

Evaluation of Antimicrobial Activity of Water Infusion Plant-Mediated Silver Nanoparticles

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Abstract

Phytochemical analysis revealed that the infusion of *Rumex dentatus* is rich in flavonoids, phenolics, reducing sugars and total sugars. The plant infusion found to possess a significant antioxidant activity. The aqueous extract of *Rumex dentatus* has been used as a green reducing and stabilizing agent in addition to its desirable biological activity. The biosynthesis of reduced silver nanoparticles was very fast, and silver nanoparticles were synthesized by exposure of a mixture of different ratios of 4 mM AgNO₃ and *Rumex dentatus* aqueous extract to UV-irradiation at room temperature. Ultraviolet-visible absorption spectroscopy, transmission electron microscopy and X-ray diffraction confirmed reduction of Ag⁺ ions and formation of silver nanoparticles. Varied morphology of the bioreduced silver nanoparticles was recorded. On contrast to the main plant infusion, the resulting silver nanoparticles were found to possess wide spectrum of antimicrobial activity increasing proportionally with the increase of the concentration of the silver nanoparticles against wide variety of bacterial and fungal pathogenic strains.

Keywords: Antimicrobial activity; Phenolics; Flavonoids; Antioxidant; Silver nanoparticles

Introduction

Nanoparticles are atomic or molecular aggregates with at least one dimension between 1 and 100nm [1-3], that can drastically modify their physico-chemical properties compared to the bulk material [3,4]. Nanoparticles can be made from a variety of bulk materials and can explicate their actions depending on the chemical composition and on the size and/or shape of the particles [3,5]. Nanostructure materials have generated intense scientific and technological interest because of their potential applications in areas as diverse as electronics, optics, sensors, information and communication technology, transparent sunscreen lotions, stain-resistant fabrics, scratch free paints for cars, products labeling, textiles, biomedicine, health care, antibacterial dressings [6,7].

The noble metal nanoparticles such as gold (Au), platinum (Pt) and silver (Ag) nanoparticles have gained a considerable interest over the last decade owing to their important applications [8-11]. It is now well understood that the intrinsic characteristics of noble metal nanoparticles are dependent on their composition, size, crystallinity, shape, and structure (either hollow or solid) [12]. Ag nanoparticles are excellent components for the Surface Enhanced Raman Scattering (SERS) to probe single molecules [13], in addition to their desirable role as catalysts for accelerating some chemical reactions [14]. Moreover, they have been used in other various applications as antimicrobial, electrical conducting, and in sensing/optical applications [15].

An important branch of biosynthesis of nanoparticles is the application of plant extracts to the biosynthesis reaction. With increasing focus on green chemistry, natural compounds like glucose [16], chitosan [17], soluble starch [18] and some microorganisms [19-22], etc., have attracted considerable research interest as safer alternatives, reducing and stabilizing agents to synthesize the silver nanosphere. Synthesis of nanoparticles through biochemical routes, using plant extracts as reducing and capping agents, has received special attention among others, due to maintaining an aseptic environment

during the process [23-29]. Therefore, medicinal plants having well established therapeutic importance are being widely used for the size- and shape-controlled synthesis of silver nanoparticles [30-33].

Rumex dentatus L. (Polygonaceae) is a weedy plant known as toothed dock. It is widely distributed in many countries including Egypt. It occurs in waste places, shores and cultivated fields. It has been used as a leafy vegetable in the Mediterranean diet. It responds to unfavourable environmental conditions by including Reactive Oxygen Species (ROS) and malonyldialdehyde [34-38]. Its leaves are diuretic, refrigerant, and used as cooling agent, while the roots are used as an astringent and in cutaneous disorders [38-40]. It showed antibacterial, antifungal, insecticidal, molluscicidal, antitumor, astringent, anti-inflammatory, antidermatitis and allelopathic activities [37,41-46]. Its roots are used in folk medicine for treating acariasis, eczema, diarrhea and contipation [37,47]. Previous phytochemical studies on *Rumex dentatus* have demonstrated the presence of anthraquinones, flavonoids, phytosterols, phytoesteryl esters, free fatty acids, chromones and anthrones [37,48,49]. Furthermore, some phenolics have been detected in *R. dentatus* [38,41,50].

Materials and Methods

Plant material and Preparation of the infusion

The aerial parts of the wild *Rumex dentatus* plants were collected

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from their original habitats Mansoura, Egypt. The plant was taxonomically identified according to Boulos [51].

Fresh parts of *Rumex dentatus* were washed thoroughly and chopped into fine pieces. About 20 gm of the chopped *Rumex dentatus* were transferred into a 500 ml Erlenmeyer flask containing 100 ml deionized water, mixed well, and boiled for 10 minutes in water bath at 80°C. The extract obtained was filtered through Whatman no. 1 filter paper, and the filtrate was collected and stored at 4°C for further use.

A part of the plant material was air dried in shadow for 15-20 days and then grinded into fine powder for phytochemical analysis.

Phytochemical analysis

Determination of total phenolic compounds: The determination of total phenolic compounds in the extracts was carried out using Folin Ciocalteu method developed by Wolfe et al. [52].

Gallic acid was used as a standard, where total phenolics content expressed as mg gallic acid equivalent /100g dried plant material.

Determination of total flavonoids content: The total flavonoids were determined using aluminum chloride colorimetric assay developed by Zhishen et al. [53].

Catechin was used as a standard, where total flavonoids content expressed as mg catechin equivalent /100g dried plant material.

Determination of the radical scavenging activity using DPPH assay: The antioxidant activity was estimated by the Free radical scavenging method (DPPH) as described by Kitts et al. [54] with slight modifications by Liyana- Pathirana et al. [55] and Parejo et al. [56].

Gallic acid, catechin and ascorbic acid were used as standards.

Total soluble sugars and total carbohydrates content: Carbohydrates content and total soluble sugars were determined spectrophotometrically using the method described by Thayumanavan and Sadasivam [57].

Glucose was used as a standard, where total soluble sugars and carbohydrates content were expressed as g glucose equivalent /100g dried plant material.

Synthesis of silver nanoparticles

Aqueous silver nitrate solution (4 mM) was prepared and used for the synthesis of a series of silver nanoparticles in presence of the prepared *Rumex dentatus* water extract upon exposure of the mixture to UV irradiation as a reducing agent for the Ag⁺ ions. Different v/v ratios of the extract and the aqueous AgNO₃ solution were mixed thoroughly (15:60, 25:50, 37.5:37.5, 50:25, and 60:15) and the mixtures were subjected to UV-irradiation using different time intervals from 2 min up to 10 min.

Instrumental analysis

ATI Unicom UV-Vis. Spectrophotometer: The reduction of pure Ag⁺ ions into silver nanoparticles was monitored by recording the UV-Vis spectra of the reaction mixture at different time intervals. The UV-Visible spectra of the resulting silver nanoparticles were recorded in the range of 200-800 nm using ATI Unicom UV-Vis. spectrophotometer with the aid of ATI Unicom UV-Vis. vision software V 3.20. The analysis was performed at room temperature using quartz cuvettes (1 cm optical path), and the blank was the corresponding *Rumex dentatus* aqueous solution.

Fourier transform infrared spectroscopy: FT-IR measurements were carried out for both the *Rumex dentatus* aqueous extract and the silver nanoparticles synthesized in the presence of the *Rumex dentatus* to identify the possible biomolecules in the *Rumex dentatus* extract that can participate in the reduction process of the Ag⁺ ions and capping of the resulting silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on Mattson 5000 FTIR spectrometer in the range of 400–4000 cm⁻¹ at a resolution of 8 cm⁻¹ at 25°C.

Transmission electron microscope measurement: The size and morphology of the resulting silver nanoparticles was investigated by transmission electron microscopy, TEM (JEOL TEM-1230) attached to a CCD camera at an accelerating voltage of 120 kV. The samples were prepared by placing few drops of the nanoparticles suspension on carbon coated copper grids, followed by allowing the solvent to slowly evaporate under the sun light before recording the TEM images.

Microbial susceptibility testing

The antimicrobial activity of the plant infusion was estimated by filter paper disc method (9) using inoculums containing 10⁶ bacterial and fungal cells or 10⁸ yeast cells/ml to spread on nutrient agar, Czapek Dox agar and Sabouraud agar plates, respectively.

The sterilised filter paper discs (Whatman no.1, 6mm in diameter) were saturated with infusion obtained from the plant and another set of filter paper discs were soaked in water served as controls. The discs were placed on the surface of agar plates seeded with the test organisms. The plates were incubated at 28°C for fungi, at 37°C for bacteria and at 30°C for yeast. Diameters of inhibition zone (mm) were measured after 18-24 hours for bacteria, 24-48 hours for yeast and 4-7 days for fungi [58].

Paper discs impregnated with 20 µl of a solution of 10 mg/ml of Ampicillin, Penicillin and Tobramycin (for bacteria) and Nystatin and Clotrimazole (for fungi) as standard antimicrobials were used for comparison.

Tested organisms

Bacteria: *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella spp.*, *E. coli*, *Proteus vulgaris*.

Fungi: *Aspergillus niger*, *Erwenia carotovora*, *Fusarium solani*, *Aspergillus flaviries*, *Aspergillus ochraceous* and *Trichothecium spp.*

Stock cultures of the tested organisms were obtained from the microbiological lab at Faculty of Medicine in Mansoura University.

Results

Phytochemical analysis

Rumex dentatus is well known to be rich in diverse phytochemicals like phenolics, flavonoids, carbohydrates, and vitamins that might play a significant role in bioreduction of silver nanoparticles.

Total phenolics content

The phenolic compounds, ubiquitous in plants are an essential part of the human diet and are of considerable interest due to their antioxidant properties. The antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings [59,60].

The total phenolic compounds of the *Rumex dentatus* examined

infusion, was reported as gram of gallic acid equivalents/100 gram of the dried plant material with reference to the standard curve ($y=0.0063x$, $r^2=0.987$). The total phenolics content was 175 ± 4.20 mg gallic acid equivalent per gram of *Rumex dentatus* extract and 2.70 ± 0.05 gram of gallic acid equivalents/100 gram of the dried plant material.

The total Flavonoids content

The total Flavonoids content were expressed as gram catechine equivalent per 100 gram of the dried plant material by reference to standard curve ($y=0.003 x$, $r^2=0.994$). Among the different plant extracts, the total flavonoids contents was 43.75 ± 1.30 mg catechine equivalent per gram of *Rumex dentatus* extract and 0.675 ± 0.013 gram of gallic acid equivalents/100 gram of the dried plant material.

Evaluation of the antioxidant activity of the plants water infusions

The free radical scavenging activity using DPPH assay: The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (EC50) is a parameter widely adopted to measure the antioxidant activity [61]. The lower the EC50, the higher the antioxidant power. Another parameter was defined as antiradical efficiency ($AE=1/EC50$) or antiradical power (ARP) was used [62]. The higher the AE, the higher the antioxidant activity. Catechine, ascorbic acid and gallic acid were employed as reference compounds in this experiment. EC50 value is calculated as mg of the tested extract or compound/ mg of DPPH where the inhibition of test activity reached 50% using the exponential curve obtained from plotting the DPPH % against mg sample/mg DPPH.

The water extract of the wild *R. dentatus* plants showed $EC50=3.23$ mg extract/mg DPPH and $AE=0.134$. Catechine, ascorbic acid and gallic acid showed $EC50=0.268$, 0.610 and 0.525 mg extract/mg DPPH and $AE=3.73$, 1.64 and 1.91 , respectively.

Total soluble sugars and total carbohydrates content

The total soluble sugars and total carbohydrates were 2.61 ± 0.25 and 3.26 ± 0.02 gram glucose equivalent/100 gram of the dried plant material, respectively (Figure 1).

Synthesis of nanoparticles

The well known yellowish brown color of silver nanoparticles arises due to excitation of Surface Plasmon Resonance (SPR) with an absorption maxima at 460 nm.

UV-Vis spectroscopy: The synthesis of the silver nanoparticles has been confirmed by measuring the UV-Vis spectra of the reaction mixture (Figure 2). As apparent from Figure 2, the absorption peak appeared at about 460 nm is corresponding to the characteristic surface plasmon resonance of the resulting Ag NPs. The silver nanoparticles formed employing *R. dentatus* extract were found to be very stable, possibly because of the starch present in the extract that prevented agglomeration (Figure 2).

FTIR spectroscopy: FTIR analysis was carried out in order to identify the possible reducing and stabilizing biomolecules, described in Figure 1, in the *Rumex dentatus* extract. FTIR absorption spectra of *R. dentatus* water extract before and after reduction of silver are shown in Figure 3. FTIR spectrum of *Rumex dentatus* extract shows bands at 517, 773, 825, 1078, 1384, 1635 and 3432 cm^{-1} . The band around 1078 cm^{-1} corresponds to the C-O stretching vibration whereas, the band

appeared at about 1635 cm^{-1} can be assigned for aromatic rings. The strong broad band appearing at 3432 cm^{-1} can be associated to the stretching vibrations of alcoholic and phenolic O-H (Figure 3).

Transmission electron microscopy: Transmission electron microscopy (TEM) has been utilized to characterize the size and morphology of the formed silver nanoparticles. Figure 4 shows the TEM micrographs of the silver nanoparticles developed from a *Rumex dentatus* and $AgNO_3$ after UV-irradiation reduction. From the Figure, the obtained nanoparticles are predominantly spherical, polydispersed and are surrounded by a thin layer of organic material which tends

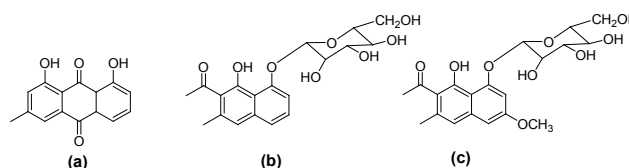


Figure 1: The chemical structure of the three main compounds in the *Rumex dentatus* extract.

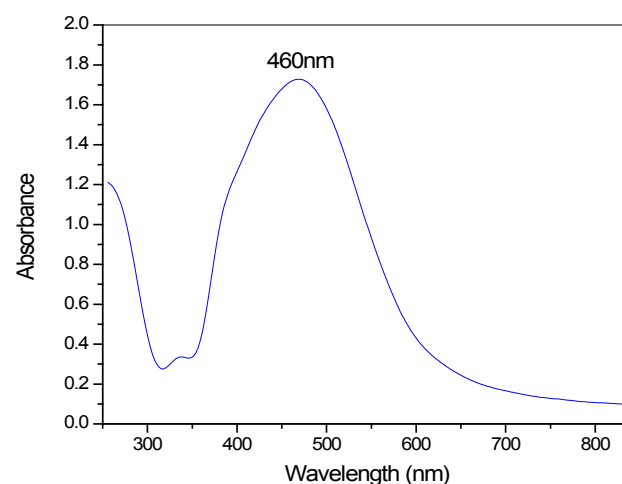


Figure 2: UV-vis spectra of Ag nanoparticles recorded as a function of reaction time.

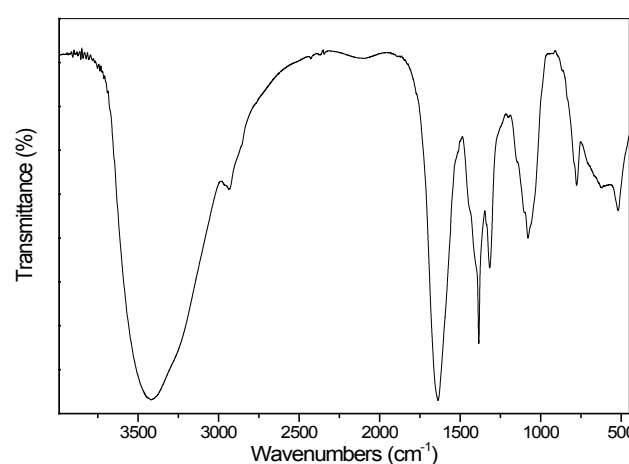


Figure 3: FTIR spectrum of *Rumex Dentatus* extract.

to be characteristic of silver nanoparticles developed in plant extracts. The Figure also propose the presence of two distinct size regions, small nanoparticles with diameters in the range between 5 and 18 nm and larger particles of about 30 nm sizes.

In order to verify the crystalline nature of the nanoparticles the selected area electron diffraction (SAED) patterns were obtained for the sample containing 4 mM of AgNO_3 . The presence of bright circular rings in the SAED patterns confirms the crystalline nature of the silver nanoparticles. The spots corresponding to various orientations appearing inside the concentric rings also show that the obtained silver nanoparticles has a good crystallinity [63].

Evaluation of the antimicrobial activity

Microbial Susceptibility Testing (Disc diffusion assay): Silver is well known as one of the most universal antimicrobial substances. The results of the antimicrobial activity of wild *Rumex dentatus* water extracts and the prepared silver nanoparticles using different v/v ratios of the water extract and the aqueous AgNO_3 solution (15:60, 25:50, 37.5:37.5, 50:25, and 60:15) that were mixed thoroughly and subjected to UV-irradiation tested against pathogenic microbial strains by disc diffusion assay are summarized in Table 1.

The water extract of wild *R. dentatus* plants was found to possess antimicrobial spectrum against *Staphylococcus epidermis*, *Klebsiella pneumonia* and *E. coli*.

As the percent of synthesized silver nanoparticles increased as the antimicrobial spectrum increased. The silver nanoparticles synthesized from the mixture with the ratio 4 AgNO_3 : 1 *R. dentatus* water extract showed the broadest antimicrobial spectrum against the tested pathogenic strains among all the other prepared extracts as shown in Table 1 and Figure 5.

Discussion

We have successfully demonstrated a rapid and efficient route for synthesis of silver nanoparticles using *R. dentatus* extract. The development of yellowish brown color owing to the surface Plasmon resonance with absorption maxima at 460 nm confirmed the formation of silver nanoparticles [64,65]. *R. dentatus* is a rich source of flavonoids and phenolics. Flavonoids play a vital role in the reduction process for synthesis of silver nanoparticles [65,66]. Thus the high flavonoid and phenolics content in *R. dentatus* water extract revealed in the phytochemical analysis strongly support the potential of *R. dentatus* to bioreduce Ag^+ to Ag^0 . Similarly the reducing sugars that are known

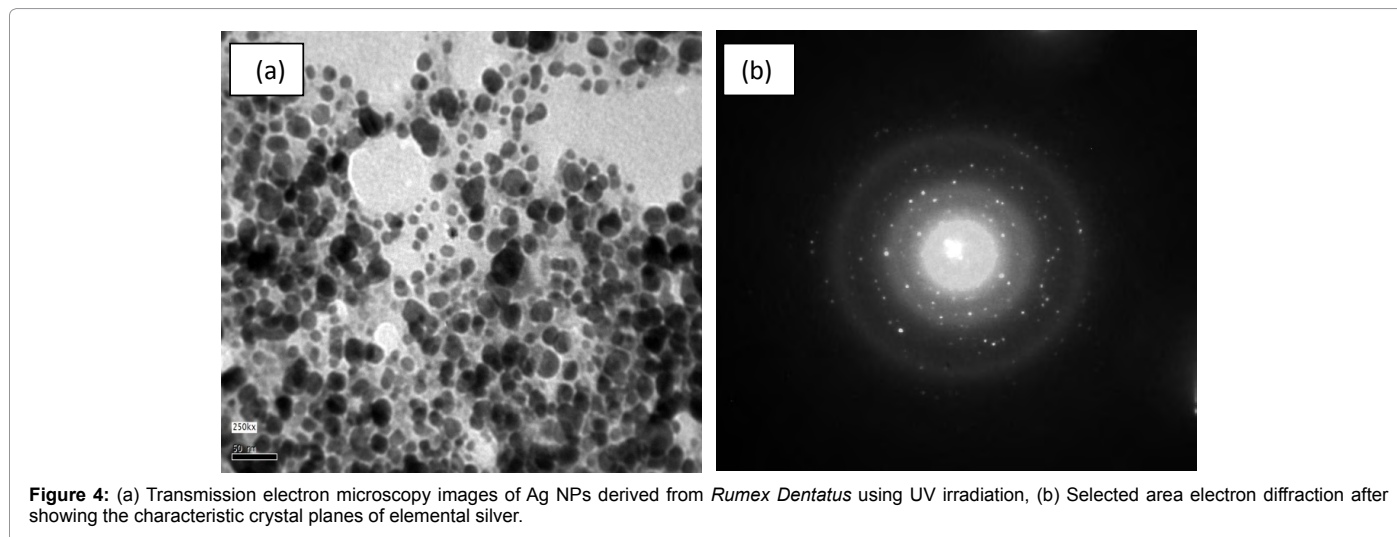
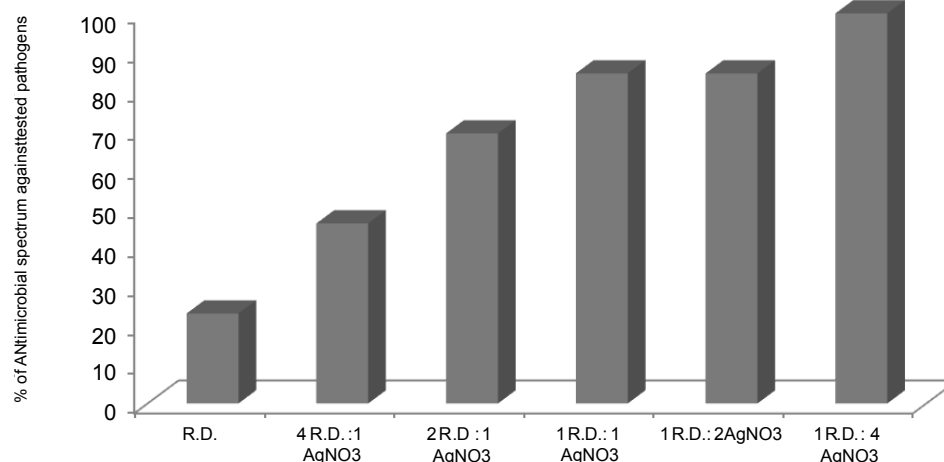


Figure 4: (a) Transmission electron microscopy images of Ag NPs derived from *Rumex Dentatus* using UV irradiation, (b) Selected area electron diffraction after showing the characteristic crystal planes of elemental silver.

Tested Pathogenic Microbial strains	<i>Rumex dentatus</i> extract (R. D.)	Zone of Inhibition (ZOI)				
		4 R.D. : 1 AgNO_3	2 R.D. : 1 AgNO_3	1 R.D. : 1 AgNO_3	1 R.D. : 2 AgNO_3	1 R.D. : 4 AgNO_3
<i>Staphylococcus epidermis</i>	8	-	11	9	13	16
<i>Klebsiella pneumonia</i>	7	10	9	8	-	11
<i>Pseudomonas aurignos</i>	-	9	15	13	23	15
<i>Staphylococcus aureus</i>	-	6	13	13	8	10
<i>Shigells spp.</i>	-	14	10	10	18	18
<i>E. coli</i>	10	8	9	15	12	20
<i>Staphylococcus biogenesis</i>	-	-	-	14	15	18
<i>Erwenia carotovora</i>	-	-	9	18	2	10
<i>Proteus vulgaris</i>	-	8	12	-	13	13
<i>Fusarium solani</i>	-	-	-	-	-	7
<i>Asperigillus flaviries</i>	-	-	-	10	9	12
<i>Asperigillus ochraceous</i>	-	-	14	13	11	10
<i>Trichothecium</i>	-	-	-	14	15	22

Zone of inhibition, including the diameter of the filter disc (6.0 mm)

Table 1: Antimicrobial activity of the wild *R. dentatus* water extract and the synthesized silver nano particles using mixtures of different ratios of the plant extract and silver nitrate solution using disc diffusion assay.



R.D. = *Rumex dentatus*

Figure 5: % of the antimicrobial spectrum of *R. dentatus* water extract alone and in combination with different concentrations of AgNO₃ from which nanoparticles synthesized against tested pathogenic strains.

to play a vital role in bioreduction were found to be predominant in the extract [65,67]. Likewise the non soluble carbohydrate content like starch reflects the capping properties of the extract, and starch is widely used in the synthesis and stabilizing silver nanoparticles [68].

The FTIR absorption spectra offered information regarding chemical changes in the functional groups involved in the bioreduction of precursors and evolution of shape in nanoparticles [65,69].

Silver ion and silver based compounds are highly toxic to microorganisms, showing strong biocidal effect against microbial species because these are highly reactive species with large surface area [65,70-73]. Silver nanoparticles produced using plant extracts are known to exhibit antimicrobial activity [74-75]. Antimicrobial activity determined using disc diffusion method confirmed that synthesis of nanoparticles using *R. dentatus* plant extract and UV-irradiation resulted in a greater bactericidal and fungicidal effect with increasing the concentration of the synthesized nano particles on the test pathogens than either the water extract alone or the silver nanoparticles with lower concentration. This experiment provides solid evidence of synergy between *R. dentatus* water extract and silver nanoparticles.

More recently, it was shown that silver (I) chelation prevents unwinding of DNA. Silver nanoparticles are composed of silver (0) atoms [65,76]. Silver nanoparticles are larger in size than silver (I) ions, which make them react with more molecules, leading to more antimicrobial activity.

In conclusion, In an attempt to find natural, environmentally benign, and easily available plant-based agents for synthesis of metal nanoparticles, we have demonstrated the efficiency of *R. dentatus* tuber extract in the rapid synthesis of silver nanoparticles possessing a variety of fascinating morphologies owing to its diverse groups of phytochemicals like phenolics, flavonoids, reducing sugars, anthraquinones, sterols, phytoesteryl esters, free fatty acids, chromones and anthrones. Based on our kinetic studies, together with evidence obtained from FTIR, we propose that the main biomolecules responsible for nanoparticles synthesis were polyphenols or flavonoids.

The results of our study show that the combination of silver

nanoparticles and water extract of *R. dentatus* had improved antimicrobial efficiency against the tested pathogenic microbes. Overall, this combinational approach seems to be one of the best strategies for therapeutic management in infection control.

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