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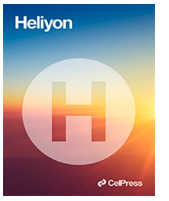

Research article

Hierarchical plant extracts in silver nanoparticles preparation: Minuscular survey to achieve enhanced bioactivities

Aroosa Habib [a], Yamin Bibi [b,*], Iqra Qayyum [a], Muhammad Farooq [c,**]

[a] Department of Plant Sciences, Quaid- i- Azam University Islamabad, 45320, Pakistan
[b] Department of Botany, PMAS-Arid Agriculture University Rawalpindi, 46300, Pakistan
[c] Pakistan Council of Scientific and Industrial Research (PCSIR), Ministry of Science and Technology, 1-Constitution Avenue, Sector G-5/2, Islamabad, 44000, Pakistan

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ABSTRACT

Extracts obtained from *M. longifolia* (Lamiaceae) and *R. ellipticus* (Rosaceae) were selected to utilize in the reduction and stabilization of silver nanoparticles (AgNPs) for achieving remarkable bioactivities. In brief, the cytotoxic potential of the as synthesize AgNPs was high at higher concentrations. In DPPH assay, maximum antioxidant potential was shown by AgNPs synthesized from *M. longifolia*. Meanwhile, Methanolic extracts exhibited more antioxidant potential than chloroform based extracts.

Further, brine shrimp lethality assay was carried out to achieve 34.6 μg/mL & 25.65 μg/mL LD_{50} values against the NPs prepared from *M.* and *R.,* respectively. In addition, antioxidant activities were carried by ABTS Radical cation assay where 38.6 μg/mL and 47 μg/mL IC_{50} values were obtained for the NPs obtained from *M. longifolia* and *R. ellipticus*, respectively. Reducing power assay (0.370–0.15 and 0.37–0.26 mean absorbance) and DPPH (% scavenging: 88.91–46.48 and 88.91–44.78) percentages were recorded for *M.* and *R.* synthesized AgNPs, respectively.

In brief, *M. longifolia* functionalized particles performed better in comparison to *R. ellipticus* treated particles. In addition, the nano assembly dispersed in polar solvent demonstrated better results in comparison to non-polar solvents.

In conclusion, the as synthesized AgNPs were better in bioactivities than crude extracts of the selected plants.

In future, this work could be extended to isolating active components for the nanofabrication of biologically intelligent nanoparticles for pharmacological interest. In the proposed investigation, the purified bioactivities fractions would be highlighted for further consideration in various medical treatments.

1. Introduction

Nanoparticles (NPs) are practiced in many fields of life due to having size dependent properties for example, excellent penetration power, high surface area, and exceptional morphologies [1,2]. The most targeted use of NPs is their utilization in biomedical field for

* Corresponding author.
** Corresponding author.
E-mail addresses: dryaminbibi@uaar.edu.pk (Y. Bibi), mfaruq752@gmail.com (M. Farooq).

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example, in drug loading, drug delivery, antioxidant, antidiabetic, antimicrobial etc. due to the above cited characteristics. One of the major problems associated with NPs is how to synthesize the desired nanomaterial for desired applications [3]. Nanotechnology is therefore emerged to cope with the purposes to achieve the above cited goals [4]. This technology is used to assemble, manipulate, and apply structures by controlling shape and size at nanoscale [5]. In the current scientific era, metal NPs have been commercially used in extensive areas including electronics, energy contact actions, and medicines. Optimization is the basic requirement as the conventional NPs synthesis methods are linked with toxic chemicals that are lethally harmful to the environment, as well as, living organisms which thereby retard the applicability of targeted material in biomedical field [6,7]. It is therefore desired to develop safe methods based on environment friendly process rather than associated with toxic chemicals [8]. Development of environment-friendly green nano-technology has led to exploring various routs to investigate in natural reducing agents for metal NPs reductive synthesis [9]. Environment-friendly green nanotechnology is therefore found robust to fabricate metallic NPs for utilization in biomedical fields. Particularly, green nanotechnology transfers biological talent to the nano assembly for robust contact with the human body. Plants extracts have thus treated as essential target due to possessing biological and nearer to natural compounds in large quantity [10]. The synthesis of NPs via green nano-technological treatment has shown various applications by transforming textural stability appropriate granular dimensions. However, NPs readily destabilize even upon exposure to safe environment and therefore the targeted activity is nullified gradually. To coup with this issue, various stabilizing methods, such as, dendrimers, polymeric gels, and some natural entities are incorporated to protect NPs active surface for enhanced efficacy. Natural entities used for NPs reduction purpose are various plants, fungi and bacteria to carry out biosynthesis of NPs under green nano-technological protocols [11,12].

Silver NPs have owned profitable applications in the field of pharmacological and other remedial sciences owing to having unique size, wavelength dependent properties, and ultra-safer nature [13–15]. Silver NPs based materials have been found nonpoisonous and safe bactericidal agents that have been used from a decade against microbes oriented diseases. Silver has been labeled as oligodynamic since it has capability to exert a microbial consequence at very small concentration.

Biosynthesized AgNPs are more adequate for remedial applications owing to greater biocompatibility compared to chemically synthesized counterparts.

Several conducted studies have revealed the use of different plants extracts for green synthesis of silver nanoparticle. Among these, *Aloe vera* [16], *Piper nigrum* [17], *Eucalyptus globules* [18], *Sida cordofolia* [19], *Rosa damascene* [20], and *Cinnamomum camphora* [21] have been identified as potent plants obtain biologically intelligent Silver NPs.

Plants are latent source of approved anticancer medications and it has been observed that more than 60% of presently used carcino defensive have been directly or indirectly invented or initiated from the plants [22].

The genus *M.* (mint) is one of the most significant taxa of the family *Lamiaceae,* and includes 25 to 30 species grown in different parts of the world [23]. Due to chilling, pleasant fragrance and taste, the essential oils of mint are utilized in perfumery, beauty care products, candy parlor, and the pharmacological enterprises [24]. Similarly, genus *R.* is very diverse and comprises above 750 species in 12 subgenera found in all continents except Antarctica [25]. *R. ellipticus* is a feeble raspberry and well recognized in disturbed wet forests. Various parts of the plant have been claimed to be beneficial in diseases like diabetes, diarehea, gastralgia, wound healing, dysentery, antifertility, antimicrobial, analgesic and epilepsy [26,27].

Herein, we report the use of *R. ellipticus Sm.* and *M. longifolia* L. plants leaf extract for the synthesis of AgNPs. As reported, silver NPs have achieved numerous applications in biological domain. However, the stability, and cytcompatibilty still require further studies to achieve optimized response in biological activities. To achieve this, the cytotoxicity and biological activities of the well-characterized NPs prepared from the two plants species of thrilling medicinal interest were carried out *in vitro*. The as synthesized NPs were found hierarchically non-cytotoxic, and robust in antioxidant potential. The demonstrated biological potential might be due to synergistic effect of the organic-inorganic counterparts of the hybrid Nano-assembly, whereby, the natural products based benefits were transferred to the brilliant AgNPs. The obtained NPs might be considered for medicinal interest keeping in view the above mentioned biological potential.

2. Materials and method

Fresh leaves of selected plants were collected from Holar, Azad Kashmir. The plant parts were thoroughly washed with deionized water, and kept for drying under shade for at least two weeks to preserve sensitive phytochemicals by preventing UV radiation and heat degradation.

The dried leaves were then ground into fine powder by using an electric grinder. The plants powder was kept at a safe place in air tight container, and maintain at 4 °C until use [28].

2.1. Extraction preparation, and silver nanoparticles green synthesis

The extraction was carried out by cold maceration technique with a slight modification keeping in view the nature of plant. The plant powder was soaked separately in conical flask containing solvent (20 g/100 mL). The flasks were wrapped tightly with aluminum foil, and kept at room temperature with occasional shaking for at least two weeks. Then filtration was carried out by using Whatman filter paper No. 1 and solvent was evaporated whereas, the residue was maintained at 4 °C for pharmacological activities [28]. The obtained extract was further used in AgNPs synthesis by following the protocol described by Hussain et al. with some modification [29]. Briefly, $AgNO_3$ (0.17 g) was dissolved in 1 L distilled water to prepare 1 mm resultant solution. The solution was boiled for 5 min, and reduced stepwise by the addition of plant extract with continuous boiling until the color of solution turned to dark brown. The reaction mixture was then centrifuged at 14000 rpm for 10 min. After discarding the supernatants, the pellets were re-suspended in

deionized water, and the centrifuged for 10 min at 14000 rpm. The process was repeated for four times. Different concentration for example, 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm were freshly prepared and used in future experiments.

2.2. Characterization of green synthesized silver nanoparticles

To observe the electronic response of the synthesized AgNPs, UV–Visible absorption analysis was carried out by using SUV-S2100 spectrophotometer in 200–800 nm range. Functional group of the surface functionalized NPs were investigated via FT-IR spectroscopy performed by using a JASCO FTIR (680 plus, Japan) spectrometer with KBr pellet in the range of 4000–400 cm^{-1}. The crystalline nature of NPs was investigated through powdered XRD analysis. The morphology of AgNPs was examined by through TEM (cm30-Philphs). Furthermore, SEM (HITACHI S-4160) study was carried out to investigate the shape and size of the fabricated NPs.

2.3. Cytotoxicity test

Cytotoxic investigation was carried out via designing brine shrimp lethality assay [30]. In brief, a rectangular plastic pot was partitioned with two unequal parts and holes were dug in the divider. Artificial seawater was poured into the pot. Approximately 20 mg eggs of shrimp were poured in the larger dark portion, whereas, smaller was lighted with the help of lamp. After 48 h, the phototropic nauplii was picked from the lighter portion with glass capillary tube. Different concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm) of synthesized NPs and for plant extracts (10 μg/mL, 125 μg/mL, 250 μg/mL, 500 μg/mL and 1000 μg/mL) were prepared. Artificial sea water was used as negative control and vincristine sulphate used as positive control.

Then 500 μL of each concentration will be poured in each glass vials. Then, 10 shrimps were added to each along with 2 μL of sea water. These were put under the illumination of electric bulbs at room temperature. & then numbers of alive shrimps were counted after 24 h. Sea water & DMSO serves as blank & negative control while two commercial anticancer drug vincristine and etoposide were used as positive control. The third positive control was potassium dichromate [31]. The percentage Morality was calculated via the following well known equation:

$$\text{Percentage death} = [(\text{pc} - \text{pt}) / \text{pc}] \times 100$$

where pt is dead naupii in treatment & pc is dead naupii in control respectively.

2.4. Anti-oxidant activity

2.4.1. DPPH free radical scavenging assay

The DPPH assay was performed by using reported by Arbaayan et al. with some modifications. DPPH (24 mg) was dissolved in 100 mL of methanol to prepare stock solution, and stored at 20 °C. To prepare the working solution, stock solution was diluted with methanol to attain an absorbance of 0.980 at 517 nm. Briefly, 3 mL of diluted DPPH solution was separated in a test tube, and mixed with 1 mL AgNPs suspension of varying concentrations (50–10 ppm) as well as of plant extract at different dilutions. Test tube containing working solution and sample solution were incubated in dark room for 15 min. After incubation, absorbance was measured at 517 nm. Ascorbic acid used as positive control with same concentration as that of plant sample. The radical scavenging activity was calculated from co-relation curve obtained from the plot (between concentrations versus scavenging activities) of equation given below:

$$\%\ \text{scavenging} = [(\text{Abs control} - \text{Abs sample}) / \text{Abscontrol}]\text{x}100$$

2.5. ABTS radical cation assay

ABTS radical Cation scavenging activity was determined by protocol used by Re et al. with a slight modification. ABTS (7 Mm) was added into potassium persulphate solution (2.45 mM) and kept in dark overnight. Thereafter, 50 % methanol was used for dilution ABTS solution, to set the absorbance of around 0.700 (at 745 nm). After that 3 mL of ABTS solution was coupled with different concentration of AgNPs, and plant sample to examine the free radical scavenging effect. Decrease in absorbance was observed after every 1 min of the mixing of solutions, whereas, final absorbance was recorded after 6 min gap. The percentage of scavenging activity of sample and ascorbic (standard) were calculated by following formula:

$$\text{Scavenging activity } (\%) = (\text{abs of control} - \text{abs of sample})/(\text{control abs}) \times 100$$

Correlation curve was applied for IC_{50} value calculation by plotting graph between scavenging activity and the concentration of the AgNPs and plant samples.

2.6. Reducing power assay

We used slightly modified method of Ebrahimzadeh et al. Each AgNPs concentration and 3 mL methanolic extract was taken, mixed with potassium ferricyanide (2.5 mL), and the mixture was diluted with sodium phosphate buffer, transferred into incubator, and kept at 50° for 20 min. Resultant mixture was treated with trichloroacetic acid (2.5 mL, 10 %). The final product was then centrifuged at 3000 rpm for 10 min. The supernatant was treated with distilled water and ferric chloride $FeCl_3$ (0.5 mL of 0.1 %). For positive control,

ascorbic acid solution was used and absorbance was measured at 700 nm.

3. Results and discussion

3.1. Synthesis and characterization of synthesized silver nanoparticles

Fabricated AgNPs were initially characterized via UV–Vis spectroscopy in the spectrum range of 300–700 nm. The broad absorption peak appeared at around 430 manifests the presence of nano-particulate material excitation [32]. The characteristic UV–Vis spectrum recorded in 300–700 nm spectrum is shown in Fig. 1a.

Further, the surface active NPs represented irregular to regular pattern of the dried and agglomerated AgNPs, as can be seen from SEM micrograph (Fig. 1b). The prepared NPs were further characterized via XRD technique. In brief, the sharp diffraction peak in XRD pattern of powdered Ag silver based Nano particulate material gives an insight of the grown Ag NPs crystallinity (Fig. 1c) [33].

Elemental distribution of reduced Ag was confirmed by recording EDX analysis against the respective elements. Recorded typical sharp peak of Ag spectrum manifest the dispersion of Ag ion in the range of 2.6–3.8 (Fig. 1d).

To assess the cytotoxicity, pesticidal, phytotoxicity, and many other activities [34–36], potent and cost effective brine shrimps lethality was carried.

The bioassay is an indicator of general toxicity that figures out effects on cancer cells [37]. The brine shrimps lethality test assessed the toxicity of leaves of *R. ellipticus* and *M. longifolia,* as well as, of synthesized Ag NPs using vincrinstine as positive control. The LD_{50} of

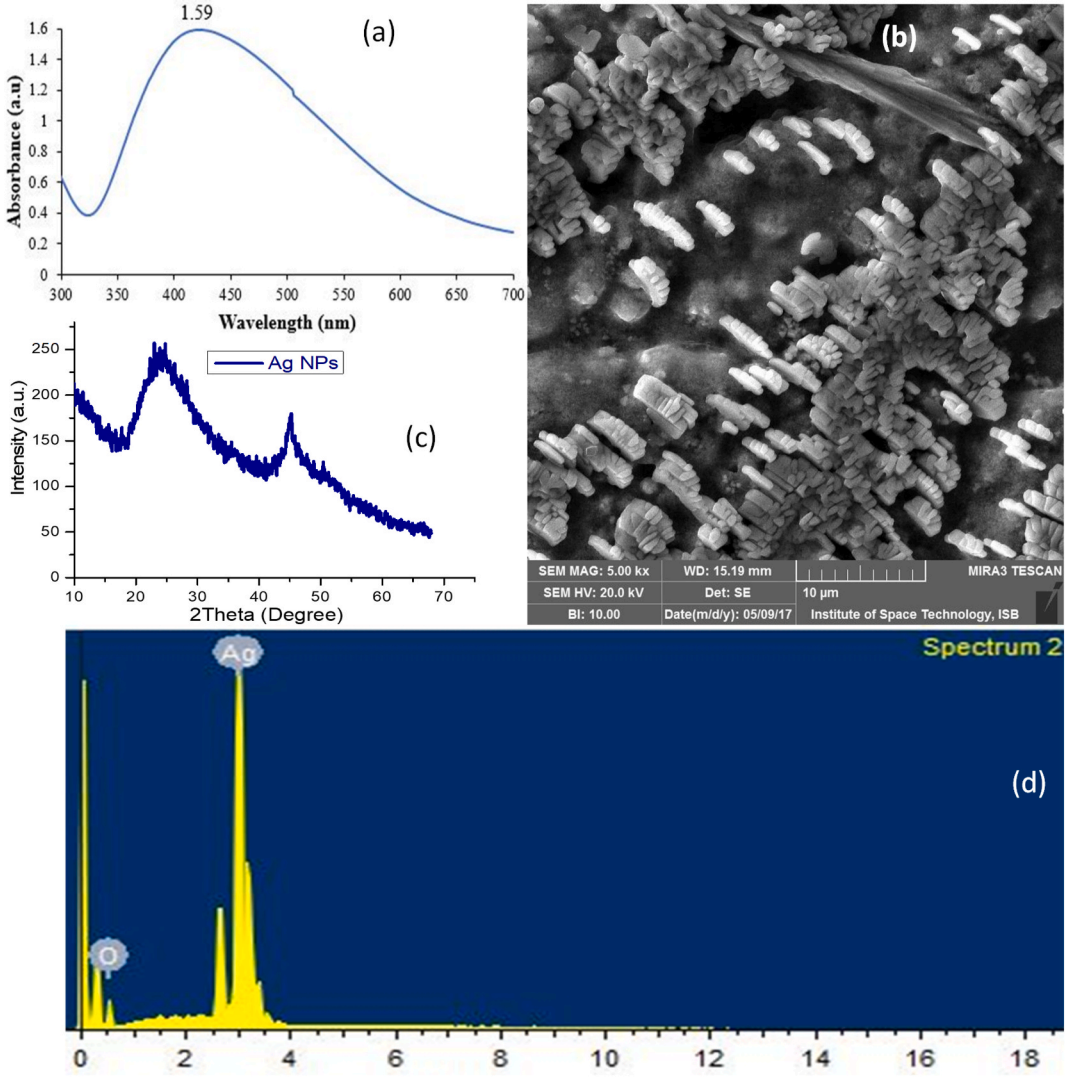

Fig. 1. a. UV–Vis spectrum, SEM micrograph (b), XRD profile (c), and EDX spectrum of green synthesized AgNPs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

green synthesized NPs was recorded as 34.6 μg/mL, and 25.65 μg/mL from *M.* and *R.*, respectively (Fig. 2a). The LD_{50} values recorded against Ag NPs has been reported as 33.92 μg/mL in earlier researches [38].

The LD_{50} values of leaves extract of *M. longifolia* was observed between 27.07 μg/mL and 350.82 μg/mL for methanol and chloroform extraction, respectively. In earlier research LD_{50} was reported as 40 μg/mL [39]. The LD_{50} of *R. ellipticus* was 32.12 μg/mL and 270.97 μg/mL for methanol and chloroform, respectively (Fig. 2b). Similar results were found in previous literature [40]. The insignificant results obtained in chloroform extracts may be due to the presence of bioactive compounds which are nearly always lethal in high doses [41]. The results evidenced that methanol is comparatively more potent solvent than chloroform. The lethality of methanol extract of selected plants might be due to the presence of comparatively more bioactive compounds in it [42]. This study justified that the plant may be potential source of anticancer bioactive molecules [43]. Comparison of LD_{50} value of different plants extracts and LD_{50} of AgNPs synthesized from the various extracts is outlined in Fig. 2c.

Cytotoxicity of nano material depends on the size, shape, coating agent and type of pathogens against which their toxicity is investigated [44]. The cytotoxicity by AgNPs is believed to be produced through reactive oxygen species as a consequence of which a reduction in glutathione level is recorded, whereas, an increase in ROS level occur [45,46].

The percentage scavenging ranged from 88.36 μg/mL to 39.60 for methanolic extract of *R. ellipticus* leaves and for chloroform it was measured from 86.30 to 15.79 at 250 to 15.62 μg/mL concentrations (Fig. 2d).

In present study the IC_{50} value was obtained as 21.41 for methanolic extract of *R.* leaves, whereas, in earlier research it was reported as 6.96 μg/mL [47]. This activity is due to the occurrence of chemical compounds like flavonoids, and phenolics etc. Between the phenolics compounds and antioxidant activity of plant species, there is positive relationship, due to possessing good redox characteristics and hydrogen donating ability [48]. The IC_{50} for chloroform extract was recorded as 116.65 μg/mL whereas, in literature same was noted as 270.27 μg/mL [47].

M. longifolia percentage scavenging ranged between 88.18 and 40.18 μg/mL for methanol extract and 57.23 to 23.55 μg/mL for chloroform extracts at different concentrations (250–15.62 μg/mL). The IC_{50} value of *M.* was 21.41 and 186.28 μg/mL for methanolic and chloroform extract, respectively (Fig. 2c). The value of IC_{50} was mentioned as 41 μg/mL for *M. arvensis* in polar solvent in previous study [39].

By increase in concentration the percentage scavenging activity of silver nano particles were also increases in similar pattern (Fig. 2a). The percentage scavenging was found between 88.91 to 46.48 and 88.91 to 44.78 for nano particles synthesized from *M.* and *R.* at different concentration (50–10 ppm) (Fig. 3d). It has been evaluated that AgNPs have ability to scavenge the free radicals either by accepting or donating electrons which is seen by change in color [49]. This DPPH scavenging is possibly due to the presence of

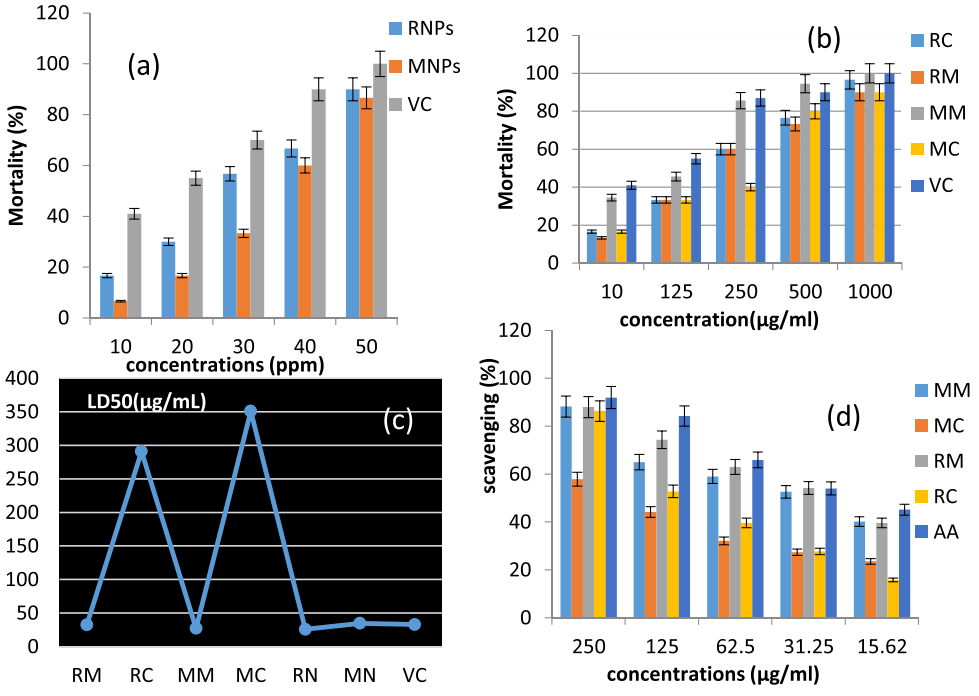

Fig. 2. (a)Percentage of mortality at different concentrations of silver nano-particles (b) percentage of mortality at different concentrations of plants extract, (c) Comparison of LD_{50} value of different plants extracts and LD_{50} of plant samples with AgNPs (d) Scavenging percentage of plant extracts. ** MN: AgNPs synthesized from *M. longifolia*, RN: AgNPs Synthesized from R. ellipticus, MM: *M. longifolia* leaf methanolic extract, MC: *M. longifolia* leaf chloroform extract, RC: R. ellipticus leaf chloroform extract, RM: R. ellipticus leaf methanolic extract, VC: Vincristime).

phyto-consituents in selected plants capping nanoparticles [50].

Results of the study indicated that free radical scavenging activity of leaf extract and ascorbic acid increased in a concentration dependent manner (Fig. 3b). This was in accordance with previously conducted study [51]. At higher concentration high inhibition percentage is associated with higher antioxidant potential of plant extract [52]. Lower the absorbance, higher will be the antioxidant activity as DPPH is a radical that get reduced by accepting electron from antioxidant compound for conversion into colorless final compound [53].

The percentage scavenging of *M. longifolia* and *R. ellipticus* were observed as 56.66–45.5 and 55.16–42.46 in methanol extracts with IC_{50} value 108.67 and 160.52, respectively (Fig. 3c). Percentage scavenging in chloroform extract was recorded as 49.95–42.46 and 53.62–40.19 for *M. longifolia* and *R. ellipticus* respectively. The IC_{50} was measured as 243.26 and 162.43 for *M.* and *R.* (Fig. 4b). Our findings was similar with findings of [54]. According to the study, in polar solvents plants showed high percentage scavenging as compared to non-polar solvents. Nickavar et al. (2008) reported the IC_{50} of *M. piperita* as 153.80, that is in close agreement with our study. In case of AgNPs the percentage scavenging was recorded as 54–48.55 and 53.98–48.11 for *M.* and *R.* synthesized AgNPs (Fig. 4a). The IC_{50} value was noted as 38.6 and 47 μg/mL for nanoparticles synthesized from *M.* and *R.* respectively as compared to control that was ascorbic acid having IC_{50} value as 4.49 μgmL. The absorbance of sample decreases with increase in concentrations of sample. Same trend was observed by R. Bonilla et al. [55]. The ABTS assay was proved as dose dependent for nanoparticles. Higher the concentrations of nanoparticles higher the percentage scavenging observed [56].

In present study, the mean absorbance of synthesized AgNPs from *M. longifolia* and *R. ellipticus* was ranges from 0.37 to 0.15 and 0.37 to 0.26 respectively as shown in (Fig. 4c). *M. arvensis* AgNPs exhibit same pattern [57]. By increasing concentration of AgNPs, the percentage or mean absorbance increases.

The mean absorbance of *M. longifolia* leaves was recorded between 0.78 to 0.393 and 0.62 to 0.213 for methanol and chloroform extracts, respectively (Fig. 4d). Same pattern of mean absorbance was observed by earlier researchers [40]. Absorbance values for *R. ellipticus* leaves were found between 0.94 t- 0.3 and 0.81 t- 0.23 for methanol and chloroform, respectively. Similar absorbance values were recorded for *R. ellipticus* fruits in ethanol, aqueous and in petroleum ether in previous study [58].

4. Conclusion and recommendations

In this study, *M. longifolia* and *R. ellipticus* were used in AgNPs fabrication for possibly enhanced biological potential. The prepared NPs were less cytotoxic at lower doses. The DPPH assay based maximum antioxidant potential was demonstrated by AgNPs synthesized from *M. longifolia*. Likewise, antioxidant activities were carried by ABTS Radical cation assay where 38.6 μg/mL and 47 μg/mL IC_{50} values were obtained for the NPs obtained from *M. longifolia* and *R. ellipticus*, respectively. Methanolic extracts presented higher

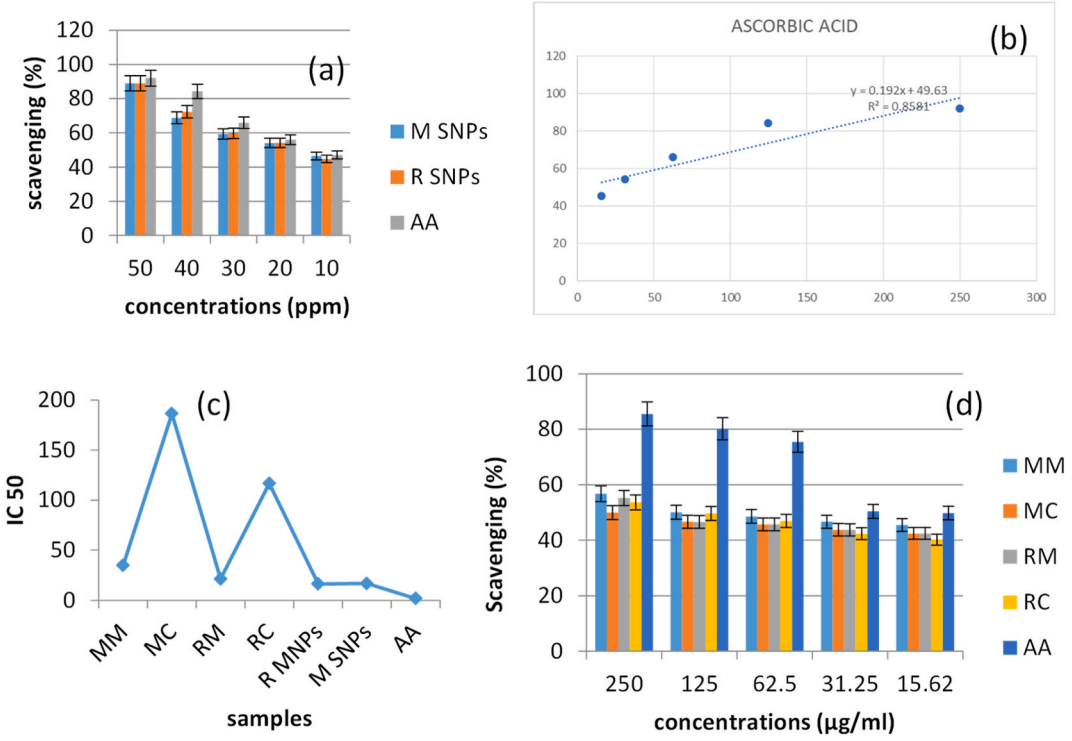

Fig. 3. (a) Scavenging percentage of AgNPs at different concentrations. (b) Ascorbic acid graph (used as control). (c) LC_{50} value of methanolic plant extracts and silver nanoparticles. (d) Scavenging percentage of plant extracts and Ascorbic acid at different concentrations.

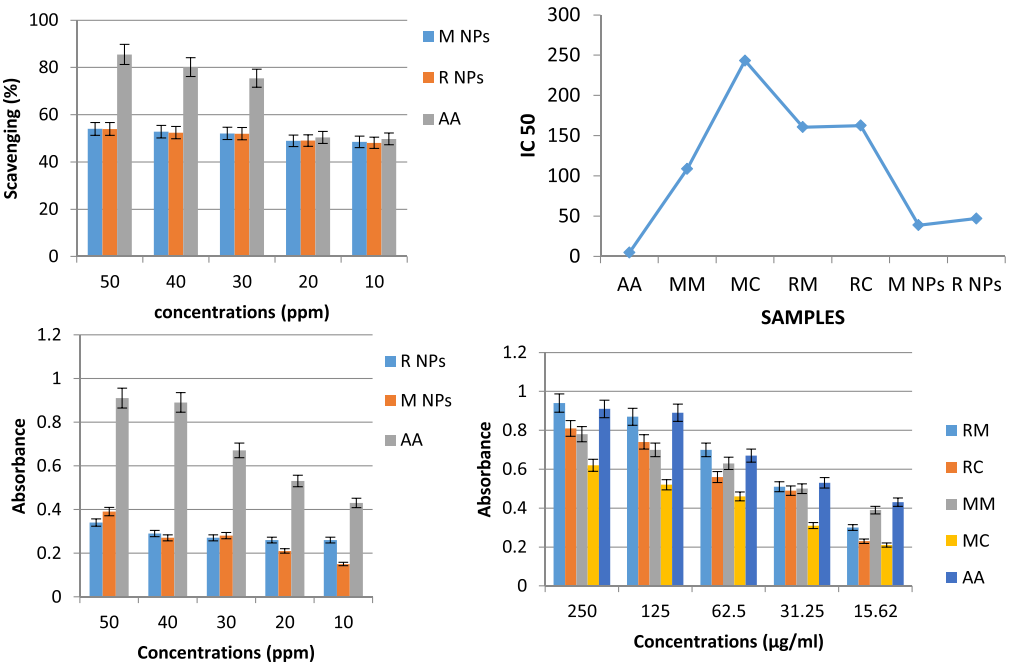

Fig. 4. (a). % Scavenging of nano particles and Ascorbic acid. (b) IC_{50} values of plant extracts and nano particles. (c) Absorbance of nano particles at different concentrations. (d) Absorbance of plant extracts and Ascorbic acid.

antioxidant potential than chloroform based extracts. Reducing power assay (0.370–0.15 and 0.37–0.26 mean absorbance) and DPPH (% scavenging: 88.91–46.48 and 88.91–44.78) percentages were recorded for *M.* and *R.* synthesized AgNPs, respectively.

Briefly, *M. longifolia* functionalized particles performed better in comparison to *R. ellipticus* treated NPs. The nano assembly dispersed in polar solvent demonstrated better results in comparison to non-polar solvents.

Further, the bioactivities of the fabricated NPs were higher than crude extracts of the selected plants.

The proposed work could help in evaluating active ingredients for bio-based NPs production. The purified bioactive fractions would be highlighted for further consideration in various medical treatments associated with various kinds of NPs.

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Availability of data and materials

Data will be made available on request.

CRediT authorship contribution statement

Aroosa Habib: Writing - original draft, Methodology, Conceptualization. **Yamin Bibi:** Writing - review & editing, Writing - original draft, Validation, Supervision, Resources, Investigation, Data curation, Conceptualization. **Iqra Qayyum:** Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Muhammad Farooq:** Writing - review & editing, Visualization, Supervision, Software, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper"

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