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Green Synthesis of Silver Nanoparticles Using Muntingia Calabura. Lextract and Evaluvation of Anti-Diabetic Activity

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ABSTRACT: The present work aim to investigate a green synthesis of silver nanoparticles using Muntingiacalabura.L extract.TheAgNPs formation was monitoring using UV-Visible spectroscopy obtained at 449.6nm for M.Calabura leaf extract and 461nm for M.Calaburaseed extract conforming the formation of silver nanoparticles. Scanning electron microscopy analysis showed the spherical shape of silver nanoparticles. Fourier transforms infrared (FTIR) spectroscopy result predict functional group of Muntingiacalabura .Lextract and silver nanoparticles functionalized with with MC extract explaining the interaction between them. Total phenolic content in extract of M calabura.Lalso determined. Further, the anti-diabetic activity against the silver nanoparticles was established.

KEY WORDS: Muntingiacalabura, silver nanoparticles, Plant extract, Anti-diabetic.

I. INTRODUCTION:

Muntingiacalabura(MC) is a shrub whose leaves, stems and roots are documented to be used for traditional medicinal purposes in various forms. Medicinal uses of MC leaves and flowers: Used to treat headaches, colds, stomach ulcers, low blood pressure, abdominal cramps, prostate swelling, antiseptic, diabetic and antispasmodic. This plant has been scientifically proven to have cardioprotective effects and anti-inflammatory [1], antipyretic, antibacterial, antinociceptive [2] and antioxidant activities. Previous studies have shown the in vitro antibacterial activity of MC leaf extract against Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus and Bacillus subtilis[3]. It also exhibits good antifungal activity against Alternariasolani, Fusariumoxysporumf.splycopersici, Pythium sp., Phytophthora sp., Rhizoctoniasolani, Aspergillusniger, Colletotrichum sp., Candida neoformans and Candida albicans [4-6]. Flavonoids, phenolics, chalcones, and terpenoids are the main constituents of MK plant extracts, and these compounds are responsible for the various medicinal properties of the plant [7]. Medicinal plants are a source of therapeutic agents for ameliorating human diseases. About 80% of people in developing countries rely on traditional treatments for primary care, and about 85% of traditional medicines contain plant extracts[8]. Interest in the use of plants and plant-derived medicines has increased globally due to increasing awareness of the health risks and toxicity resulting from the misuse of synthetic medicines. However, the pharmacological effects of many medicinal plants have not been studied. A plant that has recently been recognized as a medicinal plant is Muntingiacalabura L. M. calabura is widely conserved and has become a common roadside tree in Indonesia, where it is called "cherry". M. calabura (Elaeocarpaceae), commonly known as phenolic cherry, is a species of the genus Muntingia[9]. In addition, although the use of M. calabura is still limited in Indonesia, it is used to treat various diseases overseas. The roots of M. calabura have been consumed as an abortifacient in Malaysia and as an emetic in Vietnam. In another country, Colombia, the flowers are administered and consumed as a tonic and sedative. The methanolic extract of M. Calabura fruit has shown strong DPPH scavenging ability [10]. In addition, phytochemical studies of various plant components have identified many bioactive flavonoids, sesquiterpenes, chalcones and phenolic compounds. A class of secondary metabolites is found in crude extracts of M. Calabura, which generally belongs to the flavonoid family, is known to be reliable for various plant physiological activities [11]. Previous studies have shown the presence of flavones, flavanones, flavans and non-flavansin Some of the M.calabura showantiplatelet aggregation and cytotoxic activities [12]. However, the above studies are mainly conducted in countries other than Indonesia. The different locations where M. calabura plants are grown result in different phenolic and flavonoid contents. In addition, this

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study aimed to find the highest content of quercetin, phenol and flavonoids at different variations of solvent concentration.

Muntingiacalabura.L (Family: Muntingiaceae) is a slender tree native to America and widely cultivated in warm regions of Asia, including Malaysia and India. The plant has several local names such as strawberry, Jamaican cherry (English), sakkaraipajam (Tamil) and chetu cherry (Telugu) [13]. This plant is well known for its antiseptic and antispasmodic properties and is also an antihypertensive agent [14]. Each part of this tree has different recorded medicinal uses. The leaves, bark and flowers are rich in flavonoids, flavones and flavones, which have medicinal value and contribute to strong anti-cancer activity[15]. In addition, the flowers of Muningiacalabura have been used as an antiseptic, antispasmodic, indigestion, diaphoretic, sedative, tonic and headache treatment, while the roots have been used as an antiemetic and abortifacient. The flowers of this plant are decocted and consumed as a sedative and tonic [16]. This herb has been scientifically proven to have nociceptive, anti-inflammatory and antipyretic properties, potential antibacterial activity, strong tyrosinase and antioxidant activity, and cardioprotective properties. effect. Stigmasterol isolated from the roots of Muntingiacalabura showed strong antifungal activity against A at a minimum inhibitory concentration of 1 mg/ml. Solani [17-21]. One of the traditional medicinal uses of Muntingiacalabura leaves is as an antidiabetic agent. This is because synthetic diabetes drugs were more toxic. Due to the abundance of plants in subtropical regions and the lack of documentation, we set out to investigate and compare the antidiabetic activity of leaves and seeds.

ABOUT PLANT:

Botanical information

Taxonomy (scientific classification)

Taxon :Muntingiacalabura Linn
Kingdom :Plantae
Division :Spermatophyta
Class :Dicotyledonae
Order :Malvales
Family :Muntingiaceae
Genus :Muntingia
Species :Muntingiacalabura

Fig 1: Muntingiacalabura

Vernacular name:

Tamil	Chakkaraipazham
English	Panama –berry

Nanotechnology is the most promising leading science in the development of modern basic technologies. The synthesis of green nanoparticles aims to reduce the generated waste and implement sustainable processes. Recent developments in nanotechnology have focused on green processes that use less toxic materials to ensure environmental sustainability. Nanoparticles have attracted attention because their very small size and large surface area lead to chemical and physical differences in properties such as mechanical properties, biological and catalytic activity, thermal

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and electrical conductivity, light absorption, and melting point. Attached Same chemical composition. Most of the physical and chemical methods to synthesize silver nanoparticles (AgNPs) have been found to be too expensive and present several biological hazards [22]. Nanoparticles (NPs) are particles between 1 and 100 nm in size. In this form, it has special properties that distinguish it from bulk materials. In particular, silver nanoparticles (AgNPs) exhibit antibacterial activity and are widely used in medical products. Proposed mechanisms for the antibacterial activity of AgNPs: attachment of AgNPs to the cell wall and cell membrane surfaces, disruption of metabolic processes, cellular penetration of AgNPs, disruption of intracellular structures (mitochondria, vacuoles, ribosomes) and biological structures. Molecule (protein) AgNPs have cytotoxic effects by generating oxidative free radicals. In the context of bacterial resistance to existing antibiotics, AgNPs are considered a new hope for humanity. Two common methods are used to synthesize NPs. These are bottom-up (ions are reduced to atoms and these atoms then combine to form NPs and top-down (bulk substances are broken down into NPs). In the bottom-up method, the reducing agent can be electromagnetic waves, chemical reducing agents (citric acid, ascorbic acid) or plant extracts (green synthesis).[23]

Recently, the attractive properties of silver nanoparticles (AgNPs) have led to the increase of their use in various fields, especially in the textile, medical and food industries. Its antibacterial properties have been tested against bacteria, viruses and fungi. Nanoparticle synthesis generally uses physical methods, such as pyrolysis and thermal/laser ablation, or chemical methods, such as electrochemical deposition, sol-gel processes, and aerosol pyrolysis. It can also be performed using biological methods such as plants, bacteria, mold, algae and yeast. Compared to physical and chemical methods, biological methods are considered environmentally friendly and economically efficient [24].

ABOUT DIABETIC MELLITUS

Diabetes has a significant negative impact on quality of life, social, psychological and physical health. Complications of diabetes are mainly associated with oxidative stress, such as increased production of reactive oxygen species (ROS) or impairment of antioxidant defense systems. Increased lipid peroxidation, changes in antioxidant enzymes, and impaired glutathione metabolism are major factors in the development of diabetes. The production of free radicals isalso involved in the development of several diseases, including diabetes. The formation and increased accumulation of advanced glycation end products (AGs) are also involved in the complications of diabetes such as retinopathy, neuropathy, nephropathy and renal dysfunction through several pathological changes. Different hormones are involved in regulating blood sugar levels. The most important are insulin and glucagon. When there is an imbalance in the hormone levels in the body, blood sugar levels rise, sugar begins to accumulate in the blood and is eventually excreted in the urine along with other minerals. In most cases of diabetes, T cell-mediated actions underlie the destruction of pancreatic islet beta cells. Serological markers such as islet cells and glutamic acid decarboxylase (GAD) have also been associated with beta-cell destruction [25-27].

TYPE 1 DIABETESMELLITUS

The disease is characterized by absolute insulin deficiency due to massive beta-cell necrosis or loss of beta-cell function. This usually refers to an autoimmune-mediated process against beta cells and may be caused by viral invasion or the effects of chemical toxins involved in beta cell destruction. The pancreas does not respond to glucose due to lipolysis, proteolysis, and lipolysis. Breakdown of glycogen. Type 1 diabetes has typical symptoms, including polydipsia, polyuria, bulimia, and weight loss. The onset and progression of neuropathy, nephropathy, and retinopathy are directly related to the degree of glycemic control (as measured by blood sugar and/or hemoglobin A1C [HbA1c] levels). Today, the goal of treatment is to use appropriate doses of exogenous insulin. Subcutaneous administration. Hyperglycemia and life-threatening ketoacidosis are caused and prevent the described catabolic states.[28]

TYPE 2 DIABETES MELLITUS

Type 1 diabetes is a multifactorial dysfunction of pancreatic beta cells that is more likely to be due to genetic factors, aging, obesity and peripheral insulin resistance rather than autoimmune diseases or viruses. The goal of treating type 2 diabetes is to keep blood sugar levels within normal limits and prevent the development of long-term complications of the disease. Weight loss, exercise, and dietary changes can reduce insulin resistance and correct hyperglycemia in type 2 diabetes in some patients.

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However, most patients rely on pharmacological intervention using oral hypoglycemic agents. As the disease progresses, beta cell function declines and insulin therapy is often required to achieve satisfactory serum glucose levels [29].

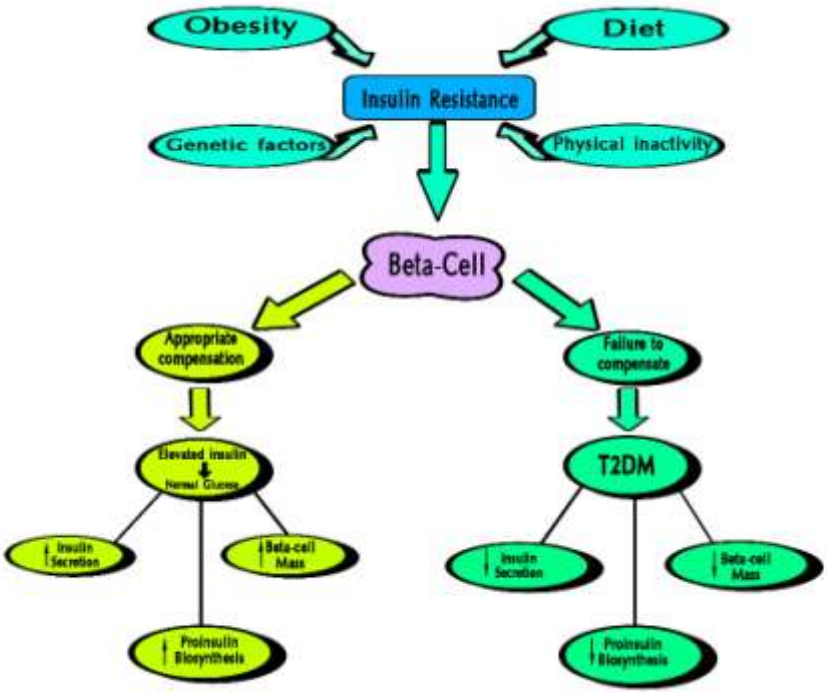

Fig 2 : Pathophysiology of Diabetic Mellitus

MECHANISM OF SILVER NANOPARTICLES

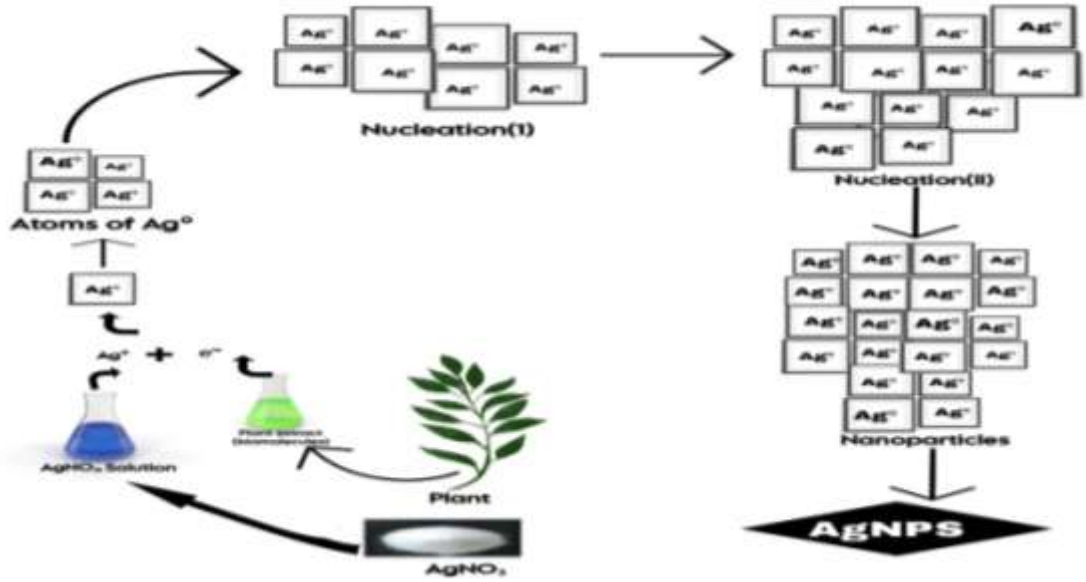

Fig 3 : Mechanism of formation of Silver Nanoparticles

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II. MATERIALS AND METHODS:

Collection of plant:

The leaves of Muntingiacalabura L. is collected from the surrounding area of Coimbatore district, Tamil Nadu, India and the plant was identified and authenticated.

Preparation of Extract:

To 0.5 ml of sample powder using martel and position to grained the sample and add 20 ml of sterile distilled water. Then kept for 15minutes in water bath. And add 0.5 ml of sample kept in shaking incubator for over night.

Phytochemical analysis:

- **Test for alkaloids (Mayer's test)**

To 1ml of extract, 1 ml of Mayer's reagent (Potassium iodide solution) was added. Formation of whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

- **Test for terpenoids(Salkowski test)**

To 1 ml of extract, 2ml of chloroform and few drops of sulphuric acid were added. Formation of reddish brown ring indicates the presence of terpenoids.

- **Test for phenol (Lead Acetate test)**

To 1ml of extract, 1 ml of lead acetate solution was added. Formation of precipitate indicates the presence of phenols.

- **Test for tannins (Lead acetate test)**

To 1ml of extract, 1ml of lead acetate was added. A formation of white precipitate indicates the presence of tannins.

- **Test for saponins(Froth test)**

To 1 ml of extract, 5 ml of distilled water was added and shaked vigorously. Formation of froth indicates the presence of saponins.

- **Test for steroids (LiebermannBurchard test)**

To 1ml of extract, 2ml of acetic anhydride and 2ml of concentrated sulphuric acid were added. Formation of violet to blue or green colour indicates the presence of steroids.

- **Test for flavonoids (Alkaline reagent test)**

To 1 ml of extract, few drops of dilute ammonium solution and few drops of concentrated hydrochloric acid were added. A yellow colouration indicates the presence of flavanoids.

- **Test for reducing sugars(Fehling's test)**

To the 1ml of extract, equal quantities of Fehling solution A and B were added and heated. Formation of brick red precipitate indicates the presence of reducing sugars.

Total Phenol estimation:

To 1ml of sample extract was mixed with 0.2 ml of foling phenol reagent and 1ml of 20% of sodium carbonate. The solution Was incubate at 45° C for 45 mins in water bath . This was detected at 765 nm

Thin Layer Chromatography:

50µl of the extracts were spotted on a TLC plate (silica gel 60 F_{254} 20X20cm). After spotting the sample TLC sheet was dried and kept in the saturated chamber containing Formic acid: Acetic acid: water: Methanol in the ratio of 1:1:1:2(v/v) as a mobile phase and developed up to 7cm length. The plate was removed and dried. The development of the sample spots were finally confirmed and the R_f value was calculated.

CHARACTERIZATION OF SILVER NANOPARTICLES :

UV –Visible Spectrophotometric Analysis

The green synthesised silver nanoparticle was primarily examined with UV Visible spectrophotometer – Labtronics LT291. The sample were scanned from the wavelength of 300-600 nm and the characteristic peaks were detected.

FTIR

FTIR study was done for the identification of the functional group which is present in the synthesized silver nanoparticle. Using shimadzu instrument from 4000^{cm-1} to 400^{cm-1} scanning was done and finalised the compound.

FESEM

After synthesizing the sample was made to powder form by drying. Followed by scanning electron microscope (ZEISS) examination was done to study the morphology and size of green synthesized silver nanoparticle.

Anti-diabetic activity:

Alpha –amylase inhibition assay

To 1ml of the sample was mixed with 0.1% starch solution in 16mM of sodium acetate buffer and 0.2 ml of the alpha –amylase enzyme which is prepared by mixing of 27.5gm in 100ml distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3,5 di nitro salicylic acid solution in the concentration of 96mM. After adding the tubes were incubated in alkaline condition at 25^0C for 3-5minutes. The generation of maltose was quantified by the reduction of 3,5 dinitrosalicylic acid to 3-

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amino-5-nitrosalicylic acid. This was detected at 540nm using spectrophotometer.

Alpha – glucosidase inhibition Assay

The inhibitory activity was determined by mixing of 1ml of 2% starch solution and 1ml of the sample with 1ml of the 0.2M tris buffer (pH-8), incubated at 37^0C for 5minutes. The reaction was initiated by adding 1ml of the alpha-glucosidase enzyme (1U/ml) incubated for 40minutes at 35^0C. The reaction was terminated by adding 2ml of 6N HCl and the reading was measured by 540nm using spectrophotometer.

III. RESULT AND DISCUSSION:

Phytochemical Analysis: The synthesis nanoparticles were subjected to the phytochemical analysis and all test showed positive**(TABLE 1)**M.calabura extract

S.NO	TEST	OBSERVATION	RESULT
1.	**Alkaloids** Mayer's test	Formation of whitish yellow or cream coloured precipitate	+
2.	**Terpenoids** Salkowski test	Formation of reddish brown	+
3.	**Phenol** Lead acetate test	Formation of precipitate	+
4.	**Saponins** Forth test	Formation of forth	+
5.	**Flavonoids** Alkaline reagent test	Formation of yellow colour	+
6.	**Steroids** Liebermann test	Formation of violet to blue or green colour	+
7.	**Reducing of sugar** Fehling's test	Formation of brick red precipitate	+
8.	**Tannins** Lead acetate test	Formation of white precipitate	+

TABLE 1: PHYTOCHEMICAL ANALYSIS OF EXTRACT

Phytochemical analysis of aqueous extract of Muntingiacalaburaclearly shows the presence of several important phytochemical.

TLC STUDY OF MUNTINGIA CALABURA.L:

TLC study of aqueous extraction of Muntingiacalabura.L is given below

- Solvent system : Methanol, Formic acid, Acetic acid
- No. of spots : two

The **Fig 4** TLC plate shows the solute and solvent traveled front

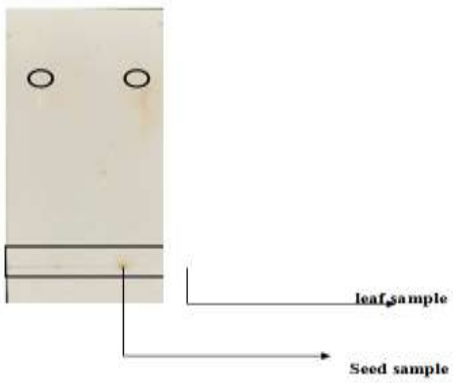

Fig 4 : TLC plate of Extract

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Characterization of Synthesis of Silver nanoparticles:

The Silver nanoparticles were synthesised and after 24hrs of incubation the colour change from pale green to dark brown colour indicates the formation of AgNPs

UV –Visible Spectrophotometric Analysis

Maximum absorbance of M.Calabura leaf scanned at range 200 to 600nm and absorbance at 449.6nm.(**Fig 5).** Confirm the formation of silver nanoparticles.

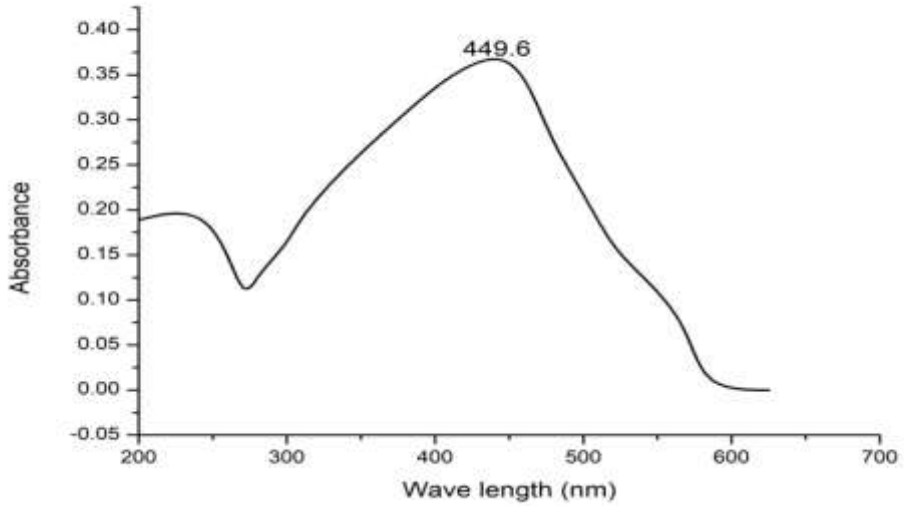

Fig 5:Spectrum of leaf extract silver nanoparticles

ʎ max of M.Calabura seed obtained at range 200 to 600nm and absorbance at 461.3nm.**Fig 6.** Shows the UV-Visible spectroscopy.

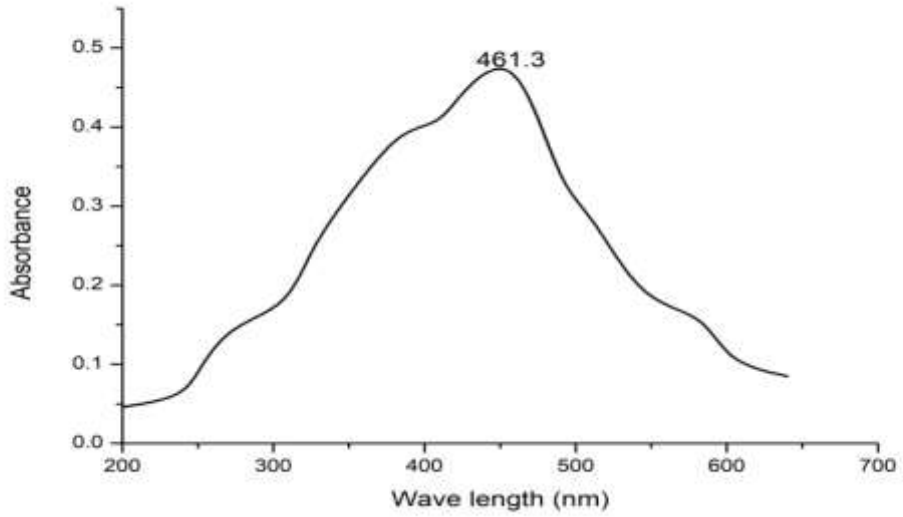

Fig 6: Spectrum of seed extract silver nanoparticles

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FTIR:

FTIR ANALYSIS FOR SEED SAMPLE:

FTIR spectrum was recorded both extract and silver nanoparticles. The functional group were confirm the formation of silver nanoparticles shown in (**Fig 7&8**).

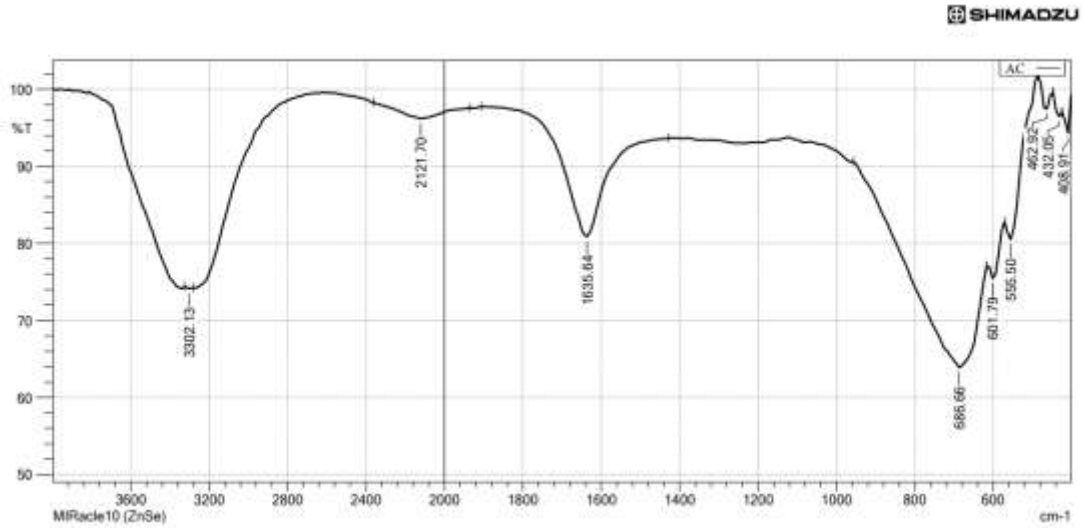

Fig 7:FTIR for seed extract

FREQUENCY (cm-1)	FUNCTION GROUP	INTENSITY
3302.13	=C-H Stretch	Strong
2121/70	C=C Stretch	Variable
1635.64	C=C Alkene	Weak
686.66	C-CI	Strong
601.79	C-CI	Strong
555.50	C-I	Strong
462.92	C-I	Strong
432.05	C-I	Strong
408.92	C-I	Strong

TABLE 2: FTIR Functional group

FTIR ANALYSIS FOR SEED NANOPARTICLES:

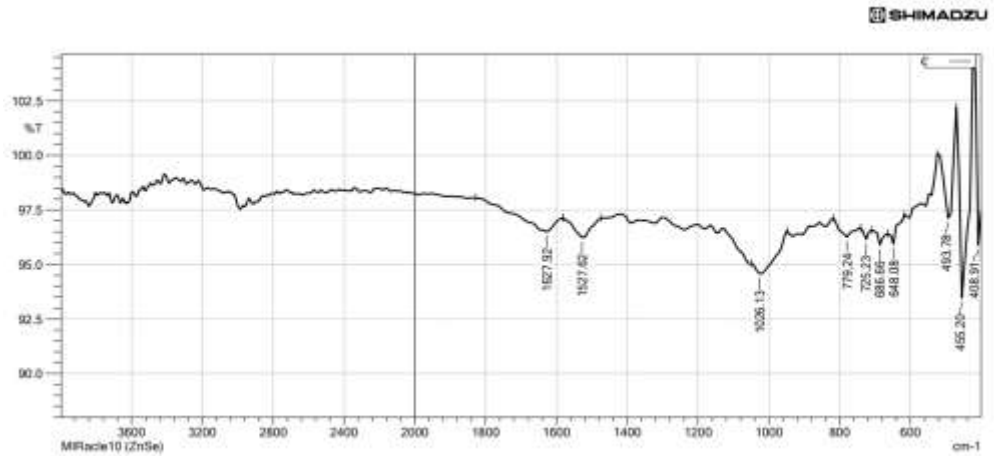

Fig 8: FTIR for silver nanoparticles

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FREQUENCY (cm-1)	FUNCTION GROUP	INTENSITY
1627.92	C=C Aromatic	Weak
1527.62	C=C Aromatic	Weak
1026.13	C-OH Stretch	Strong
779.24	C-CI	Strong
725.23	C-Br	Strong
686.66	C-CI	Strong
648.08	C-CI	Strong
493.78	C-I	Strong
455.20	C-I	Strong
408.91	C-I	Strong

TABLE 3:FTIR Functional group

SEMAnalysis :

The SEM analysis was carried out by using Zeiss with 20um resolution at 50 kV with 10.0nm wide. The size and shape of the nanoparticles were in the range of 80 to 120nm round and spherical in shape. The synthesized silver nanoparticles were further characterized by SEM analysis where it was noted that the particles were spherical in shape.(**Fig 9).** Shows the SEM analysis of nanoparticles.

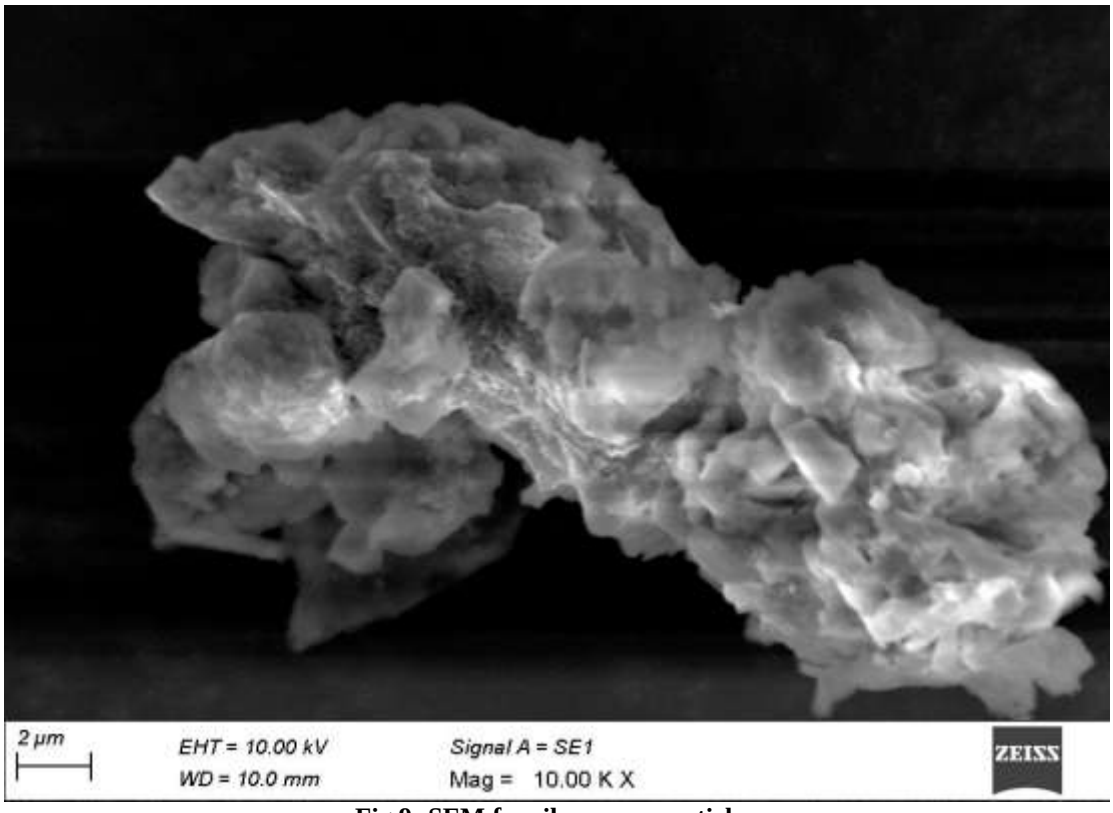

Fig 9: SEM for silver nanoparticles

TOTAL PHENOL CONTENT:

The total phenolic content of M.calabura.L extract **Fig 10** indicates that total phenolic content

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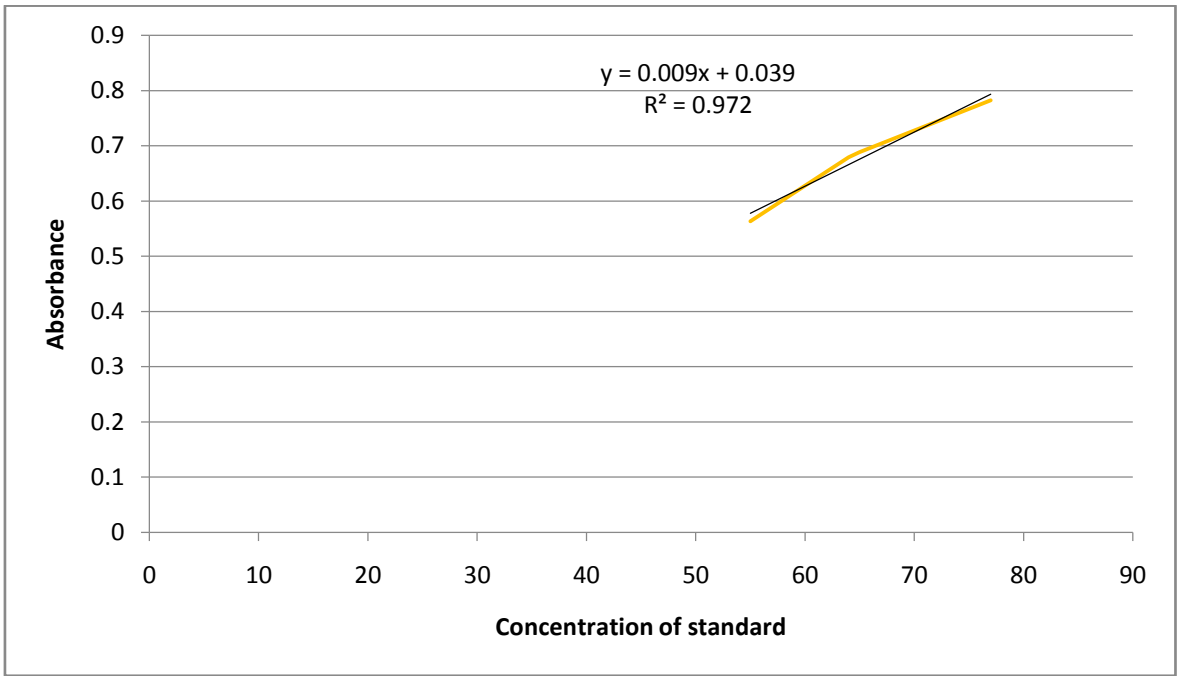

Fig 10: Total phenolic content

The total phenolic content value R^2 is 0.9724

Sample	OD Readings	Concentration (µg/ml)
Seed	0.563	55
Seed nanoparticles	0.688	65
Leaf	0.679	64
Leaf nanoparticles	0.782	77

Table 4: Total Phenolic Content

ANTI-DIABETIC :

The Anti-Diabetic activity of M. calabura.Lsilver nanoparticles was analysed by α-amylase and α-Glycosidase.The Anti-Diabetic activity showed higher inhibition activity in leaf nanoparticles them seed nanoparticles. Overall to activity is higher in nanoparticles formulation from Extract.**Fig 11&12**

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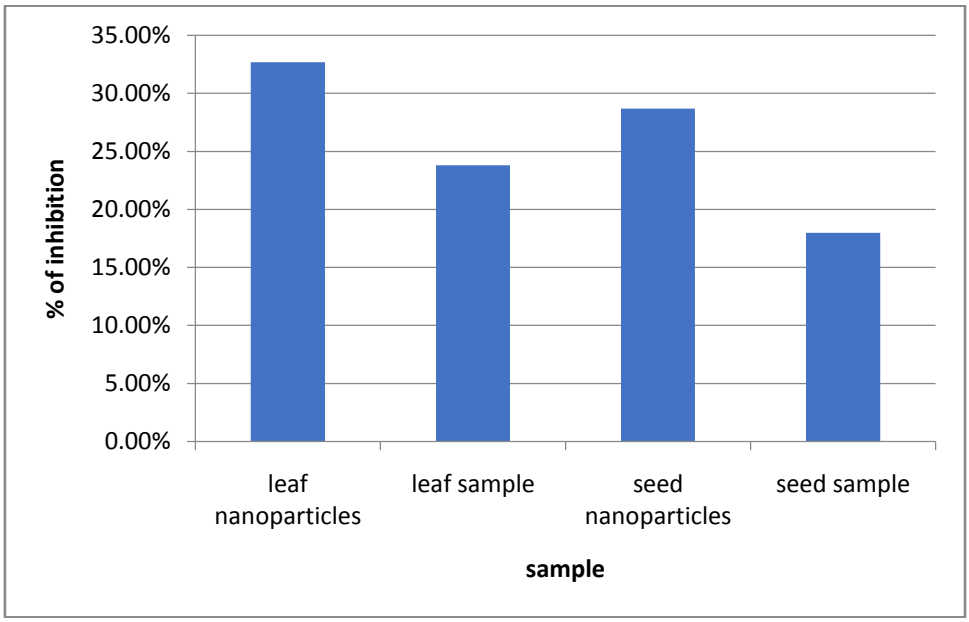

Fig 11:Anti-diabetic activity for α-Amylase

TABLE 5: Percentage of inhibition of Anti-diabetic activity for α-Amylase

Sample	OD reading	% of inhibition
Leaf	0.352	23.80%
Leaf nanoparticles	0.521	32.68%
Seed	0.493	17.97%
Seed nanoparticles	0.604	28.68%

Table 5: Anti-diabetic activity for α-Amylase

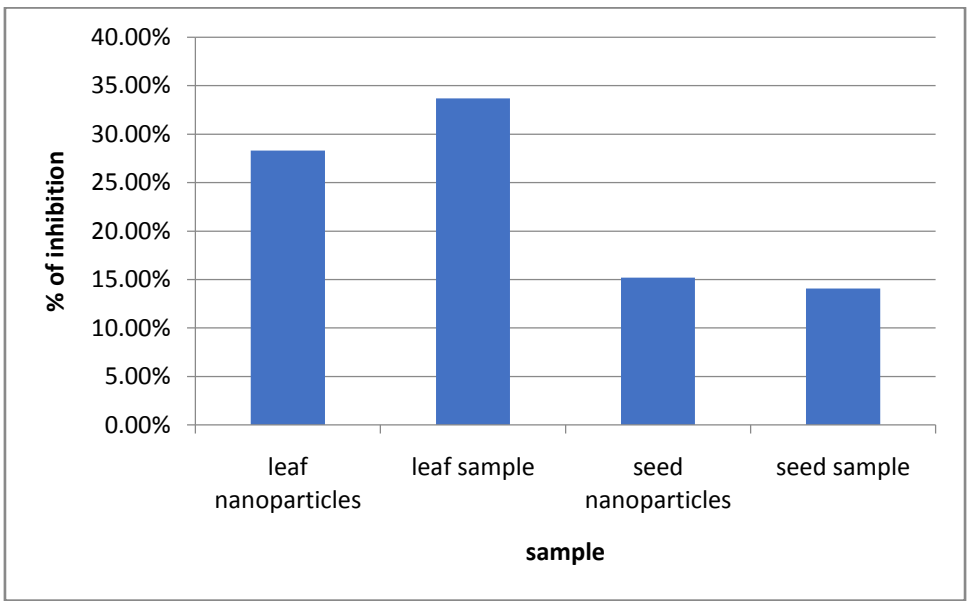

Fig 12: Anti-diabetic activity for α-Glycosidase

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TABLE 6: Percentage of inhibition of Anti-diabetic activity for α-Glycosidase

Sample	OD reading	% of inhibition
Leaf	0.210	33.70%
Leaf nanoparticles	0.752	28.31%
Seed	0.397	14.06%
Seed nanoparticles	0.497	15.21%

Table 6: Anti-diabetic activity for α-Glycosidase

IV. SUMMARY AND CONCLUSION:

The aim of the present study was evaluate the in-vitro Anti-diabetic activity of silver nanoparticles of aqueous extract of Muntingiacalabura.L. Aqueous Muntingiacalabura .L extract was found to contain flavonoids, phenol, saponins, tannins, steroids, alkaloids and terpinoids. The presence of phenolic compound and flavonoids provide the substantial anti-diabetic activity for the plant extract, and this made the plant to be successfully used for the synthesis of silver nanoparticles from $AgNo_3$. The brown color appeared after incubation of the mixture for 24 h. The formation of silver nanoparticles is confirmed by UV-Vis. UV-Visible spectroscopy for leaf showed absorption maxima at 449.6 nm. UV-Visible spectroscopy for seed showed absorption maxima at 461.3 nm. Silver nanoparticles were characterized using FTIR and SEM. In-Vitro anti-diabetic activity of Muntingiacalabura.L extract. The percentage of anti-diabetic activity of Mutingiacalabura.L extract silver nanoparticles is higher than sample extract. Green synthesized silver nanoparticles showed anti-diabetic activity.

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