Revista de Chimie
https://revistadechimie.ro
https://doi.org/10.37358/Rev.Chim.1949

Green Synthesis of Silver Nanoparticles Using *Ajuga parviflora* Benth and *Digera muricata* Leaf Extract: Their Characterization and Antimicrobial Activity

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Abstract. *The aim of the present study is to search out nontoxic silver nanoparticles synthesized from the leaf extract of two plants Ajuga parviflora Benth and Digera muricata for antimicrobial activity. The plants used in this investigation are rich in alkaloids, flavonoids, steroids, terpenoids, protein, amino acids, carbohydrate, quninones, phenols and tannins. The formation of nanoparticles were confirmed by UV/Visible spectroscopy, peaks at 423nm for Ajuga parviflora Benth and 408nm for Digera muricata. The morphology of the silver nanoparticles was established through state of the art spectroscopic tools. SEM analysis reveals average size of AgNPs 18 nm for Digera muricata and 22 nm for Ajuga parviflora Benth respectively while transmission electron microscopy confirms that AgNPs are spherical in shape. The synthesized nanoparticles were subjected to Escherichia coli, Staphylococcus aureus, Salmonella typhimurium and Pseudomonas aeruginosa. The results suggest that the silver nanoparticles have promising activity against all the bacterial strains and can be used an effective bactericides.*

Keywords*: green synthesis, silver nanoparticles, Ajuga parviflora Benth, Digera muricata, antimicrobial activity*

1. Introduction

Nanotechnology is a multidimensional field which interfaces the physical, chemical, medical, and environmental sciences. This versatile field has numerous applications in the engineering fields; some prominent applications are progress of biosensors and biomedical devices, alternate energy production and ecological restoration [1]. Nanoparticles serve as the basic building blocks for a variety of nanotechnology applications. Nanoparticles are considered as distinct group of chemical materials with unique features and wide applications in various arenas [2]. Nanoparticles of different metals are being used in number of fields which include mechanical, pharmaceutical, electrical, biological, chemical fields and also in field of textiles.

Conventional procedures for nanoparticle synthesis incorporate physical or chemical routes, involving toxic chemicals as chemical precursors to transform macro or bulk materials into the nanoparticles form [3]. Silver nanoparticles are synthesize by using biological methods, which are considered to be superior due to the fact that AgNPs are synthesized without the use of any punitive, toxic and expensive chemical substances [4, 5]. Thematic approach to synthesized silver nanoparticle by using plant extracts which act as reducing agent, the plant's metabolites also aid to stabilization of these nanoparticles by emulsifying activity. Among the green synthesized nanoparticles, silver nanoparticles gain attention of researchers due to versatile characteristics of silver and wide-ranging

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applications of AgNPs in different areas such as filters, integrated circuits, sensors [6-8], bio-labeling, antimicrobial deodorant fibers, cell electrodes [9], low-cost paper batteries (silver Nano-wires) [10] and antimicrobials [11-14].

Ajuga parviflora Benth is bugleweed, commonly known as Neel Kanthi. *Ajuga parviflora Benth* belongs to *Ajuga* genus and *Lamiaceae* family. It has worldwide distribution commonly found in dry, low temperature hilly areas [15]. It is found in Pakistan, India Afghanistan and Kashmir. It is an annual or short live perennial herb. It has unbranched spreading or ascending stem. Its leaves are green rosette forming and are of variable size. It has small purple flowers.

Due to broad spectrum application of *Ajuga parviflora* in folk medicines it got attentions of chemists to analyze for phytochemical analysis. It contains flavonoids, saponines and different amino acids [16-18]. Phytochemical screening also reveal the presence of alkaloids, terpenoids, aromatic compounds, dions, aminophenols, glycosides, tannins, polyphenols, quinines and steroids/sterols [18]. Philip S. Beauchamp and Albert T. Bottini [19] reported isolation of neo-clerodane diterpenoids, deoxyajugarin-I, ajugarin-I chlorohydrin, and 3β-acetoxy-clerodinin C, from Ajuga parviflora. Essential oil of *Ajuga parviflora* contain α-humulene, δ-cadinene, β-caryophyllene, caryophyllene oxide, γ-muurolene, γ-terpinene, α-amorphene and β-selinen [20] different types of quinoles are also isolated [21].

Digera muricata is commonly known as false Amaranth. It is annual herb, commonly found as weed in fields. It is distributed in both tropical and subtropical regions. it occurs naturally in semi-arid and moist fields. It is distributed in Africa Asia and Madagascar.

It had been reported that *Digera muricata* have great medicinal and nutritional value [22]. Traditionally is used for the treatment of stomach ache and related gastrointestinal problems. Extracts of *Digera muricata* is also used for the treatment of urinogenital tracts. Phytochemical analysis reveals the presence of flavonoids, alkaloids, sterols, terpenoids, tannins and saponin [23]. Antioxidant analysis confirms the presence of active phytochemicals such as flavones and glycosides [24].

Keeping in view of the important constituents of the *Ajuga parviflora Benth* and Digera muricate plants, the present study is aim to synthesize the silver nanoparticles through the leaf extract of these plants and to establish their antimicrobial potential.

2. Materials and methods

2.1 Materials

Silver nitrate and ethanol were purchased from Merck, Germany and were used without further purification. *Ajuga parviflora Benth* and Digera muricate plants were obtained from Moonan and Changi Bandi district Haripur, KP, Pakistan. Distilled water and deionized water was used throughout in the whole study.

2.2 Preparation of *Ajuga parviflora* Benth and *Digera muricate* leaf extracts

Fresh leaves of *Ajuga parviflora Benth* and *Digera muricata* were washed with tap water to remove dust followed by double distilled water in order to remove any kind of ions sourced by dust or tap water from the leaf surface. The leaves were crushed into small pieces, 10g of cut leaves were taken in double distilled water and heated on 90°C until the colour of solution turn into dark yellowish green. The solution then was filtered through Whatman No.1 filter paper; the filtrate obtained was further centrifuged for 30 min in order to remove any kind of heavy biomaterial present in extract, which can hinder the formation of nanoparticles. Thus obtained extract was stored at 4°C for further use in nanoparticles synthesis. The same procedure was followed for both plants.

2.3 Synthesis of Silver nanoparticles

Biogenic synthesis of silver nanoparticles was done according to the method of Haq et al. [26] with slight modification. 50 mL of each plant extract and 50 mL of 1 mM $AgNO_3$ solution was taken into two 250 mL Erlenmeyer flask. The mixture was heated at 90ºC on hotplates with magnetic stirrer for 4 h, change in color was observed, which is dark yellow to brown. This color change indicates the formation

of AgNPs. So obtained nanoparticles were centrifuged for 10000rpm for 30-40 min in order to obtain maximum yield.

For finding the best ratio that is fruitful in maximum formation of AgNPs, reaction was design with change in ratio of salt (1:1, 1:2, 1:3, 1:4) and by changing concentration of plant 2:1, 3:1, 4:1 are made color change is observed as basic parameter for detection of synthesis of Ag-NPs then established by UV-Vis spectroscopy. In which 1:1 shows maximum absorbance value 3.7 at 408nm in case *of Ajuga parviflora* Benth and 423nm in case of *Digera muricata.*

2.4 Antimicrobial activity

Agar well diffusion process [25] is used for the determination of antimicrobial activity of silver nanoparticles and studied by using four different bacterial strains which are *Staphylococcus aureus*, *Escherichia coli*, *Pseudo aeruginosa*, and *Salmonella typhimurium*. The bacterial culture was spread on agar nutrient petri dishes with swabs and well duged with sterile borer. The silver nanoparticles suspension was prepared by ultrasonic dispersion of 1mg in 2mL deionized water. The wells in each dish were loaded with prepared suspension and plates were incubated at 37°C. The zone of inhibition was measured in millimeter (mm) after 24h of incubation.

2.5 Characterization of NPs

UV/Visible spectra were recorded on UV-1800, Shimadzu, Kyoto, Japan at a resolution of 1nm. SEM 5910 (JEOL) was used to record SEM images while FTIR analysis of silver nanoparticles were carried out on KBr using Nicolet 6700 USA (range of 4000-400 cm^{-1}).

3. Results and discussions

3.1 UV/Visible spectroscopy

The change in colour of aqueous solution indicates synthesis of Ag-NPs, which was confirmed from the UV–Vis spectra of aqueous colloidal solution. The UV-Vis spectra of Ag-NPs solution, a characteristic surface plasmon resonance (SPR) band at 423 nm in case of *Ajuga parviflora* Benth (Figure 1) whereas the bands for *Digera muricata* were observed around 408 nm (Figure 2). These two characteristic SPR band at 423 and 408 nm thus showing the synthesis of silver nanoparticles in the aqueous solutions. The difference in colours of two AgNPs solution was due to variation in morphologies of the nanoparticles. Variation in size and shape results in difference in observed colours. Communal oscillation of the electrons in the conduction band is major reason of colour production by the respective nanoparticles. Silver nanoparticles shows brown coloration and their oscillation frequency lies in the visible range, hence give rise to strong SPR absorption [22, 23, 25].

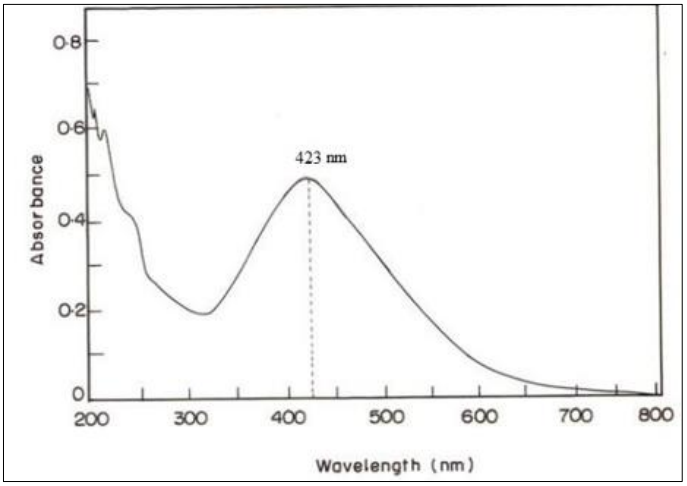

Figure 1. UV-Vis Spectra of *Ajuga parviflora* Benth AgNPs

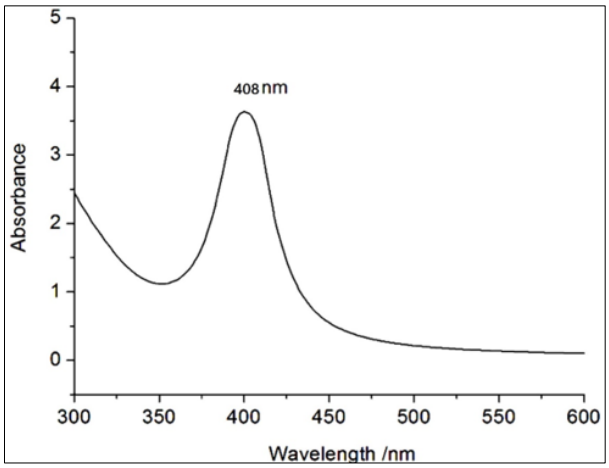

Figure 2. UV-Vis Spectra of *Digera muricata* AgNPs

3.2 SEM analysis

SEM was carried out to determine the structure, size and forms of nanoparticles. It was obvious through the SEM images that the biosynthesized Ag-NPs were in the range of 10-35 nm. The result showed that the particles were of spherical in shape in case of *Ajuga parviflora* Benth and average size was 22 nm (Figure 3) while in case of *Digera muricata* the average size was 18 nm and particles were spherical in shape (Figure 4). AgNPs was uniformly distributed and of small size which means plant extracts have strong ability to Reduce Ag+ ion to AgNPs while capping agents are also in excess that make the AgNPs Stable [24].

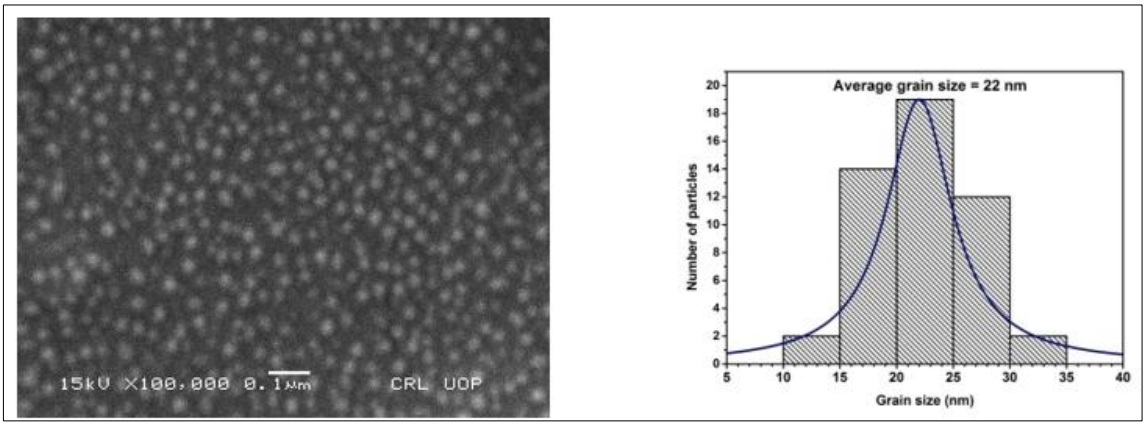

Figure 3. SEM of *Ajuga parviflora* Benth AgNPs

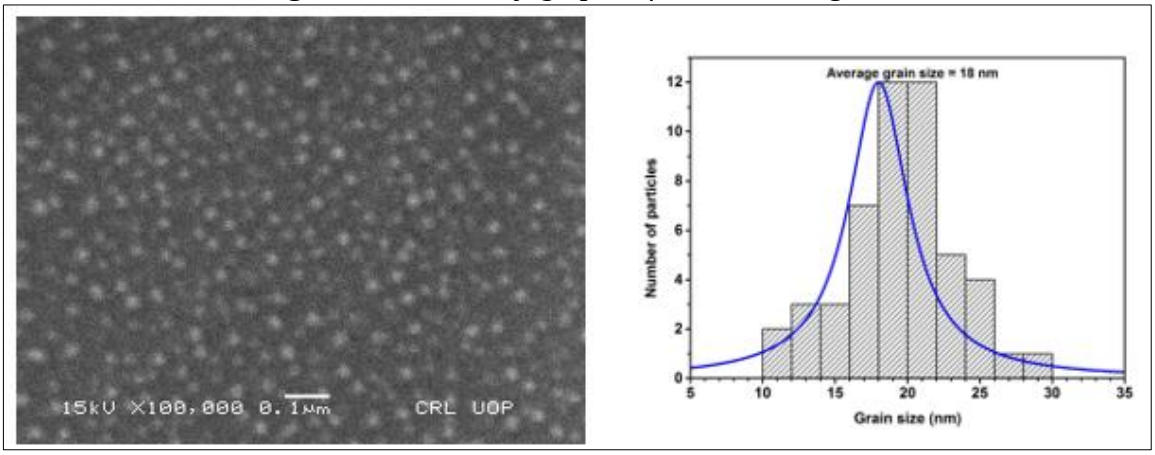

Figure 4. SEM of *Digera muricata* AgNP

3.3 Fourier Transforms Infrared Spectroscopy (FTIR)

FTIR spectra of Ag-NPs derived from *Ajuga parviflora* Benth plant extract are given below (Figure 5). It is obvious from spectra that plant metabolites have an extensive O-H stretching band at 3290cm^{-1}. At 2830 cm^{-1} is observed that was because of C-H bond stretching. C=O bond stretching is represented by an intense peak at 1715 cm^{-1}. Conjugated C=C bond shows an intense peak at 1610 cm^{-1}. This peak indicates the existence of flavonoids and carotenoids.C-H bond bending vibration and O-H were detected at 1418 cm^{-1} and 1374 cm^{-1} respectively. C-O stretching vibration gives intense peak at 1036 [25, 26].

In synthesis of Ag-NPs, the participation of O-H groups in the process of reduction and capping is clearly depicted by the shifting of bonded O-H stretching from 3290 cm^{-1} to higher frequency of 3301 cm^{-1}. Disappearance of carbonyl carbon peak at 1715 cm^{-1} shows the involvement of carboxylic group in the reduction and stabilizing. Bending vibrations at 1415, 1374 cm^{-1} also vanished because of the attraction by the silver nanometal. A slightly fused peak at 2838 cm^{-1} in the FTIR spectra of bio-synthesized silver nanoparticles were due to the dealings of carboxylic group with Ag-NPs. These outcomes established that O-H, C- O and carbonyl groups reduced the Ag metals as well as proven as a stabilizing agent [26].

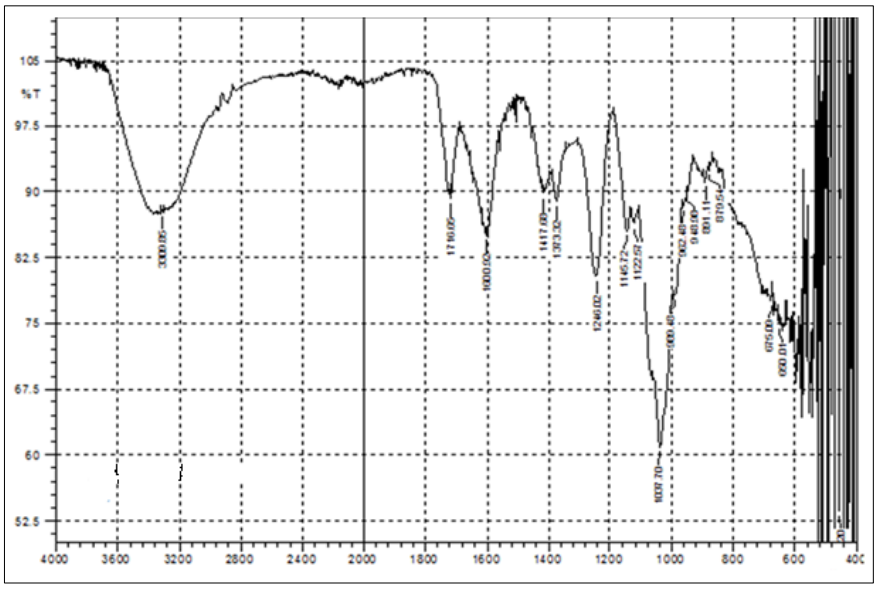

Figure 5. FTIR spectra of Ag-NPs derived from *Ajuga parviflora* Benth plant extract

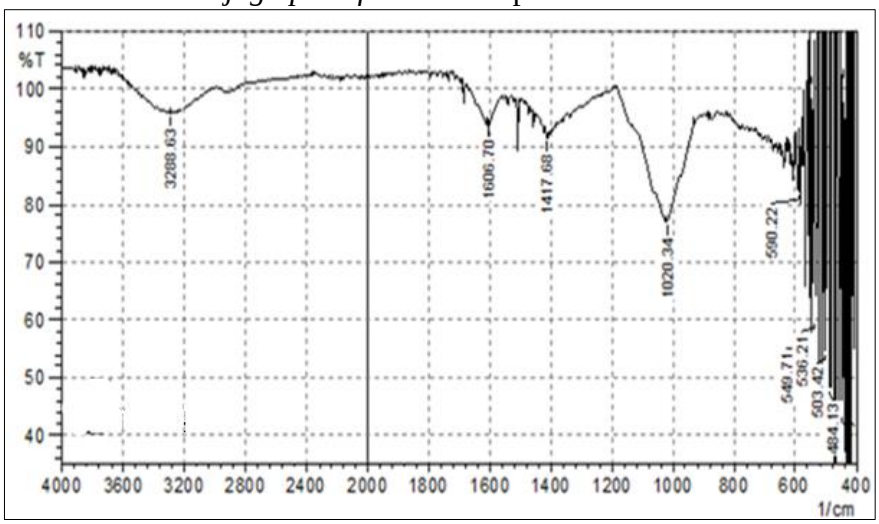

Figure 6. FTIR spectra of Ag-NPs derived from *Digera muricata* plant extract

Important deviations were detected after nanoparticles synthesis in FTIR spectra. Ag-NPs absorption peaks were observed at 3316, 2933, 1604, 1401, 1353, 1021 cm^{-1} (Figure 6). For silver nano particles, the attached O-H stretching shifted from 3324 cm^{-1} to inferior frequency of 3315 cm^{-1}, and thus exhibited the participation of O-H groups throughout the reduction and capping scheme. The small peak at 2932 cm^{-1} because of C-H stretching continued unaffected. A sharp peak of carbonyl carbon at 1604 cm^{-1} was moved to 1601 cm^{-1} for the silver nanoparticles, representing the participation of carboxylic groups in nanoparticles synthesis as reducing and stabilizing agent. Important variations were detected in the bending vibrations at 1408, 1327, 1257, and 1022 cm^{-1} in the spectrum of plant extract because of probable attraction by the silver metals [26].

3.4 Antimicrobial activity

The silver nanoparticles results from the aqueous extract of *Ajuga parviflora Benth* and *Digera muricata* leaf were further subjected to Staphylococcus aureus, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. Agar well diffusion method was used to check the antimicrobial potential of silver nanoparticles derived from *Ajuga parviflora* Benth and *Digera muricata* leaf extract. As it is very much establish from the literature that silver ions and silver salts/compounds possesses promising antimicrobial potential but their used was limited due to their larger size, in the recent past the researchers were interested to prepare nanoparticles of Ag and Ag based compounds and use them as antimicrobial agents. It was seen that these Ag in the form of nanoparticles have advantages over the conventional chemical based antimicrobial compounds. But later on the toxic effects of chemical based conventional nanoparticles was proved due to which the researchers search the alternate ways to overcome the toxic effects of chemical originated nanoparticles as well the conventional methods for the synthesis of nanoparticles were much expensive, therefore it was essential to synthesize nanoparticles with low cost and ecofriendly method with enhanced antimicrobial potential.Therefore during the recent times researchers focused the ecofriendly synthesis of nanoparticles through plant based extracts which is cost friendly as well non-toxic [26]. Keeping in view the theme, in present study the silver nanoparticles are originated from the leaf extract of *Ajuga parviflora* Benth and *Digera muricata*. It can be seen from the results that the silver nanoparticles are most effective against gram positive bacteria as compared to the gram negative bacteria. One of the possible reasons for the silver nanoparticles activity may be due to electrostatic attraction of silver nanoparticles with bacterial cell membrane which forms pits on the surface resulting the structural changes in bacterial and ultimately cause the death of bacteria [27].

Table 1. Antibacterial activity of *Digera muricata* and *Ajuga parviflora* Benth loaded Ag-NPs (zone of inhibition in millimeter)

Sample	*Staphylococcus aureus (Gram positive)*	*Escherichia coli (Gram negative)*	*Salmonella typhimurium (Gram negative)*	*Pseudomonas aeruginosa (Gram negative)*
Ag Nps Derived from *Ajuga parviflora Benth*	18.7 ± 0.4	14.2 ± 0.7	14.7± 0.5	13.1 ± 0.2
Ag Nps Derived from *Digera muricata*	16.4 ± 0.7	13.9 ± 0.6	14.3± 0.3	15.1 ± 0.4
Streptomycin	23.6 ± 0.8	21.8 ± 0.2	16.6 ± 0.3	18.6 ± 0.3

4. Conclusions

Herein, we reported the biosynthetic method to prepare silver nanoparticles. In this aspect we synthesized AgNPs from two plant extracts. The method reported is an easy, economical and eco-friendly. We used the leaves extract of (*Digera muricata* & *Ajuga parviflora* Benth) to synthesize metallic nanoparticles. The secondary metabolites in plant extract act as a reducing and capping agent. The synthesized AgNPs, which had an average size of 18-22 nm. Antimicrobial essay shows that, these

nanoparticles showed antibacterial activity against *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

To overcome these limitations and implement this procedure for large scale productions, a long-term research is required.

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Manuscript received: 1.06.2020