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Exploring the antioxidant potential of a notorious weed using a microwave-based transformation to gold nanoparticles: The case of *Mikania micrantha*

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Abstract

Purpose: We used a notorious weed, *Mikania micrantha* (Asteraceae), to develop and optimize a microwave-assisted quick, single-step, cost-effective, eco-friendly, and safe approach for the biosynthesis of gold nanoparticles (AuNPs) to investigate its antioxidant potential. **Methods:** *M. micrantha* AuNPs (MMAuNPs) were produced in 75 seconds under microwave irradiation. The UV-Vis spectroscopy, SEM, HR-TEM, SAED, EDX, FTIR, XRD, and DLS analysis was used to describe the AuNPs. DPPH and ABTS radical scavenging assays showed antioxidant activity. Anticancer potential was assessed by MTT assay against breast cancer cells. Swiss albino mice were tested for BBB impact by actophotometer and tail immersion. **Results:** The synthesis of MMAuNPs was confirmed by a characteristic ruby red coloration and surface plasmon resonance peak at 534 nm. The MMAuNPs are predominantly spherical in shape, small (107.82 nm), and crystalline in nature. The antioxidant properties of the MMAuNPs were found to be better than *M. micrantha* aqueous extract. MMAuNPs showed anticancer potential with IC₅₀ values of 157.1 and 174.2 µg/mL against MCF-7 and MDA-MB-231, respectively. MMAuNPs did not show a significant response of locomotor count and antinociceptive activity. **Conclusion:** Natural creations always have a purpose, and even the most renowned weeds have something worthwhile to impart on society. The results may provide unforeseen opportunities in terms of maximizing trash usage. Nevertheless, more investigation is necessary to evaluate the *in vivo* effects of MMAuNPs prior to their widespread use in the field of medicine, whether as independent therapies, antioxidant, or anticancer substances, or as drug carriers.

Keywords: Gold nanoparticles, Green synthesis, *Mikania micrantha*, Microwave assisted synthesis, Antioxidant, Anticancer

Statement and Declaration

Conflicts of interest

The authors declare that there is no conflict of interests.

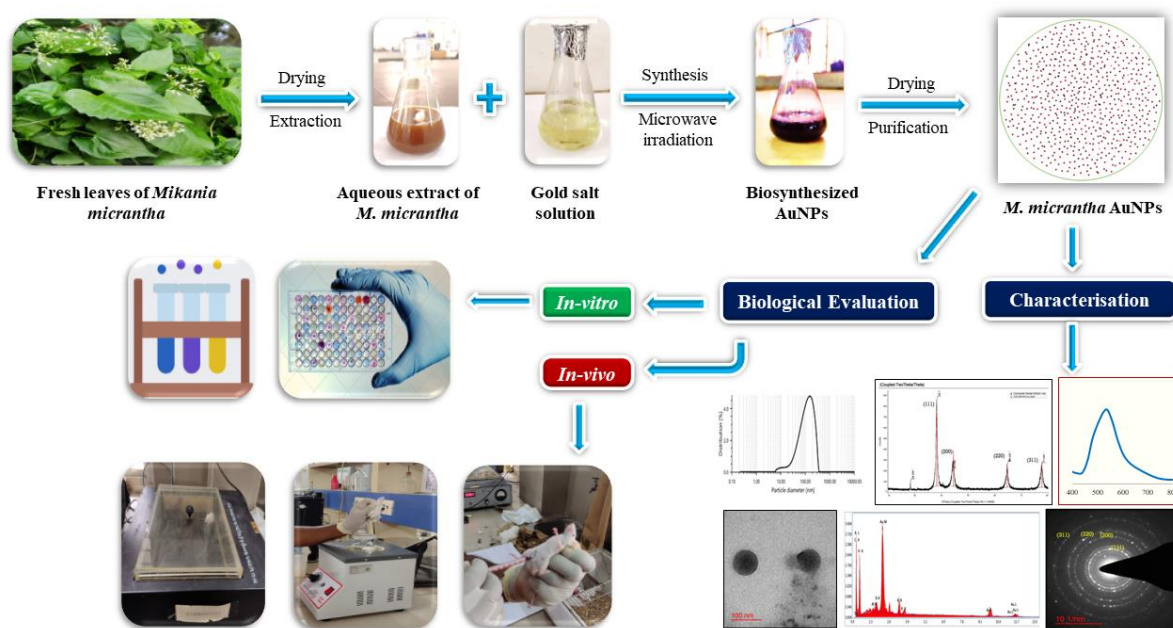
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Highlights

- This study conclusively demonstrates that the creation of nature is never futile, and even one of the most notorious weeds have something valuable to impart for humanity.
- We developed and optimized a microwave-assisted approach for the biosynthesis of gold nanoparticles (AuNPs) using *Mikania micrantha*, an internationally recognized weed.
- In addition, the process is quick, cost-effective, eco-friendly, and safe as well as consumed nine times less electricity and liberated nine times less CO₂ to the atmosphere as compared to the conventional green synthesis of metal nanoparticles.
- Our aim was to investigate the antioxidant potential and anticancer activities of the *M. micrantha* in the form of AuNPs.

Graphical Abstract



Introduction

Mikania micrantha is a perennial climber that originated in tropical America. It is a prominent invasive species in portions of Southeast Asia and the Pacific Islands. It was introduced to India in the 1940s as ground cover in tea plantations and is now a major danger to various plantation crops as well as forest regions throughout the nation. It produces thousands of lightweight seeds that are distributed by the wind, and it may also reproduce vegetatively through its roots, resulting in quick and broad invasion of any disturbed area [1]. *M. micrantha* is a tropical member of the Asteraceae family. It is also known as climbing hemp vine, bitter vine, or American rope. Various chemical constituents of a different class, mainly flavonoids, sesquiterpene dilactones, terpenoids (essential oils), sesquiterpene lactones, phenolics, tannins, glycosides, quinones, alkaloids, saponins, etc. have been isolated from *M. micrantha* by various methods of extraction using a variety of solvents [2, 3]. It is customary to use the leaf juice of this plant to cure a variety of illnesses, such as skin rashes and itching, whereas serious bug bites are treated with a poultice made from the leaves [4]. It is most often used in Jamaica as folk medicine to assist in the healing of sores and as a bandage for wounds. It is also used to treat respiratory illnesses, rheumatism, fever, stomach-aches, and jaundice [3]. Scientific data from contemporary pharmacological investigations show that bitter vine has exceptional medicinal potencies, including antibacterial, anti-inflammatory, cytotoxic, anticancer, antidiabetic, antioxidant, and wound-healing effects [2]. Different sesquiterpenes like 3 β -acetoxy-1,10-epoxy-4-germacrene-12,8;15,6-diolide, potassium mikanin 3-sulfate, alpinetin, 1,10-epoxy-4-germacrene-12,8;15,6-diolide, mikanin, ergosta-7,22-dien-3 β -ol, dihydromikanolide, 11H β -11,13-dihydromicrantholide, and 8-epi-mikanokryptin have been isolated from the aerial portions of *M. micrantha*. Some compounds showed modest antiviral and cancer activity against respiratory syncytial virus and parainfluenza type-3, while sesquiterpene lactones showed marginal anti-inflammatory activities in a mouse ear oedema model [5-7]. Sixty-six components from the *M. micrantha* flower essential oil were identified. The lead phytoconstituents were zingiberene, germacrene-D, β -bisabolene, δ -cadinene, trans-caryophyllene, debromofiliformin, and isolodene. The oil contains a complex blend of oxygenated sesquiterpenes and sesquiterpene hydrocarbons, as well as other

terpenes. Flower oil exhibited cytotoxicity against cervical, ovarian, and pancreatic cancer cell lines [8]. The plant extract also exhibited moderate to good antioxidant activity [9-11]. The ethanolic extract of *M. micrantha* exhibits sufficient cytotoxicity and thrombolytic activity but no anthelmintic properties [12]. Up to a dose of 2000 mg/kg, the n-butanolic fraction of *M. micrantha* leaves showed no adverse responses or death. The cytotoxicity and anticancer activity of n-butanolic extract against the Ehrlich ascites carcinoma (EAC) cell line induced in Swiss albino mice are probably attributed to rich flavonoids [13]. Additionally, the biosynthesized silver nanoparticles (AgNPs) and Fe₃O₄ NPs using *M. micrantha* leaf extract showed antimicrobial activity [14-16].

Nanoscience and nanotechnology are novel scientific advances that have emerged in this century. Nanotechnology is a multidisciplinary area that integrates basic science and engineering with applied fields such as biophysics, molecular biology, and bioengineering to develop, synthesize, characterize, and apply of materials and systems on the nanoscale [17, 18]. A nanoparticle (NP) or ultrafine particle is a matter particle having any shape with a diameter of one to one hundred nanometers (nm). This term is often used to describe bigger particles with sizes of up to 500 nm, along with fibers and tubes having diameters of 100 nm or smaller in just two dimensions [19]. NPs are classified into several types based on their morphology, size, and chemical properties. Based on the type of fabricated material, NPs are categorized as lipid-based NPs, inorganic NPs, polymeric NPs, hybrid NPs and bioinspired NPs [20]. Metallic NPs are flexible nanostructures that can modify their composition, size, shape, structure, assembly, optical characterization, and encapsulation. They have unique surface features like plasmon excitation and dielectric properties, making them suitable for biomolecular ultrasensitive target identification, protein labelling, targeted delivery, cancer treatment, and micro-RNA expression, that alters various physiological and metabolic processes [21]. Metallic NPs are highly relevant in research due to their size-dependent behaviour, low particle size distribution, and easy surface functionalization. They are biocompatible, inert, nontoxic, and easy to synthesize, making them attractive substrates for drug delivery mechanisms due to their rich stacking of targeting ligands on the outermost layer at a small core dimension [22]. In addition to their many uses, metal NPs have additional benefits such as high catalytic activity, recycling capabilities, and good selectivity [23]. Among all metal NPs, gold nanoparticles (AuNPs) are widely used metal nanoparticles with excellent applications in various fields like delivery carriers, imaging technology, diagnostics, therapeutic agents, catalysts, and drug release tools. AuNPs have nontoxic and inert gold cores and are easy to synthesize in small sizes (1 to 150 nm) with different shapes [20]. AuNPs have the ability to specifically target cancer cells that have an excessive number of cell surface receptors. They can also bind to a range of medications and proteins [22]. In immunochemical research, AuNPs are employed to identify protein interactions. Additionally, AuNPs are used to identify drugs such as aminoglycosides, including neomycin, gentamicin, and streptomycin, and as lab tracers in DNA fingerprinting. Gold nanorods are being employed for cancer diagnosis, cancer stem cell detection, and the identification of different bacterial species [24].

Different techniques are employed to synthesize metal NPs. Chemical and physical synthesis processes are costly and produce hazardous residues, as these methods involve the use of expensive and toxic chemicals, which eventually pollute the environment and make things worse. However, the biological approach, is affordable, easy to implement, less toxic to the environment, and does not involve any extra processing during synthesis. The term "biogenic nanoparticles" refers to NPs made from biological components, and the synthesis method used is called "green synthesis of nanoparticles". The conventional methods for producing metal NPs are at room

temperature or with a hot plate magnetic stirrer. However, a novel approach for the fast and environmentally friendly production of metal NPs is microwave-assisted one-pot synthesis, or microwave-assisted synthesis (MAS), which uses plant extracts as both reducing and capping agents [25]. Compared to convection-based heating, MAS demonstrates reliable distribution of energy throughout the reaction medium [26]. It offers several appealing qualities, including a quick reaction time, less energy use, and higher product output. Microwave irradiation quickly and evenly heats the reaction media, which creates homogenous conditions for NP nucleation and growth [27]. MAS has not been widely used yet, because only a small number of solvents are microwave active. In MAS, water is a microwave active solvent where, molecular rotation is caused by the immediate introduction of microwave energy into the mixture of reactions via a thermocouple driven by a magnetron [26]. The hot plate magnetic stirrer consumes more electricity, which is more expensive, and room temperature synthesis takes a substantially longer time compared to MAS. Considering the sustainability and cost-effective aspects of microwave based AuNPs synthesis using plant extract, it was thought worthwhile to impart value addition to a notorious weed *M. micrantha* by exploring its AuNP form in maintaining the body's oxidative balance.

Materials and methods

Chemicals and reagents

The Folin-Ciocalteu (FC) reagent, aluminium chloride, sodium carbonate, and chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) were acquired from Central Drug House (P) Ltd., New Delhi, India. The ABTS, DMEM, DPPH, and MTT solutions were acquired from Sigma-Aldrich Chemicals Private Limited, Bangalore, India. Methanol and ethanol were acquired from Thermo Fisher Scientific India Pvt. Ltd. All the other chemicals used were of analytical grade.

Collection and preparation of plant materials

Fresh leaves of *M. micrantha* from the East Medinipur district of West Bengal, India, were collected in December 2021. The Plant Identification Cell, Department of Botany, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur, Chhattisgarh, India, verified the authenticity of the plant (No.: Bot/GGV/2022/09). The leaves were carefully cleansed with tap water to remove dust and contaminants, and then they were rinsed with distilled water. It was then let to air dry for two weeks in the shade. Before being utilised, the dried leaves were crushed and stored in a container that was firmly sealed.

Preparation of plant extract

We heated the aforementioned powdered material (10 g) to boiling for 30 minutes in a beaker with 200 mL of distilled water. We cooled the mixture and then filtered it using Whatman filter paper. We heated the remaining solid residue for an additional 15 minutes with 100 mL of distilled water to ensure thorough extraction. After cooling down, we passed it through a Whatman filter paper. Subsequently, the two filtrates were combined, and the final volume was adjusted to 120 mL using distilled water. The *M. micrantha* aqueous extract (MMAE) in a sealed glass container and refrigerated it until we needed it.

Preliminary phytochemical screening (PPS)

Different groups including carbohydrates (Molisch's test), proteins (Millon's test and Biuret test), amino acids (Ninhydrin test), steroids (Salkowski reaction), alkaloids (Mayer's test, Dragendorff's test, and Hager's test), flavonoids (Shinoda test and zinc test), phenolic compounds, and tannins (acetic acid test, ferric chloride test, and lead acetate test) were screened in MMAE [28-31].

Quantitative phytochemical estimation

Determination of total phenolic content (TPC)

The TPC in MMAE was determined using the technique previously reported [32], with a slight modification involving the use of FC reagent. A solution containing 0.5 mL of MMAE (1 mg/mL in methanol) was combined with 2.5 mL of a 10% FC reagent and allowed to sit for 5 minutes. Subsequently, a 2 mL aliquot of Na₂CO₃ solution (10% w/v) was introduced into the mixture. The solution was agitated using a vortex mixer and incubated in a light-free environment for a duration of 90 minutes at ambient temperature. The absorbance was quantified at a wavelength of 765 nm using a UV-Spectrophotometer (UV-1800, Shimadzu, Japan), using a blank solution containing methanol instead of the extract. A range of gallic acid concentrations (10, 20, 30, 40, 50, and 60 µg/mL in methanol) were utilised to construct the standard curve. The TPC of the MMAE was calculated from the standard curve of gallic acid (GA) using the equation $y = 0.0127x + 0.0623$ ($R^2 = 0.9992$). The assays were conducted three times, and the TPC of the sample was measured in milligram of gallic acid equivalent per gram of dried extract (mg GAE/g DE).

Determination of total flavonoid content (TFC)

The TFC of the MMAE was measured using the previously established technique [33], with a minor adjustment involving the use of an aluminium chloride colorimetric assay. In summary, 500 µL portions of the sample solution (1 mg/mL in distilled water) were combined with 1.5 mL of 95% ethanol. Next, 100 µL of a solution containing 10% w/v aluminium chloride, 100 µL of a solution containing 1 M potassium acetate, and 2.8 mL of distilled water (DW) were added and mixed well by vortexing. We allowed the solution combination to react in the absence of light for 45 minutes. The absorbance was measured at a wavelength of 415 nm using a UV-spectrophotometer, with distilled water as a blank. A calibration curve was produced using various concentrations of quercetin (6.25, 12.5, 25, 50, and 100 g/mL in methanol) and their corresponding absorbances, acquired using the above-described procedure. The calibration curve equation was, $y = 0.0016x + 0.021$, yielding an R^2 value of 0.9992. This calibration curve was then utilised to compute the TFC. The studies were performed three times, and the TFC was measured as milligram of quercetin equivalent per gram of dried extract (mg QE/g DE).

Determination of total tannin content (TTC)

TTC was calculated using the FC technique, with slight modifications to the previously described process [34]. A volume of 1 millilitre (1 mL) of MMAE solution with a concentration of 1 mg/mL was combined with 500 µL of FC-reagent solution, which had a concentration of 10% (v/v). Additionally, 1 mL of sodium carbonate solution with a concentration of 35% (w/v) and 7.5 mL of DW were added to a 10 mL volumetric flask. The solution containing the combination was vigorously agitated at the temperature of the room and left undisturbed for a duration of 30 minutes. The absorbance of the solution was measured at a wavelength of 700 nm, using distilled water as a reference instead of the extract. The trials were conducted three times. The TTC was quantified

as milligram of tannic acid equivalent per gram of dried extract (TAE/g DE) using a standard curve of tannic acid (TA). The standard curve equation was $y = 0.0007x + 0.0015$, with an R-squared value of 0.995. The standard curve was generated by measuring absorbance of different concentrations of tannic acid (20, 40, 60, 80, and 100 $\mu\text{g/mL}$).

Synthesis of gold nanoparticles (AuNPs)

Microwave-assisted synthesis

In the beginning, a trial synthesis was carried out in a microwave (Catalyst™ CATA R) by mixing an aqueous leaf extract of *M. micrantha* with 1 mM aqueous solution of chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) in 1:9 ratio. The solution combination turned from light yellow to ruby red after 60 seconds of microwave irradiation, and the produced AuNPs were confirmed using a UV-Vis spectrophotometer. The reaction parameters, including microwave power, irradiation time, and the ratio of extract to metal salt solution were optimised to achieve small and stable nanoparticles. This was done using the Taguchi L9 (3,3) or orthogonal arrays experimental design, which involved varying the reaction parameters based on the results of the trial experiment. Three distinct variables corresponding to the aforementioned three factors were utilised, and a grand total of nine experiments (each conducted three times) were carried out in order to acquire the optimised circumstances for the production of AuNPs (as shown in Table 1). To optimise the formulation, many parameters were examined, including the hydrodynamic diameter (HD), polydispersity index (PDI), and mean zeta potential (ZP) of the biosynthesized AuNPs.

Table 1 Processing and materials constrain at various levels with preparation batches of AuNPs.

Control Factors	1	2	3
A= Time (Sec)	45	60	75
B= Microwave power (watt)	320	480	640
C= Extract: Metal salt solution	3:7	2:8	1:9
Run	A	B	C
1	45	320	3:7
2	45	480	2:8
3	45	640	1:9
4	60	320	2:8
5	60	480	1:9
6	60	640	3:7
7	75	320	1:9
8	75	480	3:7
9	75	640	2:8

Synthesis by using a digital magnetic hot plate stirrer

A solution containing MMAE and chloroauric acid at a ratio of 3:7 was prepared and heated to 60°C using a digital magnetic hot plate stirrer (SPINOT™ Digital Magnetic Stirrer Hot Plate, TARSON). Ultimately,

the enduring presence of the vivid ruby red hue indicates the formation of AuNPs. The generated AuNPs were also verified by UV-Vis spectroscopy [35].

Purification of green synthesized gold nanoparticles

To remove the uncapped plant extract from the surface of the synthesized AuNPs generated using the aforementioned process, the NP suspension was centrifuged (REMI, Mumbai, India) for 10 min at a speed of 10000 rpm. Then, redistributing the NPs in DW after decanting the supernatant liquid, and repeating the process for two more times and finally, the obtained NP pellets were dispersed in DW. The NPs suspension was lyophilized overnight to obtain powdered *M. micrantha* AuNPs (MMAuNPs) [36].

Characterization of synthesized gold nanoparticles

UV-Vis spectroscopy

It is most accepted technique for the determination of surface plasmon resonance (SPR) peak, which confirms the synthesis of AuNPs. A double-beam spectrophotometer (UV-1800 Shimadzu, Japan) was used to track the SPR peak after the synthesis of the AuNPs. The AuNPs were scanned within the wavelength range 400-800 nm against distilled water as a blank. Further, UV-Vis spectroscopy was also used for the optimization and stability evaluations of the AuNPs.

Dynamic light scattering (DLS) analysis

The particle size, zeta potential, and size distribution of the biologically synthesised AuNPs were assessed using a particle size and zeta potential analyzer (Litesizer500, Anton Paar, Austria). This allowed for the determination of the HD, average ZP and PDI. In summary, the sample was diluted by a factor of 50 using ultrapure water and then subjected to analysis using Dynamic Light Scattering (DLS).

Fourier transform infrared (FTIR) spectroscopy

Different nature of compounds as the functional groups were determined in the MMAE and biosynthesized MMAuNPs by FTIR analysis. Briefly, the MMAE and the powdered MMAuNPs were positioned on the FTIR sample holder, and the spectra were recorded using an FTIR spectrophotometer (Perkin Elmer Spectrum II, IR Version 10.7.2, Singapore) within the wavenumber range of 4000 to 450 cm⁻¹.

X-ray diffraction (XRD) analysis

The degree of crystallinity and purity of the synthesised MMAuNPs were examined using an X-ray diffractometer (XRD, Bruker® D8 ADVANCE, USA) equipped with Cu K α radiation, which has a wavelength of 1.5406 Å, operating at 40 kV and 25 mA. The diffraction angle (2 θ) at which the X-rays are incident on the specimens varied from 20° to 80° with a step size of 0.05° and 2.0 s in each step.

Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis

Using a scanning electron microscope (JSM-6084LV, JEOL®, USA), morphological examinations were performed on the powdered MMAuNPs. Electron photomicrographs with a 1000x magnification were taken. Additionally, EDX spectroscopy was used to analyse the MMAuNPs' elemental composition.

High-resolution transmission electron microscopy (HR-TEM) and selected area electron diffraction (SAED) analysis

To examine the particle shape and crystallinity, HR-TEM and SAED investigations were performed using a HAADF detector-equipped HR-TEM running at 200 kV and a carbon-coated copper grid (400 mesh, 3.05 mm diameter, treated Pella). Twenty microliters of the sample were put on the carbon side of the TEM grid, dried at 40°C, and then put through a 0.45 µm syringe filter after being diluted five times with Millipore water. The sample was then submitted to HR-TEM analysis.

Biological evaluation of synthesized gold nanoparticles

Antioxidant activity

Antioxidant activity reduces or prevents nutritional oxidation, particularly of lipids and proteins, by limiting oxidative chain reactions. Conclusions about the antioxidant activity should not be based just on one antioxidant test model. The DPPH method is the most widely used free radical scavenging technique because it is rapid, simple (i.e., requires few steps and reagents), and fairly priced when compared to other test models of similar effectiveness. Conversely, hydrophilic, and lipophilic antioxidants can both be utilised with the ABTS decolorization test.

DPPH radical scavenging activity: The test for DPPH radical scavenging was used to evaluate the antioxidant potential of MMAuNPs [37]. We combined 250 µL of MMAuNPs (1 mg/mL) with a 4750 µL aliquot of the 1 mM DPPH stock solution in methanol. The reaction mixture was mixed and then allowed to sit at room temperature for 30 minutes in the dark before measuring the absorbance at 517 nm. The percentage of DPPH inhibition was measured and compared to the control. Ascorbic acid was used as a positive control. The IC₅₀ (concentration of MMAuNPs needed to quench 50% of the initial DPPH radical) and the percentage radical scavenging activity (using Equation 1) for each sample was calculated.

$$\% \text{ Scavenging activity} = \frac{(A_c - A_T)}{A_c} \times 100 \quad (1)$$

where A_C is the absorbance of the control, and A_T is the absorbance of the test sample.

ABTS•+ radical scavenging assay: The previously described approach, with slight modifications [38, 39], was employed to assess the ABTS radical cation scavenging activity of AuNPs. A solution containing 2.5 mM potassium persulfate and 7 mM ABTS was prepared. The solution was then kept in the dark at room temperature for 16 hours to allow the formation of ABTS radical cations. Methanol was introduced into the resulting ABTS working solution to achieve further dilution, resulting in an absorbance of 0.85±0.04 at 734 nm. A total of 3 mL of the ABTS working solution was combined with 1.0 mL of different concentrations of MMAuNPs that had been diluted with methanol. The absorbance was measured at a wavelength of 734 nm after allowing the reaction mixture to incubate at room temperature for 4 min, in comparison to a control sample. As a reference standard,

ascorbic acid was used. The percentage of radical scavenging activity and the IC₅₀ value of the sample were determined using Equation 1.

Anticancer activity

The cytotoxicity of the biosynthesized AuNPs against the breast cancer cell lines (MDA-MB-231 and MCF-7) was evaluated using the MTT test at dosages ranging from 6.25 to 400 µg/mL. The cells were cultured in DMEM medium with 10% foetal bovine serum and 1% penicillin/streptomycin. The cells were placed onto 96-well plates at a density of 3000 cells per well. The plates were cultured for 24 hours at 37 °C in a 5% CO₂ environment, after which the medium was changed with new media containing different concentrations of MMAuNPs. To serve as a control, culture material that did not contain any medicines was utilised. After 72 hours of incubation, the medium was cautiously removed from the wells, and the cells were then washed twice with sterile PBS (phosphate buffer saline). Subsequently, 50 µL of MTT solution (0.5 mg/mL in DMEM medium) was introduced into each well and incubated for an additional four hours under identical conditions. After discarding the medium from each well, 100 µL of DMSO was injected to dissolve the purple formazan crystal generated by the MTT assay. The measurement of absorption intensity at a wavelength of 570 nm was conducted using a microplate reader [40]. Equation 2 was utilised to calculate the percentage of cell viability under different treatment conditions.

$$\% \text{ Cell viability} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100 \quad (2)$$

Sedative Activity

Actophotometer test: A sound-attenuated, dimly lighted locomotor box or activity cage (420×420×200 mm) with six integrated photo sensors and a four-digit digital counter was used to assess the locomotor activity of mice for 5 min. It uses photoelectric cells, which are linked in a circuit with a counter, to work. An animal's movement causes the light beam that is falling on the photocell to be interrupted, which results in a count that is referred as a locomotor count. Group 1 (control) received DW (10 mL/kg b.w., i.p.), Group 2 (standard) acquired diazepam (2 mg/kg b.w., i.p.), and Groups 3 and 4 (test groups) received MMAuNPs (10 and 20 mg/kg b.w., i.p., respectively) prior to 30 minutes of exposure to the actophotometer. Each mouse had a five-minute window on the actophotometer that was recorded for the analysis [41].

Antinociceptive Activity

Tail immersion test: The tail immersion test was used to quantify the nociceptive response in accordance with a previously described procedure [42]. The constant temperature of the water in a beaker was 50 ± 0.5 °C. The tail of each mouse (from the tip of the tail) was dipped (3 cm) into the beaker, and the time required (in sec) to visibly pull the tail out of the water was recorded as the tail flick latency time with a cut-off time of 15 sec to prevent tissue damage. The mice were first given a baseline correction before receiving therapy. The animals were split up into four groups: groups 3 and 4 (test groups) got MMAuNPs (10 and 20 mg/kg b.w., i.p., respectively), group 2 (standard group) received aspirin (50 mg/kg b.w., i.p.), and group 1 (control group) received DW (10 mL/kg b.w., i.p.). After 30 min of various treatments, the tail flick delay time was observed.

Statistical analysis

The data are represented as the mean $\pm$ standard error of means (SEM) (n= 3, in the case of *in vitro* biological study and n= 6, in case of *in vivo* biological study). One-way analysis of variance (ANOVA) and then the Newman-Keuls post hoc test was used for the statistical analysis, to find significant variations using the GraphPad Prism software. Statistical significance was set at $P < 0.05$.

Results and Discussion

Preliminary phytochemical screening

Numerous researches have documented that the reduction of metal ions in plant decoctions rich in polar secondary metabolites is primarily responsible for the stability of the NPs [43]. Significantly promoting the creation of AuNPs is also the reduction of metal salts in turn. Results showed the presence of phenolic compounds, amino acids, carbohydrates, flavonoids, and tannins, all of which are likely to have been important in the reduction of metal salts to produce AuNPs.

Quantitative phytochemical estimation

The phenolic, flavonoid, tannin content of MMAE was evaluated and the results are shown in table 2.

Table 2 Quantitative phytochemical estimation of MMAE.

SL No.	Test	Quantity (Per gram of dried MMAE)
1	Total phenolic content	46.72 mg GAE
2	Total flavonoid content	28.33 mg QE
3	Total tannin content	56.43 mg TAE

MMAE, *Mikania micrantha* aqueous extract; GAE, Gallic acid equivalent; QE, Quercetin equivalent; TAE, Tannic acid equivalent.

The formation of metallic nanoparticles has been found to rely heavily on phenolic chemicals (caffeic acid, gallic acid) and flavonoids (anthocyanidins, isoflavones, flavones, and flavols). This is owing to the strong nucleophilicity of hydroxyl and carbonyl groups, as well as their exceptional metal ion binding affinity. Polyphenolic chemicals, such as flavonoids and phenols, are key antioxidant ingredients in most plant species, and their antioxidant action is mostly attributed to their redox characteristics [44]. As a result, they can function as reducing agents, assisting in the reduction of metallic ions into nanoparticles. Plants with a greater TPC have significant antioxidant activity and, as a result, superior reductive capacity, which is required for the production of nanoparticles. Phenols and flavonoids have long been known to have anticancer and apoptosis-inducing properties. They are also powerful antioxidants and free radical scavengers [33]. Similar explanation also provided in the past by researchers who reported high content of phenolics, flavonoids, and tannins in the plant extract which had been used for the preparation of AuNPs [45, 46].

Synthesis of gold nanoparticles

Phytoconstituents are crucial in the green synthesis of AuNPs. According to a widely recognized model, the synthesis of NPs in this method occurs as a result of a phytochemical process in which complex-reducing

phytoconstituents from the plant extract, such as enzymes, antioxidants, and phenolic moieties, convert gold cations into AuNPs. To create zerovalent gold, HAuCl_4 is hypothetically reduced in the presence of phytochemicals. This hypothetical process then causes gold atoms to aggregate into nanoparticles, which are then stabilized by the phytochemicals to produce isotropic AuNPs [47]. The potential reduction methods explained earlier [48] are provided below (Eq. 3 and 4).



Microwave-assisted synthesis

The present study revolves around the rapid microwave-assisted synthesis of AuNPs using an extract derived from *M. Micrantha* leaves. The evident change in colour and distinct absorption peaks observed in the UV-Vis absorption spectra of the nanoparticles provided conclusive evidence that this extract functioned as a reducing and stabilising agent for the creation of the nanoparticles. Carbohydrates, amino acids, flavonoids, phenolics, and tannin groups are secondary metabolites in the extract reduce metal ions, resulting in the formation of colour-coordinated nanoparticles. The reaction combination of leaf extract and chloroauric acid solution (1 mM) in the ratio of 1:9 turned ruby red from light brown after 60 seconds of microwave exposure in the trial run and exhibited an SPR peak at 536 nm in UV-Vis spectroscopy.

To minimize the cost and time, and to obtain the smaller particle size, by considering the basic Taguchi L9 (3,3) or orthogonal arrays, an experimental design by variation of the reaction parameters obtained in the trial experiment was performed. Initially, the validity and consistency of all nine syntheses were verified by visually seeing the presence of a ruby red colour and a SPR peak in the synthesised gold nanoparticles (AuNPs) within the wavelength range of 520-560 nm [33]. The following performance evaluation parameters mainly HD, PDI, and mean ZP of the synthesized AuNPs were used for optimizing the synthesis. A microwave power of 480 W, an extract: metal salt solution (1 mM) ratio of 3:7 (v/v), and irradiation time of 75 sec (Run 8) was found to be the optimal set-up for the synthesis of MMAuNPs (Table 3).

Table 3 Characteristic responses of AuNPs synthesized by the basic Taguchi L9 experimental design.

Run	Colour before synthesis	Colour after synthesis	SPR peak (nm)	Hydrodynamic diameter (nm)	PDI (%)	Mean zeta potential (mV)	Colour after 15 days of synthesis	Particle settle after 15 days
1	Light brown	Reddish brown	532	154.46±5.20	27.9±1.60	13.2±2.03	Light Ruby Red	No
2	Light brown	Light Ruby Red	538	135.29±2.71	26.0±2.33	30.0±2.13	Light Ruby Red	No
3	Light brown	Ruby Red	540	162.83±5.76	24.4±2.96	4.6±0.22	Ruby Red	No
4	Light brown	Ruby Red	534	121.07±3.47	25.9±1.98	-25.0±1.95	Ruby Red	No

5	Light brown	Ruby Red	536	146.76±6.85	26.9± 2.75	-1.9± 0.76	Ruby Red	No
6	Light brown	Light Ruby Red	532	178.60±5.82	24.7± 1.21	-59.4± 2.11	Light Ruby Red	No
7	Light brown	Ruby Red	536	145.06±3.86	28.5± 2.89	67.1± 3.80	Ruby Red	No
8	Light brown	Ruby Red	534	107.82±2.40	26.2± 1.71	-24.6± 1.09	Ruby Red	No
9	Light brown	Ruby Red	534	149.16±4.12	28.8± 1.39	-21.9± 1.36	Ruby Red	No

The stability of the synthesized MMAuNPs was checked by keeping them in a refrigerator (4-8°C) for 15 days. There was neither any color change nor aggregation of particles in the biosynthesized AuNPs after 15 days. Further, no significant variation in particle size or SPR peak was observed after storage for 15 days which indicates that the AuNPs are stable for two weeks.

Synthesis by using a digital magnetic hot plate stirrer

When the reaction mixture of MMAE and 1 mM chloroauric acid in the ratio 3:7 was allowed to react at 60 °C on a digital magnetic hot plate stirrer, the initial light brown color changed to ruby red after 38 min, and the SPR peak was obtained at 532 nm indicating the synthesis of AuNPs.

Environmental impact

For the success of any process its impact on the environment is a critical factor. In order to ascertain the eco-friendliness of the proposed microwave method, its impact on the environment in terms of power consumption and CO₂ emission have been reported below. The power consumption of the microwave process was found to be 0.0099 kWh which is 8.9 times less when compared to the power consumption of the hot plate method of MMAuNPs synthesis. Similarly, the CO₂ emission of microwave process was found to be 7.92 g, which is 8.93 times less than the CO₂ emission cause by the hot plate method (70.72 g). All calculation related to CO₂ emission were based on standard conversion data (1 kWh energy = 800 g of CO₂) as reported in the literature [49]. As per the electricity tariff for non-domestic consumption released by Chhattisgarh State Power Distribution Company Limited for the financial year 2023-2024, the cost of microwave synthesis of 50 mL MMAuNPs is 5.79 paisa whereas, in the heating method the cost involved was 51.7 paisa. In light of the above facts, the choice of microwave process for the synthesis of MMAuNPs stand fully justified.

Characterization of synthesized gold nanoparticles

UV-Vis spectroscopy

Sharp and strong surface plasmon absorption peaks were found in the UV-Vis spectrum because of the interaction of conduction electrons of metal nanoparticles with incoming photons. It is unique to each metal and is determined by the size, shape, and distribution of nanoparticles [50]. AuNPs were synthesized utilizing chloroauric acid and MMAE under various circumstances such as extract-to-metal salt solution ratio, microwave

power, and irradiation time. The colour shift in the reaction solution to ruby red, on the other hand, suggests the formation of AuNPs from MMAE. The SPR absorption peak of all nine synthesized MMAuNPs was observed as a distinctive peak between 530 and 540 nm, which confirmed the formation of AuNPs. There was no such peak detected in the UV spectrum of gold salt solution and MMAE. Further, run-8 was optimized due to the tiny size and stability of the AuNPs, with colour shifts from light brown to ruby red and SPR peak at 534 nm (Fig. 1a).

Dynamic light scattering (DLS) analysis

This analysis was basically performed for the evaluation of HD, PDI, ZP of biogenic AuNPs. The optimised MMAuNPs had a HD of 107.82 nm (Fig. 1b), PDI of 0.262, and ZP of -24.6 mV. The HD of the optimized formula is lower than that of the other formulations. The PDI value of 0.262 represents a near homogeneous and monodisperse particle size distribution [51]. Nanoparticles having ZP greater than ± 30 mV are stable in solution and do not agglomerate [44]. In addition to this, based on surface charge of NPs colloidal dispersion, its stability may be classified as highly unstable (ζ , between -10 and 10 mV), less stable (ζ , between 10 and 20 mV or -10 and -20 mV), moderately stable (ζ , between 20 and 30 mV or -20 and -30 mV), and highly stable (ζ , $>+30$ or <-30 mV) [52]. Experimental findings revealed a ZP value of -24.6 mV indicating that the synthesized MMAuNPs can be grouped under moderately stable nature. The stability of the particles is directly related to its negative charge which produces electrostatic repulsion among them, hence prevent the aggregation.

X-ray diffraction (XRD) analysis

XRD was employed to ascertain the crystalline structure of AuNPs. The XRD analysis of MMAuNPs revealed characteristic peaks at angles of 38.194° , 43.956° , 64.632° , and 77.445° within the 20° to 80° diffraction angle range. These peaks corresponded to the (111), (200), (220), and (311) planes of the face centred cubic (fcc) structure of AuNPs, as shown in Figure 1c. This alignment provides strong evidence for the crystalline nature of the AuNPs. The presence of nanoparticles was also evidenced by the enlargement of Bragg's peaks. The collected data exhibit a high level of concordance with the standard database maintained by the Joint Committee on Powder Diffraction Standards, specifically JPCDS file No: 004-0784 [53]. A peak at 38.194° indicated that there was a higher rate of development in the (111) faces of MMAuNPs. The presence of bio-organic phase on the surface of nanoparticles might potentially lead to unanticipated peaks [54].

Selected area electron diffraction (SAED) analysis

SAED analysis was used for the phase identification and structural growth of the particles. Fig. 1d depicts the SAED pattern of the produced AuNPs. Inside the rings, the SAED pattern revealed bright circular dots corresponding to the (111), (200), (220), and (311) orientations. The fcc structure of gold suggested that MMAuNPs were made of pure crystalline gold [44], which also supports the XRD data.

Energy dispersive X-ray (EDX) analysis

EDX is a widely used technique for the analysis of elemental composition. The EDX spectrum of AuNPs produced using *M. micrantha* leaf extract is shown in Fig. 1e. The presence of a significant Au signal in the samples provides confirmation that AuNPs were synthesized. The prominent peak of the Au signal occurred at 2.3 KeV. Additionally, faint indications for elements such as oxygen, potassium, and carbon are seen. These

signals are likely connected to the biomolecules in the extract used to create AuNPs and might also be the result of human errors during the handling and washing of the AuNPs.

Scanning electron microscopy (SEM)

Real time evidence regarding shape and surface nature of biogenic AuNPs were generated through SEM studies. MMAuNPs revealed the spherical shape and smooth structure with an average diameter of 97.86 nm (Fig. 1f) by calculating the average size of 30 MMAuNPs in the SEM images. The diameter was found to be smaller than that revealed by the DLS method.

High resolution transmission electron microscopy (HR-TEM)

Anisotropic character of NPs was demonstrated by the HR-TEM images of the MMAuNPs shown in Fig. 1g. It is the primary proof of the morphology structure of MMAuNPs. The presence of primarily spherical morphology structures has confirmed the anisotropic nature and morphological structure of AuNPs [55]. The particle size of MMAuNPs was 80-105 nm, which in contrast is relatively smaller than that determined by DLS approaches.

The size reported by the DLS approach is a hydrodynamic diameter. The particle size assessed by SEM and HR-TEM is the actual diameter of the particle as it is measured in the dry state. The particle will therefore display a greater hydrodynamic volume as a result of the solvent effect in the hydrated state [56]. Therefore, the particle size measured by DLS techniques is larger than that found by SEM and HR-TEM analysis. During the synthesis of MMAuNPs, the organic molecules found in MMAE enveloped the gold core as a thin coating. Additionally, these molecules served the dual purpose of reducing, capping, and stabilising agents, leading to the creation of stabilised NPs (Fig. 1h). The disparity in particle size between the DLS technique and SEM and HR-TEM may be attributed to this factor.

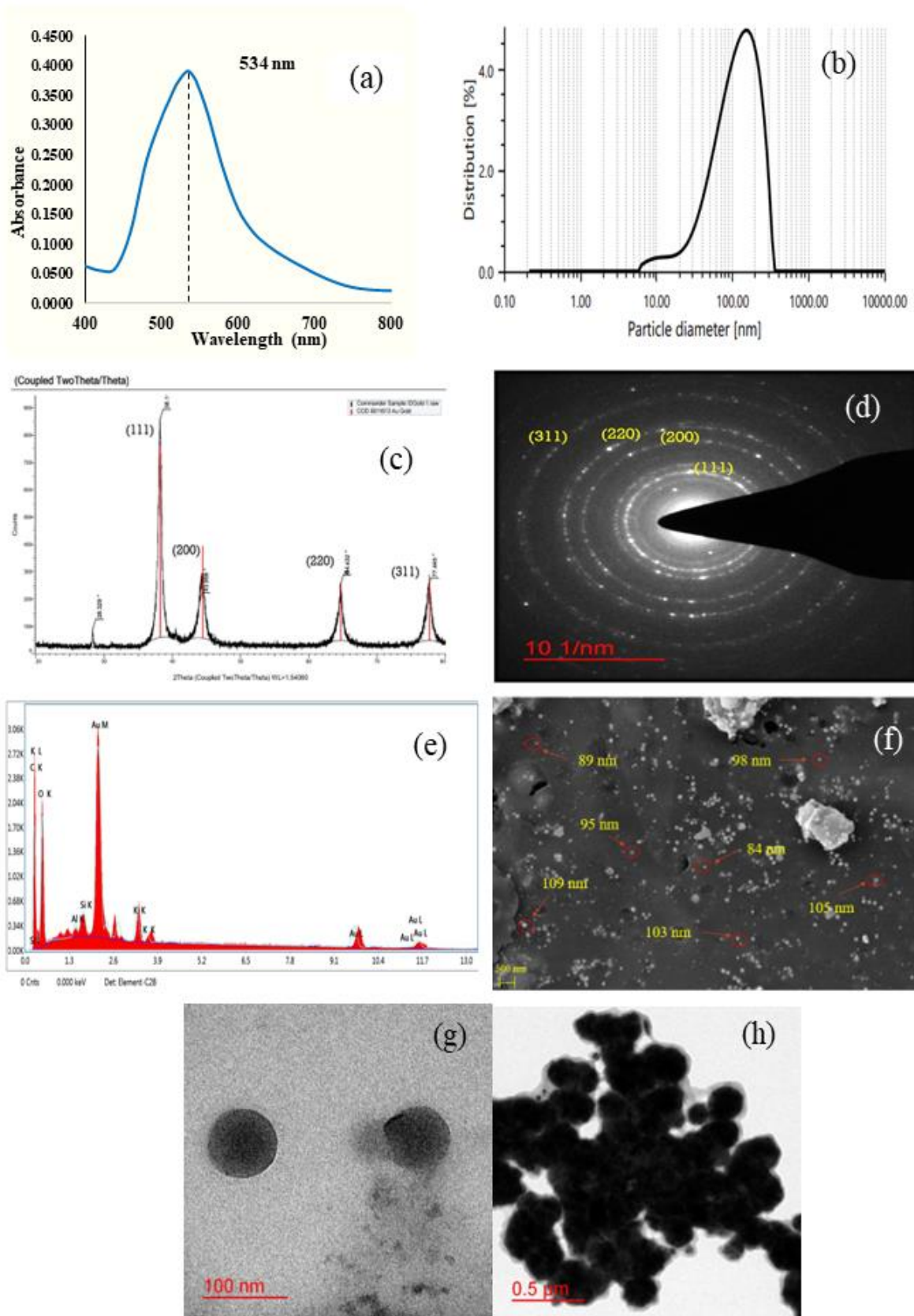


Fig. 1. Characterization of synthesised AuNPs at optimum condition. (a) SPR peak, (b) Hydrodynamic particle size and size distribution, (c) XRD diffractogram, (d) SAED pattern, (e) EDX spectra, (f) SEM images, and (g and h) HR-TEM images.

Fourier transform infrared (FTIR) spectroscopy

The optimised MMAuNPs' FTIR spectra showed the existence of strong peaks at 343.52, 1375.20, 1025.48, 3036.13, 2818.36, 2881.36, 1964.57, 1638.20, 1508.40, and 669.56 cm⁻¹ (Fig. 2a). The -OH link of an alcohol group may be the cause of the large peaks at 3036.13 and 2818.36 cm⁻¹. The alkane group's C-H stretching was the cause of the peaks at 2881.36 cm⁻¹. Allene's C=C=C stretching may be the cause of the peak at 1964.57 cm⁻¹. The existence of the alkene group's C=C bond was the cause of the 1638.20 and 669.56 cm⁻¹ peaks. The nitro compound of the N-O group was the cause of the existence of 1508.40 cm⁻¹. The primary cause of the absorption peaks at 1438.52 and 1375.20 cm⁻¹ was the carboxylic acid and phenol groups' O-H bending, respectively. The stretching of the aliphatic amine chain at position C-N is responsible for the peak at 1025.48 cm⁻¹. These peaks resembled those in the FTIR spectra of MMAE (Fig. 2b) quite a little, suggesting that many MMAE biomolecules are involved in the production of MMAuNPs. Functional groups that operate as reducing, capping, and stabilising agents of the metal ions, such as phenolics, flavonoids, tannins, amino acids, and carbohydrates, may be involved, according to this FTIR investigation. The phytochemical analysis results verified that these phytoconstituents were present in the MMAE. Many phytochemicals, such as terpenoids, ketones, flavonoids, proteins, tartaric acid, alkaloids, and aldehydes with functional groups like hydroxyl, amide, carboxylic acid, and methylene, capped AuNPs synthesised from *Nepenthes khasiana* leaf extract [51], which is quite similar to our findings.

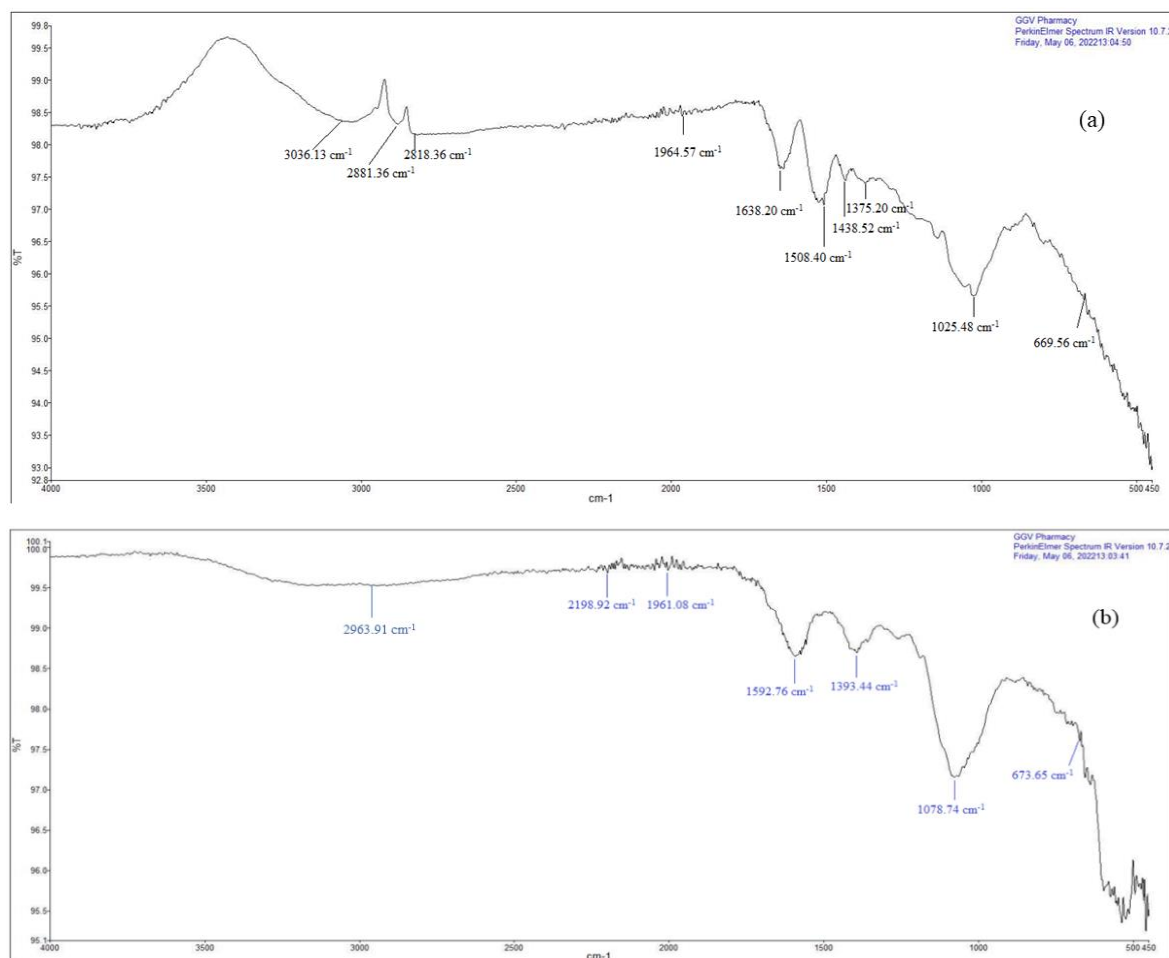


Fig. 2. FTIR spectra of (a) *M. Micrantha* AuNPs and (b) *M. micrantha* aqueous extract.

Biological evaluation of synthesized gold nanoparticles

DPPH radical scavenging activity

The antioxidant potential of MMAE and green synthesized MMAuNPs at various concentrations (15.625, 31.125, 62.50, 125, 250, and 500 $\mu\text{g/mL}$) was studied in this work. Both MMAE and MMAuNPs produced dose-dependent antioxidant potential, as shown in Fig. 3a. According to percentage scavenging activity, the antioxidant activity increased as the extract and MMAuNPs concentrations increased. The IC_{50} values of MMAuNPs and MMAE were 273.37 and 398.25 $\mu\text{g/mL}$, respectively. The hydrogen donating activities of antioxidant materials are recognised to be responsible for DPPH scavenging capacities. When DPPH free radical scavenging activity was examined, it was determined that AuNPs boosted their activity in a dose-dependent manner. Green-mediated AuNPs by using medicinal herbs have greater antioxidant actions against the generation of free radicals in the biological system. The green-formulated AuNPs have outstanding redox characteristics and are significant in the deactivation of free radicals. A previous study has shown that flavonoids and phenolic chemicals bound to metallic nanoparticles have considerable antioxidant effects [57].

ABTS•+ radical scavenging assay

The production of ABTS free radicals from the oxidation of ABTS with potassium persulfate is a great way to assess the antioxidant nature of compounds which donate hydrogen and breaks the oxidizing chain reaction [39]. By boosting the concentration of produced AuNPs, the ABTS radical cation scavenging activity steadily increased. The antioxidant potential of MMAE and MMAuNPs in the concentration range of 15.625-500 $\mu\text{g/mL}$ was evaluated through ABTS assay model. The findings are depicted in Fig. 3b. The IC_{50} values clearly indicate improved antioxidant potential of the corresponding MMAuNPs (264.57 $\mu\text{g/mL}$) when compared to MMAE (413.35 $\mu\text{g/mL}$). The findings were found to be in-sync with the earlier published report where the AuNPs of *Camellia sinensis*, *Thymus vulgaris*, *Gundelia tournefortii* L., *Falcaria vulgaris* [57], *Citrus aurantifolia* [58], *Elaeocarpus ganitrus* [59], and *Ziziphus nummularia* [38] Showed improved antioxidant potential when compared to their mother extract.

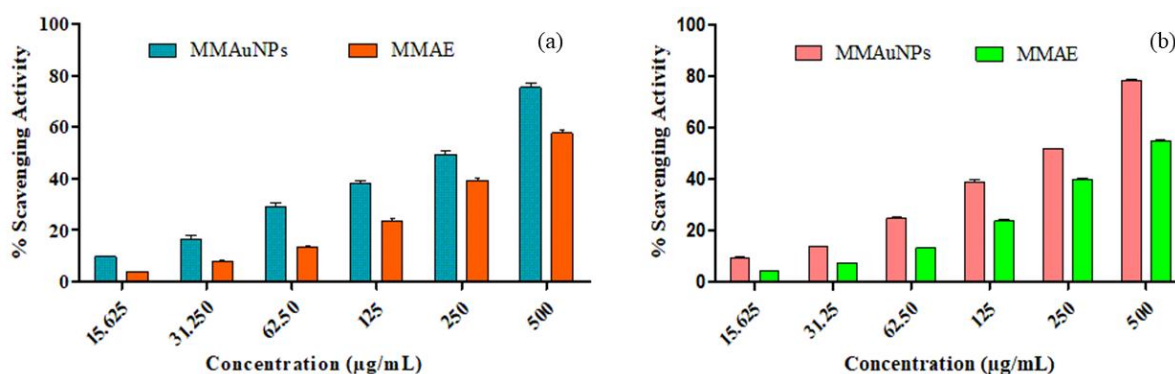


Fig. 3. Antioxidant activity of *M. Micrantha* AuNPs and *M. micrantha* aqueous extract by (a) DPPH free radical scavenging assay and (b) ABTS free radical scavenging assay. The values are represented as mean $\pm$ SEM (n=3).

The higher concentration of biomolecules on the surface of the MMAuNPs improved antioxidant potential of NPs can be attributed to its tiny size and high surface-to-volume ratio which proved effective in the termination of oxidizing chain reaction resulting in neutralization of unstable free radicals [38]. Due to their various oxidation states, such as Au^{+1} - Au^{+3} , gold nanoparticles readily receive or give electrons to scavenge free radicals. DPPH and ABTS assays showed that AuNPs made from onion peel extract (OPE) had strong antioxidant activity even stronger than the standards. The phytoconstituents of OPE on the NP surface may be the cause of the increased antioxidant effectiveness of AuNPs [60]. The antioxidant results provided a major source of information for taking forward the assessment of MMAuNPs as a potential anticancer agent which is described in the later section.

Anticancer activity

The effectiveness of various doses of MMAuNPs (ranging from 6.25 to 400 $\mu\text{g/mL}$) against breast cancer cells (MCF-7 and MDA-MB-231) was assessed using the MTT test. The generated MMAuNPs exhibited dose-dependent cytotoxicity, meaning that as the concentration of nanoparticles grew, the proportion of viable cells dropped. Fig. 4 illustrates the efficacy of bioengineered gold nanoparticles against MDA-MB-231 and MCF7 cells at different doses. The synthesised MMAuNPs exhibited strong cytotoxicity, with IC_{50} values of 157.1 and 174.2 $\mu\text{g/mL}$ against MCF-7 and MDA-MB-231 cells, respectively. The results of this investigation were superior than those reported by Oueslati M. H. and colleagues. In their work, AuNPs produced from the extract of the aerial portion of Lotus (Leguminosae) did not show 50% inhibition even at a concentration of 1000 $\mu\text{g/mL}$ [61]. The IC_{50} value obtained in the present work was found to be similar to the IC_{50} value (160 $\mu\text{g/mL}$) of AuNPs produced from broccoli extract, as reported by Khoobchandani M. and colleagues [62]. The presence of positively charged surface functional groups on AuNPs enhances their potential to interact with cells, making them a main contributor to their cytotoxic properties.

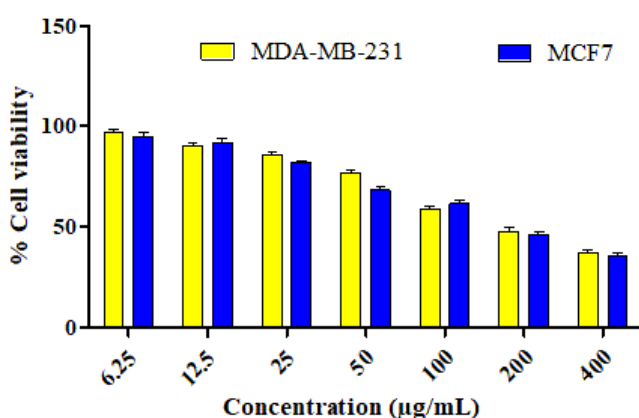


Fig. 4. Anticancer activity of *M. micrantha* AuNPs against MDA-MB-231 and MCF-7 (breast cancer cells). The values are represented as mean $\pm$ SEM (n=3).

Impact of MMAuNPs on Blood Brain Barrier

The sedative and antinociceptive action of MMAuNPs was evaluated in order to determine their capacity to pass the blood-brain barrier. When compared to the control group, MMAuNPs treatment had no discernible

impact on the locomotor counts of the mice. Nevertheless, Fig. 5a illustrates that mice given diazepam (2 mg/kg) exhibited a noteworthy increase in locomotor activity in contrast to the control group ($P < 0.001$). Since hydrophilicity is by no means a prerequisite for MMAuNPs to penetrate the blood-brain barrier, it is possible that this study's results reflect the fact that MMAuNPs did not exhibit a statistically significant response in locomotor count. As a result, MMAuNPs did not show any effect on CNS and also did not exhibit sedative response as well. On the other hand, the standard drug diazepam showed four times decrease locomotor count due to CNS depressant properties. The tail immersion test was used to measure antinociceptive activity. In the tail immersion test, mice treated with MMAuNPs (10 and 20 mg/kg) did not significantly alter tail flick latency time when compared to the control group, showing no antinociceptive-like action (Fig. 5b). In contrast to the control group, mice administered with the conventional medication (aspirin, 50 mg/kg) exhibited significant activity ($P < 0.001$), with a four-fold increase in tail flick latency time (Fig. 5b). The MMAuNPs treated mice did not show a significant response in the tail immersion test. May be the water soluble MMAuNPs cannot cross the blood brain barrier, and hence there was no engagement with the opioid receptors as a result, no central pain perception effect on experimental mice treated with MMAuNPs was observed [63].

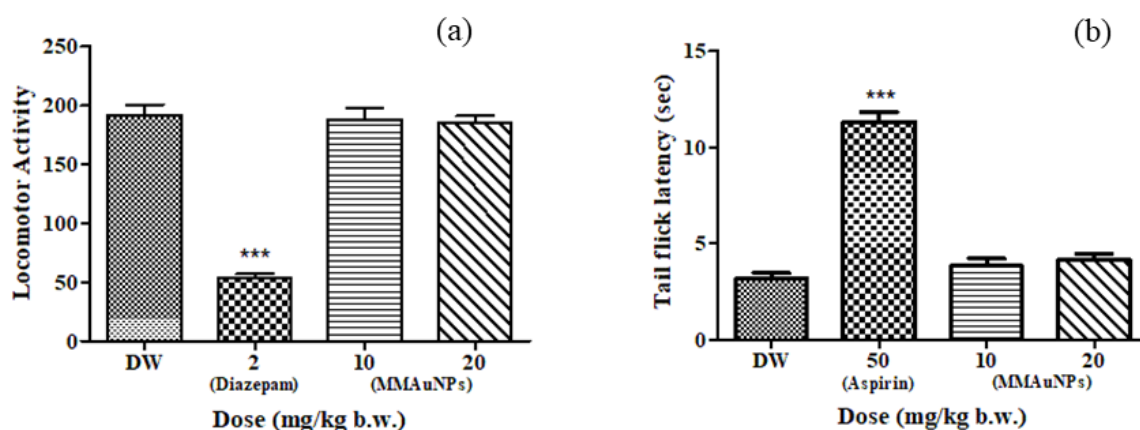


Fig. 5. *In-vivo* activities of MMAuNPs in Swiss Albino mice. (a) Locomotor activity using actophotometer, and (b) antinociceptive activity by tail immersion test. Each bar represents the mean $\pm$ SEM of 6 animals. *** $p < 0.001$ vs DW (One way ANOVA followed by Neuman-Keuls multiple comparison test).

Conclusion

This study clearly highlights that microwave based AuNPs synthesis approach can be effectively used as the valorisation strategy for the management of worst weed of the environment, *M. micrantha*. Results are very promising as it was seen that *M. micrantha* in its AuNPs form exhibited potential antioxidant activity with IC_{50} value less than 34% when compared with crude extract form. Noteworthy, lesser is the IC_{50} value higher is the antioxidant potential. The findings of the antioxidant studies laid an interesting foundation for assessing the anticancer potential of the one of the notorious weed *M. micrantha*. The current study's anticancer activity assessment was based on the significant link between antioxidant activity and cancer treatment [64, 65]. The MMAuNPs showed promising cytotoxic activity and results were comparable with other published plant based AuNPs. However, intense scientific intervention is required before establishing its anticancer properties. Through

this research it is clearly evident that nature creation never goes in vain and even one of most notorious weeds has something to offer to the mankind. The research could be an eye opener in terms of making the best out of the waste. However, further research is required to assess the *in vivo* actions of MMAuNPs before making them widely available in the area of medicine, either as stand-alone treatments or as drug carriers.

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Author Contribution

SD, AD, VT, and SS: Investigation, Methodology, Experimental work, and Writing - original draft; VY, DM, SM, and AK: Data analysis, Visualization, and Statistical analysis; ANS, MK, MDH, MG, and VM: Investigation, Writing - review & editing; AP: Conceptualization, Writing - original draft, review & editing, Supervision. All authors read and approved the submitted version.

Data availability

All data are described and available in the article.

Statement and Declaration

Conflicts of interest

The authors declare that there is no conflict of interests.

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