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PLUMERIA OBTUSA (L) - MEDIATED BIOSYNTHESIS OF ZINC OXIDE (ZnO) NANOPARTICLES: CHARACTERIZATION, BIOCHEMICAL AND ANTIBACTERIAL PROPERTIES

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Abstract

Plumeria obtusa (L) is a traditional herb with medicinal properties used worldwide. Plant extracts are the potential precursors for the synthesis of nanomaterials. In this study, we used *Plumeria obtusa* (L) methanolic leaves extracts to biosynthesize zinc oxide nanoparticles. GC-MS, HPLC, UV-visible spectrophotometer, FTIR, SEM-EDX were used to analyze the phytochemical variations, physicochemical alterations, antibacterial properties. GC–MS and HPLC revealed the presence of strong antioxidant substances like octadecadienoic acid, hexadecanoic acid and benzofuran compounds with the polyphenolic compounds like coumaroylquinic acid, quercetin rutinoside, kaempferol rutinoside and chlorogenic acid. UV–visible spectra of ZnO NPs resulted with absorption at 383 nm. FTIR spectra showed the nearness of extending vibrations of O–H, C=Cl, N=O, C≡C groups at the side C–H and N–H including biomolecules extricates deformation and stabilization of ZnO nanoparticles. SEM images showed irregular and mostly hexagonal shaped morphologies, EDX analysis determined its elemental compositions. ZnO NPs displayed antibacterial activities on *Bacillus*, *Klebsiella*, *E.coli*, *Staphylococcus* and *Enterobacter*. In the present study, callus culture was raised for *Plumeria obtusa.* (L). The results illustrated that leaves obtained forms *in vitro* raised bulging of tissues with maximum 1 mg/L 2, 4 D and 1 mg/L IAA.

Keywords: *Plumeria obtusa* (L) leaves, phytochemical, ZnO nanoparticles, antioxidant, *invitro* callus.

1. INTRODUCTION

Plumeria obtusa. L. is a lactiferous tree species, belonging to Apocynaceae family, native to Central America, and is commonly known as the temple tree. Various species are now widely distributed in temperate areas of around the world and are renowned for having curative properties including anti cancer, antidepressant, antibacterial, anti-inflammatory, antioxidant, and neuroprotective properties. The flowers are white with a yellow throat, each with five petals, flowers bloom with clusters with fragnance and the leaves are obovate. Various sections of the plant are traditionally used to treat various medical conditions like wounds and skin disorders, as a diuretic, laxative, and abortifacient, and are also used in cosmetics and aromatherapy. It was traditionally used to cure a variety of illnesses, including *Diabetes mellitus* (Semanya *et al*., 2012). In India, it is commonly called Champa or Chafa; in the state of Tamil Nadu, it is called Neela Champangi, known for its fragnant flowers and ornamental purposes The current study purpose is to estimate the phytochemical presence by qualitative and quantitative assessment of methanol and chloroform aqueous leaves extracts of *Plumeria obtusa L. leaves* and confirmation by GC-MS and HPLC methods. The biosynthesis and characterization ZnO nanoparticles of using UV-Visible spectrophotometer, FTIR, SEM-EDX and evaluation of the antimicrobial activity and to finally initiate callus from the leaf explant.

2. MATERIALS AND METHODS

2.1 Preparation of leaf extracts

Plumeria obtusa.L leaves samples were gathered from Salem, Tamilnadu, India. To get removal of associated dust and debris, the fresh leaves were thoroughly rinsed three to four times under running tap water. Following that, in order to preserve the plant's active components, the collected samples were allowed to air dry at room temperature in the shade and away from the sun. (Thakare, 2004). Using an electric grinder the fully dried leaves were chopped into small pieces and ground them into a fine powder. The ground leaves samples were kept in sterile air tight containers separately at 4˚C until further usage. The aqueous extracts of resulted powders were collected by using two different solvents methanol and chloroform respectively.

2.2 Preparation of methanol and chloroform extraction

The air-dried sample of about 10g was weighted and 70 ml of methanol and chloroform was measured, mixed with samples separately and incubated overnight in mechanical orbital shaker. Then the extracts filtered with whatman no 1 filter paper to collect the liquid sample which is used for phytochemical screening. Several qualitative experiments were performed to analyse the phytochemicals that is in raw crude methanolic and chloroform extracts of leaves. (Aqsa Hanif *et al*., 2023). Following standard protocols, the presence of alkaloids, carbohydrates, glycosides, phenols, tannins, terpenoids, proteins and amino acids were determined.

2.3 PHYTOCHEMICAL QUALITATIVE ANALYSIS

2.3.1 Test for alkaloids: (Hager's test)

The presence of alkaloids is indicated by the production of a yellow precipitate, after dissolving 0.25 ml of picric acid in 25 ml of distilled water and adding 1 ml of plant extract.

2.3.2 Test for terpenoids: (H_2SO_4 test)

The presence of terpenoids can be identified by adding few drops of concentrated sulphuric acid with 1 ml of plant extract, by the formation of yellow colour.

2.3.3 Test for phenols and tannins: (folin-ciocalteu method)

The presence of phenol and tannins can be confirmed by the formation of blue colour by adding few drops of sodium hydroxide (NaOH) to 1 ml of plant extract and kept for 3-5 minutes in boiling water bath followed by further cooling and adding few drops of folin's phenol reagent.

2.3.4 Test for protein and amino acid: (Ninhydrin test)

The presence of protein is indicated by few drops of ninhydrin solution added to 1 ml of plant extract by the formation of blue or violet colour.

2.3.5 Test for glycosides: (Keller killani test)

Formation of green to blue colour ring indicates the presence of glycosides by adding glacial acidic acid with 1 drop of ferric chloride solution to 1 ml of plant extract, followed by mixing well and by adding 1 ml of sulfuric acid along the sides of the test tube.

2.3.6 Test for carbohydrates:

a) *Molisch's test*

Formation of purple ring indicates the presence of carbohydrates by adding few drops of Molisch's reagent to 1 ml of plant extract followed by mixing well and by adding few drops concentrated sulphuric acid.

b) *Benedict's test*

The presence of carbohydrates can be indicated by the formation of brick red color by adding Benedict's test reagent of 2 ml to 1 ml of plant extract and by keeping it in boiling water bath for 3-5 minutes.

2.4 PHYTOCHEMICAL QUANTITATIVE ANALYSIS

To analyse the total metabolites, 10 grams of each powdered leaves sample were dissolved in 100 ml of methanol in an airtight Erlenmeyer flask covered with cotton and foil paper to prevent contamination. To achieve higher concentration of yields a mechanical orbital shaker was used to keep the sample extracts at room temperature for 24 hours. Extracts were then filtered through a double muslin cloth and Whatman No. 1 filter paper; filtrates collected in an airtight bottle. Lastly, the filtrate placed in an oven at 40°C to allow the solvent methanol to evaporate. After evaporation, a paste was produced that was partially water-containing and it was again then dried in water bath at 60°c for 1 hr After that, the concentrated and dried crude extracts were kept in sterile vials at 4°C until they were needed for additional quantitative research. (Jeyaseelan *et al.*, 2012).

2.4.1 The Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

Methanolic extracts of *Plumeria obtusa.L* was experimented using a GC-MS Instrument: GCMS-3, Capillary Column: Agilent DB5MS, Column Length: 30m / 0.25mm internal dia / 0.25micron film thickness, Library: NIST the total GC/MS running time was 36min. By comparing the average peak area of each compound to the total areas, the relative percentage that constitutes each compound is identified. The Gas Chromatography/Mass Spectrometry (GC/MS) system is used to separate mixtures of compounds to identify their molecular composition. It is among the most exact tools to evaluate natural samples. The GC operates on the idea that, when heated, a mixture will split into distinct substances. The hot gases are sent through an inert gas column (like helium). The separated substances

transfer into the MS as they emerge from the column opening. Compounds are identified using mass spectrometry based on the mass of the substrate molecule. Mass spectrometry is considered the only defective analytical detector. GC/MS is a technique that can be used to separate volatile organic compounds.

2.4.2 High Pressure liquid Chromatography (HPLC):

Plumeria obtusa.L methanolic extracts were analysed using HPLC consisted of a two-pump system liquid chromatography shimadzu model LC-20A Prominence equipped with a SIL-20A auto sampler, a CTO20A column oven, a CBM-20A communications bus module, an in-line degasser DGU-20A3, and a photodiode array detector (SPD-M20A). The LC solution software (version 1.25) was used for data processing. Analyses were conducted on a Shim-pack CLC-ODS column (25 cm × 4.6 mm i.d, 5 μm; Shimadzu). To estimate the optimal conditions, preliminary experiments were conducted using the gradient scouting run evaluation described by Snyder and Dolan (Snyder and Dolan, 1996). After the final adjustments, the optimized conditions included an isocratic elution system consisting of 90% acetonitrile in water and 0.1% acetic acid (v/v), at a flow rate of 1.0 ml.min-1. The temperature of the column was set at 40°C; absorbance was read at 201 nm.

2.5 BIOSYNTHESIS OF ZINC OXIDE NANOPARTICLES (ZnO NPs)

Based on the procedure followed by by Kalaiarasi *et al* (2015) 10 grams of leaf powder samples were added to 50 ml of distilled water and by heating the mixture to 60 °C, and then filtering the mixture through filter paper. Zinc sulfate ($ZnSO_4$) of 0.2M concentration was prepared. To the aqueous solution of 50 ml of *Plumeria obtusa L.* leaf extract 50 ml of 0.2M Zinc sulfate was added and 0.1M NaOH was added to until the pH reaches 12 which stabilize the nanoparticles, the sample liquid was covered and stored in a dark room for 24 hours' incubation, to minimize the photoactivation of zinc oxide and the reduction of Zn+ ions into ZnO. The sample's color changed from green to brown during the incubation time, denoting the synthesis of ZnO NPs. Following by centrifugation at 8000 rpm for 10 min the pellet was collected, NP pellet was dissolved in sterile distilled water and washed thrice by centrifugation to remove impurities and dried for an hour at 60°C in an oven, and then stored.

2.6 CHARACTERIZATION OF ZnO NPs

2.6.1UV-Visible spectroscopy

The initial confirmation of the synthesis of ZnO NPs were confirmed using UV-Vis spectra which were recorded as a function of the reaction time on a Systronics 2203 spectrophotometer. The bioreduction of zinc ions into zinc oxides formation and stability of ZnO NPs in an aqueous colloidal solution was examined periodically with UV-Vis spectroscopy (200–700 nm) at room temperature.

2.6.2 Fourier transforms infrared spectroscopy (FTIR)

Identification of the types of functional groups present in compounds can be done using Fourier Transform Infrared Spectrophotometer (FTIR), which is an effective tool acts by analysis of the wavelength of light absorbed and it is the characteristic feature of the chemical bond that can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be identified. (R.Ashokkumar and M. Ramaswamy., 2014)

2.6.3 Scanning Electron Microscopy (SEM)

The prepared nanoparticles were observed on a ZEISS EVO 40 EP Electron microscope by generating a drop of solution into a clean electric stub and allowing the water evaporate completely the suspension of nanoparticles in water was utilized for SEM investigation.

2.7 ANTIMICROBIAL ACTIVITY

2.7.1 Antibacterial activity

The antibacterial activity of the ZnO nanoparticles was evaluated with the agar diffusion method with standard antibiotic chloramphenicol against *Escherichia coli* (Gram negative), *Bacillus* (Gram positive), *Staphylococcus* (Gram positive), *Enterobacter* (Gram negative), *Klebsiella* (Gram negative). The microorganisms were raised in nutritive media and kept at 37^0C for 24 h in petri dishes containing Muller-Hinton agar. An amount of 20 µL of ZnO NPs, plant extract, methanol and chloroform aqueous extract was administrated respectively into the wells and the zone of inhibition was measured after 24 h. (Parandis Ehsania *et al.,* 2021)

2.8 INVITRO CALLUS INDUCTION

The callus induction and regeneration of *Plumeria obtusa. L* has been developed in the culture medium. Young leaf region were used as explant for callus induction on MS medium, newly formed healthy nodes were collected (Prithviraj *et al.,* 2015) (Kalaiarasi *et al*., 2014). The plants were thoroughly rinsed for half an hour under running water and then treated with Teepol, a surfactant, four to five times; they were rinsed with distilled water. After that, they were surface sterilized for three minutes using 0.1% mercuric chloride and 70% alcohol for 45 seconds. Ultimately, the explants were inoculated into MS media containing plant growth regulators (auxins) 2,4 D and IAA 1mg/L after being rinsed three to five times with sterile distilled water to eliminate any remaining unwanted chemical traces (Karthikeyan *et al*., 2018).

2.8.1 Culture conditions and maintenance:

The growth room conditions were maintained for the culture at 25±2ºC under 7 hours' photoperiod with photo flux of 30-40 µM m^{-2} s^{-1} (40 W, white-cool fluorescent bulbs and 50– 60% range of relative humidity for the growth) and sleeping time 7/17 hours. Each experiment was conducted at thrice with 10 replicates per treatment.

2.9 STATISTICAL ANALYSIS:

All statistical analyses were carried out using one-way ANOVA with Dennett's post-test using Graph Pad InStat and Prism version 3.00 for Windows (Graph Pad Software).

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical analysis of methanol and chloroform extraction

The methanol and chloroform aqueous extract of *Plumeria obtusa.L* leaves contains phenolic compounds, tannins, flavonoid, alkaloids, steroids, glycosides, and proteins which are visually observed by colour change and precipitate formation. Various phytochemicals have different physical and chemical characteristics, such as the ability to reduce and stabilize metal ions, lipid peroxidation, and antioxidant activity. Summary of the phytochemical tests procedures were described below.

a) Alkaloid (Hagers test): Results with presence of yellow Precipitation
b) Terpenoids (H_2SO_4 test): Absence of yellow Formation
c) Phenols and Tannins (folin-ciocalteu method): Absence of black precipitation
d) Protein and Amino acid (Ninhydrin test): Absence of Violet colour
e) Carbohydrate (Molisch's test): Presence of purple ring
f) Carbohydrate (Benedict's test): Presence of redbrick colour
g) Glycosides (Keller killani test): Presence of brown ring formation

Mostly found phytochemical constituents of *Plumeria obtusa.L* is glycosidic derivative compounds like flavonoid glycosides, alkaloid, phenol and tannins, terpenes/terpenoid, and carbohydrates (Ahmad ghorbani *et al.*, 2017). The specific biomolecule therapeutic compounds without any side effects and with fast action during drug target delivery are needed. Plants are a wide source of biomolecules, which have enormous amounts of unbelievable medicinal properties. And now the world is changing to replace synthetic drugs because of their side effects. Among the different kinds of biomolecules, secondary metabolites are the key components for drug discoveries. Our current study mainly focuses on polyphenols, where limited research has been published. The methanolic leaves extract of *Plumeria obtusa.L* revealed the presence of tannins, phenols, glycosides, and terpenoid, while the chloroform leaves extract showed only the presence of alkaloids and glycosides, refer Table 2 ; This demonstrates that, in comparison to the non-polar chloroform extract, the polar methanol extract is more effective at extracting more secondary metabolites from the plant leaves extract. (Rutuba *et al*., 2021).

3.2 GC-MS Analysis- Identification of compounds:

Analysis of the mass spectrum was done by the National Institute of Standards and Technology (NIST) database, which contains more than 62,000 patterns. A comparison was made between the spectra of the unknown molecule and the recognized compounds kept in the NIST collection. The components of the test materials' names, molecular weights, and structures were determined for the methanolic extracts of *Plumeria obtusa .L.* According to the result, 30 compounds were isolated, among these the major compounds with four highest peaks were obtained with the largest percentage of area of about 14.28 and retention time 17.76 with the molecular formula $C_{18}H_{30}O_2$, Octadecatrienoic acid with the molecular weight 278.4.The second peak obtained was the percentage of area of about 12.52 with and retention 16.35 molecular formula $C_{16}H_{30}O_2$, 1 n-Hexadecanoic acid, with the molecular weight 254.41.The third

peak was with the percentage of area of about 7.79 and retention time 8.897 molecular formula C_8H_8O, 1 Benzofuran, 2, 3-dihydro with the molecular weight 120.15.The fourth peak obtained was with the percentage of area of about 6.6, retention time 22.27 molecular formula $C_{24}H_{38}$, Tetracosahexaen with the molecular weight 326.6. In this study, the main metabolite produced by *Plumeria obtusa .L* extract was Octadecatrienoic acid respectively, refer Table 4; which is a fattyacid molecule responsible for antioxidative properties. The 9, 12-Octadecadienoic acid has the bioactivity of Phosphatidyl glycerophosphatase inhibitor, Acylcarnitine hydrolase inhibitor and Mucomembranous protector (Siregar *et al*., 2023). The fatty acid hexadecanoic acid, often known as palmitic acid, has antioxidant, antibacterial, nematicide, anti-inflammatory, hypocholesterolemic, chemopreventive, flavourful, anti-androgenic, Lipo-oxygenase and suppression of hemolytic 5-reductase blocking, preventing cancer, and boosting the immune system (Javaid *et al*., 2023).The most well-known, Benzofuran compounds such as amiodarone, angelicin, bergapten, nodekenetin, xanthotoxin, and ursnic acid have a variety of medical uses. These substances have been widely used in dermatology, anticancer, and antiarrhythmic treatments, highlighting the critical role that benzofuran compounds play in clinical applications like neurogenerative and the enormous promise that these compounds represent for the creation of new therapeutics (Yu-hang Miao *et al*., 2019). Most of the identified compounds were saturated fatty acids & alkane hydrocarbons.

High Performance liquid Chromatography (HPLC)

The validation of an analytical approach to identify and quantify compounds in *P. obtusa* L extracts which in response creates an idea to perform HPLC analysis. Since the validation criteria were successfully satisfied and assessed. (Javaid *et al.*,2021). The presence of Coumaroylquinic acid, Quercetin rutinoside, Kaempferol rutinoside and Chlorogenic acid were confirmed from the samples. refer Table 3. The flavonoids kaempferol 3-O-rutinoside, quercetin 3-O-rutinoside, and kaempferol 3-O-glucoside have antihypertensive and anticancer effects. According to Liu Zhongying *et al.*, (2023), quercetin suppresses oxidative stress, osteoblast apoptosis, osteoclastogenesis, and inflammatory response. In summary, our findings demonstrated that the phytochemical-rich *P. obtusa.* L extract can be considered as an analytically supported substitute for creating innovative and potent medications.

3.2 Characterization of ZnO Nanoparticles

Plumeria obtusa. L aqueous leaves extract was used to the capped Zn2+ions, which led to the nucleation of the reaction mixture to produce ZnO NPs, which were then stabilized and subjected to a various technical procedure for characterization.

3.3 Fourier transforms infrared spectroscopy (FTIR)

Plumeria obtusa.L nanoparticles showed distinct absorption bands at the following wavelengths: 555.24 cm-1 for alkyl halides (C-Cl Stretch), 624.00 cm-1 for alkynes (C-triple bond) C-H: C-H bond) 851.86 cm-1 for alkyl halides (C-Cl Stretch), 1063.10 cm--1 for alcohols, ethers, carboxylic acid (C-O Stretch), 1397.18 cm--1 nitro groups (N=O Stretch), 1578.40 cm-1 for Strong (NO_2 Stretch), 1863.33 cm-1 for aldehydic (C-H Stretch), 2231.84 cm-1 for alkynes (C triple bond, C- Stretch), 2507.63 cm-1 for

carboxylic acid (O-H Stretch), 2627.63 cm-1 for carboxylic acid (OH Stretch), 2624.36 cm-1 for carboxylic acid (O-H Stretch), 2767.73 cm-1 for aldehydes (H-C=O : C-H Stretch), 2888.47 cm-1 for alkanes (C-H Stretch), 3045.85 cm-1 for alkanes (=C-H Stretch), 3138.41cm-1 for carboxylic acid (O-H Stretch), 3219.40 cm-1 for carboxylic acid (O-H Stretch), 3435.04 cm-1 for alcohols, Phenols (O-H Stretch, H-bonded), 3572.28 cm-1 for alcohols, carboxylic acid (OH Stretch), 3703.32 cm-1 for water (OH Stretch), 3875.54 cm-1 for water (OH Stretch). *Plumeria obtusa.L* contains a variety of flavonoids. Flavonoids, which are polyphenolic chemicals frequently found in plants contain with a wide range of biological activity and antioxidant properties. It's possible that these polymers' functional groups contribute to the reduction of Zn+ to Zn0. It was known that these functional groups allowed all biological constituents to interact with metal salts and mediate their reduction to nanoparticles. It is clear that for synthesized ZnONP, there may be variations in the absorption bands, band position shifts, and capping series. (Kalaiarasi *et al*., 2015).

3.4 SEM-EDX

ZnO NP's shape and size were examined using SEM-EDX and SEM pictures at various magnifications. A cluster of moderately spherical, hexagonal shaped unevenly distributed ZnO NPs is seen by SEM-EDX examination. Utilizing SEM-EDX, the chemical profile of the synthesized ZnO NPs was assessed. The ZnO NP's EDX pattern exhibits strong emission energy at 1.743keV Peaks below 8.020 keV indicates the existence of a pure zinc ion. The pattern also shows peaks that coincide with the binding energies of carbon and oxygen, which are likely caused by impurities that entered the nanoparticles during drying. The ecologically friendly and effective biological production of ZnO-NPs utilizing *P. obtusa* L leaf extract UV-visible absorption spectroscopy and FT-IR methods was used to characterize the produced ZnO-NPs, which had an average high yield of 97%. ZnO-NPs were discovered to have an average particle size of 27.23 nm, indicating a distribution of uniformed size dispersed SEM spectra of the particles of *P.obtusa L* leaves extract. (Halanayake *et al.,* 2021).

3.5 *Invitro* callus induction

P.obtusa. L. leaves extract showed that combinations of MS media (Bahadur *et al*., 1991) with 1 mg/L 2, 4 D and 1 mg/L IAA showed callus initiation when compared to other concentrations (Kalaiarasi *et al*., 2012) there were no frequently published articles about callus initiation in the species *P. obtusa.* L. *plumeria aclitifolia* explants cultivated on baseline media were previously reported to have no reaction. Within a week, the medium showed bulging of tissues with low concentrations of auxins 1 mg/L 2,4 D and 1 mg/L IAA.

3.6 **Antibacterial activity**

The antibacterial activity shows the nanoparticles with ZnO was found to be more effective than the methanolic and chloroform plant extracts against all the bacterial cultures namely *Bacillus* (0.5±0.1cm), *Klebsiella* (1.5±0.05cm) followed by *E.coli* (1.9±0.1 cm) *Staphylococcus* (1±0.1 cm) and *Enterobactor* (1.2±0.05 cm). It was effective against *Staphylococcus* (1±0.1 cm) and it was more effective when compared with the microorganism *Enterobactor* (1.2±0.05 cm) which was found to be more susceptible.

This research reports an efficient green synthesis of ZnO NPs with significant physical, chemical and antibacterial properties (Khanam *et al.,* 2023).

3.1 DISCUSSION

The specific biomolecule therapeutic compounds without any side effects and with fast action during drug target delivery. In the Plants are wide sources of biomolecules, which have enormous amounts of unbelievable medicinal properties. And now the world is changing to replace synthetic drugs because of their side effects. Among the different kinds of biomolecules, secondary metabolites are the key components for drug discoveries. Our current study mainly focuses on polyphenols, where limited research has been published. The presence of tannins, phenols, glycosides, and terpenoids was observed in the methanolic leaf extract of *P. obtusa* L. whereas, the chloroform leaf extract revealed a lower quantity of alkaloids and glycosides. This indicates that the polar methanol extract is more effective at extracting secondary metabolites from the plant sample than the non-polar chloroform extract. (Rutuba C *et al*., 2021).

SEM-EDX analysis of the produced ZnO nanoparticles revealed a cluster of irregularly dispersed ZnO NPs with relatively square and hexagonal shapes. The ecologically friendly and effective biological production of ZnO-NPs utilizing *P. obtusa* L leaf extract of UV-visible absorption spectroscopy methods was used to analyze the produced ZnO-NPs, which had an average high yield of 97%. ZnO-NPs were discovered to have an average particle size of 27.23 nm, indicating distribution of uniformed size dispersed SEM spectra of the particles. (Halanayake, K.D. *et al.,* 2021). Silver nanoparticles (AgNPs) from P. obtusa *L* at four distinct concentrations of leaf extracts were used to investigate synthesized AgNPs with FTIR, SEM, and UV-visible absorption spectrophotometers.The functional groups in charge of decreasing and stabilizing the AgNPs were identified using the FTIR analysis. The aggregated AgNPs are visible in the SEM micrograph. (Bibi Raza Khanam *et al.,* 2023)

HPLC analysis of the various phytoconstituents was proven in different amounts by the aqueous-methanolic leaf extract of *P. rubra* L the phytochemicals rutin, isoquercetin, kaempferol, and plumericin were found to be in the aqueous-methanolic leaf extracts (Javaid *et al*., 2021). Among these, the recognized essential phytochemicals were rutin, isoquercetin, kaempferol, and plumericin (Khan *et al.,* 2021). Using LC/ESI-QToF, 37 compounds were discovered from the methanolic fraction of *Plumeria obtusa* L extracts. These compounds included 10 plumeria-type iridoids, 18 phenolics, 7 quinoline derivatives, 1 amino acid, and 1 fatty acid. Additionally, five chemicals were isolated: 13-O-caffeoylplumieride, glochiflavanoside B, plumieride, kaempferol 3-O-rutinoside, and quercetin 3-O-rutinoside. (Eloutify *et al*., 2023). The presence of Coumaroylquinic acid, Quercetin rutinoside, Kaempferol rutinoside and Chlorogenic acid were confirmed in this study.

The synthesized ZnO nanoparticles of *P. obtusa* L leaves were found to exhibit antimicrobial properties activity on fungus (*Aspergillus niger*) and harmful bacteria (*Klebsiella pneumoniae*), with inhibition

zones ranging from 1.9 to 2.6 cm. This study denotes the productive green synthesis method for ZnO NPs, which have significant physicochemical and antimicrobial qualities. (Khanam *et al.,* 2023).

Polyphenols such as flavonoids and non-flavonoids, such as resveratrol, curcumin, coumarin, and phenolic acids, are thought to the therapeutic agents in the treatment of mental diseases because of their anti-inflammatory, antioxidant, and neuroprotective properties. The effects of polyphenols on immune cells, the induction of apoptosis, which reduces DNA damage, the release of pro-inflammatory cytokines and the regulation of genes involved in their production and the effects on immune cells all contribute to their anti-inflammatory properties. In conclusion, the present study clearly demonstrated that the methanolic extract of *P. obtusa L. leaves,* which contains polyphenolic compounds with synthesized ZnO particles, will be used in the treatment and could significantly prevent anxiety and depression. However, for other behavioral effects and mechanisms of action, further preclinical investigations are necessary.

CONCLUSION

The current research was to characterize the initial phytochemical nanoparticle synthesis by using the Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) and to experiment with antibacterial actions. Phytochemical Screening of leaves extract using GC-MS and HPLC analysis of methanol extract of the plant leaves, revealed the presence of carbohydrates, flavonoid glycosides, terpenoids, chlorogenic acid/ neochlorogenic acid, coumaroylquinic acid. The comparative results with methanolic and chloroform extracts showed best results with the polar solvent methanol. The ZnO nanoparticles were efficient in exhibiting activity against *Escherichia coli, Enterobacter, Klebsiella pneumonia* and *Staphylococcus aureus* Plant tissue culture media prepared from stock in MS media and 2,4 D+IAA hormones added in plant inoculated for initiation suggests positive approach towards plant tissue culture where this was our new study on *invitro* callus induction. Findings from current study support the use of *Plumeria obtusa*.L in traditional medicine for treatment of various bacterial infections, the isolated compounds present in the plant may use for drug formation in future for skin lesions, wound, anticancer treatment, and will help further to treat and cure neurodegenerative disease when used in combined formation of ZnO NP drug and regarded as complementary therapeutic substances in the treatment of mental illnesses. However, for other behavioral effects and mechanisms of action, further preclinical investigations are necessary.

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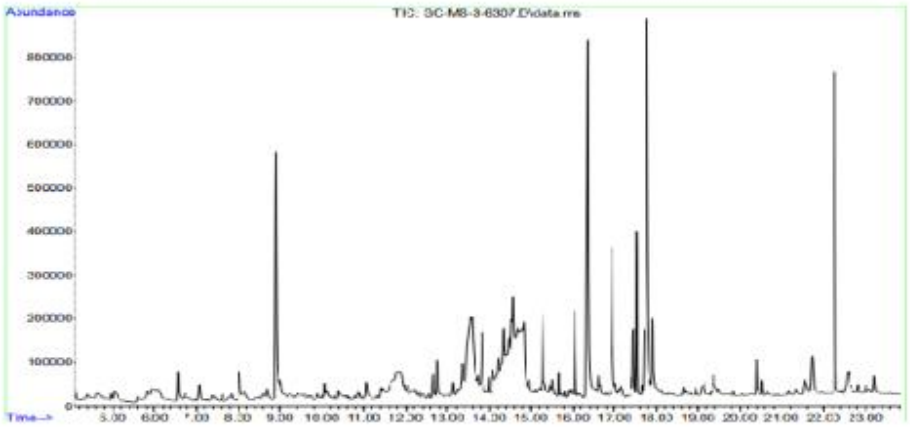

Figure 1: GC-MS Chromatogram of methanolic extracts of *P.obtusa.L* leaves

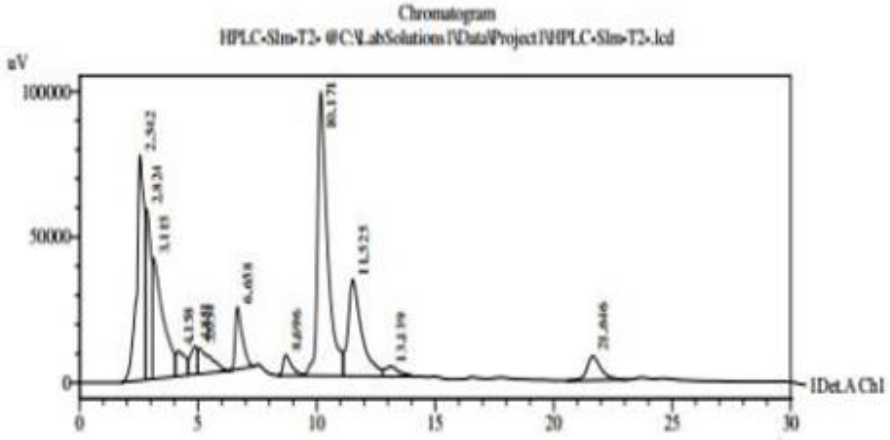

Figure 2: HPLC Chromatogram of methanolic extracts of *P.obtusa.L* leaves

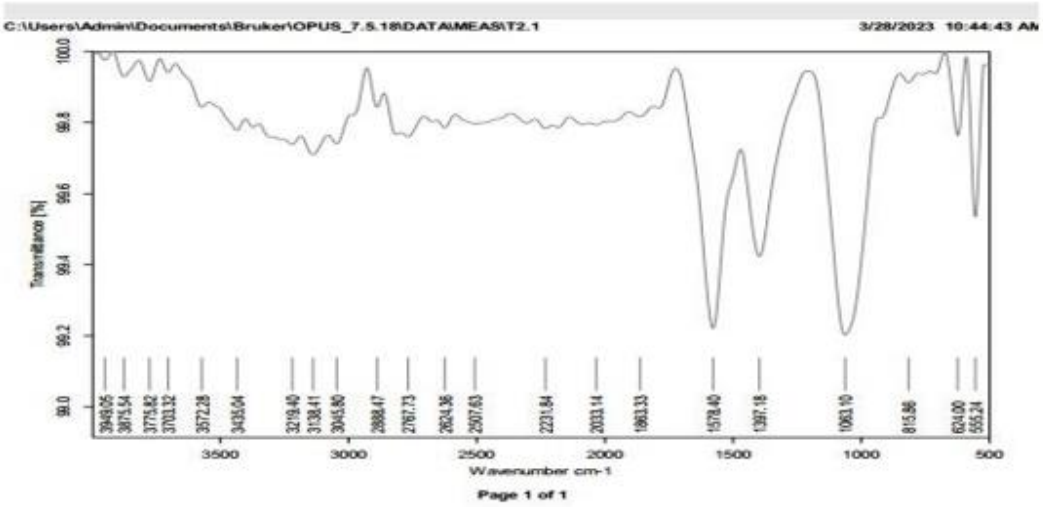

Figure 3: FTIR spectra of methanolic extracts of *P.obtusa.L* leaves

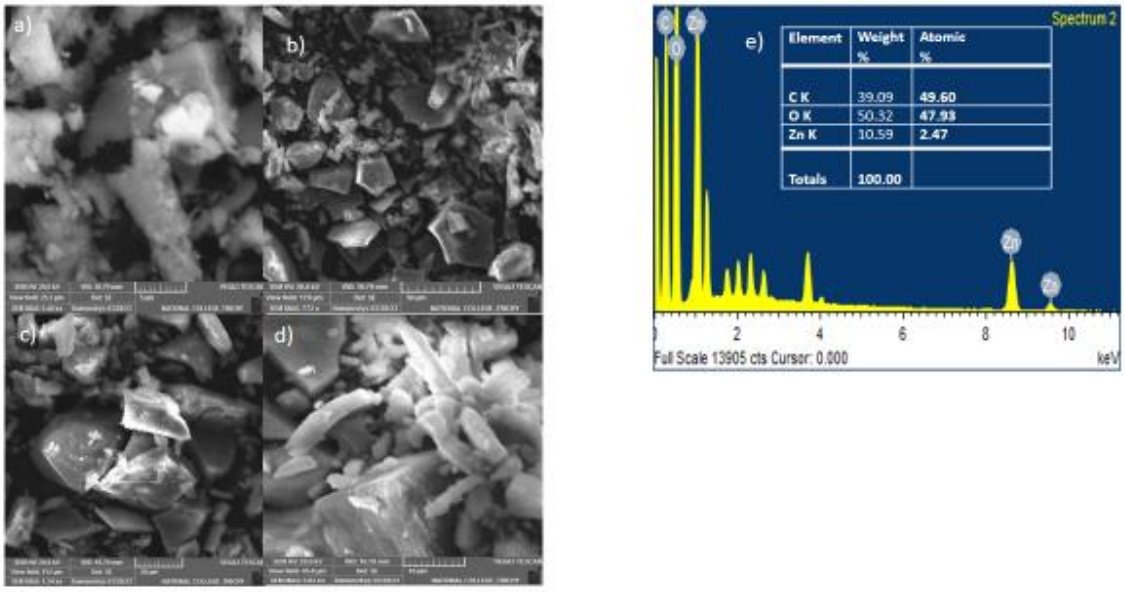

Figure 4: (a), (b),(c), (d) Scanning electron microscopy (SEM), (e) The EDX spectra of biofabricated ZnONps

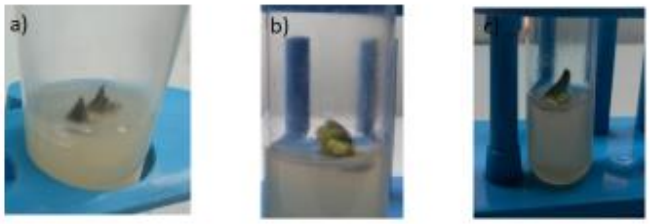

Figure 4: (a) Meristem inoculated in media (b),(c) bulging of tissues from explant after 7 days

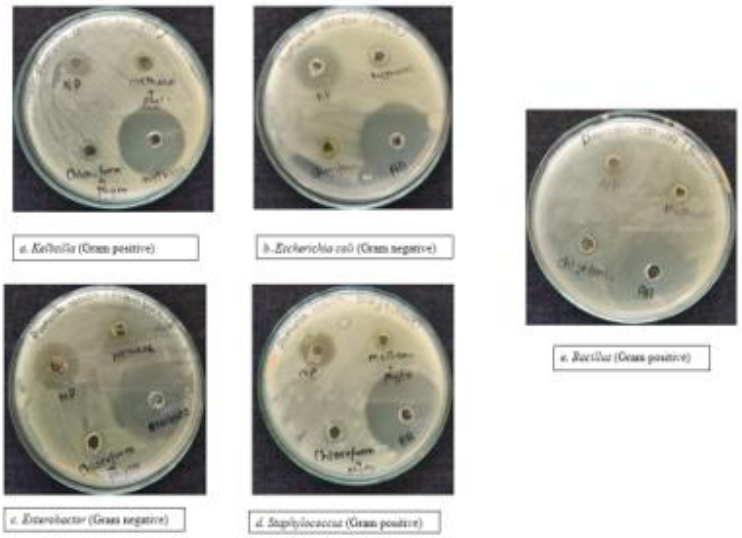

Figure 6: Antibacterial activity of ZnO NPs' ; PE-20µl of Plant Extract, AB-20µl of Chloramphenicol, ME- 20µl of Methanol Extract, CE- 20µl of Chloroform Extract, NP- 20µl of Nanoparticles

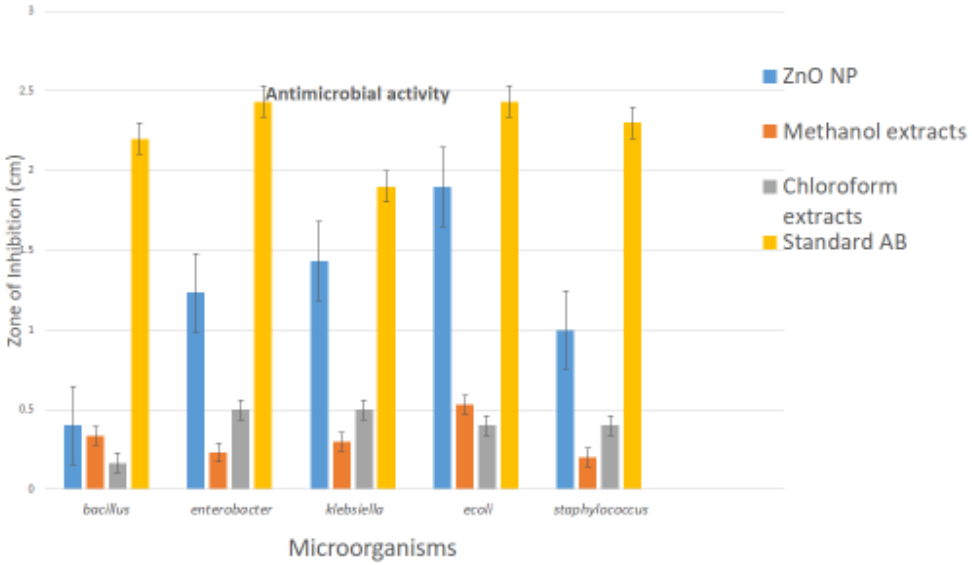

Figure 5: Antimicrobial activity of different microorganisms with Zn O nanoparticles, methanol and chloroform extracts of *Plumeria obtusa.L*

Class	*Angiosperms*
Kingdom	*Plantae*
Class	*Trachephytes*
Class	*Eudicots*
Class	*Asterids*
Order	*Gentianales*
Family	*Apocynaceae*
Genus	*Plumeria*
Species	*P.obtusa*

Table 1. Scientific classification of *P.Obtusa.*

Microorganism		**Methanol extracts**	**Chloroform extracts**
Alkaloid		+ + +	+ + +
Terpenoids		+	_ _ _
Phenols & Tannins		+ + +	_ _ _
Carbohydrate	Molisch's test	+ + +	+ + +
	Benedict's test	+ + +	+ + +
Glycosides		+ + +	+ + +
Amino acids		+ + +	+ + +

Presence of phytochemical (+++) (- - -) Absence of Phytochemical

Table 2 : Preliminary phytochemicals screening tests of the methanol and chloroform extraction from *plumeria obtusa L* leaves

S.No	RT	Name of the compound	Molecular formula	MolecularWeight	Peak %Area
1	5.042	2- Pyrrolidinemethanolamine, 1-ethyl	$C_7H_{16}N_2$	128.2153	0.76
2	5.809	1H-Imidazole, 1-phenyl-	$C_9H_8N_2$	144.1732	0.62
3	5.901	1 Diglycerol	$C_6H_{14}O_5$	166.17	0.55
4	6.075	1 Diglycerol	$C_6H_{14}O_5$	166.17	0.72
5	6.553	1 Benzyl Alcohol	C_7H_8O	108.14	1.01
6	7.064	1 Thymine	$C_5H_6N_2O_2$	126.115	0.61
7	8.009	1 4H-Pyran-4-one, 2, 3-dihydro-3, 5-…	$C_6H_8O_4$	144.1253	0.97
8	8.164	1 Ethane, 1,2-bis(methylthio)-	$C_4H_{10}S_2$	122.3	0.63
9	8.897	1 Benzofuran, 2, 3-dihydro	C_8H_8O	120.15	7.79
10	8,997	2-Furancarboxaldehyde, 5- (hydrox…)	C_6H_6O	126.111	0.95
11	11.05	5-(1-Hydroxyethyl)-2-thioxo-4-im…	$C_{12}H_{12}NO_2S$	236.29	0.86
12	11.85	1 Cyclopentanol, acetate	$C_7H_{12}O_2$	128.17	4.48
13	12.66	1 Dodecanoic acid	$C_{12}H_{24}O_2$	201.31	0.64
14	12.74	1 2, 5-Dimethoxy-4- ethyl amphetamine	$C_{13}H_{21}NO2$	223.31	0.91
15	13.35	1-Naphthalenecarboxaldehyde, 4-m….	$C_{12}H_{10}O_2$	186.21	1.73
16	13.56	1 n-Butyric acid 2-ethylhexylester	$C_{12}H_{24}O_2$	200.32	10.68
17	13.73	1 Bicyclo [3.1.0] hex -2-ene, 4, 4, 6, 6….	$C_{10}H_{16}$	136.1248	0.66
18	13.83	1 Ethyl methyl ethylphosphonate	$C_5H_{13}O_3P$	152.13	1.64
19	14.09	1 Phenol, 2, 6-dimethoxy-4-(2-prope….	$C_{11}H_{14}O_3$	194.23	0.82
20	14.22	1 2 , 2-Diethoxytetrahydrofuran	$C_8H_{16}O_3$	160.213	0.68
21	14.35	1 Naphthalene, 1-propyl-	$C_{13}H_{14}$	170.25	1.22
22	14.47	1 Thiophene, tetrahydro-2-methyl	$C_5H_{10}S$	102.2	0.71
23	14.57	1 Tetradecanoic acid	$C_{14}H_{28}O_2$	255.54	2.17
24	15.29	1 Bicyclo [3.1.1] heptane, 2, 6, 6-tri.	$C_{10}H_{18}$	138.25	1.88
25	16.04	1 Hexadecanoic acid , methyl ester	$C_{17}H_{34}O_2$	270.4507	1.77
26	16.35	1 n-Hexadecanoic acid	$C_{16}H_{30}O_2$	254.41	12.52
27	16.63	1–[1-Hydroxybutyl]-2, 5-dimethoxy…	$C_{12}H_{18}O_3$	210.27	0.75
28	16.94	1Dibenzofuran,3-intro-	$C_8H_5NO_4$	179.13	4.67
29	17.76	Octadecatrienoic acid	$C_{18}H_{30}O_2$	278.4	14.28
30	22.27	Tetracosahexaene…	$C_{24}H_{38}$	326.6	6.6

Table: 4 GC-MS Peak details of methanolic extracts of *P.obtusa L* leaves

S.NO	Peak	Name of the Compound
1	2.542	2-undecenyl-4-hydroxyquindine)
2	2.824	Phenylalanine
3	3.115	Dehydroplumeridoid A
4	4.158	Hydroxyquinoline
5	4.851	quinic acid
6	5.054	Chlorogenic acid/ neochlorogenic acid
7	6.658	Coumaroylquinic acid
8	8.696	Quercetin rutinoside
9	10.171	Kaempferol rutinoside
10	11.525	Glochiflavanoside B
11	13.139	Dicaffeoylquinic acids
12	21.646	Trildroxy-octadecadienoic acid

Table 3: HPLC peaks details of successive methanolic extracts of *P.obtusa L* leaves

frequency, cm–1	***Bond***	***functional group***
555.24	C-Cl Stretch	Alkyl halides
624.00	C(triple bond)C-H : C-H bond	Alkynes
851.86	C-Cl Stretch	Alkylhalides
1063.10	C-O Stretch	Alcohols,ethers,carboxylic acid.
1397.18	N=O Stretch	Nitro groups
1578.40	NO2 Stretch	Strong
1863.33	-C-H Stretch	Aldehydic
2231.84	-C(triplebond)C-Stretch	Alkynes
2507.63	OH Stretch	Carboxylic acid
2627.63	O-H Stretch	Carboxylic acid
2624.36	O-H Stretch	Carboxylic acid
2767.73	H-C=O : C-H Stretch	Aldehydes
2888.47	C-H Stretch	Alkanes
3045.85	=C-H Stretch	Alkenes
3138.41	O-H Stretch	Carboxylic acid
3219.40	O-H Stretch	Carboxylic acid
3435.04	O-H Stretch, H-bonded	Alcohols, Phenols
3572.28	OH Stretch	Alcohols, Carboxylicacid
3703.32	OH Stretch	water
3875.54	OH Stretch	water

Table 5: FTIR analysis of methanolic extracts of *P.obtusa L* leaves

CATEGORY	**CHEMICALS**	**AMOUNT (1 lit)**
MACRO ELEMENT	Potassium nitrate (KNO_3)	1.900ml
	Calcium chloride dihydrate($CaCl_2$)	0.440ml
	Magnesium sulphate ($MgSO_4$)	0.370ml
	Potassium dihydrogen phosphate (KH_2PO_4)	0.0170ml
	Ammonium nitrate (NH_4NO_3)	1.650ml
	Myo-inositol	100mg/litter
MICRO ELEMENT	Manganese sulphate ($MnSO_4$)	22.3ml
	Zinc sulphate heptahydrate ($ZnSO_4$)	0.086ml
	Sodium molybdate ($Na_2. MoO_4. 2H_2O$)	0.25ml
	Copper sulphate pentahydrate ($CusO_4. 5H_2O$)	0.25ml
	Cobalt chloride ($COCI_2 6H_2O$)	0.025ml
	Potassium Iodide (KI)	0.83ml
	Boric acid	6.20ml
	Iron	10ml
ORGANIC SUPPLEMENT	Thymine Hcl	2ml
	Pyridoxine Hcl	0.5ml
	Nicotinic acid	0.5ml
	Glycine	5ml
	Sucrose	20g/1 lit
HORMONES	IBA	1mg/1 lit
	IAA	1mg/1 lit

Table 6: MS media composition for *invitro* callus induction

S.NO	Types of TestMicroorganism	ZnONanoparticles (cm)	Antibiotics(cm)	Methanol(Aqueousextract)(cm)	Chloroform(Aqueousextract) (cm)
1	*Bacillus*	0.5±0.1	2.3±0.1	0.4±0.11	0.2±0.05
2	*Enterobactor*	1.2±0.05	2.5±0.057	0.3±0.05	0.4±0.1
3	*Klebsiella*	1.5±0.05	2±0.1	0.4±0.1	0.5±0.1
4	*E.coli*	1.9±0.1	2.4±0.057	0.6±0.05	0.4±0.1
5	*Staphylococcus*	1±0.1	2.3±0.1	0.3±0.1	0.5±0.1

Table 7: Antibacterial activity with the respective zone of inhibition
Values expressed as mean±standard deviation of three replicates

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