Book Chapter

Green Synthesis and Biomedicinal Applications of Silver and Gold Nanoparticles Functionalized with Methanolic Extract of Mentha Longifolia

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Published **April 23, 2024**
This Book Chapter is a republication of an article published by Ajmal Khan, et al. at Artificial Cells, Nanomedicine, and Biotechnology in February 2021. (Rauf, A., Ahmad, T., Khan, A., Maryam, Uddin, G., Ahmad, B., … Al-Harrasi, A. (2021). Green synthesis and biomedicinal applications of silver and gold nanoparticles functionalized with methanolic extract of Mentha longifolia. Artificial Cells, Nanomedicine, and Biotechnology, 49(1), 194–203.
https://doi.org/10.1080/21691401.2021.1890099)

How to cite this book chapter: Abdur Raufa, Touqeer Ahmad, Ajmal Khan, Maryam, Ghias Uddin, Bashir Ahmad, Yahia Nasser Mabkhot, Sami Bawazeer, Nadia Riaz, Bates Kudaibergenova Malikovna, Zainab M. Almarhoon, Ahmed Al-Harrasi. Green Synthesis and Biomedicinal Applications of Silver and Gold Nanoparticles Functionalized with Methanolic Extract of Mentha Longifolia. In: Dr. Zahoorullah S MD, editor. Prime Archives in Biotechnology. Hyderabad, India: Vide Leaf. 2024.

Acknowledgements: The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through Research group Project under grant number [R.G.P. 2/26/42].

Disclosure Statement: No potential conflict of interest was reported by the author(s).s

Correction Statement: This article has been republished with minor changes. These changes do not impact the academic content of the article.

Abstract

This study deals with facile and rapid synthesis of silver nanoparticles (AgNPs) and Gold nanoparticles (AuNPs) using *Mentha longifolia* leaves extracts (MLE). The synthesized AgNPs and AuNPs were characterized by UV-visible spectroscopy (UV-Vis), Fourier transformed infra-red spectroscopy (FT-IR), atomic force microscopy (AFM) and transmission electron microscopy (TEM) techniques. The phytochemical analysis showed the presence of bioactive secondary metabolites, which are involved in the synthesis of nanoparticles (NPs). The surface plasmon resonance (SPR) observed at 435 and 550 nm, confirmed the green synthesis of AgNPs and AuNPs, respectively. The TEM images showed poly dispersed and round oval shapes of Ag and Au NPs with an average particles size of 10.23 ± 2 nm and 13.45 ± 2 nm, respectively. TEM results are in close agreements with that of AFM analysis. The FT-IR spectroscopy revealed the presence of OH, $-NH_2$ and C = O groups, which involved in the synthesis of NPs. The MLE and their AgNPs and AuNP exhibited good *in vitro* antibacterial and anti-oxidant activities. Moreover, MLE and NPs also showed *in vivo* analgesic activities in mice, and excellent sedative properties in open field test paradigm.

Keywords

Green Synthesis; AgNPs; AuNPs; Antibacterial; Antioxidant; Analgesic; Sedative

Introduction

Nanotechnology is considered as a significant tool to unveil the hidden secrets of nature, such as natural products coated metallic nanoparticles (MNPs) are nowadays considered for their extensive use in scientific arena to decode the natural enigmas. Green synthesis of MNPs has gained momentum in the last decade due to its cost-effectiveness. Moreover, it is environmentally friendly, and easily scalable for commercial purposes [1–5]. Various types of molecules are used for the green synthesis of MNPs, among them natural products are considered as the best candidates, as they can be used effectively

for reduction as well as stabilization of the MNPs [6–8]. Moreover, natural products coated MNPs have significant medicinal and pharmaceutical applications [9–13]. A large number of medicinal plants have been used for the synthesis of AgNPs and AuNPs, including leaves of *Dodonaea genus* [14] *Cinnamomum camphora*, [15] *Aloe vera* [16] and Amla (*Emblica officinalis*) [17]. Triangular synthesis of AuNPs has been performed using extract of *Aloe vera* plant [16]. *Magnolia kobus* and *Diopyros kaki* leaf extract has also been used for successful synthesis of metallic AuNPs [18]. Sun dried *Cinnamomum camphora* leaves [15] and purified apiin compound from henna leaves [19] are famous for photosynthetic synthesis of AuNPs. Vijayan *et al.* have reported green synthesis of AgNPs and AuNPs from *Bauhinia purpurea* and examined their potential against lung carcinoma cell line A549 in a dose-dependent manner with IC_{50} values of 27.97 μg/mL and 36.39 μg/mL, respectively. Synthesized NPs showed a significant increase in anticancer in contrast to plant extract [20,21]. Later Ghramh and co-worker used *Euphorbia peplus* leaves extract for MNPs synthesis and tested their anticancer activity against Hela and HepG2 cell lines, antimicrobial, haemolytic and insecticidal activities [22]. Different fractions of *Musa acuminata* colla flower extract were used to synthesise AgNPs and AuNPs, and their antibactrial activity against spectrum beta-lactamase (ESBL) gene-producing bacteria and anticancer efficacy considerable after NPs formation [23,24]. AgNPs exhibit immense application in agriculture and medicine as antibacterial, antifungal and antioxidants [21,25].

In the current studies, MLE was used for the green synthesis of AgNPs and AuNPs [26,27]. *Mentha longifolia* is distributed in southern Africa, Botswana, Namibia, Zimbabwe [28], Europe, Western and Central Asia. *M. longifolia* is a fast-growing perennial herb with a peppermint-scented fragrance. It has a creeping rhizome, with a stem 40–120 cm tall. The leaves vary from oblong-elliptical to soft, lanceolate (long and narrow with a sharp point) 5–10 cm long and 1.5–3 cm broad, and the colour varies from green to greyish-green above and white below. The purple and white colour flowers are produced at the tip of the branched, tapering and tall spikes in the form of cluster.

Flowering starts from mid to late summer. It also forms colonies by scattering through rhizome [29].

Mentha longifolia like other members of this family is used as a domestic herbal remedy, the whole plant is used as a medicine and sometimes rhizomes of the plant is also used [30]. A tea made from the leaves has traditionally been used to treat of fevers, headaches, digestive disorders and various minor ailments [31]. Moreover, the essential oil of this plant has numerous applications like anti-microbial activity, decongestant, anti-spasmodic effects, etc. The medicinal properties include diuretic effects [32], carminative, stomachic, digestive health improver and anti-inflammatory agent in folk medicines [33]. The essential oils and methanol extract of *M. longifolia L.* have reported for their antimicrobial and antioxidant properties [34]. The essential oil of *M. longifolia* L. also possess calcium channel blocking activity [35], hepatoprotective [36] and insecticidal activities [37]. Literature showed that two main classes, terpenoids and flavonoids, have been reported from *M. longifolia* [38–40]. The antimicrobial activities for these two class have also been reported [41]. Javed et al. synthesized AgNPs of *M. longifolia* to check their inhibitory potential against various bacterial strains [42]. Here, we report the rapid green synthesis of AgNPs and AuNPs using *M. longifolia* leaves extracts and examined their *in vitro* and *in vivo* pharmacological activities and also study their analgesic activities in mice.

Material and Methods

All solvents used were HPLC grade and purchased from Sigma-Aldrich. Silver nitrate ($AgNO_3$) and gold chloride ($H[AuCl_4]$) were purchased from Merck. Sodium hydroxide (NaOH), hydrochloric acid (HCl), and sodium citrate were purchased from Sigma Aldrich. Double-distilled water was used throughout the experiment. The pH effect was checked using a pH metre (Oakton, Eutech) equipped with glass working electrode.

Collection of Plant Material and Extraction

Fresh leaves of *M. longifolia* were collected from different location of Barawal, District Upper Dir, KPK, Pakistan at attitude of 5000–7000 m (35° 00′ 45.27″N and 71° 50 40.14″E).

Shade dried leaves of *M. longifolia* were ground with a grinder. The dried leaves (1kg) were then soaked in methanol for 5 days. The obtained extract was filtered and concentrated by using negative pressure in rotary evaporator at 40 °C to get a dark green extract (22.5 gm).

Phytochemical Screening

The extracted plants crudes were analysed for the presence of secondary metabolites (alkaloids, tannins, anthraquinones, reducing sugars, glycosides, saponins, flavonoids, phlobatanins, steroids, terpenoids, coumarin, emodins, anthocyanin, betacyanins and carbohydrates) using standard procedures [43,44].

Preparation of Stock and Salt Solutions

A 0.1 g MLE was dissolved in 100 ml of methanol to prepare a stock solution. Salt solutions having 1 mM concentration were separately prepared by using $AgNO_3$ and $H[AuCl_4]$ in deionised water. Both salt solutions were then placed in the refrigerator.

Synthesis of AuNPs and AgNPs

Around 1 mM solution of $AgNO_3$ and $H[AuCl_4]$ were prepared by dissolving 42.46 mg and 84.94 mg in 250 ml of double-distilled water, respectively. Similarly, 0.1 g methanolic extract of plants *M. longifolia* was dissolved in 100 ml of methanol and dilute the solution up to 500 ml by adding of water. The extract solution was filtered to remove undissolved extract component. Reaction was optimised using different metal to extract ratio. After mixing plant extract and metal at 70 °C at different ratios, reaction mixture was kept on stirring. Slight change in solution colour (greenish for AgNPs and reddish for AuNPs) was noticed at early moments (2–3 min) and reaction colour become more intense over constant stirring with the passage of time. Change in solution colour is primary indication of NPs formation. Later NPs syntheses was further confirmed via recording UV-Vis spectra of AuNPs and AgNPs. The ratio which gives best absorbance peak was used to synthesise NPs. The AgNPs and AuNPs were synthesized by mixing separately salt solution with a stock solution in 1:1 ratio at 70 °C; a colour change indicated

the reduction process showing the synthesis of AgNPs and AuNPs. In contrast to previous studies, we synthesized AuNPs and AgNPs using *M. longifolia* plant extract without altering reaction pH. More importantly NPs syntheses were carried out at lower salt concentration in comparison to previous studies where higher concentration of silver salt was used. Synthesized NPs were centrifuged at 1500 rpm for 20 min, followed by washing with double-distilled water and vacuum-dried. Vacuum-dried NPs were used for further characterization, that is, TEM, AFM and FT-IR spectroscopy.

Characterization of AgNPs and AuNPs

The synthesized AgNPs and AuNPs were characterized by UV-Vis spectroscopy (Shimadzu UV-240, Hitachi U-3200, Japan), FT-IR (Prestige-21 Shimadzu, Japan), AFM (Agilent Technologies 5500, USA) Agilent 5500 operated in tapping mode and TEM (TEM-1400 Electron Microscope, JEOL), operated at an accelerating voltage of 110 kV. Few drops of AuNPs and AgNPs solution were placed on the carbon grid and allowed to dry prior to measurement.

Antioxidant Activity

The antioxidant activity was performed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, through previously reported protocol [45]. The electron donation abilities of the corresponding crude extracts, synthesized NPs and standards (*n*-Propyl gallate) were measured by changing the purple-coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). About 9.5 mg DPPH was dissolved in 50 ml methanol to make a solution. The stock solution of plant crude extract was prepared by dissolving 25 mg of the extract in 50 ml methanol, while NPs solution was prepared by adding 5 mg of NPs in 5 ml methanol. Different concentrations were made from a stock solution of crude extract and from NPs, that is, (10, 20, 40, 80, 100, 150, and 250 µg/mL) and were mixed with 1 ml of DPPH solution, while the control has only methanol and DPPH solution. The solutions were kept for 30 min in the dark then absorbance was recorded at 517 nm. The decrease in the absorbance of DPPH solution indicates increase in the antioxidant activity of samples. Antioxidant activity by DPPH as

percent radical scavenging activities (% RSA) was calculated as follows.

% DPPH = (OD control - OD sample) / OD control × 100

Antibacterial Activity

The MLE and synthesised NPs were checked for their antibacterial activities against various bacterial strains, that is, *Klebsiella pneumoniae* (ATCC: 13882) *(K. pneumoniae)*, *Staphylococcus aureus* (ATCC: 25923) *(S. aureus)*, *Bacillus subtilis* (ATCC: 21332) *(B. subtilis)*, using the standard protocol [46]. *K. pneumoniae*, a Gram-negative, while *S. aureus* and *B. subtilis* as Gram-positive bacteria were subjected to trials. These organisms were stored in Muller-Hinton agar (MHA) in the refrigerator at 4 °C. Modified agar well diffusion procedure was followed to study antibacterial activity of MLE and synthesised NPs. (MHA) agar was used as a growth medium. The cultures were cultivated and then incubated at 37 °C for 24 to 72 h, and taken as triplicate. Sterilized Petri-dishes were used for broth culture, 0.6 ml of the broth culture was added and Petri-dishes were sterilized first and then 20 ml sterilized molten MHA was added to each petri-dish. Wells were bored in the medium and 0.2 ml volume of crude extract was added to each well with the help of a micro pipette, while 2 mg/mL of synthesized NPs were used to examine their antibacterial ability. Streptomycin (2 mg/mL) was used as a standard drug. Petri-dishes were kept in a laminar flow hood for 1 h for proper diffusion, later plates were incubated at 37 °C for 24 h. Next day the zone of inhibition was measured [47].

Animals

BALB/c mice (20–28 g) were used in all experiments purchased from the National Institute of Health (NIH), Islamabad. Pakistan. They were fed with standard laboratory food and water *ad libitum*. Animals were kept under the standard condition of temperature and light. Before the start of the experiment, animals were acclimatized with laboratory conditions. The rulings of the institutes of Laboratory Animal Resources, Commission on Life Sciences, National Research Council were maintained during all the experiments. The experimental protocols were approved by

the ethical committee (5/pharm) of the Pharmacy Department, University of Peshawar, Peshawar, Pakistan.

Acetic Acid Induced Writhing Test

The peripheral nociceptive activity of crude extracts and synthesized NPs was investigated by acetic acid-induced abdominal constriction test [48–50]. The pre-screened animals were segregated into various groups ($n = 6$) and each group contain six animals of weighing 18–22 g. The pain was induced by intraperitoneal injection of 0.9% acetic acid (*v/v*, 0.1 ml/10 g body weight). One group of six animal received normal saline (10 ml/kg, i.p.) as control, second group received diclofenac (10 mg/kg i.p.) as a standard (a mostly commonly used standard drug for the antinociceptive test). The remaining groups received extracts and synthesized NPs at the dose of 50 and 100 mg/kg, i.p., respectively. The number of muscular contractions was counted over a period of 20 min after acetic acid injection. The number of writhes in each treated group was compared with control (Saline treated group).

Sedative Activity

The apparatus design for sedative potential consists of an area of white wood (150 cm diameter) enclosed by stainless steel walls and divided in 19 squares by black lines. The open field was placed in a light and sound-attenuated room. Balb/c mice of either sex ($n = 6$) weighing 26 ± 4 g were used in this study. Animals were acclimatized under red light (40 Watt red bulb) one hour before the commencement of the experiment in laboratory with food and water available *ad libitum*. Animals were administered with distilled water as a blank (10 ml/kg) MLE (50 and 100 mg/kg), AgNPs and AuNPs (5 and 10 mg/kg, respectively) or diazepam (0.5 mg/kg, i.p). After 30 min each animal was placed in the centre of the box and the number of lines crossed was counted for each mouse [48,50].

Statistical Analysis

Results were expressed as mean ± SEM. One-way ANOVA was used to compare test of significant differences among groups, followed by Dunnet's multiple comparison post-test. A level of

significance (p<.05 or .01) was considered for each test. The results of antibacterial and antioxidant activities are also presented as mean ± SEM of three different readings and their statistical analysis was carried out using the GraphPad program (GraphPad, San Diego, CA, USA).

Results and Discussion

The results of phytochemical screenings of MLE are presented in Table 1. The phytochemical analysis showed the presence of bioactive secondary metabolites. Results of MLE indicated the presence of tannins, reducing sugar, saponins, terpenoids, steroids, cardiac glycosides, coumarin, anthocyanin, carbohydrates and soluble starch.

Table 1: Effect of phytochemical tests of MLE.

Chemical components	Methanolic extract
Alkaloids	_
Tannins	+
Anthraquinone	_
Glycosides	_
Reducing sugar	+
Saponins	+
Flavonoids	+
Phlobatanins	_
Steroids	+
Terpenoids	+
Cardiac glycosides	+
Coumarins	+
Emodins	_
Anthocyanins	+
Carbohydrates	+
Monosaccharide's	_
Free Reducing Sugar	_
Combined reducing sugars	_
Soluble starch	+

Synthesis of NPs

The colour change easily indicates the reduction process followed by synthesis of AgNPs and AuNPs for methanolic and aqueous extracts. The colour of MLE was changed from green to brown for AgNPs (Figure 1(A–C)), and green to pink while in case of AuNPs (Figure 1(D–F)), which indicates the formation of

these MNPs [51]. The UV-Visible spectra of AgNPs and AuNPs revealed their characteristic SPR at 435 nm and 550 nm, respectively as shown in Figure 2 [51,52]. The aqueous extract did not form any stable NPs at any ratio.

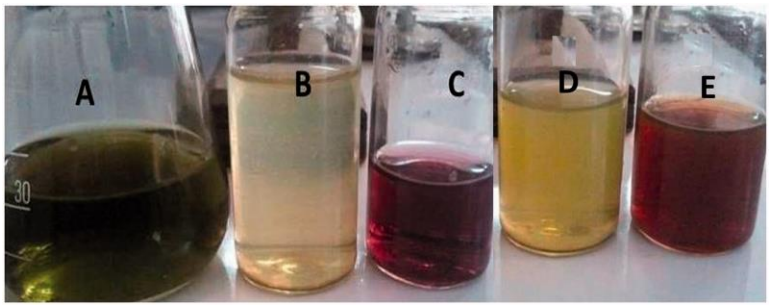

Figure 1: Image of (A) MLE have green colour due to the presence of UV active natural product, (B) Silver Solution are colourless due to the UV inactive ions (C) AgNPs having brown colour due to the surface plasmon resonance (D) Gold solution having no colour due the UV inactive ions of gold and (E) AuNPs having pink colour due to the surface plasmon resonance.

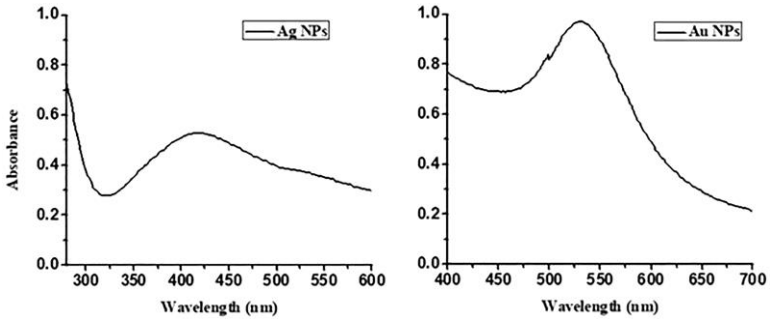

Figure 2: UV-Visible spectrum of (A) AgNPs and (B) AuNPs of MLE.

FT-IR Analysis

The plant contains a large number of chemical constituents such as flavonoids, tannins, alkaloids, monoterpenes and saponins, which plays important role in the synthesis of AgNPs and AuNPs. These compounds may actively involve in the reduction of Ag and Au ions to metallic silver and gold [51]. The crude methanolic extract showed a broad peak at 3400 cm^{-1} that confirmed the presence of –OH and $–NH_2$ group (flavonoids [53], carbohydrates, terpenoids [54], tannins [53], saponins

[54,55], reducing sugar [56], cardiac glycosides [56], steroids [53], and coumarine) and a sharp peak at 2900 cm^{-1} corresponds to $–NH_2$ stretching. A characteristic peak at 1700 cm^{-1} was observed by C = O stretching (carbonyl group of carbohydrates, glycosides, coumarine and tannins) [53]. In case of AuNPs and AgNPs, the intensity of –OH peak further increased, while the peak at 3000 cm^{-1} completely disappeared, indicating that –OH and $–NH_2$ were involved in the formation of NPs [57]. The peak observed for C = O at 1700 cm^{-1} in the extract was also enhanced and red shift was noticed 1650 cm^{-1}, which strongly supported the formation of Ag and Au NPs (Figure 3). Phytochemical analysis indicated the presence of flavonoids, steroids, saponins, cardiac glycosides, tannins, alkaloids, terpenoids and anthraquinones. FT-IR analysis revealed the presence of phenolic flavonoids, saponins, tannins as major functional groups [53]. Yassin et al. studied methanolic extract against cancer cells and confirmed the presence of above compound with certain functionality via GC-MS analyses [58]. Therefore, our results are in close agreement with the findings of Yassin et al. FTIR spectral analysis of *M. longifolia* plant extract and silver and gold NPs are given in Table 2.

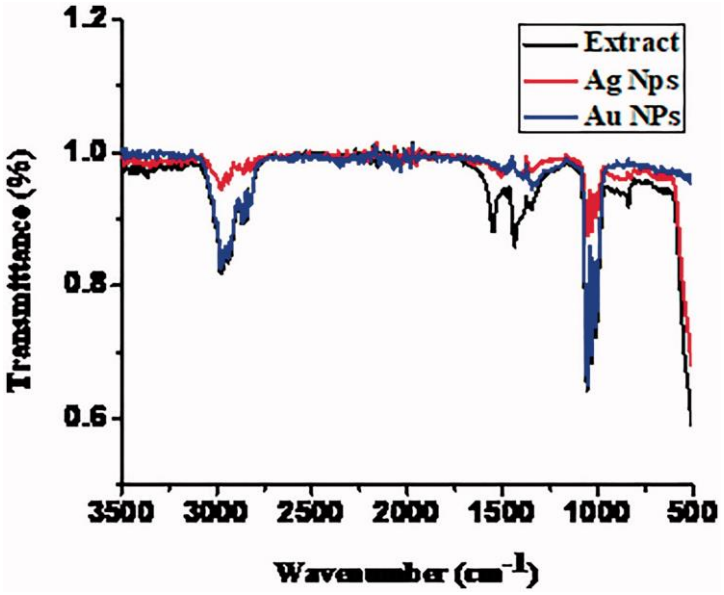

Figure 3: FTIR spectrum recorded for (A) MLE, (B) AgNPs and (C) AuNPs.

Table 2: FTIR spectral analysis of MLE, and AuNPs and AgNPs.

MLE	AgNPs	AuNPs	Assignment	Ref
3600–3100 cm^{-1}	3600–3100 cm^{-1}	3600–3100 cm^{-1}	–OH (H-bondong)	[Citation13]
2900 cm^{-1}	---	---	$–NH_2$	[Citation59]
1700 cm^{-1}	1650 cm^{-1}	1650 cm^{-1}	–C = O	[Citation60]
1500 cm^{-1}	---	---	COO of protein side chain	[Citation61]
1000 cm^{-1}	---	---	C–H and –OH deformation	[Citation59]

AFM Imagining

AFM used to examine the morphology, size and shape of the synthesised AgNPs and AuNPs [51,62]. AFM images of AgNPs and AuNPs are shown in Figure 4. AgNPs and AuNPs were round oval in shape with the size ranges from 10–15 nm and 20–60 nm, respectively.

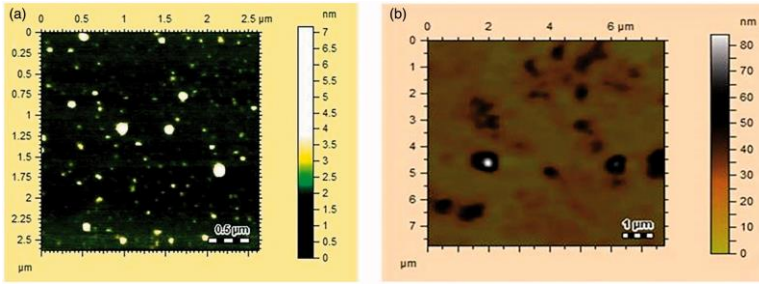

Figure 4: Atomic force microscopy images of (A) AgNPs) and (B) AuNPs of MLE.

TEM Analysis

The size of AuNPs and AgNPs of *M. longifolia* was studied through transmission electron microscopy (TEM, TEM-1400 Electron Microscope, JEOL, Japan) [11]. Average particles sizes of AgNPs and AuNPs were calculated using Image J software. As depicted in Figures 5(A,B) and 6(A,B), TEM showed that AuNPs and AgNPs were round oval in shape with an average particles size of 10.23 ± 2 and 13.45 ± 2 nm, respectively, which is in close agreement with AFM analysis.

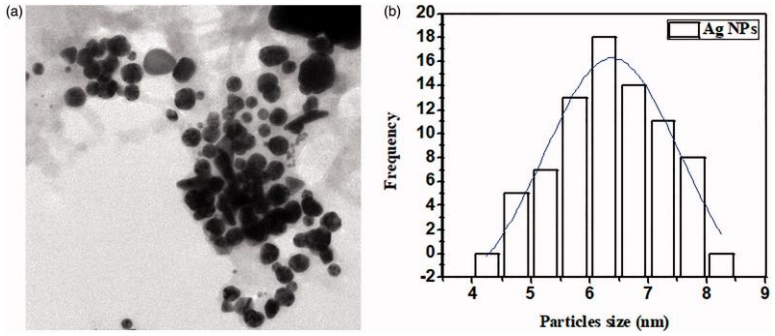

Figure 5: TEM images of (A) showing particles size distribution of AgNPs (B) Average particles size and size distribution of AgNPs.

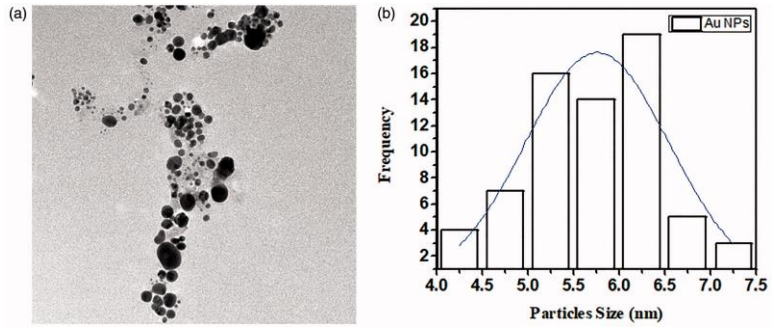

Figure 6: TEM images of (A) showing particles size distribution of AuNPs (B) Average particles size and size distribution of AuNPs.

DPPH Radical Scavenging Assay

Plant source antioxidants have the potential to reduce different diseases. Most of the antioxidant compounds in a typical diet are derived from plant sources caring wide range of physical and chemical properties [63,64]. DPPH is a stable free radical compound with ability to trap free radicals, and use to study the scavenging effect of plants with a characteristic absorption at 517 nm. The antioxidant profile of MLE and AuNPs and AgNPs are presented in Figure 7 which display the maximum antioxidant potential of crude extract and NPs at different concentration. The methanolic extract of the plant along with NPs exhibited varying degrees of scavenging abilities at dose depended manners (Figure 7). Result showed that highest RSA

was observed in AgNPs as compared to AuNPs at 80 µg/mL. The degree of discolouration indicates the scavenging potentials of the antioxidant present in MLE and both NPs.

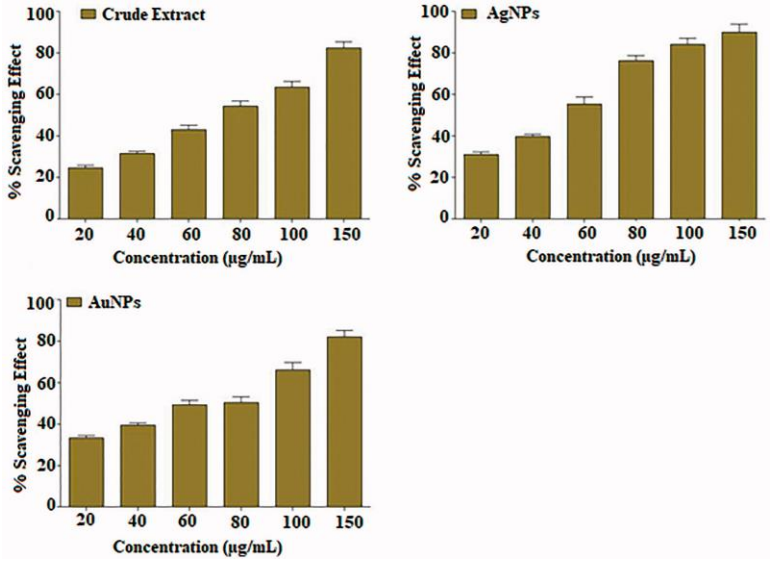

Figure 7: DPPH radical scavenging activity of MLE and NPs with 20–150 µg/mL. The results are presented as mean ± SEM of three different readings SEM: standard error mean.

Antibacterial Assay

Antibacterial activity of MLE and their NPs are presented in Table 3 in comparison with the standard drug, Streptomycin [52]. MLE did not show any activity; however, their synthesized NPs exhibited some degree of antibacterial activity against human pathogenic microorganisms such as *K. pneumoniae, S. aureus* and *B. subtilis* bacterial strains. The zone of inhibition for NPs of MLE ranged from 10 to 12 mm. The MLE and their respective AuNP's and AgNP's did not show any potential activity against *K. pn.* While both AuNPs and AgNPs of the MLE showed good inhibition of *S. aureus* with zone of inhibition = 10 ± 0.01 and 12 ± 0.03 mm, respectively. Whereas, only AgNPs showed good inhibitory activity for *B. subtilis* with zone of inhibition 10 ± 0.01 mm.

Table 3: Antibacterial activity of MLE and AuNPs and AgNPs.

Microorganisms		Zone of inhibition (mm at 3 mg/mL) ± S.E.M.			
	Gram	MLE	AgNP's	AuNP's	Streptomycin
K. pneumoniae	–	0	0	0	26 ± 0.02
S. aureus	+	0	12 ± 0.03	10 ± 0.01	28 ± 0.02
B. subtilis	+	0	10 ± 0.01	0	28 ± 0.05

The results are presented as mean ± SEM of three different readings, Streptomycin: Standard drug used.

Effect in Acetic Acid-Induced Writhing Test

The acetic acid-induced writhing test is one of the primary tools used for characterization of analgesic potential of test compounds. Injection of acetic acid caused the release of various endogenous pain producing mediators such as bradykinin, serotonin, histamine, substance P. The local peritoneal receptor cause abdominal pain (writhing) which is symbolised by contraction of the abdominal muscle accompanied by an extension of the forelimbs and body elongation. The antinociceptive effect of MLE and both NPs are presented in Figure 8. Pre-treatment of MLE (*p<.05, **p<.01) and AgNPs (**p<.01) and AuNPs (**p<.01, ***p<.001) evoked dose-dependent antinociceptive effect in acetic acid-induced writhing test. The overall effect of both NPs was better than the corresponding extract, while less than standard drug, diclofenac. However, the antinociceptive potential of AuNPs (100 mg/kg i.p.) was comparable with standard drug (10 mg/kg i.p). The unwanted effects of synthetic analgesic drugs are already been reported [49,50]. The current antinociceptive findings, therefore, suggested the AuNPs as an effective alternative to synthetic drugs. The pain-relieving property of a compound or extract is related to lot of receptors/enzymes including cyclo-oxygenase (COX-I and COX-II), opioids receptors and GABA ionic channel receptors. These receptors and enzymes are widely distributed in the body including central nervous system.

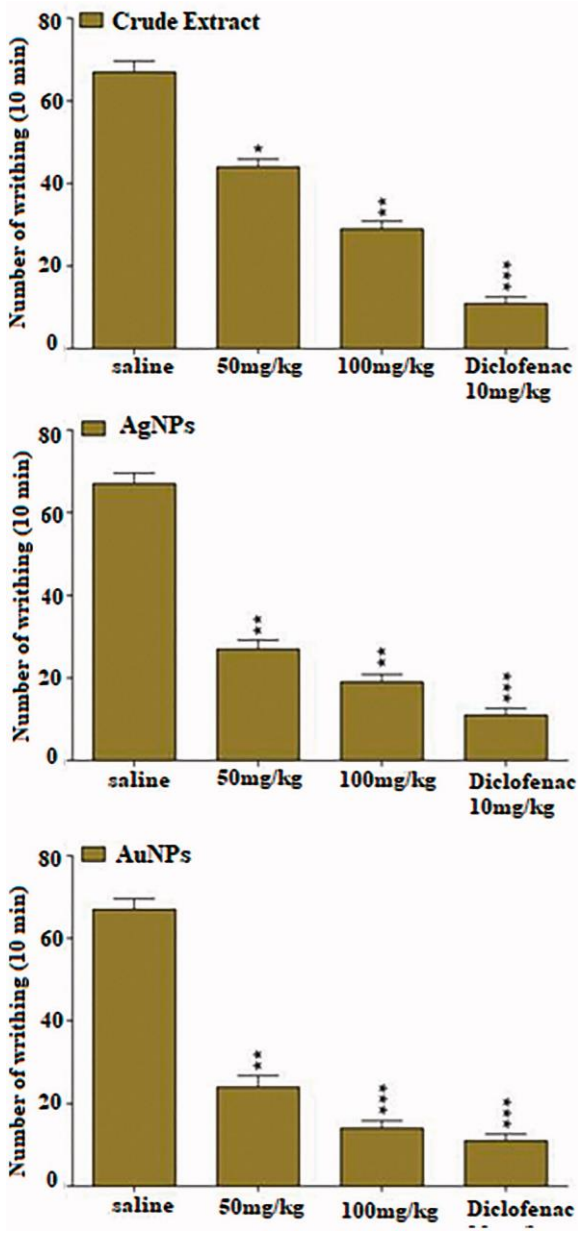

Figure 8: Effect of intraperitoneal administration of AuNPs and AgNPs in acetic acid induced test. Values are expressed as mean ± SEM *p<.05, **p<.01, ***p<.001 as compared to control. n = 6.

The MLE and synthesized NPs were also evaluated for their sedative potential. The results of open field, that is, sedative effect is presented in Table 4. Diazepam, a reference drug, exhibited maximum sedation, which was significantly (p<.001) different from saline control group. The methanolic crude extract exhibited mild sedative effect (p < .05) at the doses of 50 and 100 mg/kg, as compared to diazepam. AgNPs and AuNPs also exhibited significant (p<.01) sedative effect in doses of 5 and 10 mg/kg as compared to MLE and standard drug (diazepam). Diazepam showed superior sedative effects then NPs and MLE (Table 3). The sedative effect may be due to increase action of GABA binding with GABA channels. GABA channels stimulates the cell by keeping them polarised and decrease the release of neurotransmitter including (substance P), therefore, acts as potential pain desensitisers [65]. It is hypothesized that MLE and NPs may follow similar interaction, thus act as painkiller and sedative.

Table 4: Sedative effect of MLE and NPs in open field test (Locomotive activity).

Treatment	Dose (mg/Kg)	No of line crossed in 10 min
Distilled water	10 (mL/Kg)	126 ± 1.23
Diazepam	0.5	6 ± 0.12***
MLE	50	123.38 ± 2.99
	100	115.84 ± 2.22*
AgNPs	5	100.34 ± 0.87***
	10	88.56 ± 1.88***
AuNPs	5	98.89 ± 1.22***
	10	89.44 ± 1.99***

Values represent the number of lines crossed by an animal in a box, 30 min after treatment with distilled water (10 ml/kg, control), MLE (50 and 100 mg/Kg), AgNPs and AuNPs (5 and 10 mg/kg) or diazepam (0.5 mg/kg). Data presented as mean ± SEM, (n = 6). *p<.05, **p<.01, ***p<.001, all compared with control.

Conclusion

Current study present synthesis and characterization of AuNPs and AgNPs of MLE by using advance techniques, such as UV, FT-IR, AFM and TEM. The phytochemical analysis reflects the

presence of several bioactive secondary metabolites. The synthesis of AgNPs and AuNPs was confirmed *via* UV-Vis spectroscopy with SPR at 300–500 and 500–700 nm, respectively. The FT-IR spectra showed the presence of chemical constituents like flavonoids, tannins, alkaloids, monoterpenes and saponins, which play a key role in the synthesis of AgNPs and AuNPs. The size and shapes of both NPs were confirmed by AFM and TEM techniques, which showed poly dispersed and spherical shapes of AgNPs and AuNPs of 10.23 ± 2 nm and 13.45 ± 2 nm, respectively. As compared to MLE, the NPs significantly increase the antibacterial, antioxidant, antinociceptive, analgesic and sedative activities.

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