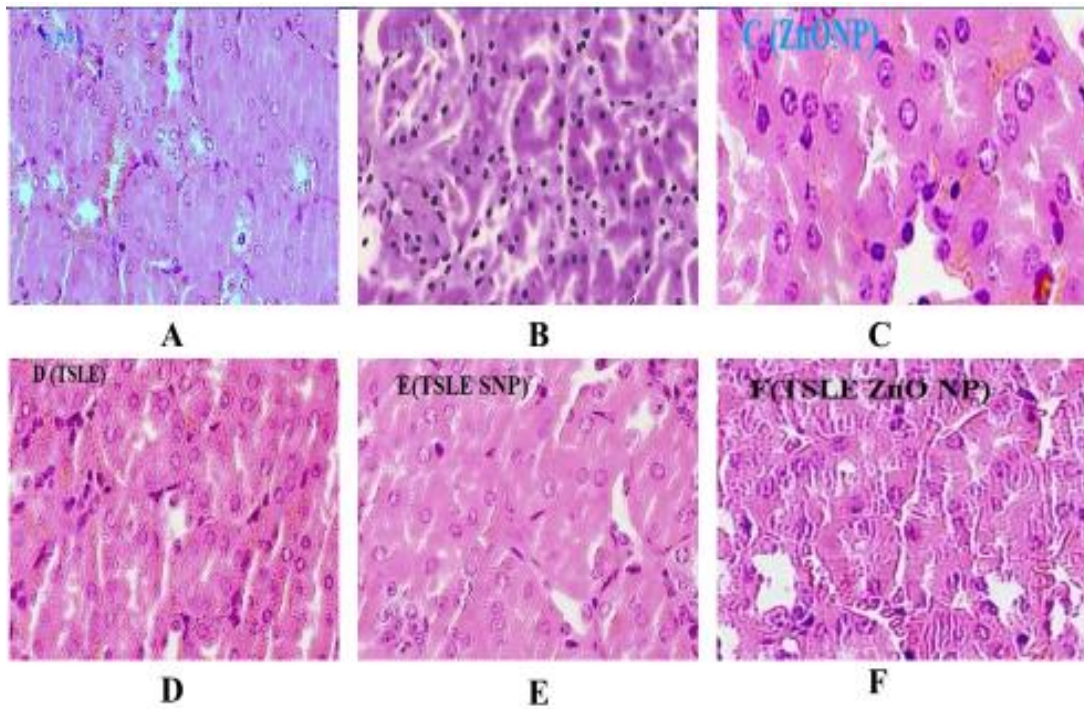


Figure 60: Kidney histopathology, A (VC): Nothing ab-normal detected; B(SNP): Mild tubular epithelial cell degeneration; C (Zn ONP): Moderate tubular epithelial cell degeneration; D (TSLE): Mild tubular epithelial cell degeneration. E (TSLE SNP): Moderate tubular epithelial cell necrosis and granular degeneration. F (TSLE Zn ONP): Moderate tubular epithelial cell necrosis and granular degeneration.

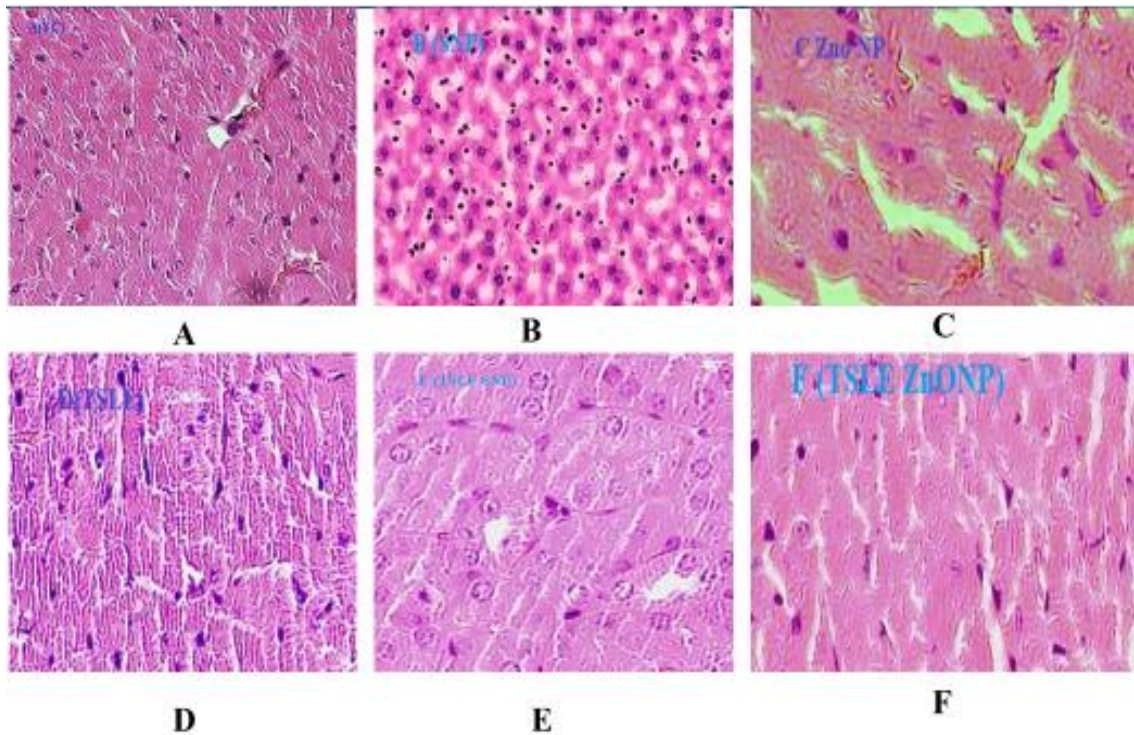


Figure 61: Histopathology observations of the Heart, A (VC): Not detected any abnormal growth; B (SNP): Moderate myocardial fatty fiber infiltration; C (Zn O NP): Mild to moderate myocardial fatty fiber infiltration; D (TSLE): Myocardial fibers have somewhat deteriorated; E (TSLE SNP): Myocardial fibers have somewhat deteriorated; F(TSLE Zn ONP): Myocardial fibers have Mild granular degeneration.

7.2.16 In-vivo Antidiabetic activity

7.2.16.1 Effect on Body weight

The body weight of the animals was measured on the 1st, 15th, 22nd, and 29th days (**Table 51**). After two weeks of nutritional modification, a significant change in body weight was found on the 15th day in rats fed the High fat diet versus rats fed the Normal pellet diet. On the 22nd day following one week of STZ injection, there is a modest loss in body weight in all groups fed a high fat diet, most likely related to the development of diabetes. In comparison to the 22nd day body weight, no significant drop in body weight was noticed on the 29th day.

Table 51: Effect of HFD and STZ model on to the body weight in Albino Wister rats

Group number	Drug treatment	1 st Day	15 th Day	22 nd Day	29 th Day
I	Saline 0.5% w/v of CMC (p.o.)	254.01±6.49	258.89±6.85	260.71±6.28	259.90±5.21
II	Metformin (50 mg /kg b.w) (p.o.)	249.46±8.71	296.14±7.49	271.73±6.10	258.09±6.45
III	STZ (40g /kg b.w) (i.p.)	249.76±9.71	293.16±8.90	278.86±9.62	256.20±6.12
IV	TSLE 200 mg /kg b.w (p.o.)	252.55±8.93	300.34±6.02	279.29±5.76	268.94±6.40
V	TSLE 400 mg /kg b.w (p.o.)	225.06±5.77	257.48±7.80	253.12±4.66	251.01±5.28
VI	TSLESNP 100 mg /kg b.w (p.o.)	211.97±5.30	230.9±4.52	240.02±9.82	229.54±6.13
VII	TSLESNP 200 mg /kg b.w (p.o.)	210.50±6.58	223.86±5.64	239.33±6.22	230.86±4.37
VIII	TSLE Zn ONP 100 mg /kg b.w (p.o.)	210.84±6.09	231.33±5.60	239.97±6.68	229.41±6.68
IX	TSLE Zn ONP 200 mg /kg b.w (p.o.)	210.07±4.06	231.88±6.00	239.86±4.91	234.63±5.74

NPD - Normal pellet diet, while HFD - high fat diet. STZ stands - streptozotocin, TSLE stands - *Tradescantia spathacea* extract, TSLE SNP stands - *Tradescantia spathacea* extract silver nanoparticles, and TSLE Zn ONP stands - *Tradescantia spathacea* extract zinc oxide nanoparticles. The values are given as Mean ± SD, n=6. Statistical analysis was carried out using one way ANOVA; # denotes p< 0.05 compared. the first day of therapy.

7.2.16.2 Biochemical evaluation

Table 52 shows 22nd and 29th-day plasma glucose, cholesterol, and triglycerides. All HFD+STZ treated groups have a significant increase in sugar, cholesterol, and triglycerides on day 22 after 7 days of STZ injection, indicating the presence of type 2 diabetes. Compared to the experimental group, the glucose, cholesterol, and triglyceride levels of TSLE SNP and TSLE Zn ONP at 200mg/kg, p.o., after one week of therapy was significantly higher more effective than ordinary metformin. The TSLE 200 mg/kg, 400 mg/kg, SNP 200 mg/kg, and Zn O NP 200 mg/kg p.o. Groups did not vary from the experimental group. Based on the results, the combination of the TSLE SNP and TSLE Zn ONP is a powerful antidiabetic compound that is efficacious at both doses, but more so at 200 mg/kg compared to the standard.

Table 52 *Tradescantia spathacea* extract and nanoparticles on Albino wister rats' plasma glucose, total cholesterol & triglycerides.

Group number	Plasma glucose level (mg/dL)		Total Cholesterol level (mg/dL)		Triglyceride level (mg/dL)	
	22 Day	29 Day	22 Day	29 Day	22 Day	29 Day
I	104.68±4.20	105.68±2.71	75.60±3.92	77.39±3.87	65.27±3.15	66.65±2.40
II	334.90±4.65#	225.57±5.81#*	173.88±3.83#	116.86±5.46#*	132.01±4.40#	72.16±6.96#*
III	335.41±2.66#	335.45±6.69#	169.54±5.90#	184.29±5.61#	137.17±4.63#	147.86±6.27#
IV	333.39±5.86#	281.75±3.37#*	169.24±6.17#	143.86±3.33#*	138.83±4.10#	119.36±6.42#*
V	335.89±6.19#	280.68±5.05#*	165.38±6.35#	143.39±7.36#*	133.98±6.24#	124.56±4.26#*
VI	334.75±3.78#	250.43±5.67#*	164.33±6.10#	116.73±6.19#*	134.26±7.27#	93.49±7.17#*
VII	331.50±5.19#	244.91±3.53#*	163.80±7.07#	115.83±7.10#*	130.15±4.77#	72.61±7.62#*
VIII	335.52±5.03#	277.90±4.59#*	165.09±6.39#	115.17±7.55#*	134.86±6.14#	77.23±6.28#*
IX	335.10±5.60#	244.38±5.50#*	164.85±6.26#	115.15±4.73#*	132.74±6.32#	74.91±7.16#*

The values are given as Mean ±SD, n=6. Statistical analysis was performed using one-way ANOVA, where # denotes p 0.05 vs. Vehicle Control and * denotes p< 0.05 vs. Experimental Control.

7.2.16.3 Histopathological studies

In the experimental group, a single dosage of STZ causes type 2 diabetes and islet-cell necrosis. After a week after administration of TSLESNP and TSLE Zn NP 200mg/kg p.o., The drug's anti-diabetic potential was supported by pancreatic islets' normal structure and moderate necrosis. The 100mg/kg dose has less islet cell recovery. However, no recovery is seen in the TSLE 200 mg/kg and 400 mg/kg p.o. treated groups when compared to the experimental control group (**Figure-63**).

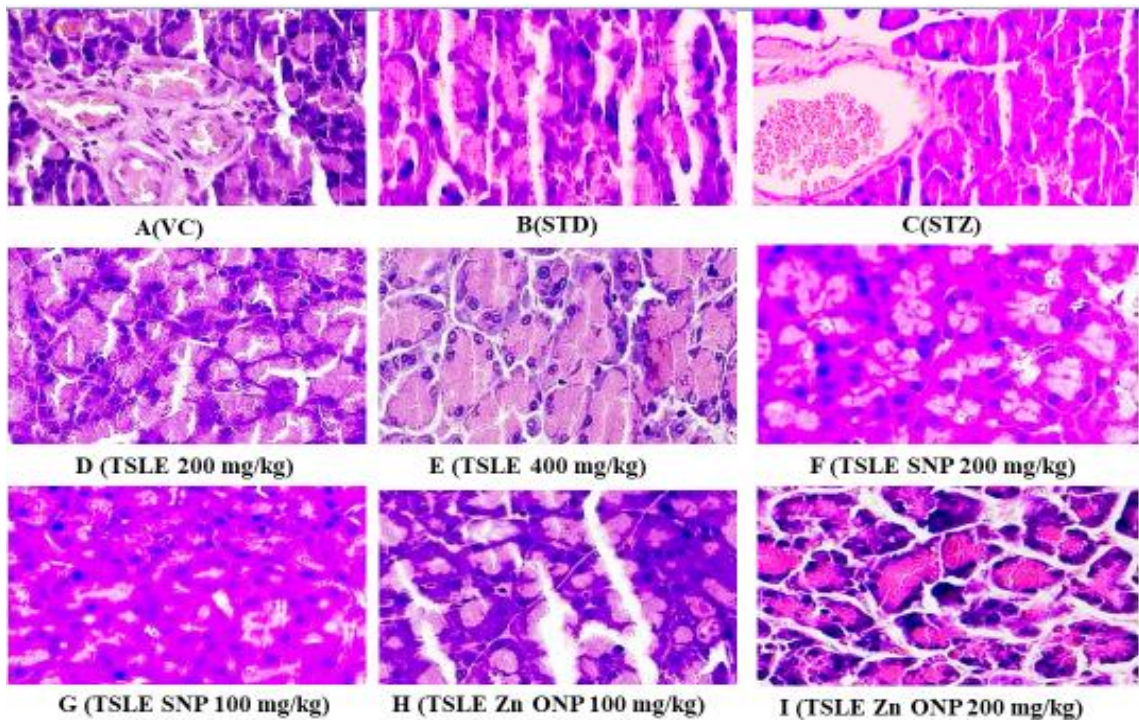
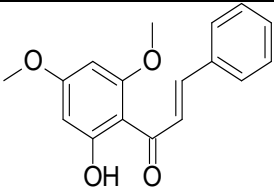
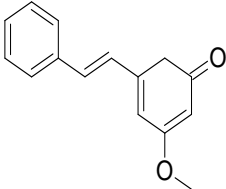
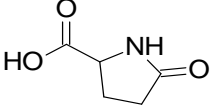


Fig-62: Analyses of pancreatic histopathology for *Tradescantia spathacea* nanoparticles. A (VC): There is no growth in tissues; B(MF): (Standard): Langerhans islets have Mild necrosis; C(STZ): Necrosis (NC) of islets of Langerhans; D (TSLE 200 mg/kg): Langerhans islets have mild-necrosis (MNC) ; E (TSLE 400 mg/kg): Mild necrosis (MNC) of Islets of Langerhans; F (TSLE SNP 100 mg/kg): Moderate degeneration and necrosis (MODN) of Islets of Langerhans.; G (TSLE SNP 200 mg/kg): Islets of Langerhans have mild-degeneration and necrosis (MODN) of; H (TSLE Zn ONP 100 mg/kg): Moderate-degeneration and Necrosis (NC.) of Islets of Langerhans; I (TSLE Zn ONP 200mg/kg): Islets of Langerhans have mid degeneration and Necrosis (NC).

7.2.17 Molecular docking studies

The primary ingredients' antidiabetic efficacy was evaluated in-silico using Human pancreatic alpha amylase (PDB: 5VA9). Among all the plant constituents the Flavokawin B showed the five hydrogen bond interactions to Arg195, Asp197, Glu233, His 299, Asn298 at the binding site of Human pancreatic alpha amylase and showed four stearic interactions to Trp 59, Ile235, Asn 298, His299 at the binding site of Human pancreatic alpha amylase. The following (Table 53 and Figures 63 a, 63 b, 63 c) reveals the binding energy of different phytochemicals.

Table 53: Molecular docking, binding affinities of phytoconstituents.

Ligand	Mol Dock Score (Kcal/Mol)	Number of Hydrogen bonds/ Hydrogen bond Residues
 <p>Flavokawin B</p>	-100.131	Arg195, Asp197, Glu233, His 299, Asn298.
 <p>5,6-Dehydrokawain</p>	-88.4749	Lys 200, Glu 233, Ile 235.
 <p>Pyroglutamic acid</p>	-81.4632	Ile 230

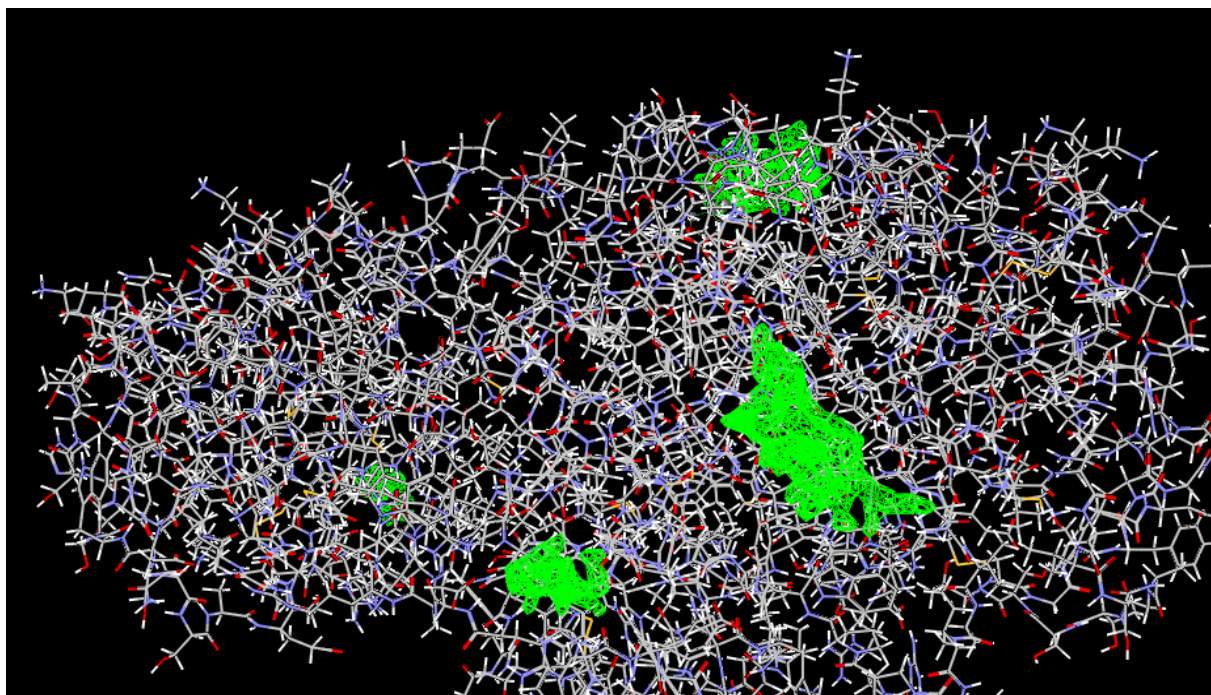


Fig-63 (a): Crystal structure of Human pancreatic alpha amylase with cavity.

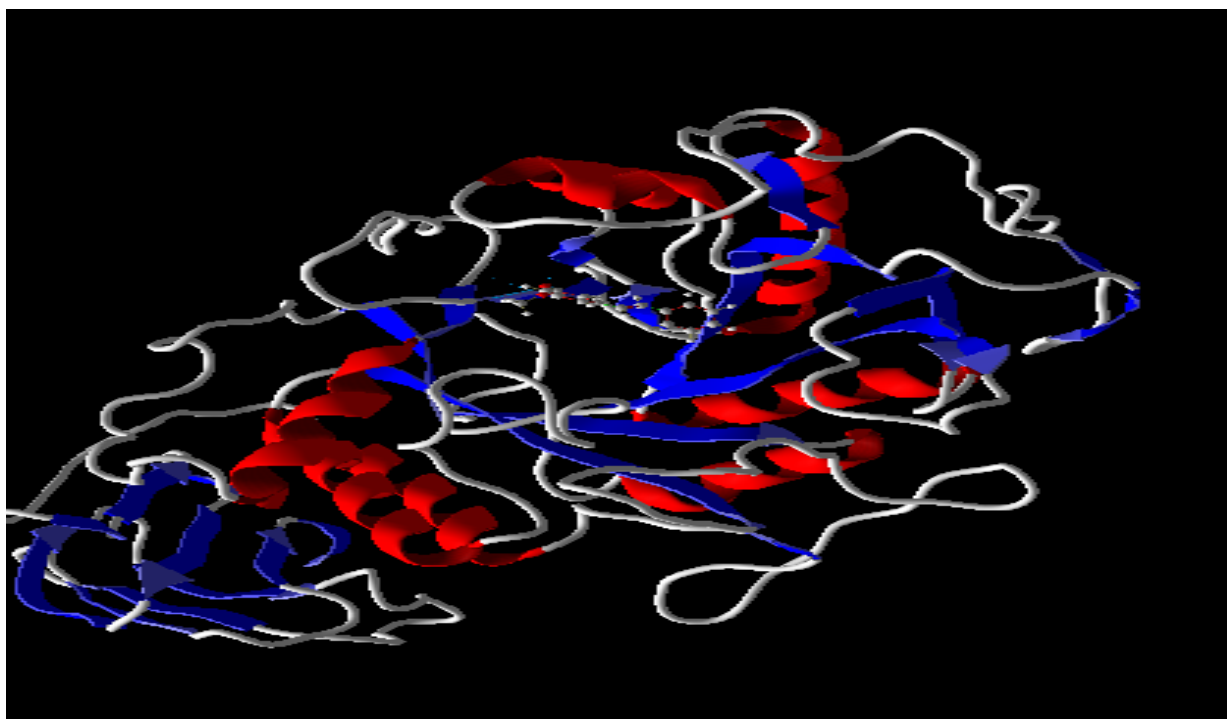


Fig- 63 (b): Hydrogen bonding interactions between Human pancreatic alpha amylase with Flavokawin B.

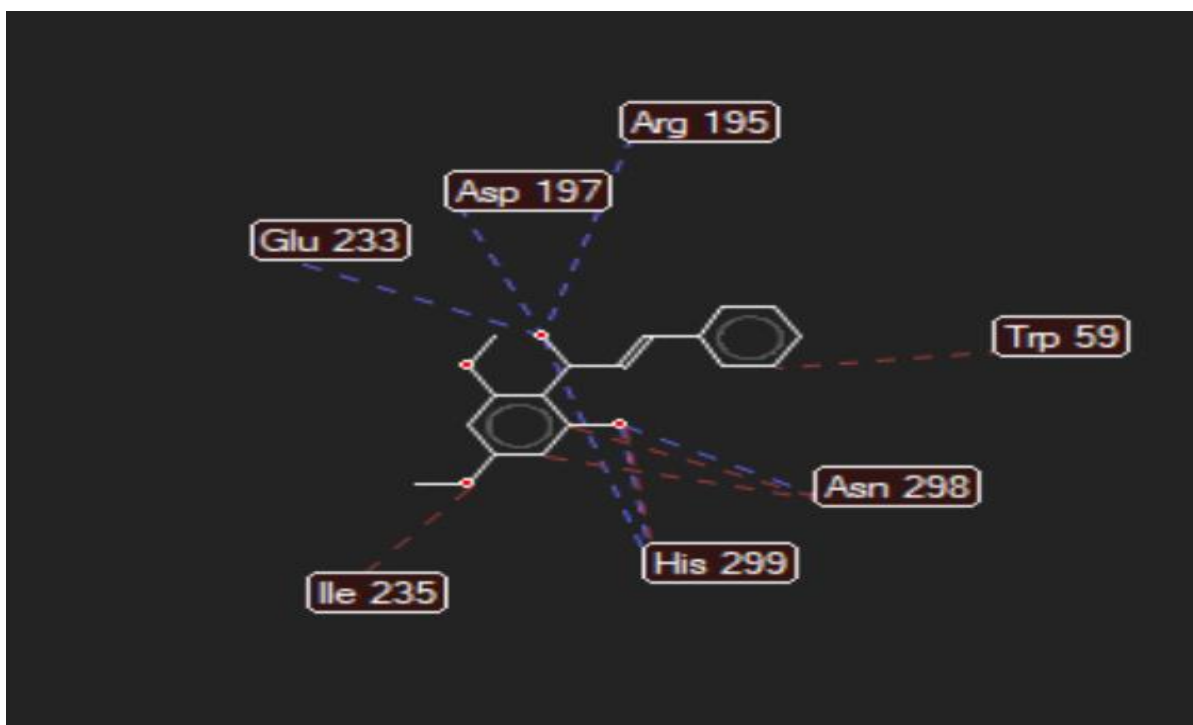


Fig- 63 (c): Hydrogen bonding interactions, stearic interactions of Flavokawin B with amino acids of Human pancreatic alpha amylase.

Discussion

Herbal materials, products, and plants are all included in the area of herbal medicine. A number of ailments are routinely treated with medicinal plants by traditional healers, and several plant species are being researched for possible medical uses [265]. Separation is essential for obtaining phytoconstituents from plant extracts for herbal medicine research. The USE approach provides several advantages over conventional methods, such as decreased solvent use, a speedier extraction procedure with improved extraction pure, and a larger yield of bio-active phyto-constituents [266]. The total phenolic content of several plant extracts was examined, and it was determined that conventional extraction was superior to an ultrasound-assisted extraction, regardless of the solvent employed [267]. The overall phenolic, flavonoid and alkaloids content rose in the ethyl acetate, a solution of hydroalcoholic extracts, with the maximum concentration in the methanol extract. This shows that USE is superior to the traditional approach for extracting plants because it uses ionic conduction and dipole rotation to convert electromagnetic energy to calorific energy, which allows the solvent to penetrate deeper into the plant's matrix [268].

Recent advances in nanotechnology have sparked renewed interest in these topics, with formulations taking the form of nanospheres, nanocapsules, liposomes, pro-liposomes, solid lipid nanoparticles [SLNs], and nano-emulsion. Furthermore, non-selective medication delivery to the target area usually results in unwanted side effects. Because it increases the loading agent's target selectivity in vivo, herbal nanoformulation shows promise as a delivery strategy for herbal medicines. A thorough investigation of nanotechnological methods will reveal fresh information about the diabetes mellitus treatment protocol. This might be a key strategy for creating a new environment for the production of unique, clinically tested pharmaceuticals derived from local sources employing cutting-edge techniques and drug designs [269] As pharmacological therapy has not been completely beneficial for diabetic patients, additional preventive and therapeutic methods are required. AgNPs are now widely used in a variety of fields, which is a huge increase over the preceding decade. In the 1990s, silver colloids were initially used as an anti-microbial agent to treat a range of ailments. Ag NPs (agglomerates of nanoparticles) have distinctive

characteristics that are influenced by their size, distribution, and form. Due to their various potential applications in a range of sectors, such as catalysis, optics, antimicrobials, and the production of biomaterials and AgNPs, nanoparticles are now receiving a lot of interest. Photosynthesis does not need a prolonged cultivation and maintenance process, hazardous chemicals, or high energy requirements, in contrast to chemical, physical, and microbiological synthesis. [270].

Silver nanoparticles with 20–15,000 silver atoms are usually smaller than 100 nm. Silver has distinct physicochemical and biological properties in its nanoscale form. Many plants, plant components, or microbes are used in biological or environmentally friendly methods to convert silver ions into silver nanoparticles. The green approach is more efficient and kinder to the environment since it uses naturally existing precursors such as vitamins, sugars, plant extracts, biodegradable polymers, and microbes as reductants and capping agents [271]. ZnO nanoparticles have a range of pharmacological properties, including those that are anti-cancer, anti-pathogenic, and antioxidants. ZnO nanoparticles' most basic uses are expanded by technical developments in areas including sensor technology, energy generation, optoelectronics, biomedicine, and drug delivery systems. ZnO composites are bioactive materials that promote biomedicine and energy storage. Its biocompatibility, biodegradability, low toxicity, and hardness make it useful in biomedicine and pro-ecological systems like cosmetics. As ZnO materials are used for a variety of applications, they have many different synthesis parameters. Due to its numerous advantages, the green synthetic technique employing natural plant extracts is prevalent, including the need for no additional chemicals, simplicity, environmental friendliness, affordability, and dependability of the procedure [272].

Also, both plants were examined using a variety of qualitative and quantitative techniques, such as macroscopy, microscopy, ash value, moisture content, extractive value, and preliminary phytochemical screening. *Alpinia mutica* and *Tradescantia spathecea* leaves were evaluated using the stomata number, index, palisade ratio, vein-islet, and termination number. All of these observations and results show that both plants adhere to the quality criteria. According to several studies, plants are a major source of natural antioxidants and contain potent antioxidants. An "antioxidant" is a vitamin, mineral, or phytochemical that protects against reactive oxygen species

(ROS). In vitro and in vivo tests for free radical elimination are available in the scientific literature. Based on their capacity to neutralise free radicals, antioxidants may be divided into three categories: strong, moderate, and weak antioxidants. [240-241]. This study used a particle size analyzer to evaluate zeta potential and distribution. FTIR at 400–4000 cm^{-1} was utilised to detect biomolecules that synthesise A.M Ag NP, A.M Zn O NP, T.S Ag NP, and T.S Zn O NPs. SEM was used to analyse A.M Ag NP, A.M Zn O NP, T.S Ag NP, and T.S Zn O NPs' morphology, particle size, shape, and elemental composition. [246-247].

DM is a type of metabolic syndrome, which is defined by hyperglycemia and insufficient pancreatic insulin production. Obesity and the possibility of cardiovascular disease are linked by the DM [273]. When compared to the synthetic medications now on the market, certain phytoconstituents have been discovered to have fewer negative effects and to be less harmful [274]. Nanotechnology improves electrocatalytic characteristics and sensor-receptor complex surface area, helping cure diabetes. [275]. Therefore, to explore a better therapy for the cure of diabetes, the leaves of the plant *Alpinia mutica* and the leaves of *Tradescantia spathecea* were selected.

Nanoformulations' antidiabetic potential in-vitro and in-vivo from *Alpinia mutica* (A.M Ag NP, A.M Zn O N.P.s) and *Tradescantia spathecea* (T.S Ag NP, T.S. Zn O N.P.) leaves was tested. The alpha-amylase inhibition test was used to evaluate the in-vitro anti-diabetic efficacy using acarbose as a standard. In order to avoid postprandial hyperglycemia, the alpha-amylase enzymes convert polysaccharides into monosaccharides (mainly glucose) [276-277]. As compared to standard acarbose, it was shown that the A.M Ag NP, A.M Zn O NPs, T.S Ag NP, and T.S Zn O NPs all exhibited extremely powerful alpha-amylase inhibitor activity. Acarbose, A.M. Ag NP, A.M. Zn O NPs, T.S. Ag NP, T.S. Zn O NPs, and their respective IC₅₀ values are 73.72, 73.49, 73.77, 73.93, and 87.26 $\mu\text{g}/\text{mL}$. Thus, an alpha-amylase test suggests that the A.M. Ag NP, A.M. Zn O N.P.s, T.S. Ag NP, and T.S. Zn O N.P.s decrease blood glucose levels and reduce the rate at which starch is converted to monosaccharides, thus metabolising starch and glycogen. In an in-vitro alpha amylase

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inhibitor study, it was discovered that A.M. Ag NP, A.M. Zn O NPs, T.S. Ag NP, and T.S. Zn O NPs produced much better results than acarbose.

Before the *in vivo* antidiabetic investigation, OECD 425 acute toxicity screening was performed on the nanoparticles. The acute toxicity investigation of *Alpinia mutica* (SNP, ZONP AMLE, A.M Ag NP, and A.M Zn O NPs) was conducted in albino mice at 2000 mg/kg, *p.o.*; Biochemical data indicated all three NPs (SNP, ZONP, and AMLE) had greater levels of total-cholesterol levels, LDL, urea, creatinine, albumin, SGOT, SGPT, and ALP levels over the vehicle control. Moreover, in A.M. Ag NP and A.M. Zn O NPs, a substantial increase in globulin and HDL levels was found. The SNP, ZONP AMLE, has been found to significantly increase HGB, HCT, RBC, MCV, MCH, MCHC levels, MPV, PLT, and P-LCR levels as per the hematology parameters. Also, it was shown that the levels of WBC, PCT, and AM Zn O NPs all increased significantly. The biochemical and haematological data, which were corroborated by the histopathology results, show that SNP and ZONP AMLE create mild to moderate organ toxicity, whereas A.M. Ag NP and A.M. Zn O NPs only produce may or may not produce mild to moderate heart, liver, and renal toxicity. According to the acute toxicity research, all of the nanoparticles from the plant *Alpinia mutica* had an LD50 greater than 2000 mg/kg.

The identical acute toxicity study approach was used for *Tradescantia spathecea* leaves, which showed no significant variations in organs' body weight index or abnormalities in any isolated organs. An increased concentration of lipids, including LDL, total cholesterol, VLDL, and triglycerides, as well as elevated levels of urea in the kidney function-test, and amino acids, albumin, and SGOT/AST in a liver function test, has been observed. Minor poisoning symptoms are seen with SNP, ZONP, and TSLE. However, TS Ag NP and TS Zn O NPs have moderate to severe symptoms. The haematological parameters also show a notable rise in platelets and other blood cells. Only the TS Ag NP and TS Zn O NPs showed a significant change in WBC compared to the control group. The histopathological data supported the biochemical and haematological findings, which showed that the NPs (TS Ag NP and TS Zn O NPs) exhibited mild or moderate granular degenerative conditions in all

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the organs studied. Therefore, the LD50 for all *Tradescantia spathecea* NPs is more than 2000 mg/kg, as shown by the acute toxicity investigation.

STZ, a glucosamine nitrosourea, destroys pancreatic beta cells, producing hyperglycemia. High-fat diets raise type 2 diabetes risk. [278]. HFD, in combination with STZ, is a commonly used method for the development of type-2 DM [279]. The plant NPs were subsequently examined for their in-vivo antidiabetic activity in HFD and Albino Wister rats with a modest dosage of STZ (35 mg/kg, i.p.). Initially, all groups (apart from the standard control) were given the HFD diet for two weeks, which considerably raised the animal's body weight by day 15 compared to the NPD-fed group. [280], after one week of STZ administration (22nd day) to HFD group rats, a fall in body weight and decrease in diet intake was observed in all the HFD groups, which is considered one of the primary symptoms of diabetes [281].

The rats received the respective treatments for 7 days from the 22nd day. In this study, two doses of Extracts and NPs (200 mg/kg and 400 mg/kg AMLE, 100 mg/kg and 200 mg/kg AMLE SNP p.o., and 100 mg/kg and 200 mg/kg p.o. AMLE Zn O NP) were tested. After a week of treatment with 400 mg/kg AMLE, 200 mg/kg AMLE SNP, and 200 mg/kg AMLE Zn o NPs, respectively, the biochemical effects of *Alpinia mutica* were observed. There was a significant decrease in glucose, triglyceride, and cholesterol levels in the control groups compared to the experimental group. AMLE SNP and AMLE Zn o NPs were more efficient than the generic version of Metformin. In contrast to the group experimenting, the AMLE SNP and AMLE Zn o NPs they considerably reduced all biochemical levels. There is no significant difference between the AMLE group, the AMLE SNP 100 mg/kg group, and the AMLE Zn ONPs group as compared to the experimental group.

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While mild necrosis was shown, supporting the possible antidiabetic impact of the AMLE SNP 100 mg/kg and AMLE Zn O NPs 100 mg/kg, significant necrosis was seen in the AMLE SNP 200 mg/kg and AMLE Zn O NPs 200 mg/kg, demonstrating that conventional form is maintained in pancreatic islets. In contrast to the experimental control group, no recovery is shown in the SNP, Zn-O NPs, and AMLE-treated groups. The anti-diabetic efficacy of *Tradescantia spathecea* NPs, SNPs, and Zn ONPs was also evaluated using the same experimental approach. After one week of treatment with 200 mg/kg p.o. of either TSLE SNP or TSLE Zn ONPs, the results reveal a statistically significant reduction in glucose, triglyceride, and cholesterol levels relative to the experimental group. TSLE SNP and TSLE Zn ONPs were discovered to be more potent than the common drug Metformin.

Furthermore, the same experimental procedure was used to test the antidiabetic potential of the NPs and extracts of *Tradescantia spathecea* (TSLE, TSLE SNP, and TSLE Zn ONPs). The findings show that after one week of TSLE SNP and TSLE Zn ONPs administration at a concentration of 200 mg/kg p.o., glucose, triglyceride, and cholesterol levels are all considerably lower than those of the experimental group. AMLE SNPs and AMLE Zn ONPs were discovered to be more potent than the common drug Metformin. Also, the same experimental procedure was used to test the antidiabetic potential of the NPs and extracts of *Alpinia mutica* (AMLE, AMLE SNP, and AMLE Zn ONPs).

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The findings show that after one week of AMLE SNP and AMLE Zn ONPs administration at a concentration of 200 mg/kg p.o., when compared to the experimental groups, the control group's glucose, triglyceride, and cholesterol levels decreased by a statistically significant amount. Even so, compared to TSLE SNP 200 mg/kg and TSLE Zn O NP 200 mg/kg NPs, AMLE SNP and AMLE Zn O NPs 200 mg/kg showed a substantial decrease in blood glucose, triglyceride and total cholesterol levels.

Results indicate that the AMLE SNP 200 mg/kg, AMLE Zn O NPs 200 mg/kg, TSLE SNP 200 mg/kg and TSLE Zn O NPs 200 mg/kg NPs are superior to the AMLE SNP 100 mg/kg, AMLE Zn O NPs 100 mg/kg, TSLE SNP 100 mg/kg and TSLE Zn O NPs 100 mg/kg NPs in the treatment of DM.

CHAPTER 8
SUMMARY & CONCLUSION

8. SUMMARY AND CONCLUSION

In this study, *Alpinia mutica* and *Tradescantia spathacea* were screened for qualitative and quantitative estimation to standardize the plant materials. Nanoparticles of silver and zinc were produced using plant extracts, and their antidiabetic potential and acute toxicity were studied in vitro and in animals. The *in-vitro* studies of all the nanoparticles were carried out using alpha-amylase inhibition assay. The *in-vitro* outcomes suggest that the methanolic TSLE Ag NPs, TSLE Zn O NP, AMLE Ag NP and AMLE Zn O NP were highly potent than standard metformin. In an in-vitro alpha-amylase inhibitor experiment, the methanolic TSLE Ag NPs, TSLE Zn O NP, AMLE Ag NP, and AMLE Zn O NP performed better than the TSLE and AMLE.

In addition, the acute toxicity profile of the various NPs from both plants was determined in albino mice using the OECD 425 standards. By all biochemical, hematological, and histopathological assessment measures, the toxicity analysis shows that all NPs cause only mild toxicity; however, TSLE Ag NPs, TSLE Zn O NPs, AMLE Ag NPs, and AMLE Zn O NPs generate mild to moderate toxicity. Acute toxicity studies on *Tradescantia spathacea* and *Alpinia mutica* showed that both plants' NPs were well tolerated at a dose of 2,000 mg/kg orally. All nano herbal formulations were evaluated with different concentrations, i.e., 100 mg/kg and 200 mg/kg, p.o. for the in vivo antidiabetic activity HFD diet and a low dose of STZ in Wister rats.

When comparing the experimental group to the control group and the Nano herbal formulations to standard metformin, the findings indicate that the TSLE AgNPs, TSLE ZnONPs, AMLE AgNPs, and AMLE ZnONPs at a dose of 200 mg/kg, p.o., result in a highly significant reduction in the sugar levels, cholesterol, and triglyceride level. To support the idea that these formulations have an anti-diabetic impact, histopathology data for TSLE AgNPs, TSLE Zn ONPs, AMLE AgNPs, and AMLE Zn ONPs reveal that the islets of pancreatic cells preserve their usual shape and exhibit very mild necrosis. In addition to both the nanoformulations, TSLE AgNPs, TSLE Zn ONPs, AMLE Ag NPs, and AMLE Zn ONPs are highly significant compared to the standard metformin at 50 mg/kg, p.o. in in-vivo antidiabetic activity.

TSLE extract was administered to diabetic rats at 200mg/kg and 400mg/kg. TSLE AgNPs and TSLE Zn ONPs at 100 and 200 mg/kg were administered to diabetic rats. Compared to crude extract, TSLE AgNPs and TSLE Zn ONPs (200mg/kg) have

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antidiabetic activity. The AMLE extract was given to diabetic-treated rats at 200mg/kg and 400mg/kg, respectively. The AMLE Ag NPs and AMLE Zn ONPs were also given to diabetic-treated rats at 100mg/kg and 200mg/kg, respectively. According to the study, AMLE Ag NPs and AMLE Zn ONPs (200 mg/kg) have much more antidiabetic potential than crude extract. The herbal nanoparticles were identified with characterized by UV, FTIR, Zeta Potential, SEM, and XRD. All herbal nanoparticles showed a significant reduction in blood glucose, triglyceride, and total cholesterol level compared to all extracts.

In conclusion, the acute oral toxicity of TSLE Ag NPs, TSLE ZnO NPs, AMLE Ag NPs, and AMLE ZnO NPs is classified as category 5 of the Globally Harmonized System of Classification has an LD50 of more than 2000 mg/kg. Compared to the standard metformin, both NPs exhibit decisive antidiabetic action in both in-vitro and in-vivo antidiabetic models. Hence, both the herbal nanoparticles can be used in the management of type 2 DM and can be explored. Additional in-vivo pharmacological studies will assist in projecting the effectiveness of recently produced all Herbal NPs as a therapy target in treating type 2 diabetes. They will fully define the mechanism of action.

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ANNEXURES

ANNEXURES

Annexure 1 : Candidacy letter of Ph.D.

Annexure 2 : List of publications, patents, awards, certificates

Annexure 3 : Certificate of analysis of Experimental Work



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Dated: 13 May 2021

Shankaraiah Pulipaka

Registration Number: 41800583

Programme Name: Doctor of Philosophy (Pharmacognosy)

Subject: Letter of Candidacy for Ph.D.

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on 09 Dec 2019 by accepting your research proposal entitled: “DEVELOPMENT, OPTIMIZATION, CHARACTERIZATION OF NANO FORMULATIONS OF ALPINIA MUTICA AND TRADESCANTIA SPATHACEA FOR ANTI-DIABETIC ACTIVITY” under the supervision of Dr. Ashish Suttee.

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

We wish you the very best!!

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

Head

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Note:-This is a computer generated certificate and no signature is required. Please use the reference number generated on this certificate for future conversations.

RESEARCH ARTICLE

Exploration of *In-vitro* Antidiabetic Activity of ZnO NPs and Ag NPs Synthesized using Methanolic Extracts of *Alpinia mutica* and *Tradescantia spathaeca* Leaves

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ABSTRACT

Diabetes might be cured with the use of medicinal herbs and environmentally friendly production of metallic nanoparticles (Ag NPs) and ZnO NPs. The methanolic leaf extracts of *Alpinia mutica* and *Tradescantia spathaeca* were used to synthesize silver nanoparticles (Ag NPs) and zinc oxide nanoparticles (ZnO NPs), respectively, for *in-vitro* evaluation.

Methanolic leaf extracts of *A. mutica* and *T. spathaeca* were used to create AgNPs and ZnO NPs under ambient conditions using ultrasound-assisted extraction (UAE). Their ability to block alpha- and beta-amylase confirmed the *in-vitro* antidiabetic efficacy of methanolic leaf extract of plant (MLEP), AgNPs, and ZnO NPs. In this study, α -amylase activity of ZnO and nanoparticles of silver produced from natural sources will be evaluated in an effort to lessen the toxicity and negative effects of the inhibitor used to treat diabetes. Antidiabetic action was especially impressive in the ZnO and silver nanoparticles produced using methanolic extracts of *A. mutica* and *T. spathaeca*. Because of their promising *in-vitro* antidiabetic action with alpha-amylase activity, MLEP of *A. mutica* and *T. spathaeca*, AgNPs, and ZnO NPs show promise for future medical uses.

Keywords: *Alpinia mutica*, Green synthesis, Phytochemical studies, Silver nanoparticles, *Tradescantia spathaeca*, Zinc oxide nanoparticles, *Alpha-amylase activity*.

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INTRODUCTION

Medicinal plants are a major source of drugs. Plants are used in health treatment by 80% of the global population¹. Natural molecules may substitute synthetic components in food and pharmaceuticals, which have adverse effects.² Because of their free radical-scavenging abilities, therapeutic plants and their phytoconstituents are becoming more popular as natural sources.

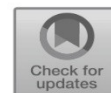
Effectively, plants are providers of natural antioxidant molecules with various pharmacological actions and few to no adverse effects that defend against many illnesses and safeguard human health.³⁻⁵ By preventing the spread of oxidative chain reactions, medicinal plant compounds delay

the deterioration of lipids or additional molecules, hence the development of oxidative stress-related illness.²

Radiation, cigarette smoke, airborne hazardous chemicals, overnutrition, shifting dietary habits, and lack of physical exercise are all examples of exogenous sources of reactive oxygen compounds (ESROS), reactive nitrogen compounds (RNS), and free radicals in the body. A few examples of cardiovascular illnesses are heart failure with congestive systolic high blood pressure, chest pain, atherosclerosis, cerebral deficiency, vein insufficiency, and ventricular fibrillation, or VF. There are many different medicinal plants that contain powerful cardioactive glycosides and have good inotropic properties on the heart; some examples are *Digitalis*

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Development of an Innovative Ultrasound-Assisted Extraction Technique to Optimize Extraction on Phytoconstituents and Compared Conventional Extraction Method



Shankaraiah Pulipaka, Ashish Suttee, M. Ravi Kumar, Kalakotla Shanker, Ramesh Kasarla, and Swamy Kasarla

Abstract In the past, people have relied on some methods like the Soxhlet and reflux device to extract plant matter. To address this problem, we use a cutting-edge extraction technique to remove the relevant plant material. There are several advantages of using ultrasonic-assisted extraction over the conventional approach, such as reduced solvent consumption, reduced extraction time, increased extraction purity, and an increased yield of bioactive phytoconstituents. The family Commelinaceae includes the Indian herb *Tradescantia spathacea* (T.S), which is used as a traditional medicine. It is the southeast Mexican region known as “Maguey Morado” are derived from *Tradescantia spathacea* (T.S) leaves extracted using traditional and ultrasonic-assisted extraction procedures using petroleum ether, ethyl acetate, methanol, hydroalcoholic, and aqueous solvents (Purple Maguey). Total phenolic

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Possible insilico exploration of alpinia mutica and tradescantia spatheca for diabetes mellitus

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Abstract

Molecular docking is computed aided tool to predict the interaction between protein and ligand. Several herbs were used in diabetic mellitus. In the current research article two medicinal plants naming *Alpinia mutica* and *Tradescantia spatheca* are screened against 4 protein to determine its *In silico* anti-diabetic potential. Fourty two constituents from *Alpinia mutica* and nineteen constituents from *Tradescantia spatheca* screened against targets namely Glutamine: Fructose-6-Phosphate Amindotransferase (GFAT,PDB ID-2ZJ3), Tetrameric 11b-HSD(PDB ID-1XU7), Aeglitaar (PDB ID-3G9E), Human SIRT6 (PDB ID-3K35) and protein tyrosine phosphatase - 1B(PDB ID-4Y14) were assessed. Molecular docking studies were performed using tool Autodock vina, biovia discovery studio and open bable, Additionally the Swiss ADME were utilized for its pharmacokinetic prediction. The docking studies with the ligands shows great inhibitory effect; In *Alpinia mutica*; 1,7-diphenyl-3-hydroxy-6-heptene5-one(-9.0kcal/mol) has the highest binding energy with protein 3K35;bisabolol(-8.1kcal/mol) with 2ZJ3; Flavokwain (-8kcal/mol) with 1XU7;1,7-diphenyl-3-hydroxy-6-heptene5-one(-6.9kcal/mol) with 4Y14 and Flavokwain (-7.8kcal/mol) with 3G9E.In *Tradescantia spatheca*, rutin (-10.1 kcal/mol),(-9.4 kcal/mol)and (-8.7 kcal/mol) respectively shows highest effect with 1XU7,2ZJ3 and 3G9E;bracteonalide A(-9.1kcal/mol) shows highest binding energy with 4Y14.

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INTRODUCTION

Diabetes is a growing metabolic disorder caused by disrupted metabolism of sugar, proteins well as fat. ¹⁻² It causes many problems because of decreased insulin secretion or working action of insulin and affect people of all age group. ³ Diabetes in children is easily identified by symptoms like excessive urine, polydipsia, blurring of vision, loss of weight etc.⁴ According to

In vitro Pharmacognostical, Phytochemical and Pharmacological evaluation of *Tradescantia spathacea*: An exploration

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Abstract

Nature has given us a vast array of medicines to treat various chronic and acute illnesses. It is estimated that over 80% of the global population is reliant on traditional medicine for their main health care. Herbal remedies have been used in medicine for thousands of years as a primary treatment in the traditional medical system and have made significant contributions to human health. Since synthetic medicines' toxicity and side effects are now well known, their limits in many therapeutic areas, their relatively expensive cost, and the lack of readily accessible raw pharmaceuticals have all contributed to a surge in worldwide interest in studying and using raw drugs during the last two decades. Because there is a growing interest in herbal medicine throughout the world, the standardization of herbal medicines is the most desired option at this time. Traditional remedies are used by a significant portion of the world's population. As a result, the economic significance of herbal treatments is quickly rising. In addition to a lack of standardization, unethical commercial practices, including adulterating and substituting real herbal drugs, are posing a serious challenge to the general acceptance of tried-and-true herbal-based traditional treatments. The number of pharmaceutical formulations produced by Vaidyas is related to the number of illnesses to be investigated. *Tradescantia spathacea* (TS) is used to treat a variety of illnesses. TS is an Indian plant that is used as a traditional medicine and belongs to the Commelinaceae family.

Keywords: Traditional medicine, *Tradescantia spathacea*, Standardisation, Antioxidant activity, Phytochemical & Pharmacognostical study.

INTRODUCTION

Natural products for the treatment and management of severe illnesses may be found in medicinal plants. The use of plant extracts and isolated pure compounds has provided the basis for the production of herbal medicines and phytopharmaceutical compounds (Evans WC, 2005). For a healthcare product to be globally accepted, it must be scientifically validated to ascertain its level of purity, potency, efficacy, and safety (Alam, F., Us Saqib, Q.N., 2015). Standard criteria, such as physicochemical and phytochemical assessment of crude medicines, have been established by the World Health Organization to evaluate herbal plants' quality, safety, and effectiveness (WHO, 1996). Setting these pharmacognostic criteria to create a crude drug monograph entails a number of stages. The evaluation of medicinal plants' quality is critical in order to justify their acceptance in the traditional medical system. The use of standards, which are numerical characteristics by which the quality of herbs can be evaluated, promotes uniformity of quality. Herbal or "botanical" remedies, which have been documented in developing nations with old civilizations such as Egypt and China, offer a vast Pharmacopoeia of items recommended for various illnesses for millennia. Natural ingredients that underpin traditional treatments have lately gained more scientific study & recognition (Han SS et al., 2002).

India has a plethora of plant life. Over 18,000 species of higher plants are thought to exist in various phyto-geological/ecological areas of the nation, with approximately a third of them being medicinally and commercially significant.



Comparison Of Ultrasound-Assisted And Conventional Solvent Extraction Techniques For Characterization Of Phenolic And Flavonoid Compounds From fresh Leaves Of *Alpinia Mutica*

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Abstract

Since a few years ago, the traditional methods, such as the Soxhlet and reflux apparatus have been used most frequently to extract plant material. However, these procedures take a long time and a lot of solvent. Today, the innovative extraction method is employed to extract plant material in order to solve this issue. The ultrasonic-assisted extraction process is superior to the traditional method in that it uses less solvent, takes less time, and produces more bioactive phytoconstituents. *Alpinia mutica* (A.M.) is an Indian herb that is a member of the *Zingiberaceae* family and is used as a traditional treatment. The *Zingiberaceae* family's largest genus, *Alpinia*, contains over 230 herbs that are widely used throughout Asia's distinctive and sub-peculiar regions. In southern India, the plant is said to contain 9 different species of plants. *Alpinia mutica* is a perennial herb that grows in Malaya and the Kingdom of Thailand. It produces horizontal, subterranean stemmed, scented plants. Although cultivation has undergone a few adjustments, the sorted types are scattered throughout northern Malaysia. The northern region of the Malayan foreland is home to a variety of species, despite the fact that there are some agricultural sources that provide alternatives to A.M. Locals utilise these plants to alleviate stomach gas issues, and the fruits are used to relieve edoema. *Alpinia mutica* leaves were extracted using both traditional and ultrasonic methods using a variety of solvents, including petroleum ether, ethyl acetate, methanol, hydroalcohol, and aqueous solvents. When compared to traditional approaches, the results show that the ultrasonic-assisted extraction procedure yields a high yield of phytoconstituent. In order to determine the overall phenolic and flavonoid content of the extracts produced by both processes, further analysis was performed on them. According to the research, the phenolic and flavonoid content of the extract has been noticeably boosted by the use of ultrasonic-assisted extraction procedures. Additionally, the ultrasonic-assisted extraction method for plant extraction proved to be a quick and effective solution.

Key Words: *Alpinia mutica*, Ultrasonic-assisted extraction technique, Optimization

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INTRODUCTION

Nowadays, pharmaceutical, food, and nutraceutical industries are studying and using more and more traditional medicinal herbs.

Since ancient times, plants have been employed as the main source of disease therapies, and many plants have been shown to have a variety

of functions today (1). Since then, all societies around the world have utilised plants, with India having one of the oldest, wealthiest, and most diverse cultures (2). The favourable action of plant drugs in the treatment of more diseases was present in the analysis and standard jurisdiction (3).



A Review on Herbal Nano Drug Delivery Systems: A New Skyline

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Abstract. Throughout history, herbal medicinal products have been commonly used in the world. Many medicinal plants have been able to clarify composition and biological activities by advancing the phytochemical and phytopharmaceutical sciences. The effectiveness of many plant species in medicines relies on the supply of active ingredients. Most natural components, such as flavonoids, tannins, and terpenoids, are soluble in water, but they can hardly pass through the cell membrane; therefore, their absorption is low. Because of these obstacles, some extracts are not used clinically. It is widely envisaged that the combination of sophisticated medicines with herbal products could be ready to enhance the action, reduce the specific dose and facet effects of plant extracts, and increase activity due to nanostructured systems. Nanosystems will deliver an active component required for all treatment volumes at a replacement concentration. Typical treatments do not meet these necessities. This is to investigate structures and herbal drugs dependent on nanotechnology.

Keywords: Natural ingredients, herbal medicine, nanotechnology, bioactivity, systems for drug delivery.

INTRODUCTION

Many populations have Full-fledged awareness of plants and used them as a seasonal remedy for human development from the time that people have learned about food plant selection and disease alleviation [1]. During the latter half of the twentieth century, however, seasonal medicines were gradually replaced by allopathic medicines, especially in the western world. Allopathic treatments in the square are now widely used, particularly in developed countries, compared to old medicines. However, most developing nations still use natural medicines because artificial medicine is likely expensive [2]. In 2010, the World Health Organization estimated that 80 developing countries lie ahead of developing countries and ranked ahead [3]

At the moment, despite pharmaceutical trade marketing and promotion of allopathic medicinal products, Complementary practices are also used to sustain the health treatment of the over-size population in many countries. Many such approaches are based on sound plants. There has been a drastic depletion of these natural resources in the hands of people who are different from the methods used [1,4].

For all scientific groups, clarification of the chemical makeup and medicinal plants has become the focus of research. This analysis could lead to more and fewer side effects of innovative products than before medicines [5]. Researchers were also impregnated with their physical and biological qualities by the wide variety of structures of

REVIEW ARTICLE

Effective use of Phytotherapy in the Management of Diabetes by Plant-based Medicine: A Review

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ABSTRACT

Objective: Herb-based restorative items have been recognized since prehistoric times, and a few therapeutic herbs and their active constituents were utilized for controlling diabetes in numerous people around the world. However, minimal toxicological data exist concerning conventional anti-diabetic plants. Several synthetic oral hypoglycemic agents are the essential treatment types for diabetes. As it may, apparent symptoms of similar medicament are the primary explanation behind an extended number of individuals looking for voluntary remedies that may have less severe or no reactions. This paper attempted to list the herbs with anti-diabetic and associated advantageous impacts from various parts of the world and polyherbal extractions. These herb's impacts can defer diabetic difficulties and give a more basis of antioxidants they are acknowledged for preventing/postponing diverse ailing states. The literature review was carried out in a scientific database using diabetes, anti-diabetic agents, and phytotherapy to manage diabetes by plant-based medicine as the keywords. To overcome the research gap, optimizing phytotherapy in the management of diabetes by plant-based medicine is regarded as a good target for anti-diabetic agents to design the treatment of type 2 diabetes mellitus (T2DM). Diabetes is the world's quick aborning emergent, and this disorder's information will increase similar additional acceptable therapies. Traditional plant medicines are used throughout the world for diabetes. Therefore, studying such drugs will provide the natural key to unlocking a scientist in the future. The review focused on alternative medicine to cure kinds of diabetes problems using herbal preparation.

Keywords: Diabetes mellitus (DM), Hypoglycemic and Medicinal herbs, Phytotherapy, Optimize
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INTRODUCTION.

Diabetes mellitus or hypoglycemia is a heterogeneous metabolic issue described by modifying sugar, lipid, and protein digestion by insulin insufficiency combined with insulin obstruction.¹ It is considered one of the five driving reasons for death on the planet.² According to WHO's report, 140 million people, are experiencing diabetes worldwide all over the world, and this figure might be multiplied continuously by 2030.³

According to studies, about 410 experimentally confirmed Indian medicinal plants with anti-diabetic activity, with 109 plants having the action elucidated or recorded. Hyperglycaemia happens because the yield of hepatic glucose is uncontrolled, and with reduced glycogen synthesis, glucose is decreased consumption by skeletal muscle. On the overreaching of the renal threshold's glucose reabsorption, there is a dropping

of glucose into glycosuria (urine) as well as polyuria (Osmotic diuresis), resulting in polydipsia (increased drinking), dryness, and dehydration. Finally, deterioration is caused by insulin insufficiency through protein reduction and breakdown synthesis.^{4,5}

Despite extensive advancement in diabetes treatment by oral hypoglycaemic agents, there is a need for more up-to-date therapeutic agents because the current engineered drugs have a few confinements.⁴⁻⁷ Oral hypoglycaemic and insulin agents such as sulphonylurea and biguanides still affect the management, but there is a search for many efficient anti-diabetic agents. Natural medications with anti-diabetic action are yet to be industrially planned as present-day drugs, regardless of the point that it has been accolade for curative properties in medication's conventional frameworks.¹ Herb provided a possible source of hypoglycaemic medications

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A REVIEW ON PHYTOPHARMACOLOGICAL ACTIVITIES OF *ALPINIA MUTICA* AND *TRADESCANTIA SPATHACEA*

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Abstract

Alpinia Mutica plant, belongs to family Zingiberaceae, is mainly scattered in tropical areas and widely known for ethno medicine. Its rhizome extract has maximum inhibitory effect against fungi as well as bacteria. *A. mutica* is also used in medicine and food preparations. Rhizome extract own more phenolic and flavonoid substances when estimated and compared to leaf extract of plant with evident antimicrobial as well as radical scavenging potential. The greater part of the crude extracts and isolated compounds indicated antimicrobial, Antioxidant activities which are determined by diphenyl picryl hydrazyl radical scavenging action test (DPPH), Bleaching of β -carotene, (SOD) superoxide dismutase. Additionally, these mixes are fit to stop the advancement of colon neoplasm cells. *Tradescantia spathacea* is an herb of India, used as conventional remedy and it is under the belonging to family Commelinaceae. In Mexican country which is called as "Maguey Morado" (Purple Maguey), elixir of the leaf is regularly free-eaten as healing of endoplasmic carcinoma. Ethanolic extract of the plant has chemical constituents like anthocyanin, flavonoids, saponins, carotenoids, terpenoids and steroid compounds. The successive solvent extract of this plant has antioxidant activity, antimicrobial properties and also found to block antiadrenergic action of bretylium tosylate and showed contraceptive effect in experimental animals (rats). It is used in cosmetics to nourish skin.

Key words: *Alpinia Mutica*, *Tradescantia spathacea*, Phytochemical and Pharmacological activities.

Introduction

Plants are used as a primary source of treatment for many diseases from the ancient times and number of plants are known to have different medicinal activities. (Kakkar *et al.*, 2014). From the olden day's plants were used by all cultures of the world wide with India that has one of the ancient, prosperous and highly multiple cultures (Tandon *et al.*, 2004). Plant drugs have beneficial activity in analysing and treating more ailments in standard jurisdiction (Steven D. Ehrlich *et al.*, 2009). Medicinal value plants have various pharmacological activities such as antioxidant, anticancer, immunostimulant, anti-inflammatory, liver protective activity and spinal reflection activities. (Chang *et al.*, 2010).

Alpinia is the largest genera of the Zingiberaceae family, with about two hundred and thirty herbs widely distributed in peculiar and sub-peculiar Asia. The plant has been reported to have 9 species of plants in southern

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India. (John Kress *et al.*, 2005, Sabu *et al.*, 2006). *Alpinia Mutica* (A.M) is a perennial herb which produce horizontal, underground stem, fragrant plant indigenous to Malayan and Kingdom of Thailand. Although a few changes can be seen in farming, the sorted varieties are spread in northern Malaysia. Although there are some alternatives to A.M in agricultural sources, a variety of species are spread in the northern end of the Malayan foreland. Importantly, these plants are used by locals to treat gas problems in stomach and fruits are used to reduce swelling (Halijah Ibrahim *et al.*, 2014).

A.M rhizomes showed the presence of flavokavain B, pinocembrins, 5, 6-dehydrokawain and 1, 7-diphenyl-5-hydroxy-6-hepten-3-one (Sirat *et al.*, 1996) and methylene chloride extract was used for lipid oxidation and observed for the inhibition of growth of *Bacillus subtilis* and *Staphylococcus aureus* species (Mohamad *et al.*, 2004).

Tradescantia spathacea sw (T.S) is vegetative plant

A Review on Nano Drug Delivery Systems of Herbal Medicine

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Abstract: Herbal medicines are widely used around the world since history. The advancement of phytochemical and phytopharmacological sciences has enabled elucidation of many medicative plant products' composition and biological activities. The effectiveness of the many species of medicative plants depends on the provision of active compounds. Most of the biologically active constituents of extracts, like flavonoids, tannins, and terpenoids, are extremely soluble in water; however, have low absorption, as a result of their unable to cross the lipoid membranes of the cells, have enormous molecular size, or poorly absorbed, leading to loss of bioavailability and effectiveness. Some extracts are not used clinically due to these obstacles. It has been wide planned to mix seasoner medication with herbal, resulting from nanostructured systems that may be ready to enhance plant extracts' action, reduce the specified dose and facet effects, and raise activity. Nanosystems will deliver the active constituent at a spare concentration throughout the whole treatment amount, directional it to the required web site of action. Typical treatments do not meet these necessities. This study aimed to review nanotechnology-based drug delivery systems and herbal medicines.

Keywords: Natural products, herbal medicines, nanotechnology, drug delivery systems, biological activity

1. Introduction

Knowledge and use of plants as seasoner medicines have occurred in numerous populations throughout human evolution, starting once the man learned to pick out plants for food and alleviate ailments and diseases.¹ However, throughout the last half of the 20th century, seasoner medicines were bit by bit replaced by allopathic medicines, particularly within the Western world. Allopathic treatments square measure presently a lot of wide used than ancient medicines, particularly in developed countries. However, most developing countries still use these natural medicines, presumably that getting a synthetic drug is pricey². in line with the globe Health Organization, eightieth of individuals in developing countries rely upon ancient healthful practices to fulfill and/or supplement their basic health desires³.

Despite marketing and encouragement from the pharmaceutical trade throughout allopathic medicines, an oversized phase of the population in several countries continues to utilize complementary practices for their health care. Several of those practices are derived from healthy plants. However, thanks to economic, political, and social changes worldwide, the therapeutic use of these natural resources, which are mainly utilized by people who cannot afford different treatments, has dramatically diminished^{1,4}.

Anti-Anxiety Activity of *Tradescantia spathacea* Assessed Using Different Experimental Anxiety Models

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Abstract: The point of present examination was to investigate the anti-anxiety activity of hydroalcoholic extracts of *Tradescantia spathacea* utilizing different animal models (elevated plus maze, open field test, light and dark test and social interaction test) of anxiety in mice. Diazepam (0.5 mg/kg) was utilized as the standard and measurement of hydroalcoholic extract of *T. spathacea* (50, 100 and 200 mg/kg) was chosen according to OECD rules. Results recommended that concentrate of *T. spathacea* at 100 and 200 mg/kg dose produced anti-anxiety effects almost similar to diazepam and at 50 mg/kg dosage did not create against anti-anxiety activity on any of the paradigm used. Additionally ponders are expected to recognize the anxiolytic mechanism(s) and the phytoconstituents responsible for the observed central effects of the hydroalcoholic extract of *T. spathacea*.

INTRODUCTION

Anxiety affects simple fraction of the whole population worldwide and has become a crucial space of analysis interest in pharmacology throughout this decade. [1] Benzodiazepines are the most important category of compounds utilized in anxiety and that they have remained the foremost unremarkably prescribed treatment for anxiety. [2] However, the belief that benzodiazepines gift a slim margin of safety between the anxiolytic impact and people inflicting unwanted aspect effects has prompted several researchers to judge new compounds within the hope that different anxiolytic medicine can have less undesirable effects. [3] The popularity of anxiolytic effects of non-benzodiazepine azapirone agents, which act as 5-HT_{1A} partial agonists, like buspirone, gepirone and ipsapirone and their therapeutic role in clinical anxiety and mood disorders has any targeted attention on the 5-HT_{1A} receptor. [4] Though the azapirone move with different neurochemical systems, like the dopaminergic and noradrenergic, they show nanomolar affinity for 5-HT_{1A} receptor sites. [5] However, the anxiolytic effects of azapirone follow a time course determined with antidepressants wherever therapeutic effects are delayed for 3-4 weeks, that is in contrast to the speedy effects determined with anxiolytic drug anxiolytics. [6] Thus, there's a requirement of strong anxiolytic compounds that have lesser aspect effects than benzodiazepines and additional immediate onset of action than presently out there 5-HT_{1A} receptor acting medicine. [7]

Tradescantia spathacea Swartz (syn. *Rhoeo discolor* L. H'ér Hance, *Rhoeo spathacea* (Swartz) Stearn) is a plant of India that is in use in traditional medicine. This plant belongs to the Commelinaceae family. [8] In the Southeastern of Mexico, it is known as "Maguey Morado" (Purple Maguey) and the decoction of the leaves is daily free-consumed as curative of cancer, without existing

scientific evidence of such property. [9] It is known that the aqueous extract of *T. Spathacea* blocks the antiadrenergic action of bretylium [10] and is contraceptive in rats. [11] The extracts of *T. Spathacea* have been incorporated in cosmetics to improve the appearance of skin. [12] Some chemicals detected in *T. Spathacea* are flavonoids, anthocyanins, saponins, carotenoids, waxes, terpenoids and coumarinic and steroidal compounds. [13, 14] On the other hand, *T. Spathacea* ethanolic crude extract evaluated in an *in-vitro* system, showed antioxidative activities [15] and antimicrobial properties. [16]

Due to the absence of scientific reports *in-vivo* that corroborate the anxiolytic activity property of *T. Spathacea*, it is evident the importance of the exploration of this plant. They additionally assessed the spontaneous activity and neuromuscular coordination. Other than this, no model(s) for anxiety (except EPM) has been used for further evaluation of anxiolytic activity of *T. Spathacea* extract, to our knowledge. The aim of the present study was to explore the anti-anxiety activity of hydroalcoholic extract of *T. Spathacea* totally different animal models (EPM, open field (OF) test, light and dark test and social interaction test) of anxiety in mice.

MATERIALS AND METHODS

Animals

Swiss albino mice (males; 20-25 g) were used in the present study. Divided into 5 groups of 6 animals per cage were used. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24 hrs). The food in the form of dry pellets and water is provided *ad libitum*. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee (Reg No: 1648/PO/A/12/CPCSEA).

Plant Material

The plant *Tradescantia spathacea* was collected within the month of Feb. 2017 from medicinal gardens of Geethanjali

¹Geethanjali College of Pharmacy, Cheryal, Keesara, Ranga Reddy District-501301, Telangana, India.

E-mail: tirumalardhi@gmail.com

*Corresponding author

²Vijay College of Pharmacy, Nizamabad-503001, Telangana, India.

³Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad-500043, Telangana, India.



भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन एवं जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण / BOTANICAL SURVEY OF INDIA
दक्कन क्षेत्रीय केन्द्र / DECCAN REGIONAL CENTRE
हैदराबाद / HYDERABAD – 500 095
तेलंगाना / TELANGANA



संख्या/No. BSI/DRC/2019-20/Tech./ 305

दिनांक/Date: 04/12/2020

सेवा मे/To

Mr. Shankaraiah Pulipaka
Research Scholar
Lovely Professional University
Phagwara- 144411
Punjab

विषय/Sub: Identification of plant material— reg.

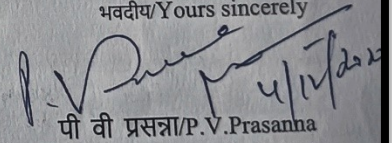
Dear Shankaraiah Pulipaka,

With reference to your letter dated 4th December 2020, the plant material brought by you has been identified by the concerned expert as *Alpinia mutica* Roxb. belonging to the family Zingiberaceae.

After identification, the plant materials are returned herewith.

धन्यवाद-/Thanking you

भवदीय/Yours sincerely


पी वी प्रसन्ना/P.V.Prasanna

वैज्ञानिक 'जी' एवम का. अ. /Scientist 'G' & HoO



भारत सरकार
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तेलंगाना / TELANGANA



संख्या/No. BSI/DRC/2020-21/Identification/Tech./ 66

दिनांक/Date: 09/06/2020

सेवा में/To

Mr. Shankaraiah Pulipaka
Assistant Professor
Department of Pharmacognosy
Geethanjali College of Pharmacy
Ceeryal (V), Keesara (M)
Ranga Reddy (D)-501301
Telangana

विषय/Sub: Identification of plant material- reg.

Dear Sir,

With reference to your letter dated 9th June 2020, the plant material brought by you has been identified by the concerned expert as *Tradescantia spathacea* Sw. belonging to the family Commelinaceae.

After identification, the plant material is returned herewith.

धन्यवाद-/Thanking you

भवदीय Yours sincerely

पी वी प्रसन्ना/P.V.Prasanna
वैज्ञानिक 'जी एम का' अ. /Scientist 'G' & HoO



Geethanjali

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Fax : +91-40-24220320
Website : www.geethanjalinstitutions.com

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Sy.No. 31, Cheeryal (V), Keesara (M), Ranga Reddy District. - 501 301.

Certificate

This is to certify that the project proposal no GCPK/IAEC/2020-21/01 entitled Development, Optimization, Characterization of Nano Formulations and Anti-Diabetic Activity of *Alpinia mutica* and *Tradescantia spathacea* submitted by Mr. Shankaraiah Pulipaka has been approved/recommended by the IAEC of Geethanjali College of Pharmacy in its meeting held on 20.11.2020 and Swiss albino mice (15g – 20g), Wister albino rats (150-200 gm) Gender: Both Sex (42 swiss albino mice and 60 wister albino rats respectively) have been sanctioned under this.

Authorized by	Name	Signature	Date
Chairman:	<u>D. M. RAVIKUMAR</u>	<u>[Signature]</u>	<u>20.11.2020</u>
Member Secretary:	<u>D. T. Srinivas</u>	<u>[Signature]</u>	<u>20.11.2020</u>
Main Nominee of CPCSEA:	<u>P. Chaitanya</u>	<u>[Signature]</u>	<u>20.11.2020</u>



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Certificate of Participation

This is to certify that **Prof./Dr./Mr./Ms. Shankaraiah Pulipaka** has successfully participated as “**Delegate and Oral presenter**” in the International Conference on “**Recent Advances in Health Sciences**” (ICRAHS-2023) on the Theme of “**Interdisciplinary Research: A key to transform Health care.**” held on 14th to 15th April, 2023 organized by School of Pharmaceutical Sciences in association with Komar University of Sciences and Technology at Lovely Professional University, Punjab.

Date of Issue : 30-08-2023

Place : Phagwara (Punjab), India

Prepared by
(Administrative Officer-Records)

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Program Chair

Prof. Dr. Kawis Aziz Faraj
Conference Co-Chair

Dr. Monika Gulati
Conference Chair



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Plenary Speakers

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(Indonesia Center of Technology Empowerment, Indonesia)

Dr. J. M. Chandra Kishen
(Professor, IISc Bangalore, India)

Keynote Speakers

Ashok Ranade
(Project Advisor, Training and Support Services for New High Tech Global Startups, Canada)

Dr. Dhananjay Tambe
(Associate Professor, University of South Alabama, USA)

Botir Usmonov
(Rector of the Tashkent Institute of Chemical Technology, Tashkent Region, Uzbekistan)

Amit Utkarsh Sinha
(CEO & Founder at BasePair Inc, New York, USA)

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(Professor, Durban University of Technology, South Africa)

Dr. Venkata Reddy Poluru
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(Founder & Director, Oztron Energy, Greater Perth Area Australia)

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Rajaram Desai
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Dr. Kashfull Orra
(Assistant Professor, IIITDM Kancheepuram)



4th International Conference
on

“Advanced Technologies for Societal Applications”

Certificate

This is to certify that SHANKARAIAH PULIPAKA of Lovely Professional University has presented a research paper entitled Development of an innovative ultrasound-assisted extraction technique to optimize extraction on Phytoconstituents and compared conventional extraction method (Paper ID-9740) in the International Conference on “Advanced Technologies for Societal Applications: Techno-Societal 2022”, held at Shri Vitthal Education & Research Institute (SVERI), Pandharpur, India during 9th - 10th December 2022.

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Coordinator

Dr. P. M. Pawar
Co-convener

Dr. B. P. Ronge
Convener

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website: www.sveri.ac.in



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from LOVELY PROFESSIONAL UNIVERSITY. has Participated/
Presented a Paper/Poster on the topic COMPARISON OF ULTRASOUND-ASSISTED
EXTRACTION WITH CONVENTIONAL EXTRACTION METHODS OF OIL & PHENOLICS FROM
ALPINTA MUTICA in the two day National Seminar on **RECENT ADVANCES IN CHEMICAL
AND ENVIRONMENTAL SCIENCES (RACES-2022)** held at Kakatiya Government
College, Hanumakonda, Telangana state, India during 21-22, September-2022

Dr. B. Ramesh

CONVENER



Dr. G. Raja Reddy

PRINCIPAL &
CHAIRMAN

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(Deemed to be University)

Kishanpura, Hanamkonda, Warangal Urban - 506001, T.S., India.

Certificate of Participation

This is to certify that Mr / Mrs / Dr. / Prof. Shankaraiah pulipaka
has participated / Presented paper on Recent update on Nano phytopharmaceuticals
in the management of Diabetes

at A National Conference on Current Trends in Pharmacy & Pharmacy Practice organized by Chaitanya Deemed to be University-Pharmacy on 1st April, 2022 in association with Indian Pharmaceutical Association (IPA), Warangal and Mybo Group.

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Dean - Pharmacy
Convener

Dr. G. Kumaraswamy
HoD - Dept. of Pharm. Analysis
Organizing Secretary

Dr. N. Chandana
HoD - Dept. of Pharmacy Practice
Co-organizing Secretary



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This is to certify that Dr/Mr/Ms/Mrs P. Shankarai ah has

given an Oral/Poster presentation on Diabetes treatment by phytonanotherapy using green Synthesis nanoparticles in

1st National Congress on Herbal Medicine & Nano Technology Inspired Novel Formulations:
An Emerging Therapeutic Target for Cancer & Neurodegenerative Disorders

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Inspired Novel Formulations: An Emerging Therapeutic Target
for Cancer & Neurodegenerative Disorders

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Department of Pharmacognosy
(25th & 26th March, 2022)


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Principal, JSSCP Ooty


Dr. Kalakotia Shanker

Organizing Secretary
HoD /c. Dept. of Pharmacognosy

DIVISION OF RESEARCH AND DEVELOPMENT

[Under the Aegis of Lovely Professional University, Jalandhar-Delhi G.T. Road, Phagwara (Punjab)]

Certificate No.240288

Certificate of Participation

This is to certify that **Mr. Shankaraiah Pulipaka** of **Lovely Professional University, Phagwara, Punjab, India** has presented paper on **A Review on Herbal Nano Drug Delivery Systems: A New Skyline** in the **International Conference on Materials for Emerging Technologies (ICMET-21)** held on February 18-19, 2022, organized by Department of Research Impact and Outcome, Division of Research and Development, Lovely Professional University, Punjab.

Date of Issue: 16-03-2022

Place: Phagwara (Punjab), India



Prepared by
(Administrative Officer-Records)



Dr. Vipul Srivastava
Convener
(ICMET-21)



Dr. Manish Vyas
Organizing Secretary
(ICMET-21)



Dr. Chander Prakash
Co-Chairperson
(ICMET-21)



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Cheeryal (V), Keesara (M), Medchal Dist, Telangana, 501301.



Certificate

This is to certify that **SHANKARAI AH PULIPAKA** of **LOVELY PROFESSIONAL UNIVERSITY** has presented e-Oral/e-Poster entitled **PHYTO NANO MEDICINES FOR THE PREVENTION OF DIABETES MELLITUS** in **DRAVYAKA 2020** the 11th National Level Virtual Conference during 11th & 12th December 2020 On “Global Burden of the Disease & Pharmacist’s Role” which is organized by **Teja Educational Society** sponsoring **Geethanjali College of Pharmacy**, in association with **APTI Telangana State Branch**.

Certificate ID ZGWW3Y-CE000013

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Pharmacy

Dr. M. SUNITHA REDDY
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Prof. Dr. M. RAVI KUMAR
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September 22nd, 2021

Mendeley Advisor(s)

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Kristin Blye
Community Manager

A handwritten signature in blue ink, appearing to be "STJ", written over a horizontal line.

Susan Tyler Jenkins
Community Manager



ELSEVIER



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DRAVYAKA 2019

"Rare Diseases - Orphan Drugs and their Prevalence in Public"

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This is to certify that Prof. / Dr. / Mr. / Ms / Mrs. P. Shankaraiah
 has participated / presented a paper in Oral / Poster entitled Herbal mediated Silver Nano particles - As Anti Diabetics
 in DRAVYAKA 2019,
 a 10th National Level Conference held at Geethanjali College of Pharmacy on 13th & 14th November, 2019
 Jointly organized by Scientific & Applied Research Center, Hyderabad (SARC) & Teja Educational Society.

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Dr. D. Yashwanth Kumar

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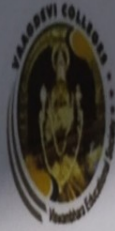
This is to certify that Prof. / Dr. / [✓]Mr. / Ms / Mrs. P. Shankaraiiah..... of Geethanjali College of Pharmacy..... has participated as judge / delegate / presented a paper in Oral / Poster [✓]session / Quiz and he/she has won II Prize..... in DRAVYAKA 2019, a 10th National Level Conference held at Geethanjali College of Pharmacy on 13th & 14th November, 2019 Jointly organized by Scientific & Applied Research Center, Hyderabad (SARC) & Teja Educational Society.

Sri G.R. RAVINDER REDDY
Secretary
Geethanjali College of Pharmacy

Prof. Dr. M. RAVI KUMAR
Principal - Convener
Geethanjali College of Pharmacy

Dr. D. Yashwanth Kumar
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Scientific & Applied Research

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DRAVYAKA- 2019



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COLLEGE OF PHARMACY

RAMNAGAR, HANAMKONDA, WARANGAL, TELANGANA

Certificate of Presentation

This is to certify that Dr/Mr/Mrs/Ms SHANKARAJAH PULIPAKA
from Lovely Professional University, Department of Pharmacy has
Presented a (Oral/Poster) Entitled NOVEL DRUG DELIVERY SYSTEM FOR
HERBAL REMEDIES AND NANOTECHNOLOGY

In the Scientific session of the TAS & TSCHE Sponsored Two day National Conference on
RECENT TRENDS IN PHARMACEUTICAL SCIENCES AND RESEARCH (RTPSR-2019)

held at Vaagdevi College of Pharmacy on 23rd and 24th November 2019.

Dr. K. SIRISHA
Convenor

Dr. CHALLA SRINIVAS REDDY
Organizing Secretary

Prof. Y MADHUSUDHAN RAO
R & D Director

Dr. CH. VAHINI DEVI
Academic Director

Certificate

This certifies that

Prof./Dr./Mr./Ms. SHANKARAJAH PULIPAKA

A Review of Anti Diabetic Herbal Drugs and NanoHerbal Formulations.

has actively participated & presented a 'poster' during

12TH INDO-MALAYSIAN CONFERENCE

Theme: "Innovations and Updates in Pharmaceutical Sciences"

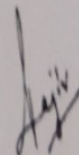
at Samskruti College of Pharmacy, Ghatkesar, Medchal, Hyderabad, Telangana

organized by APP Telangana State Branch in collaboration with APP Malaysian International Branch

on the 8th day of November 2019.



Dr. D. Venkata Ramana



Dr. Rajiv Dahiya

CONVENER & PRINCIPAL
SAMSKRUTI COLLEGE OF PHARMACY
GHATKESAR, MEDCHAL, HYDERABAD, TELANGANA

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DRAVYAKA 2018

"Emerging Innovations in Pharmaceutical Sciences"

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This is to certify that Prof. / Dr. / Mr. / Ms / Mrs. P. SHANKARAJAH has
participated / presented a paper in oral / poster entitled "Herbonano ceuticals"

..... in DRAVYAKA 2018, A 9th National Level Conference held
at Geethanjali College of Pharmacy on 2nd & 3rd November, 2018 Jointly organised by IPA,
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Sri G.R. RAVINDER REDDY
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Prof. Dr. M. RAVI KUMAR
Principal - Convener
Geethanjali College of Pharmacy

Dr. T.V. NARAYANA
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National Level Seminar on NMR Techniques and its Applications in Pharmaceutical Sciences held
at Jayamukhi College of Pharmacy on 20th & 21st September 2019.

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Convenor

Prof. S. Vasudeva Murthy
Principal



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SA, Guntur Province

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23rd October 2021

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Shankaraiah Pulipaka

has participated as an **Delegate** in the above mentioned Virtual Symposium

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Co-ordinator
Lecturer

Dr SRIKANTH JUPUDI
Co-ordinator
Lecturer



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This is to certify that, **MR. SHANKARAIH PULIPAKA** of **LOVELY PROFESSIONAL UNIVERSITY** has successfully participated in IP Awareness/Training program under

NATIONAL INTELLECTUAL PROPERTY AWARENESS MISSION

on July 08, 2022

Organized by
Intellectual Property Office, India

Date: July 08, 2022




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PATENTS, DESIGNS & TRADE MARKS



TO WHOMSOEVER IT MAY CONCERN

This is to certify that the histopathological specimens (mice) of Mr. Shankaraiah Pulipaka (Reg. No. 41800583) were performed in our department. All samples were collected in different groups and were separated as individual treatment groups.

Histopathological studies in heart of the various mice's

Group number	Treatment groups	Observation in tissues
I	Vehicle Control	There is no growth in tissues
II	SNP (2000 mg / kg (p.o.))	Moderate myocardial fatty fiber infiltration
III	ZONP (2000 mg / kg (p.o.))	Moderate myocardial fatty fiber infiltration
IV	TSLE (2000 mg / kg (p.o.))	Mayocardial fibers have somewhat deteriorated.
V	TSLESNP (2000 mg / kg (p.o.))	Mayocardial fibers have somewhat deteriorated.
VI	TSLE ZONP (2000 mg / kg (p.o.))	Mayocardial fibers have mild granular degeneration.
VII	AMLE (2000 mg / kg (p.o.))	Moderate myocardial fatty fiber infiltration
VIII	AMLESNP (2000 mg / kg (p.o.))	Mayocardial fibers have mild granular degeneration.
IX	AMLE ZONP (2000 mg / kg (p.o.))	Mayocardial fibers have mild granular degeneration.

Histopathological studies in kidneys of the various mice's

Group number	Treatment groups	Observation in tissues
I	Vehicle Control	There is no growth in tissues
II	SNP (2000 mg / kg (p.o.))	Mild tubular epithelial cell degeneration
III	ZONP (2000 mg / kg (p.o.))	Moderate tubular epithelial cell degeneration
IV	TSLE (2000 mg / kg (p.o.))	Mild tubular epithelial cell degeneration
V	TSLESNP (2000 mg / kg (p.o.))	Moderate tubular epithelial cell necrosis and granular degeneration
VI	TSLE ZONP (2000 mg / kg (p.o.))	Moderate tubular epithelial cell necrosis and granular degeneration
VII	AMLE (2000 mg / kg (p.o.))	Mild tubular epithelial cell degeneration
VIII	AMLESNP (2000 mg / kg (p.o.))	Moderate tubular epithelial cell necrosis and granular degeneration
IX	AMLE ZONP (2000 mg / kg (p.o.))	Moderate tubular epithelial cell necrosis and granular degeneration

Histopathological studies in Livers of the various mice's

Group number	Treatment groups	Observation in tissues
I	Vehicle Control	There is no growth in tissues
II	SNP (2000 mg / kg (p.o.))	Hepatocytes of the liver with mild granular degeneration.
III	ZONP (2000 mg / kg (p.o.))	Hepatocytes of the liver with moderate granular degeneration
IV	TSLE (2000 mg / kg (p.o.))	Hepatocytes of the liver with mild granular degeneration
V	TSLESNP (2000 mg / kg (p.o.))	Hepatocytes of the liver with mild granular degeneration
VI	TSLE ZONP (2000 mg / kg (p.o.))	Hepatocytes of the liver with mild to moderate granular degeneration
VII	AMLE (2000 mg / kg (p.o.))	Mild granular degeneration in hepatocytes of the liver
VIII	AMLESNP (2000 mg / kg (p.o.))	Hepatocytes of the liver with mild to moderate granular degeneration
IX	AMLE ZONP (2000 mg / kg (p.o.))	Hepatocytes of the liver with mild to moderate granular degeneration

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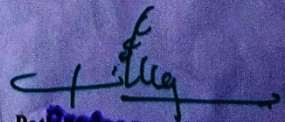


TO WHOMSOEVER IT MAY CONCERN

This is to certify that the histopathological specimens (Wister rats) of Mr. Shankaraiah Pulipaka (Reg. No. 41800583) were performed in our department. All samples were collected in different groups and were separated as individual treatment groups.

Histopathological studies in Pancreas of the various Albino -Wister rats

Group number	Treatment groups	Observation in tissues
I	Normal Control (Saline 0.5% w/v CMC (p.o.))	There is no growth in tissues
II	Standard drug (Metformin (50 mg /kg b.w) (p.o.))	Langerhans islets have mild necrosis.
III	Diabetic control (STZ (40g /kg b.w) (i.p.))	Necrosis of islets of Langerhans
IV	AMLE (200 mg /kg b.w (p.o.))	Langerhans islets have mild necrosis.
V	AMLE (400 mg /kg b.w (p.o.))	Mild necrosis of islets of Langerhans
VI	AMLESNP (100 mg /kg b.w (p.o.))	Islets of Langerhans have mild degeneration and necrosis.
VII	AMLESNP (200 mg /kg b.w (p.o.))	Moderate degeneration and necrosis of islets of Langerhans
VIII	AMLE Zno NP (100 mg /kg b.w (p.o.))	Langerhans islets have mild necrosis.
IX	AMLE Zno NP (200 mg /kg b.w (p.o.))	Islets of Langerhans have mild degeneration and necrosis.
X	TSLE (200 mg /kg b.w (p.o.)) -	Langerhans islets have mild necrosis.
XI	TSLE (400 mg /kg b.w (p.o.))	Mild necrosis of islets of Langerhans
XII	TSLESNP (100 mg /kg b.w (p.o.))	Moderate degeneration and necrosis of islets of Langerhans
XIII	TSLESNP (200 mg /kg b.w (p.o.))	Islets of Langerhans have mild degeneration and necrosis.
XIV	TSLE Zno NP (100 mg /kg b.w (p.o.))	Moderate degeneration and necrosis of islets of Langerhans
XV	TSLE Zno NP (200 mg /kg b.w (p.o.))	Islets of Langerhans have mild degeneration and necrosis.


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