IJPSR (2020), Volume 11, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 September 2019; received in revised form, 23 April 2020; accepted, 27 April 2020; published 01 September 2020

PLANT MEDIATED GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *RUMEX OBTUSIFOLIUS* LEAF EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY

M. C. Purohit [1], Mahender Singh [*2], Goutam Kumar [3] and Navneet Singh [4]

Department of Chemistry [1, 2], H. N. B. Garhwal University (A Central University), B. G. R. Campus, Pauri Garhwal - 249161, Uttarakhand, India.
Department of Chemistry [3], Cluster University of Jammu, Jammu - 180001, Jammu and Kashmir, India.
Department of Chemistry [4], Roorkee College of Engineering, Roorkee - 247667, Uttarakhand, India.

Keywords:

Silver nanoparticles, *Rumex obtusifolius*, Antibacterial activity

Correspondence to Author:
Mahender Singh

Research Scholar
Department of Chemistry,
B. G. R. Campus Pauri (H.N.B. Garhwal University (A Central University), Srinagar, Garhwal - 246001, Uttarakhand, India.

E-mail: mahender.rana143@gmail.com

ABSTRACT: *Rumex obtusifolius*, belonging to the Polygonaceae family, commonly known as 'broad-leaf-dock' is one of the most important medicinal plants. It is common wayside weeds and uses as an antidote to nettle, depurative, astringent, laxative, and tonic and in the treatment of sores, blisters, jaundice, burns, cancer, and tumors. In this study, we design to synthesize silver nanoparticles from silver nitrate *via* a green route using the aqueous leaf extracts of *Rumex obtusifolius*. This biogenic reduction of metal ion to base metal is quite rapid, readily conducted at room temperature and pressure, and easily scaled up. The use of silver nanoparticles is gaining popularity due to its antibacterial properties. The silver nanoparticles formation in the solution was monitored by UV-Vis spectroscopy, and further characterization was carried out by FT-IR (Fourier Transform Infrared Spectroscopy), XRD (X-ray Diffraction), SEM (Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy) analysis. To optimize the biosynthesis of silver nanoparticles, the effect of process variables such as extract concentrations, the mixing ratio of the reactants, time, and pH were also investigated. The antibacterial activity of the synthesized silver nanoparticles was tested against some pathogenic bacteria like *Staphylococcus aureus*, *Bacilius cereus*, *Escherichia coli*, and *Salmonella typhii* and DMSO is used as a positive control.

INTRODUCTION: The Rumex species, belonging to the Polygonaceae family, comprise about 200 species widely distributed around the World. The name Rumex originated from the Latin word for a dart, alluding to the shape of the leaves [30].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(9).4524-29

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(9).4524-29

The ethnobotanical and ethnopharmacological literature reports dealing with the occurrence and traditional uses of Rumex species [4, 11, 22].

In some areas, the leaves of Rumex species (e.g. R. acetosa, *R. acetosella*, *R. abyssinicus*, *R. crispus*, *R. sanguineus*, *R. tuberosus* and *R. thyrsiflorus*, *R. vesicarius*) are utilized as foods, mainly in the forms of sour soups (usually in milk), sauces and salads [1, 16, 21, 33]. In the case of Rumex, several species used as food reflect their gustatory characteristics, taste, and aroma. The roots of many species have been used in medicine from ancient times because of their gentle laxative effect.

Rumex acetosa is officially listed in the Korean Food Code (Korea Food & Drug Administration) as one of the main food materials, and it is used for the treatment of cutaneous diseases, and in folk medicine, it is used as a mild purgative [14]. Some of the species of this genus are cultivated, *e.g.*, *R. acetosa* and *R. vesicarius* [3]. On the other hand, several members include many invasive weeds (e.g., *R. obtusifolius* and *R. crispus*) [38].

Plants belonging to the Polygonaceae are known to produce a large number of biologically important secondary metabolites, such as anthraquinones, stilbenoids, steroids, flavonoid glycosides, leucoanthocyanidins and phenolic acids [7, 15, 17, 10]. *Rumex obtusifolius*, commonly called as the broadleaved dock or bitter dock, is a perennial herbaceous flowering plant species. It is widely distributed throughout the world. It generally grows near meadows, waste ground, roadsides, ditches, and shorelines.

It grows to a height of 50-130 cm and easily recognizable by large oval leaves with cordate bases and rounded tips. The leaves can grow to about 40 cm in length. It contains small greenish flowers that change to red as they mature. The stems of the plant are tough, often reddish, and un-branched until just below the inflorescence, and the stem leaves are alternate and are narrowly ovate-lanceolate. It shows numerous medicinal or therapeutic and pharmacological properties. This plant is used to cure jaundice, rheumatism and its roots are used as dyeing purposes.

‘‘Nanotechnology” was presented by Nobel laureate Richard P. Feynman during his well famous 1959 lecture ‘‘There’s Plenty of Room at the Bottom”, there have been made various revolutionary developments in the field of nanotechnology. Nanotechnology produced materials of various types at the nanoscale level. Nanoparticles (NPs) are a wide class of materials that include particulate substances, which have one dimension less than 100 nm at least [1]3. Depending on the overall shape, these materials can be 0D, 1D, 2D, or 3D [35]. The importance of these materials realized when researchers found that size can influence the physicochemical properties of a substance, *e.g.*, the optical properties. A 20- nm gold (Au), platinum (Pt), silver (Ag) and palladium (Pd) NPs have characteristic wine red color, yellowish gray, black and dark black colors, respectively [2, 5, 8, 24-28].

Of the noble MNPs, the AgNPs are the most widely recognized for their applications in different areas such as photonics, microelectronics, photocatalysis, medicine, and lithography. For instance, the common use of AgNPs in medicine can be credited to their powerful antimicrobial activity against a wide range of pathogenic microorganisms. Various physical and chemical techniques, including laser ablation, lithography, and photochemical reduction, are used efficiently for the production of significant amounts of AgNPs. Nevertheless, these techniques remain comparatively expensive and sometimes involve the use of some hazardous moieties. Consequently, the development of a green and effective method to develop AgNPs is of great importance [6, 12, 18, 19, 20, 23, 30, 34, 36].

MATERIALS AND METHODS:

Preparation of Plant Extract: The fresh leaves of *Rumex obtusifolius* were collected from different regions of Uttarakhand, and the collected leaves were washed with de-ionized or double distilled water to remove dust and adhering pathogens and disinfected with 0.1% mercuric chloride solution. The sample is kept for drying under shade. The dried leaves were grounded to a fine powder with the aid of grinder or mortar. Around 50 g of dried crushed or powdered leaves were boiled with 150 ml distilled water for 20 min at 60 ºC and filtered through Whatman no. 1 filter paper, and the obtained aqueous leaf extract was used for further experiments. The plant samples were authenticated from the Department of Botany, HNBGU, Srinagar Garhwal, Uttarakhand.

Synthesis of Silver Nanoparticles: Silver nano-particles were synthesized by adding 50 ml of aqueous leaf extract into 200 ml of 1mM aq. the solution of silver nitrate at room temperature. The color of the solution was started changing from light yellow to dark brown within 5 min, indicating the formation of silver nanoparticles and further, no change in color can be observed. The separation of silver NPs from the dark brown solution was carried out by centrifugation (at 7000 rpm for 20 min) process. After that, obtained silver NPs were washed four times with distilled water and acetone to remove water-soluble impurities, and then nanoparticles were stored in dry bottles for further study.

Characterization of Synthesized Nanoparticles: Characterization of plant-mediated synthesized silver nanoparticles was done using some important and advanced techniques like TEM, XRD, FT-IR & UV-Vis spectroscopy. The reduction of silver ions was monitored by measuring the UV-Vis spectrum of reaction medium after 3 h after diluting a small aliquot of the sample into double distilled water. This analysis was performed using Perkin Elmer, Lambda 25 UV-Vis spectrophotometer. Transmission Electron Microscopic (TEM) analysis was performed using JEOL JEM 1400 (Jeol Ltd., Tokyo, Japan).

It is a unique tool for characterizing crystalline structures and microstructures of materials simultaneously by diffraction and imaging techniques. It is capable of displaying magnified images of a thin specimen, typically with a magnification in the range of 10^{-3} to 10^{-6}. FT-IR analysis was carried out on a Shimadzu IR-prestige-21 (Shimadzu Corp., Japan) performed in the range of 400-4000 cm^{-1} to identify the functional groups that might be involved in nanoparticles formation. XRD analysis was performed using Bruker D2 model.

RESULTS AND DISCUSSION: In this research work, *Rumex obtusifolius* leaf extract was used for the green synthesis of silver nanoparticles and examination of the antibacterial activity of these synthesized nanoparticles. Silver nanoparticles were synthesized by adding 50 ml of aqueous leaf extract into 200 ml of 1mM aq. the solution of silver nitrate at room temperature.

The color change in the solution from light yellow to dark brown in 5-10 min indicating the formation of silver nanoparticles, and then the formed silver nanoparticles were separated from the solution using the centrifugation process at 5000-7000 rpm at 20 min. Thus, the synthesized plant-mediated silver nanoparticles were characterized by the following techniques.

UV-Vis Spectroscopic Analysis: The silver nanoparticles showed their characteristic absorption maxima between the range 400-460 nm. **Fig. 1**, showing the results of AgNPs prepared by aqueous extract of *Rumex obtusifolius*. The maximum and narrow absorption peak were observed at 460 nm, which is due to Surface Plasmon resonance (SPR).

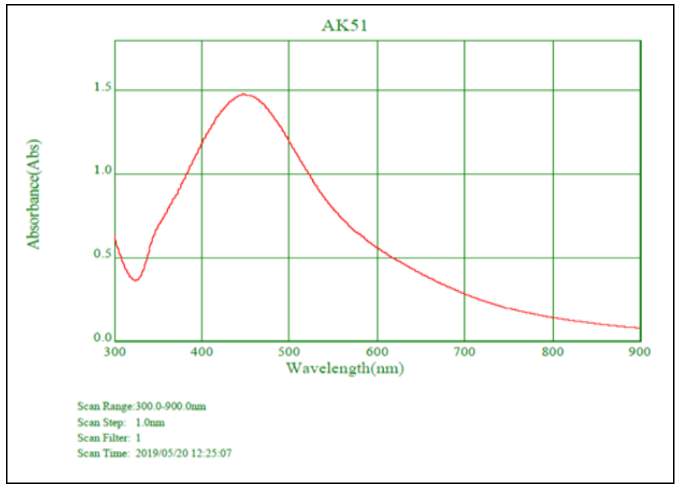

FIG. 1: UV-VIS SPECTRA OF *RUMEX OBTUSIFOLIUS* LEAF EXTRACTS MEDIATED AGNPS

Transmission Electron Microscopic Analysis: Fig. 2, showing the results of AgNPs prepared by aqueous extract of *Rumex obtusifolius*. The size of synthesized AgNPs was found in the range of 5.01 nm – 22.58 nm. The shape of NPs is spherical.

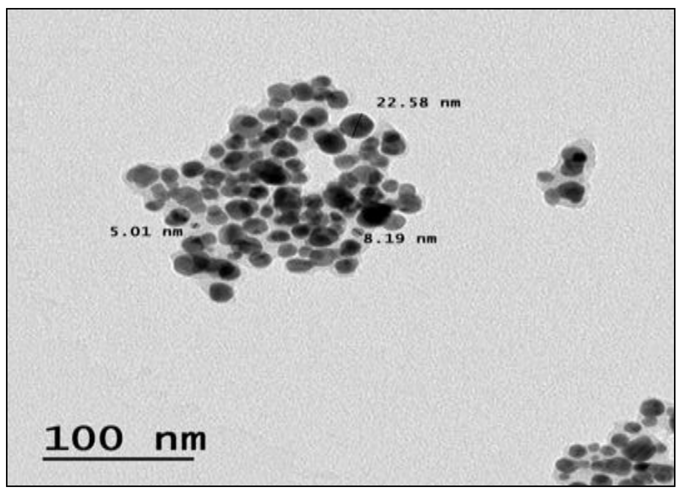

FIG. 2: TEM IMAGE OF *RUMEX OBTUSIFOLIUS* LEAF EXTRACTS MEDIATED AGNPS

X-ray Diffraction Analysis: Fig. 3, showing the results of AgNPs prepared by aqueous extract of *Rumex obtusifolius*. The spectra show 2θ (degree) values with (hkl) planes at 38.43 (111), 64.65 (220) and 77.5 (311) were recorded, which confirms the presence of silver nanoparticles AgNPs.

Fourier-Transform Infrared Spectroscopy: Fig. 4, showing the results of AgNPs prepared by aqueous extract of *Rumex obtusifolius*.

The intense peak at 3432.8 cm^{-1} corresponds to O-H stretching (strong, broad) intermolecular bonded, 2090.1 cm^{-1} corresponds to C≡C alkynes stretching mono-substituted, 1625.7 cm^{-1} corresponds to C=C stretching (medium), conjugated, 1494.4 cm^{-1} corresponds to C-H bending (medium), methylene group, 1377.3 and 1393.5 cm^{-1} correspond to O-H bending (medium), phenolic group, 1270 cm^{-1} corresponds to Ar-OH stretching (medium).

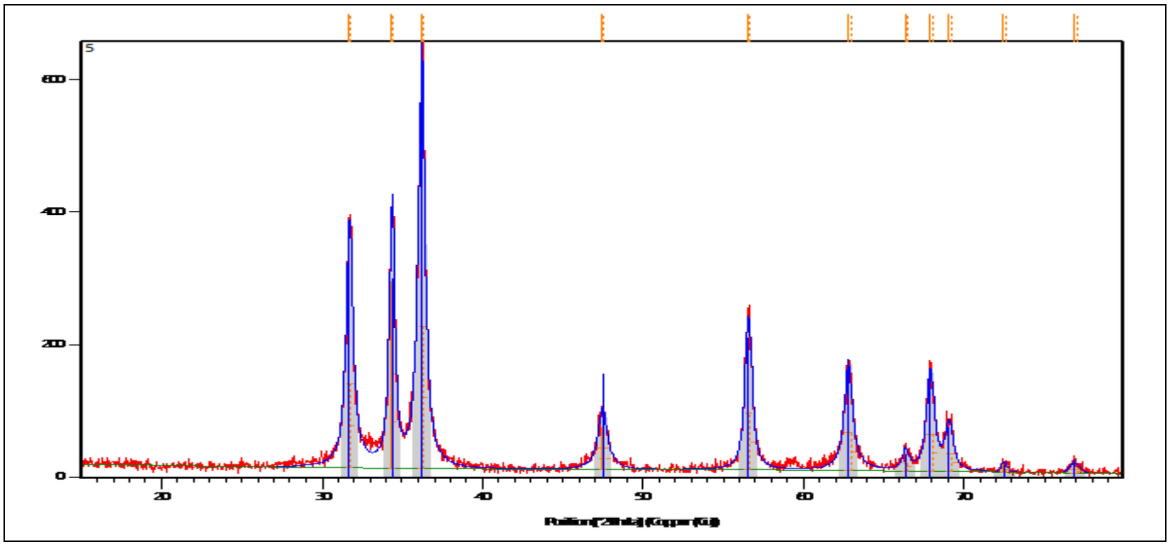

FIG. 3: X-RAY DIFFRACTION (XRD) SPECTRA OF *RUMEX OBTUSIFOLIUS* LEAF EXTRACTS MEDIATED AGNPS

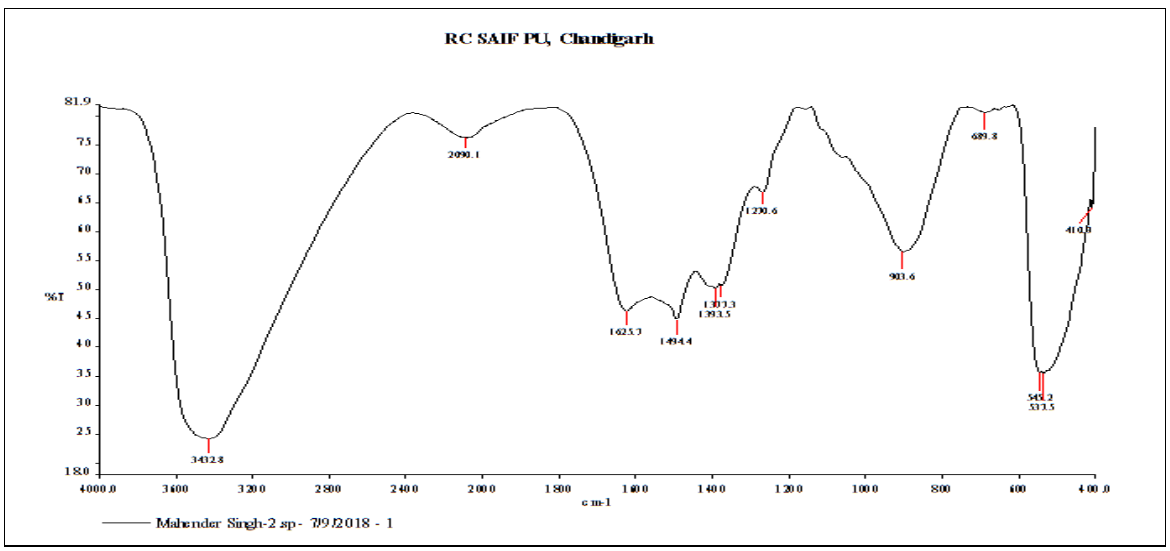

FIG. 4: FT-IR SPECTRA OF *RUMEX OBTUSIFOLIUS* LEAF EXTRACTS SYNTHESIZED AGNPS

Antimicrobial Activity:

Antibacterial Activity of Synthesized AgNPs: Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti-fungal, or antiviral. They all have different modes of action by which they act to suppress the infection. Antibacterial activity of nanoparticles is determined by their ability to effectively release metal ions and their surface area.

Nanoparticles, by virtue of their extremely large surface area, are able to establish better contact with microorganisms. In the present investigation, the antimicrobial (anti-bacterial) activity of synthesized metallic nanoparticles of aqueous extracts of *Rumex obtusifolius* was tested against four pathogenic bacteria *Staphylococcus aureus* and *Bacillus cereus* (gram's positive bacteria) and *Escherichia coli* and *Salmonella typhi* (gram's negative bacteria). The results are discussed below in **Table 1** as follows. Antibacterial activity results of synthesized silver nanoparticles prepared by aqueous extracts of *Rumex obtusifolius* are as follows: Zone of inhibition against *Staphylococcus aureus* (8.0 mm), *Bacillus cereus* (6.4 mm), *Escherichia coli* (6.6 mm) and *Salmonella typhi* (resistant to activity). DMSO, which is used as a control, is also resistant to activity.

TABLE 1: ANTIMICROBIAL (ANTIBACTERIAL) ACTIVITY OF NANOPARTICLES AGAINST GRAM'S POSITIVE AND GRAM'S NEGATIVE BACTERIA

Organisms	Zone of inhibition (in mm)	Control
Staphylococcus aureus	8.0 mm	R
Bacillus cereus	6.4 mm	5.6 mm
Escherichia coli	6.6 mm	R
Salmonella typhi	R	6.0 mm

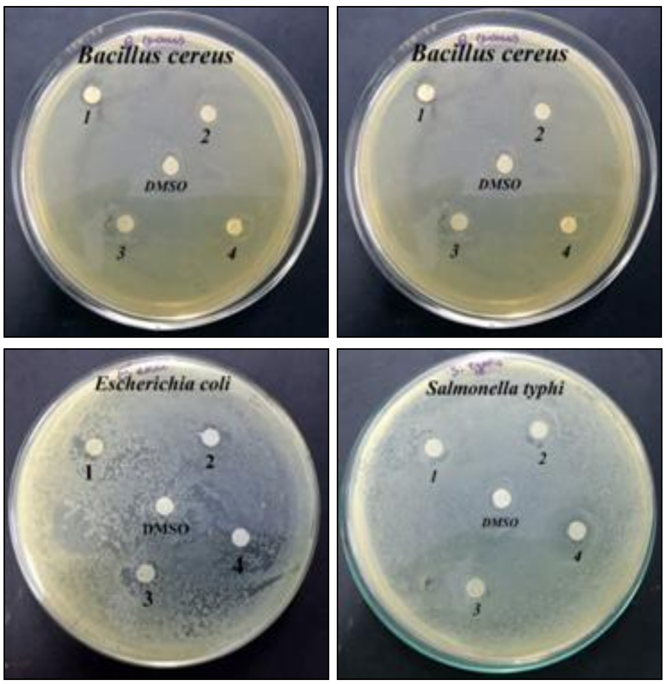

FIG. 5: SAMPLE 1 DISPLAYING THE ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES OF *RUMEX OBTUSIFOLIUS* LEAF EXTRACT

CONCLUSION: This study describes the synthesis of silver nanoparticles from the aqueous leaf extracts of *Rumex obtusifolius* and examination of antibacterial activity and their characterization. On the basis of results, it is concluded that the aqueous silver ions exposed to the aqueous extract of *Rumex obtusifolius* were reduced, and nano-particles were synthesized. The change in the color of the solution containing silver nitrate solution indicates the formation of the silver nanoparticles. The formation of silver nanoparticles firstly confirmed by UV-Vis spectroscopy and then other techniques like TEM, FT-IR, and XRD. This biogenic reduction of metal ion to base metal is quite rapid, readily conducted at room temperature and pressure, and easily scaled up. The use of silver nanoparticles is gaining popularity due to its antibacterial properties.

ACKNOWLEDGEMENT: The authors are thankful to Punjab university Chandigarh for FT-IR, thanks are due to USIC H. N. B. Garhwal University, Srinagar Garhwal for XRD and to EM-UNIT CDRI Lucknow for TEM. Authors are also grateful to the National Fellowship for Higher Education (RGNF-NFST) for funding

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:
Purohit MC, Singh M, Kumar G and Singh N: Plant mediated green synthesis of silver nanoparticles using *Rumex obtusifolius* leaf extract and their antibacterial activity. Int J Pharm Sci & Res 2020; 11(9): 4524-29. doi: 10.13040/IJPSR.0975-8232.11(9).4524-29.