# PHYTOCHEMICAL EVALUATION AND GREEN SYNTHESIS OF SILVER NANOPATICLES

# FROM

# *MORINDA CITRIFOLIA* L. AND *MUSSAENDA FRONDOSA* L.

**A dissertation submitted in partial fulfilment of the requirement for the degree of Master of Science in Botany**


**By**

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**2022**

# DECLARATION

I hereby declare that the dissertation entitled "Phytochemical Evaluation and Green synthesis of Silver nanoparticles from *Morinda citrifolia* L. and *Mussaenda frondosa* L. " Submitted is an authentic record of the research carried out by me, under the supervision and guidance of Dr. Elsam Joseph, Associate professor, Department of Botany. St. Teresa's College (Autonomous) Ernakulam, in partial fulfilment of the M.Sc degree of Mahatma Gandhi University, Kottayam, and that no part of this thesis has been presented before for any degree, Diploma, associateship or other recognition from any university.

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# CERTIFICATE

This is to certify that the dissertation titled,**"PHYTOCHEMICAL EVALUATION AND GREEN SYNTHESIS OF SIVER NANOPATICLES FROM MORINDA CITRIFOLIA AND MUSSAENDA FRONDOSA"** is an authentic record of work carried out by **FATHIMA FARZANA.P.M** under the supervision and guidance of **Dr. ELSAM JOSEPH.**, Associate Professor, Department of Botany & Centre for Research, St. Teresas's College (Autonomous), Ernakulam, in partial fulfilment of the requirement for the Master's Degree of Science in Botany.

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# ACKNOWLEDGEMENT

First and foremost, I thank God almighty for endowing me with immense blessings which helped me to overcome all hurdles, paving way for the successful completion of this investigatory project.

I express my heartfelt gratitude to Dr. Elsam Joseph., Associate professor, Department of Botany, St.Teresa's College, Ernakulam for the keen interest, valuable guidance and whole hearted support she has rendered to me throughout the course of this work.

I am also grateful to Dr.Liza Jacob, Head of the Department of Botany for providing all the necessary facilities for the work.

I express my sincere thanks to all the other teaching and non-teaching staff of the Botany department, St. Teresa's College for their kind cooperation. I would also like to acknowledge all my friends for their valuable help rendered in the completion of this study.

Last, but not the least, I sincerely thank my beloved parents who with their love, encouragement and blessings have helped in all my endeavours.

Fathima Farzana.P.M

# CONTENTS

# INTRODUCTION

Phytochemistry is the study of the chemicals produced by plants, particularly the secondary metabolites which are synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, UV exposure and environmental hazards. Phytochemistry takes into account the structural compositions of these metabolites, the biosynthetic pathways, functions, mechanisms of actions in the living systems and it's medicinal, industrial, and commercial applications. The proper understanding of phytochemical is essential for drug discovery and for the development of novel therapeutic agents against major diseases **[**Egbuna et.al (2018)]**.**

India is known for its rich diversity of medicinal plants and from ancient times these plants were utilized as therapeutic agents. Today's research is mainly focused on medicinal plants because the bioactive compounds and medicinal power mainly depends on phytochemical constituents that have great pharmacological significance. The phytochemical constituents, natural bioactive compounds, nutrients and fibers present in medicinal plants, fruits and vegetables defend us from various ailments [ Akinmoladun AC et.al (2017)].

The green belt of mother nature is the richest source of bioactive phytochemicals and natural nutraceuticals. Enormous work done during the past fifty years has shown that these phytochemicals play an important role in the routine healthcare systems worldwide. The major classes of phytochemicals like alkaloids, phenolics, terpenoids and tannins have potential to prevent diseases and act as anti-microbial, anti-inflammatory, anti-oxidant, anti-cancerous, detoxifying agent, immunity-potentiating agent and neuropharmacological agent. Each class of these functional agents consists of a wide range of chemicals with differing potency. Some of these phytochemicals are found to be multifunctional. There is, however, much scope for further systematic research in screening Indian medicinal plants for their phytochemicals and assessing their potentiality as crude drug or drug components [Koche D et.al (2018)]

Although there are tens of thousands of phytochemicals, only a small number have been isolated and identified from plants (Cao et al. 2017; Singh and Chaudhuri 2018). The most common phytochemicals in food include polyphenols, carotenoids, flavonoids, coumarins, indoles, isoflavones, lignans, organosulfures, catechins, phenolic acids, stilbenoids, isothiocyanates, saponins, procyanidins, phenylpropanoids, anthraquinones, ginsenosides, and so on (Xiao 2017; Zhao et al. 2018a).

Alkaloids are one of the important classes of secondary metabolites which are found to possess important biological properties like analgesic, muscle relaxant, antioxidant, etc. These are used for the help of mankind and found beneficial for certain life-threatening disease. Certain alkaloids have shown reverse effects such as asphyxia, paralysis or in some extreme condition patient death(Roy, Arpita 2017). The major sources of alkaloid in human diet are tea leaves (theophylline, caffeine) , coffee seeds (caffeine), cacao seeds (theobromine and caffeine), tomatoes (tomatine) and potatoes(solanine).

Flavonoids are plant pigments that are synthesized from Phenylalanine [Harborne JB, Turner BL (1984)] and generally display marvelous colors in the flowering parts of plants [Clifford AH, Cuppett SL (2000)]. Vegetables, fruits, tea (Theaflavins) and wine (procyanidin) are the main dietary sources of Flavonoids of humans. They have reported to possess antiallergic, anti-inflammatory, antiviral, antiproliferative, and anticarcinogenic activities, in addition to having effects on mammalian metabolism [Ren W et.al (2003)].

Another important phytochemical is tannin , also known as tannic acid. They are naturally present in leaves, seeds, bark, roots, fruits, vegetables, legumes, cereals, shrubs etc.[Hassanpour et al. 2011]. Tannins are responsible for the animal skin conversion into leather. Tannins can be synthesized artificially by using naphthalene, cresols and other higher hydrocarbons as primary ingredients. Chemically they are of two groups; hydrolysable tannins and condensed tannins. provide us relief from various types of ailments such as reducing the risk of diabetes by enhancing glucose uptake and thus lowering blood sugar level [Kumari and Jain ,(2015)].Diluted tannin solution is applied over an open wound as it precipitates the protein of the wound,thereby making a protective covering and prevents bleeding to aid faster healing [Ramakrishnan and Krishnan (1994)]. Condensed tannins are also effective against various types of allergies such as asthma, hypersensitive pneumonitis, allergic rhinitis, mite allergens from carpet dust and many more [Chung et al.1998].

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants.They are essential to the physiology of plants, because of their involvement in various important functions (growth,structure, defense, pigmentation, lignifications etc.).They are largely found in fruits, vegetables, cereals and beverages [Huang et al.,( 2005)]. Plant polyphenols form one of the most important and extensively used classes of plant-derived therapeutics for cancer prevention and chemotherapy. Polyphenols induce a reduction of the number of tumors or of their growth,

results in protective effect [Galleano et al., 2010; Martins et al., (2011)] at various sites including mouth, stomach, duodenum, colon, liver, lung, mammary gland or skin.

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particles that measure in nanometers (1nm = 1 billionth of a meter). People use nanotechnology and nanomaterials in everyday life because of their unique or rather different chemical and physical properties [S.H Ahmadi (2020)]

Among the various nanoparticles, silver nanoparticles have gained much attention due to their unique antimicrobial properties. However, concerns about the synthesis of these materials such as use of precursor chemicals and toxic solvents, and generation of toxic byproducts have led to a new alternative approach, green synthesis. This eco-friendly technique incorporates use of biological agents, plants or microbial agents as reducing and capping agents. Silver nanoparticles synthesized by green chemistry offer a novel and potential alternative to chemically synthesized nanoparticles [ Anupam Roy et.al (2019)]. Considering the importance of silver as an antimicrobial agent, The US Food and Drug Administration approved silver solution in 1920 for its usage in the food and drug industry. The different forms of silver, viz. silver salt, silver acetate, silver nitrate, and silver sulfadiazine, are being used for microbial inhibition (Jung et al. 2008).

For the present study two plant species from family Rubiaceae was selected; *Mussaenda frondosa* L., and *Morinda citrifolia* L.

The genus *Morinda* (Rubiaceae), including the species *Morinda citrifolia* L., is made up of around 80 species. *Morinda citrifolia* L. is a bush or small tree, 3–10 m tall, with abundant wide elliptical leaves (5–17 cm length, 10–40 cm width). The small tubular white flowers are grouped together and inserted on the peduncle. The petioles leave ring-like marks on the stalks and the corolla is greenish- white [Morton(1992); Elkins, (1998); Dixon et al.,(1999);Ross,( 2001); Cardon, (2003)].The noni fruit (3–10 cm length, 3–6 cm width) is oval and fleshy with an embossed appearance. It is slightly wrinkly, semi-translucent, and ranges in colour from green to yellow, to almost white at the time of picking. It is covered with small reddish-brown buds containing the seeds. The ripe fruit exhales a strong butyric acid-like rancid smell [Morton, 1992; Dixon et al., (1999)]. The pulp is juicy and bitter, light dull yellow or whitish, gelatinous when the fruit is ripe; numerous hard triangular reddish-brown pits are found, each containing four seeds.

The genus *Mussaenda frondosa* L. is a shrub that grows to about 1.5–2 m (4 ft 11 in – 6 ft 7 in) tall. Like most other *Mussaenda* species, they have a bract beneath their flowers, which in this species is white in color. The shrub may also grow as a scandent climber. The flowers are clusters of orange-yellow tubular flowers with one of their five sepals enlarged into a white petal-like form, set among pale green, oval leaves; berries follow the bloom. The erect, branching stem has a shrubby crown.

**OBJECTIVES**

- To detect phytochemicals present in leaf extracts of selected plants.
- Green synthesis of silver nanoparticles from the leaf extracts of selected plants.
- To conduct comparative germination study of aqueous extract of selected plants using *Vigna raditata* L. seeds.

# REVIEW OF LITERATURE

The Polynesians have been using the noni plant for food and medicinal purposes for more than 2000 years (Earle, 2001). In traditional pharmacopoeia, the fruit is claimed to prevent and cure several diseases. It is primarily used to stimulate the immune system and thus to fight bacterial, viral, parasitic and fungal infections; it is also used to prevent the formation and proliferation of tumors, including malignant ones (Dixon et al., 1999)

In 2012, Sridevi Nagalingam et.al, conducted a study to investigate the phytochemical screening of *Morinda citrifolia L.* fruit extract in three different solvent, and the results showed that *Morinda citrifolia* L.contained a broad spectrum of seccondary metabolites such as alkaloids, Saponins, steroid, phenol, tannin, terpenoids, flavonoids, Cardiac glycosides etc.

In 2018, Wahyuning Setyani and Hanny Setyowati carried out isolation of flavonoids, alkaloids, terpenoids, sterols and minerals from noni plant. They reported Iridoid, a flavonoid compound, as the main component found in the noni leaves extract. Pharmacological screening of these compounds revealed that they are potential for anti-melanogenesis therapy; melanogenesis is a multistage process involving melanin synthesis, melanin transport and melanosome release. Hence this property enables the noni plant to be used as an anti-photo aging cosmetic ingredient.

The aqueous extract of *Morinda citrifolia L.* leaves (AEMS) were subjected to pharmacological screening by Mairim Russo et.al, in 2011. Antioxidant activity was observed against lipid peroxidation, nitric oxide and hydroxyl radicals; antinociceptive effect of AEMC was observed in the acetic acid induced writhing test at higher dose; doses of 200 and 400 mg/kg showed mild antibacterial activity. Together, the results suggest that the properties of *M. citrifolia* L.leaf extract should be explored further in order to achieve newer tools for managing painful and inflammation conditions, including those related to oxidant states.

The lyophilized aqueous extract of roots of *Morinda citrifolia* L.was evaluated for analgesic and behavioral effects in mice by Chafique Younos et.al, in 1989. The extract did not exhibit any toxic effects but did show a significant dose related central analgesic activity at 800 mg/kg in the writhing and hotplate test.

In 2016, Shoeb Ahsan et. al., induced hyperlipidemia in wistar albino rats of either sex by feeding a cholesterol rich high fat diet for 45 days. Noni fruit juice administered at 50

mg/kg/day and 100mg/kg/day per oral, was compared with the standard drug Atorvastatin (10mg/kg/day oral) fed for the latter 30 days. The blood samples were then sent for complete blood lipid profile, after 30 days of treatment. The Noni fruit juice treated group showed a significant decrease in total cholesterol, and a very low density lipoprotein-cholesterol at both the doses when compared to the disease control.

Three new glycosides were isolated from the fruits of noni (*Morinda citrifolia L.*) by Mingfu Wang et.al, in 2000. Their structures were determined to be *6-O*-(β-d-glucopyranosyl)-1-*O*-octanoyl-β-d-glucopyranose (**1**), 6-*O*-(β-d-glucopyranosyl)-1-*O*-hexanoyl-β-d-glucopyranose (**2**), and 3-methylbut-3-enyl 6-*O*-β-d-glucopyranosyl-β-d-glucopyranoside (**3**) using MS and NMR methods.

It has been reported that noni inhibits the growth of certain bacteria, such as *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus morgaii, Bacillus subtilis, Escherichia coli, Helicobacter pylori, Salmonella* and *Shigella* (Atkinson, 1956). The same author claims that the anti-microbial effect observed may be due to the presence of phenolic compounds such as acubin, L-asperuloside, alizarin, scopoletin and other anthraquinones.

Another study showed that an acetonitrile extract of the dried fruit inhibited the growth of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli,* and *Streptococcus pyrogene* (Locher et al., 1995).

Shivananda Nayak et .al., in 2007 evaluated the wound healing activity on rats using ethanolic extracts of noni leaves via excision and dead space models, the extract-treated animals exhibited 71% reduction in the wound area when compared with controls which exhibited 57%.

Wang and Su in 2001 reported that commercial noni juice (Tahitian Noni Juice) prevents the formation of chemical carcinogen-DNA-adduct. In their study, rats with artificially induced cancer in specific organs were fed for one week with 10% noni juice in their drinking water and rat food (rat chow), ad libitum. They showed reduced DNA-adduct formation, depending on sex and considered organ. Reduction rates were: in female rats, heart 30%, liver 42%, lungs 41%, and kidneys 80%; in male rats, heart 60%, liver 70%, lungs 50%, and kidneys 90% .

Another Japanese team studied more specifically the influence of damnacanthal, an anthraquinone extracted from a chloroform extract of noni roots. Surprisingly, the researchers

found that damnacanthal induced the normal morphology of a particular type of cells found in human neoplasias (K-ras-NKR cells) that multiply uncontrollably and are highly malignant (Hiramatsu et al., 1993).

It has also been found that ethanol and hexane extracts of Noni have an antitubercular effect since they inhibit by 89–95% the growth of *Mycobacterium tuberculosis* (Saludes et al., 2002). The major components identified in the hexane extract were E-phytol, cycloartenol, stigmasterol, b-sitosterol, campesta-5,7,22-trien-3-b-ol, and the ketosteroids, stigmasta-4-en-3-one and stigmasta-4-22-dien-3-one.

Another study showed that commercial noni juice has a selective inhibition effect on some cyclo-oxygenase enzymes (COX-1 and COX-2) involved in breast, colon and lung cancer, and also in anti-inflammatory activity (Su et al., 2001). The inhibition of the activity of these enzymes by noni juice was compared with that of commercial traditional non-steroidal inflammatory drugs such as Aspirin, Indomethacins and Celebrexs. Noni juice showed selective inhibition of COX enzyme activity in vitro and a strong anti-inflammatory effect comparable to that of celebrexs and presumably without side effects.

T.Y.Suman et.al, successfully synthesized silver nanoparticles, from the root of *Morinda citrifolia L.*; without involving chemical agents associated with environmental toxicity. The obtained nanoparticles were characterized by UV–vis absorption spectroscopy with an intense surface plasmon resonance band at 413 nm clearly reveals the formation of silver nanoparticles. Fourier transmission infra red spectroscopy (FTIR) showed nanopartilces were capped with plant compounds. Field emission-scanning electron microscopy (FE-SEM) and Transmission electron microscopy (TEM) showed that the spherical nature of the silver nanoparticles with a size of 30–55 nm. The X-ray diffraction spectrum XRD pattern clearly indicates that the silver nanoparticles formed in the present synthesis were crystalline in nature.

Similarly, Violeta Morales-Lozoya et al., were also able to synthesise silver nanoparticles using extract of different parts of *Morinda citrifolia* L.(noni) plant. The characterization of AgNPs were performed by UV–Vis, FTIR-ATR, SEM-EDS, and TEM. The spherical AgNPs resulted well dispersed, with sizes 3–11 nm, which also shows antibacterial activity against Gram-positive and Gram-negative strains.

The *Mussaenda frondosa* Linn. Belonging to the family Rubiaceae, commonly known as Sriparnah in Sanskrit, is a scandent shrub traditionally used in the treatment of cough, bronchitis, fever, inflammation, wounds, ulcers, jaundice, leucoderma and pruritus.

In a study conducted by S.Gopalakrishnan and E. Vadivel in 2011, the ethanolic extract of *Mussaenda frondosa* L.has been subjected to GC-MS analysis. Twenty chemical constituents have been identified, The major chemical constituents identified are (-)-Quinic acid (32.87 %), 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (8.30%), Naphthalene, decahydro-2-methoxy-(7.20 %). 1, 2, 3-Benzenetriol (7.70%),

Similarly, in vitro antioxidant effects of the ethyl alcohol and aqueous extracts of whole plant of *Mussaenda frondosa L.* were tested by E.N.Siju et.al., in 2010. The ethyl alcohol extract of *Mussaenda frondosa* L. had shown good DPPH(1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity.The ethyl alcohol and aqueous extract of *Mussaenda frondosa* L. also exhibited promising result at higher concentration. BHA was used as standard antioxidant and positive control.

S. Shanthi and R.Radha in 2020 investigated antimicrobial effects of *Mussaenda frondosa* L against bacteria and fungus. In addition, Phytochemical profiling of the methanol extract of *Mussaenda frondosa L.* was done using High Performance Thin Layer Chromatography (HPTLC). The antimicrobial activity of Methanol (MEMF), Ethyl acetate (EEMF), Chloroform (CEMF) and Hexane (HEMF) extracts of *Mussaenda frondosa L.* leaves were tested against nine bacterial and four fungal strains. The Methanol extract showed significant antibacterial and antifungal activity than hexane, Chloroform, Ethyl acetate extracts which could be attributed to the presence of phenols, flavonoids and the other bioactive compounds identified through phytochemical screening. The findings in the present study offer a scientific support to the ethno medicinal use of the plant by the traditional healers.

Diuretic activity of the plant *Mussaenda frondossa* Linn. , was investigated by R.Sreelakshmi et. al., using Lipschitz method. Wistar albino rats was divided into 4 groups of 6 animals in each. Frusemide (20 mg/Kg p.o) used as a standard diuretics. Two doses of plant extract was used for the study. In urine the volume and concentration of electrolytes was measured at the end of 24 hrs. Result showed significant increase in diuretic activity.

Patil Suhas et.al., in 2012 investigated the healing effect of *Mussaenda frondosa L.* extract in comparison with Silver sulfadiazine (SSD), in second degree burn wounds. Adult albino rats

of weight around 150-200 gm. were divided into 4 groups. Standard second degree burn wounds were induced on the back of their necks. One group was treated with SSD; two groups were treated with alcoholic extract (AE) cream of *M. frondosa* L.at concentrations of 10% (AE10) and aqueous extract (AQE) cream of *M. frondosa* L. at concentrations of 10% (AQE10) and the control group which received no treatment. The duration of treatment was 16 days. Their study revealed that AE and SSD noticeably improved re-epithelization, lipid peroxide, and collagen bundle synthesis.

Leena K. Pappachen and K.S. Sreelakshmi in 2017 tested *M.frondosa L.* leaves for in vitro anticancer activity on HepG2 cell line by MTT assay method and the methanolic extract and isolated flavonoid fraction showed better activity against HepG2 cell line.

Biosynthesis of zinc oxide nanoparticles (ZnO-NPs) was achieved by Manasa Dogganal et. al., in 2020, utilizing the reducing and capping potential of leaf, stem and callus aqueous extracts of *Mussaenda frondosa L.*. The bioreduced ZnO-NPs were characterized using powder X-ray diffraction (XRD), ultraviolet–visible spectroscopy (UV–Vis spectroscopy), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), fourier transform infrared spectroscopy (FTIR) and dynamic light scattering (DLS) techniques. UV–visible spectra of ZnO-NPs showed a strong absorption peak at 370, 376 and 373 nm corresponding to the band gap energy of 3.33, 3.27 and 3.30 eV for ZnO-NPs obtained from leaf (L-ZnO-NP), stem (S-ZnO-NP) and callus (C-ZnO-NP) aqueous extracts, respectively. XRD analysis confirmed the formation of hexagonal wurtzite structures having an average grain size between 5 and 20 nm in diameter. FTIR spectra revealed the presence of stretching vibrations of –O–H, C–H, C–N, C = O groups involved in reduction and stabilization of nanoparticles. SEM images recognize the presence of spongy, spherical, porous agglomerated nanoparticles. DLS analysis and zeta potential values validated the stability of ZnO-NPs.

# MATERIALS AND METHODS

## SELECTED PLANTS FOR THE STUDY

### I .*MORINDA CITRIFOLIA* L.

**Fig.1- *Morinda citrifolia* L.**

**Systematic position**

Class - Dicotyledonae.

Subclass - Gamopetalae.

Series - Inferae.

Order - Rubiales

Family - Rubiaceae.

**Morphology**

*Morinda citrifolia* L.is a fruit-bearing tree in the coffee family, Rubiaceae. Evergreen shrub or small crooked tree with a conical crown Bark greyish or yellowish brown.Leaves dark green, opposite and simple, elliptic-lanceolate.Inflorescence globose heads.

## II. *MUSSAENDA FRONDOSA* L.

**Fig.2 – *Mussaenda frondosa* L.**

**Systematic position**

Class - Dicotyledonae.

Subclass - Gamopetalae.

Series - Inferae.

Order - Rubiales.

Family - Rubiaceae

**Morphology**

*Mussaenda frondosa* L. is an erect shrub. Leaves broadly ovate, base rounded, sparsely hairy on both surfaces.Flowers are clusters of orange-yellow tubular flowers with one of their five sepals enlarged into a white petal like form.

## QUALITATIVE ANALYSIS

I.DETECTION OF ALKALOID

For the preparation of plant extract 1 gm of powdered Sample was homogenized using 80% ethanol (10 mL). The homogenate was centrifuged at 1500 rpm for 5 minutes and supematant was collected.

For the detection of alkaloids, Mayer's, Wagner's and Dragendroff reagent were prepared

MAYER'S REAGENT

It was prepared by dissolving 1.36 g of $Hgcl_2$ in 60 mL distilled water (solution A) and 5g of kI dissolved in 10 mL distilled water (solution B). Both there solutions were mixed and diluted to 1000 mL with distilled water. The extract was acidified with Hcl before adding Mayer's rreagent.

WAGNER'S REAGENT

It was prepared by dissolving 1.27 g of iodine and 2.9 g of potassium iodide in 5 mL. distilled water and the solution were made up to 100 mL.

DRAGENDROFF'S REAGENT

It was prepared by dissolving 8 g of Bismuth Subnitrite in 20 mL of concentrated $HNO_3$ (solution A) and 27.2 Potassium iodide in 50 mL distilled water (solution B). The solutions were mixed, Supernatant solution was decanted and made up to 100 mL with distilled water.

After the preparation of reagents a small amount of plant extract was taken and tested with all the the 3 reagents and the formation of precipitate shows the presence of alkaloids. Absence of precipitate shows the absence of alkaloids.

II. DETECTION OF SAPONINS – FOAM TEST

1g of dried powdered plant material was taken in 20mL distilled water. It was shaken for 10 minutes, formation of frothy solution indicate the presence of saponins.

III. DETECTION OF FLAVONOIDS – ALKALINE REAGENT TEST.

To a little of the plant Sample add 2mL of NaOH followed by 2mL of dilute $H_2SO_4$. The yellow colour of NaOH changes when dilute acid is added. This shows the presence of flavonoids.

IV.DETECTION OF TANNIN- FERRIC CHLORIDE TEST.

Little of sample were mixed with water and filtered. The filtrate was mixed with 2 mL of 5% Ferric chloride solution in a test tube. Formation of blue, green, black color indicates the presence of tannin.

V. DETECTION OF STEROID-SALKOWSKI TEST

The ethanolic (aqueous) extract of the Samples were tested with 2 drops of conc. $H_2SO4$ . A red coloration in the lower layer of the solution indicates the presence of steroid.

VI. DETECTION OF BITTERS

1 g of powdered drug were shaken with ethyl alcohol and then with ethyl acetate. Formation of green color shows the presence of bitters.

VII. DETECTION OF RESIN

50 % $HNO_3$ was added to a little of the powdered drug sample. Brown color obtained indicates the presence of resin.

VIII. DETECTION OF PROTEIN

( a) BIURET TEST

A few drops of $CuSo_4$ solution was added to 2mL of the test solution, ( aqueous extract of leaf powder) followed the addition of 40% NaOH Solution, it was mixed thoroughly and the color was noted. Purple color indicates the presence of protein.

(b) XANTHOPROTEIC TEST

Equal volume of test solution (aqueous extract of leaf powder) and Conc. $HNO_3$ (0.5 mL) were mixed well and the solution was cooled to room temperature. The color was noted. Yellow color indicates the presence of protein. Again 40% of NaOH was added to make the solution alkaline, the color change was noted. Yellow color changes to bright orange which indicates the presence of protein.

IX.DETECTION OF CARBOHYDRATE

(a)MOLISH'S TEST

2 drops of Molisch reagent was added to 2mL of the test solution and was mixed well. 1mL of conc. $H_2SO_4$ was added along the sides of the test tube without shaking.

(b) BENEDICT'S TEST

5 mL of Benedicts reagent was added to the 1mL of test solution and the test tube was kept in boiling water bath for 5 minutes.

(C) FEHLING'S TEST

1 mL of Fehling's reagent A was mixed with 1 mL of Fehling's reagent B. To which a few drops of test solution are added and boiled.

X. DETECTION OF PHENOL.

2 drops of 5%$Fecl_3$ was added to the extract in a test tube, presence of greenish precipitate indicates the presence of phenol.

**QUANTITATIVE ANALYSIS**

It includes estimation of Tannin, Phenol content and flavonoid.

II. ESTIMATION OF PHENOLIC COMPOUND

1 g of plant sample were homogenized using 80% ethanol (25mL). The homogenate was centrifuged at 1000 rpm for 20 minutes. The Supernatant was collected and residue was again centrifuged and extract was collected. 0.2 mL aliquot was taken from this extract in a test tube and 2.8 mL distilled water was added. To this Sample,0.55 mL Foline cio-calteau reagent was added and kept for 3 minutes. After that 2 mL Sodium bicarbonate was added. The test tube was placed in a boiling water bath for 1 minute. And optical density measured against blanc using colorimeter at 650 nm.

Blanc solution was prepared by taking 3 mL distilled water. A STD graph was prepared using different concentration of Sample and the concentration of phenol was obtained from the graph. It was expressed as mg phenol/100 mg material.

III. ESTIMATION OF FLAVONOID

Sample preparation;

A ground freeze dried sample of 0.5g was weighed and flavonoid compound was extracted with 50 mL of 80% aqueous methanol on an ultrasonic bath for 20 minutes. An aliquot 2 mL of the extract was ultracentrifugated for 5 minutes at 14000 rpm.

Total flavonoid assay;

Total flavonoid content was measured by the aluminium chloride colorimetric assay. An aliquot (1mL) of extract or STD solution of catechin (20,40,60,80,100 mg/L ) was added to 10 mL volumetric flask containing 4 mL of distilled water. To the flask was added 0.3 mL 5% sodium nitrite. After 5 minute 0.3 mL of 10% aluminium chloride was added. At the sixth minute 2 mL 1 Molar NaOH was added and the total volume was made up to 10 mL with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blanc at 510 nm. It includes estimation of Tannin, Phenol content and flavonoid.

I .ESTIMATION OF TANNIN

For the estimation of tannins, first Indigo Sulphonic acid was prepared. It was prepared by adding lg Indigocarmine in 25 mL concentrated $H_2SO4$ in 100 mL beaker. The

Indigocarmine was then dissolved completely in concentrated $H_2SO4$. To this, again 25 mL of concentrated $H_2SO4$ was added. And it was made up to 1 L in volumetric flask by using distilled water.

For titration, 0.IN potassium permanganate solution was taken in a burette and 25 ml of the indigo sulphonic acid was taken in 1 L conical flask. It was then titrated and blanc value was taken. Then Ig of the sample was taken in a conical flask with a rubber cork, 100 mL distilled water was added to it. And shaken well for 10 minutes. It was filtered, 10 mL of the Sample solution was taken in 1 L conical flask and 25 mL indigosulfonic acid was added to the solution.

The above solution was titrated with 0.1N potassium permanganate solution. End point was the change of blue color to golden yellow color (Fig. 3). Then the burette reading was taken, 0.1 N KMnO4 is equal to 0.005647 g of tannins.

Total tannin content was calculated using the formula;

$$\text{Percentage of tannin} = \frac{\text{burette reading} - \text{blanc reading} \times 0.005647 \times 10 \times 100}{\text{weight of the sample.}}$$

**Fig.3 – Estimation of tannin.**

## GREEN SYNTHESIS OF SILVER NANOPARTICLES

PREPARATION OF LEAF EXTRACT

Leaf extract was prepared by taking 10 g of washed and finely cut leaves. The leaves were boiled for 5 minutes by adding 100 ml sterilized distilled water in 250 ml Erlenmeyer flask. The boiled extract was filtered using Whatman No.1 filter paper. This filtered boiled extract was used for the further synthesis of silver nanoparticles.

PREPARATION OF SILVER NITRATE SOLUTION

1 mM Silver Nitrate solution was prepared by weighing 1.6987 g of Silver Nitrate and dissolved in 100 ml distilled water. The solution was kept in dark bottles for further procedures.

SYNTHESIS OF SILVER NANOPARTICLES (AgNPs)

To 5 ml leaf extract of , 45 ml of ImM Silver Nitrate solution was added and incubated at room temperature. The solution was left undisturbed for 2 to 3 hours. The plant extract consisting silver nanoparticles were settle down gradually in the bottom of the beaker. The plant extract consisting silver nanoparticles were centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and pellets consisting silver nanoparticles were used for further processes. The pellets were transferred to petri plates and kept for drying at room temperature in dark .

CHARACTERIZATION OF SYNTHESIZED SILVER NANOPARTICLES.

UV-Visible spectroscopy (fig.4): The reduction of pure Ag+ ions was monitored by measuring the UV- visible spectrum using U-3900 spectrophotometer. The absorbance peak was recorded.

Scanning Electron Microscope(SEM) analysis : SEM analysis was carried out to determine the size and shape of the particles synthesized. Synthesized silver nanoparticles were subjected to scanning electron microscope analysis. SEM images of the synthesized silver nanoparticles were recorded.


**Fig.4 – UV visible spectroscopy.**

## GERMINATION STUDIES

To conduct comparative germination study, aqueous extract of selected plants were prepared. The *Vigna radiata* L. seeds were sterilized and soaked for 24 hours in respective solution. The seeds were then transferred to sterilized petriplates lined with sterilized whatman no.1 filter paper for germination. 10 seeds were kept in each petriplate.

The seeds were treated with the respective solution at regular intervals. The volume used for the treatment was always same. The control seeds were watered with distilled water. Triplicates were maintained for each treatment.

### Germination percentage

The seeds were kept under observation for a period of 5 days.

The number of cotyledons emerged were examined.

$$\text{Germination percentage} = \frac{\text{Number of cotyledons emerged}}{\text{Total number of seeds}} \times 100.$$

### Radicle and hypocotyle length

During germination, cotyledons are emerged as radicle and hypocotyle , where radicle is the root and hypocotyle is the shoot. The length of these two can be evaluated on the $5^{th}$ day of germination.

# OBSERVATION AND RESULTS

## QUALITATIVE ANALYSIS

Results obtained after qualitative evaluation of phytochemicals;

| | *Morinda* | *Mussaenda* |
|---|---|---|
| Alkaloid | + | + |
| Flavonoid | + | - |
| Tannin | - | + |
| Saponin | + | + |
| Steroid | + | + |
| Bitters | + | + |
| Resin | + | + |
| Phenol | + | + |

**Table 1 – Results of qualitative analysis.**

**Fig. 5(a)- 5(g) images of qualitative analysis of *Morinda citrifolia* L. leaf extract**

**Fig.5(a) – Bitters**

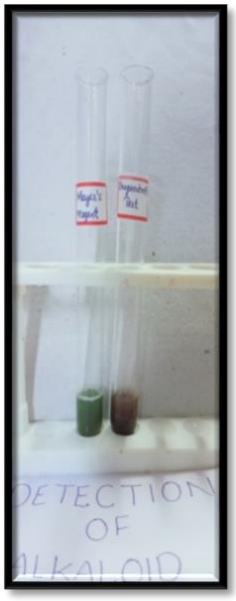



**Fig.5(b) -Alkaloid**


**Fig.5(c)-Flavonoid**


**Fig.5(d)-Saponin**


**Fig.5(e)-Resin**

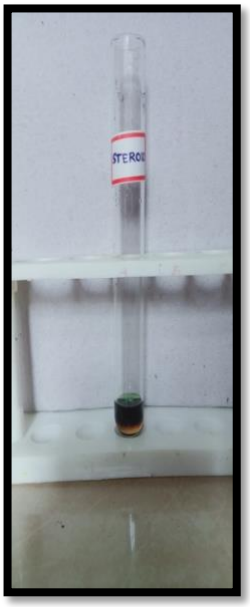


**Fig.5(f)-Steroid**

**Fig.5(g)-Phenol**

**Fig 6(a)-6(f) images of qualitative analysis of *Mussaenda frondosa* L. leaf extract**

**Fig.6(a)-Tannin**

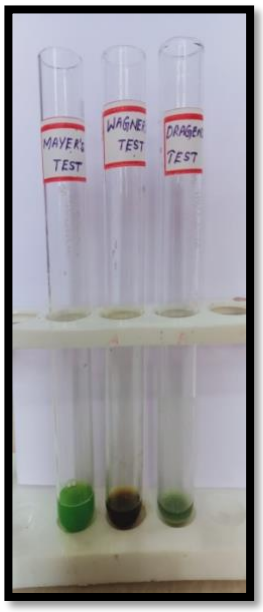



**Fig.6(b)-Alkaloid**

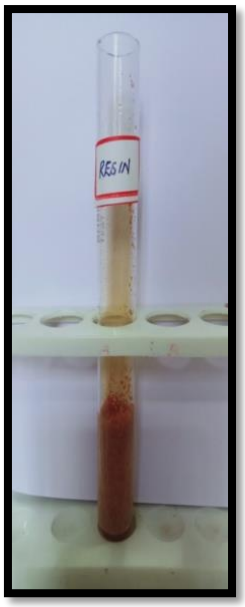



**Fig.6(c)-Resin**

**Fig. 6(d)-Phenol**

**Fig. 6(e)-Bitter**

**Fig.6(f)-Steroid**

**QUANTITATIVE ANALYSIS**

Results obtained after quantitative evaluation of phytochemicals were as follows :

Quantitative evaluation of phytochemicals – *Morinda citrifolia* L

| SI.NO. | PHYTOCHEMICAL COMPOUND | RESULT |
|---|---|---|
| 1. | Estimation of phenolic compound | 8.21 µg/L |
| 2. | Estimation of flavonoid | 4.75 mg/L |

**Table 2- estimation of phenolics and flavonoids in *Morinda citrifolia* L. leaf extract.**

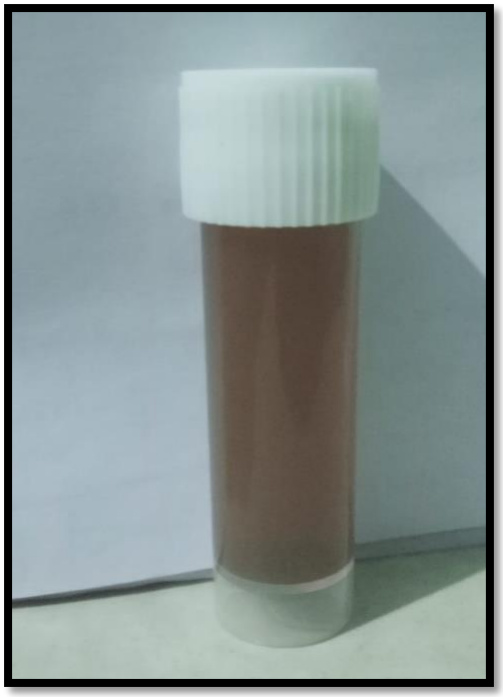
Quantitative evaluation of phytochemicals – *Mussaenda frondosa*

| SI.NO. | PHYTOCHEMICAL ANALYSED | RESULT |
|---|---|---|
| 1. | Estimation of phenolic compound | 8.0 µg/L |
| 2. | Estimation of tannin | 21.46% |

**Table 3 – estimation of phenolics and tannin in *Mussaenda frondosa* L. leaf extract**

## GREEN SYNTHESIS OF SILVER NANOPARTICLE

Green synthesis of silver nanoparticles makes use of plant constituents like carbohydrates, alkaloids etc. as reducing agents to synthesize AgNps. Synthesis of nanoparticles was confirmed by visual detection in which the colorless solution gets changed to a brown colored solution after incubation.



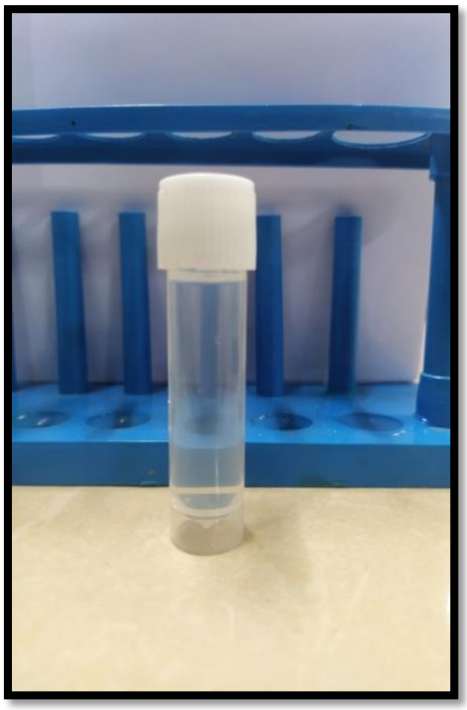


**Fig. 7(a) *Morinda* aqueous leaf extract** **7(b) 1mM Silver nitrate solution.**

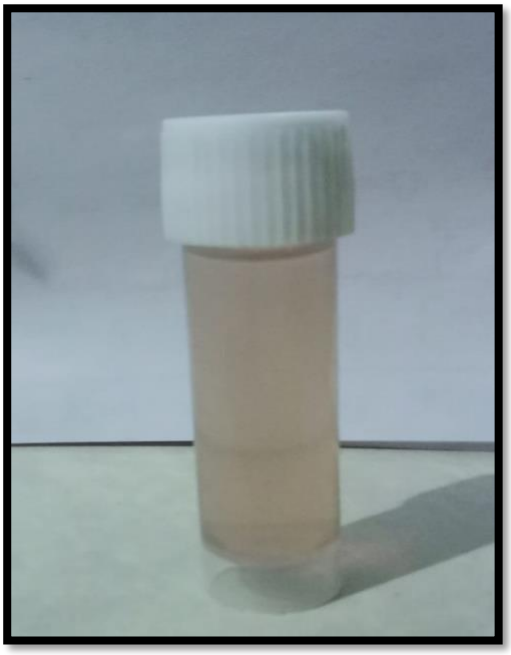


**Fig.7(c)- silver nanoparticle extract synthesized using *Morinda citrifolia* L.**

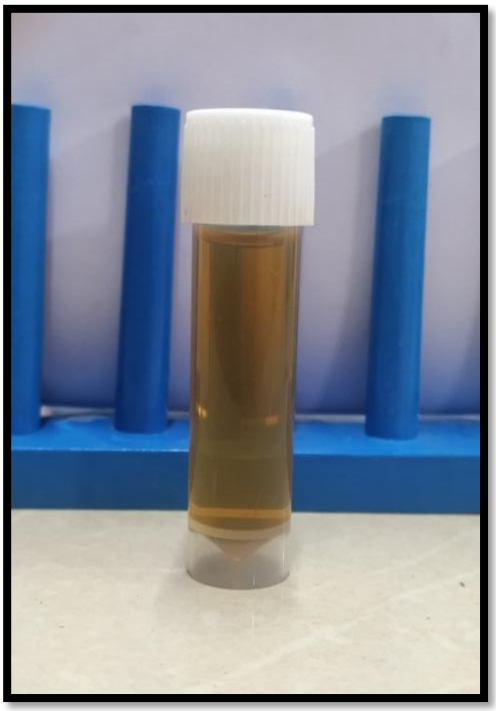


**Fig.8(a) *Mussaenda* aqeous leaf extract**

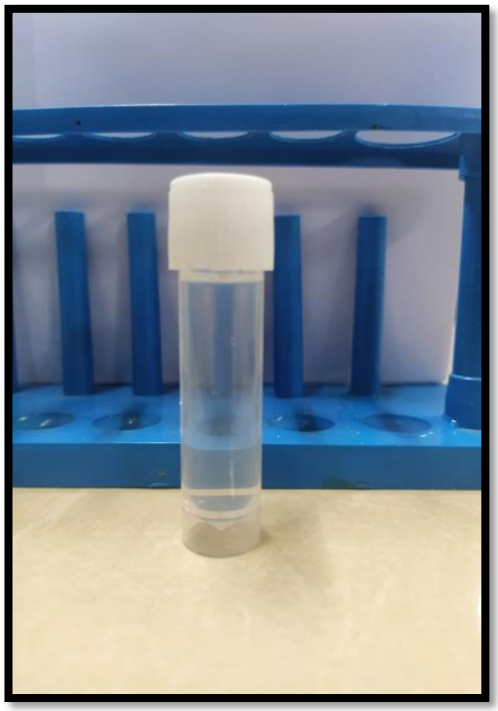


**Fig.8(b) 1m M silver nitrate solution.**

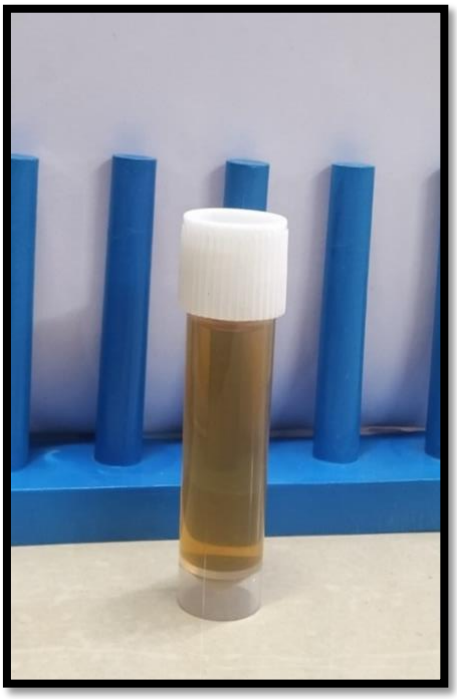


**Fig.8(c) Silver nanoparticle extract synthesized using *Mussaenda frondosa* L.**

Further characterization of green synthesized silver nanoparticles was done using UV visible spectroscopy. UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted

through a sample in comparison to a reference or blank sample. The optical absorbance was recorded in 300-450 nm for detection of synthesized nanoparticles.

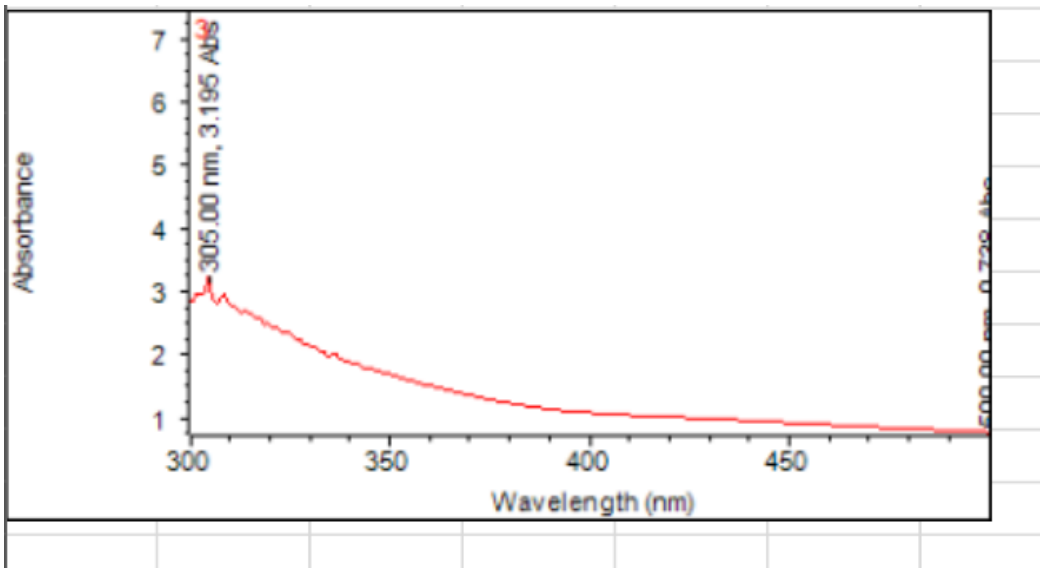



**Fig .9-UV visible absorption spectrum of Silver nanoparticles from *Morinda citrifolia* L. leaf extract**

UV visible spectroscopy analysis of synthesized silver nanoparticle extract of *Morinda citrifolia* L. was recorded in 300-450 nm, which showed a small peak at 305.00 nm (Fig. 9) which indicates the formation of green silver nanoparticles.

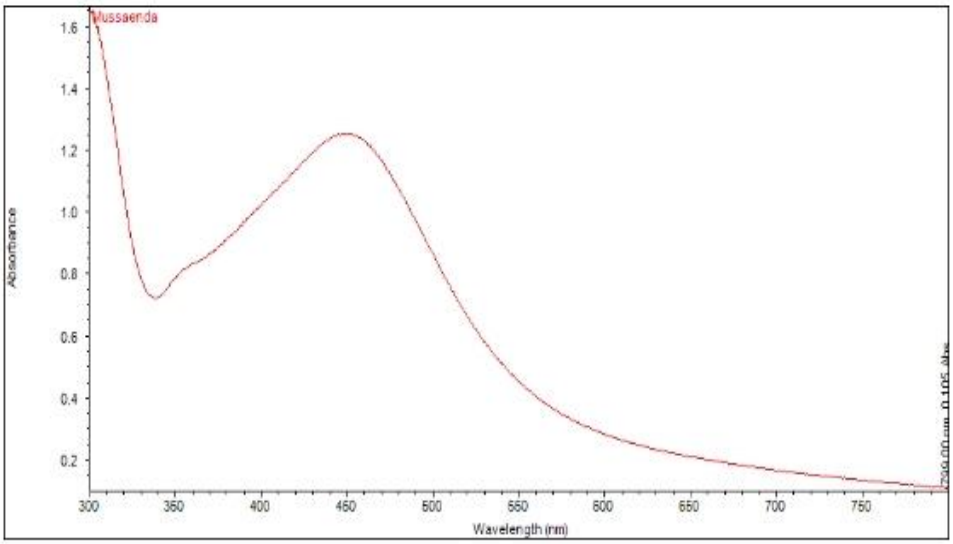



**Fig.10- UV visible absorption spectrum of silver nanoparticles from *Mussaenda frondosa* L. leaf extract.**

UV visible spectroscopy analysis of synthesized silver nanoparticle extract of *Mussaenda frondosa L.* was recorded in 300-450 nm , which showed a distinct peak at 440 nm (Fig. 10) which indicates the formation of green silver nanoparticles.

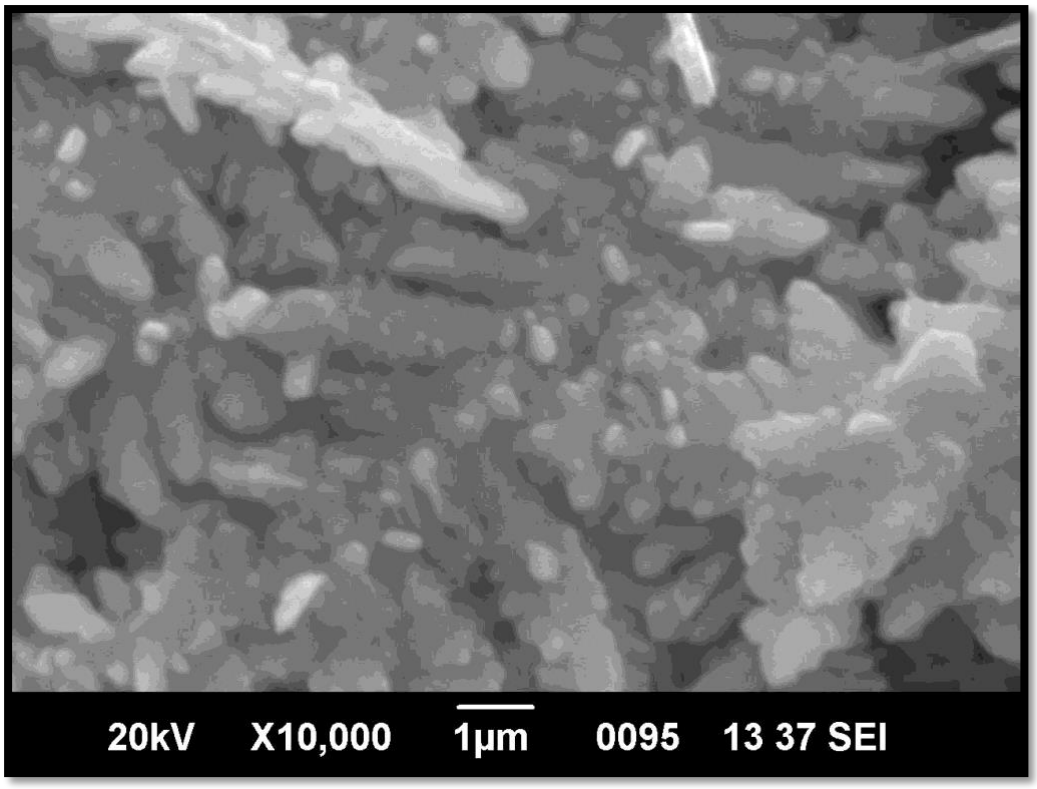



**Fig. 11- SEM image of Silver nanoparticles synthesized from *Morinda citrifolia* L. leaf extract.**

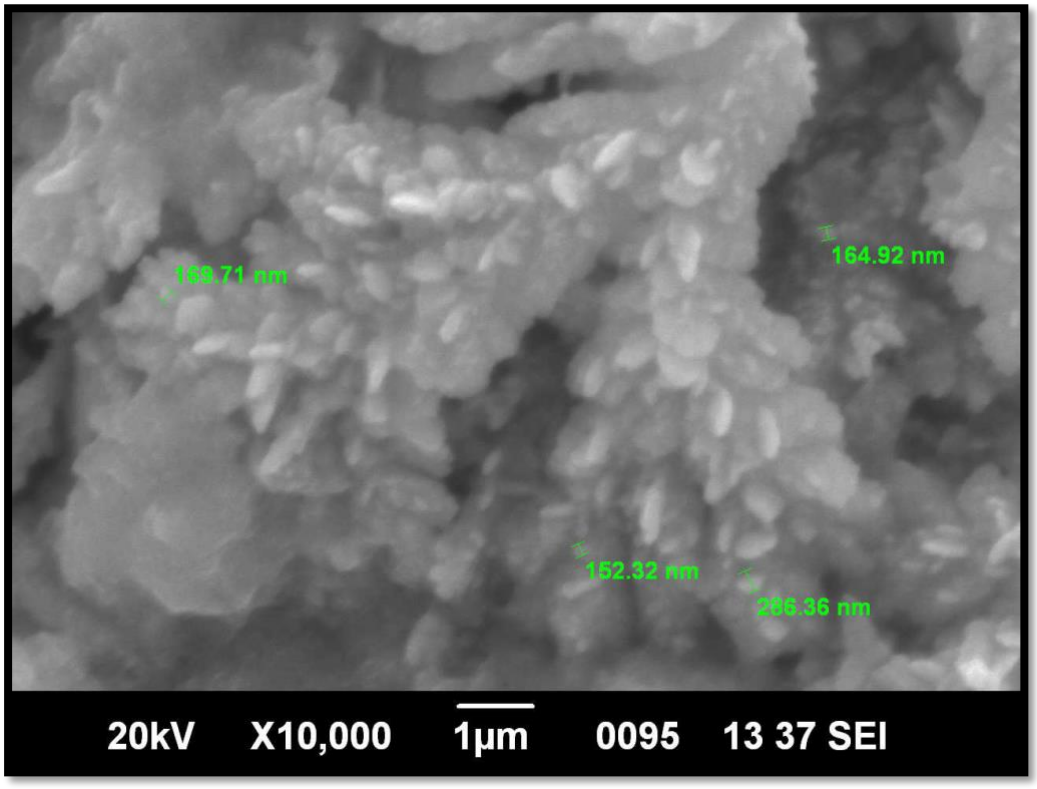



**Fig.12– SEM image of Silver nanoparticles synthesized from *Mussaenda frondosa* L. leaf extract.**

## COMPARATIVE GERMINATION STUDY

The germination percentage of the *Vigna radiate* L., seeds were found out on the 5th day of germination.

**Control;** The *Vigna radiata* L., seeds started to germinate on the 2nd day of sowing. On the 2rd day there was only 80% germination. But this increased to 100% by the 3rd day. (Fig. 14)

**Effect of *Morinda citrifolia* L. aqueous extract on seed germination.**

The seeds of *Vigna radiata* L. treated with aqueous extract of *Morinda citrifolia* L. showed 40% germination on 3rd day and it was increased to 100% by the 5th day.(Fig.15)

**Effect of *Mussaenda frondosa* L. aqueous extract on seed germination.**

The seeds of *Vigna radiata* L. treated with aqueous extract of *Mussaenda frondosa* L. showed 50% germination on 3rd day and it was increased to 100% by the 4th day.(Fig.16)

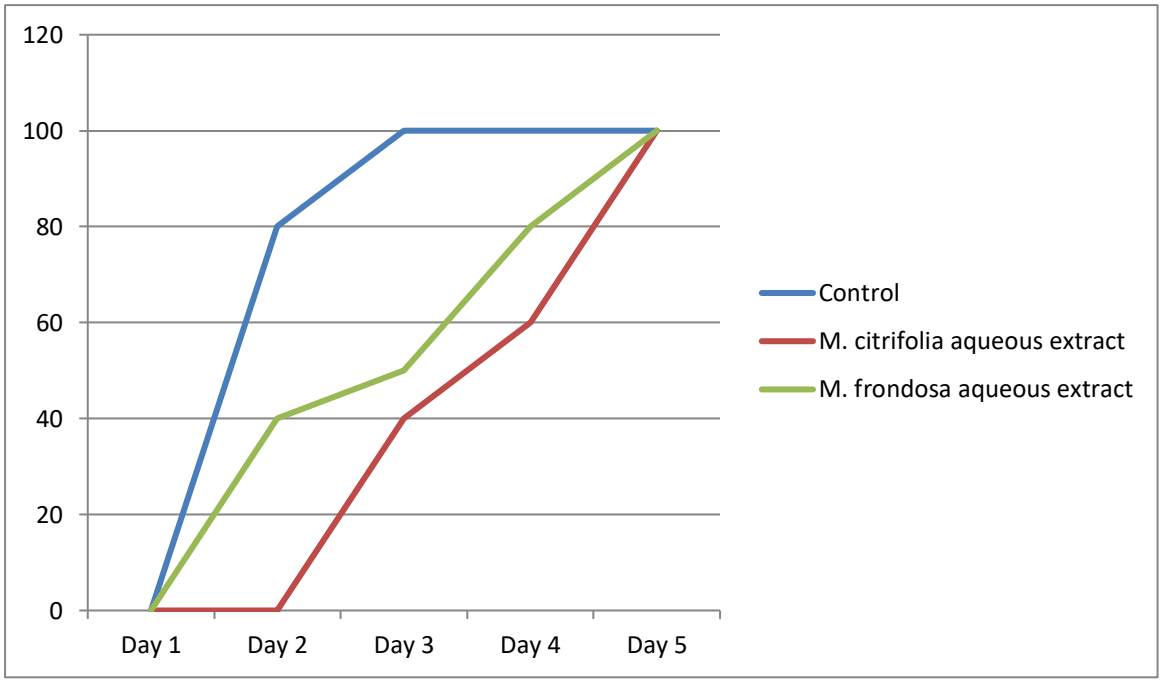



**Fig.13 – Line graph showing germination rate of *Vigna radiata* L. seeds treated with plant extracts and control (distilled water).**

## Radicle and Hypocotyle length

On the 5th day of germination , the length of the radicle and hypocotyle were measured. 10 seeds were taken for calculating the average of radicle and hypocotyle.

**Control ;** The average length of radicle on the 5th day was 3.3 cm. radicles were very healthy. The hypocotyle attained an average length of 4.75 cm by the 5th day. Hypocotyle region exhibited a dark green color. Cotyledonary leaves were well developed. ( Fig.14)

*Morinda citrifolia* L., aqueous extract treated seeds showed an average radicle length of 1.9 cm and hypocotyle length of 4.63 cm on the 5th day of seed germination. (Fig.15)

Whereas, *Mussaenda frondosa* L. aqueous extract treated seeds showed an average radicle length of 3.02 cm and averge hypocotyle length of 4.8 cm on the 5th day of seed germination. (Fig.16)

**Fig. 14- *Vigna radiata* L. seeds kept as control on the 5th day of germination.**

**Fig. 15- Aqueous extract of *Morinda citrifolia* L. leaves treated *Vigna radiata* L. seeds on the 5$^{th}$ day of germination**

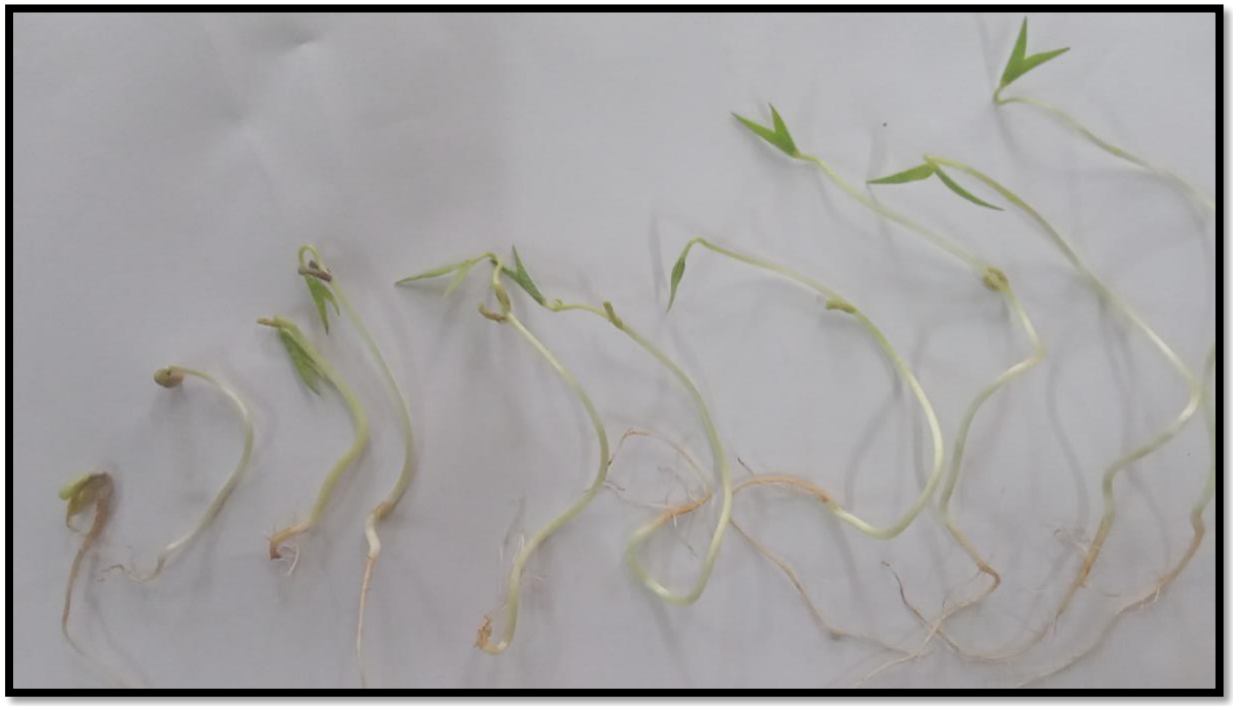


**Fig.16- Aqueous extract of *Mussaenda frondosa* L. leaves treated *Vigna radiata* L. seeds on the 5$^{th}$ day of germination**

# DISCUSSION

Plants are by far the most important source of natural therapeutics, and their role in enhancing the longevity and quality of life is gaining prominence throughout the world. Phytochemical screening of secondary metabolites in *Morinda citrifolia* L. leaves were conducted using color tests. These tests were used to determine the class of secondary metabolites, such as alkaloids, flavonoids, phenolic compounds, steroid, saponins, bitter, resin and tannins of the leaves. The active compound from noni leaves extract were flavonoids, alkaloids, saponins, bitters, phenolic compound, resin and steroids, which were indicated as symbol (+) in Table . A similar result was reported by Sheela saikumar C., et.al, in 2012. Where, in addition to the presence of above mentioned phytochemicals presence of tannin is also found to be an active constituent in *Morinda citrifolia L.* leaf extract. In another study conducted by Mairim russo et al., in 2011, phytochemical screening of aqueous extract of *Morinda citrifolia* L.showed the presence of alkaloids, coumarins, flavonoids, tannins, saponins and steroids.

Quantitative evaluation of aqueous leaf extract of *Morinda citrifolia* L. showed the presence of 8.21 µg/1 phenolic compound and 4.75 mg/l flavonoid content. Similarly, Mairim russo et al., in 2011, quantitatively analyzed the total phenolic content of the extract using slightly modified version of Singleton and colleagues method where they reported 196.8 mg of phenolic equivalents (gallic acid) per gram of extract.

Qualitative evaluation of phytochemicals in *Mussaenda frondosa* L, reported the presence of alkaloid, tannin, saponin, steroid, bitter, resin and phenol. Antimicrobial and phytochemical studies of *Mussaenda frondosa* L. by S.Shanthi and R.Radha also concluded the same result. Where they reported the presence of flavonoids, saponins, steroid, phenol and protein. Leena K. Pappachen and K. S. Sreelakshmi in 2017 conducted Phytochemical study of *Mussaenda frondosa* L. leaves along with in-vitro cytotoxicity studies using HepG2 cell line revealed the presence of various metabolites like flavonoids, glycosides, saponins, tannin and phenolic compounds.

The popularity of silver out of all metals with antimicrobial property is mainly due to efficient antimicrobial action and least toxicity (Guggenbichler et al. 1999). However, in modern scenario, the use of silver is not limited to medical applications only; it is also used for non-medical purposes such as in electronic appliances and cosmetics (Klasen 2000; Jung et al. 2008). Being an antimicrobial agent, silver has a long ancient history since Greek and

Roman civilizations, where silver was used as a disinfectant for food and water. Moreover, considering the importance of silver as an antimicrobial agent, the US Food and Drug Administration approved silver solution in 1920 for its usage in the food and drug industry.

In the present study silver nanoparticles was effectively synthesized from *Mussaenda frondosa L.* and *Morinda citrifolia* L. leaf extract using 1mM silver nitrate solution, and it's presence was confirmed by distinct peak at 440 nm and 305 nm respectively in absorbption spectra of UV spectroscopy.

A similar result was revealed by Varadavenkatesan et.al in 2016 using *Mussaenda frondosa* L. where they obtained a distinct peak at 414 nm, analysis using SEM confirmed the formation of sub-100 nm sized particles. In another work , E.sreelekha et.al, in 2021 synthesized silver nanoparticles using *Mussaenda frondosa* L. leaf extract and sodium citrate used as reducing and stabilizing agent.

Using different parts of *Morinda citrifolia* L. (noni) Violeta Morales et.al., in 2020 synthesised silver nanoparticles. The spherical AgNPs resulted well dispersed, with sizes 3–11 nm and they showed antibacterial activity against Gram-positive and Gram-negative strains.

Observations of seed germination studies suggested that aqueous extract of *Mussaenda frondosa* L. shows better seed germination potential compared to *Morinda citrifolia* L.

In future, further work can be conducted using these selected plants to determine the antimicrobial and antioxidant activities of both aqueous and silver nanoparticle extracts.

Since silver is a potent antimicrobial agent, germination studies can be carried out in future using synthesized silver nanoparticle extract on seeds of those crop plants which are highly prone to microbial attack during the seed germination

# CONCLUSION

Due to the modern civilization the resources of medicinal herbs are dwindling fast. Though, the purified plant chemicals are obtained from a significant number of studies only very few screening programs are initiated on crude plants. It is also accepted that the medicinal value of plants depends on the bioactive phytocomponents in plants associated to antibacterial activities and also have a curative property against pathogens. Therefore now there is a need to look back towards the traditional medicines which can serve as novel therapeutic agents.

The Qualitative phytochemical analysis of plant extracts in the present study, showed the presence and absence of bioactive components such as alkaloid, tannins, phenols, steroids, flavonoids, bitters, resin and saponins. Those metabolites which showed presence in qualitative analysis were quantified using various assay methods. It is also concluded that silver nanoparticles can be synthesized using aqueous extracts of plants selected for the study. Characterization of green synthesized silver nanoparticles were done using UV visible spectroscopy and scanning electron microscope (SEM) analysis. Comparative seed germination study was conducted using aqueous extract of selected plants and distilled water as control, the results suggested that *Mussaenda frondosa* L. treated seeds showed better germination potential compared to *Morinda citrifolia* L. extract.

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