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Antioxidant activity of silver nanoparticles synthesized from crud methanolic extract of rumex nervosus

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Abstract. In the present investigation, an attempt was made to prepare nanoparticles by using a medicinal plant Rumex nervosus, because the biological synthesized nanoparticles have been widely used in the field of medicine now. Silver sulfate (Ag_2SO_4) was used for the synthesis of the silver nanoparticles using the root extract of Rumex nervosus. The synthesized silver nanoparticles from silver sulfate solution through the root extract were characterized using UV-vis spectrophotometry, SEM, and FT-IR methods. The SEM analysis showed the average size of 75 nm with spherical shape and it confirmed the formation of nanoparticles in the sample. Antioxidant activity of the crude extract was evaluated by employing the DPPH radical scavenging assay. The result showed moderate antioxidant activity at various concentration as compared to standard ascorbic acid solution. The synthesized nanoparticles can be used for various applications due to its eco-friendless, nontoxic and compatibility for pharmaceutical and other applications.

Keywords: antioxidant, nanoparticles, Rumex nervosus.

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ХИМИЯ

УДК 620.3 Научная статья

Антиоксидантная активность серебряных наночастиц, синтезированных из метанольного экстракта румекса нервозуса

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Аннотация. В данном исследовании была предпринята попытка приготовить наночастицы с использованием лекарственного растения Rumex nervosus, поскольку биологически синтезированные наночастицы в настоящее время широко используются в области медицины. Для синтеза наночастиц серебра использовали сульфат серебра (Ag_2SO_4) с применением экстракта корня Rumex nervosus. Синтезированные наночастицы серебра из раствора сульфата серебра через экстракт корня были охарактеризованы с помощью методов УФ-визуальной спектрофотометрии, СЭМ и ИК-Фурье. СЭМ-анализ показал средний размер 75 нм и сферическую форму, что подтверждает образование наночастиц в образце. Антиоксидантная активность сырого экстракта оценивалась с помощью теста на поглощение радикалов DPPH. Результат показал умеренную антиоксидантную активность в различных концентрациях по сравнению со стандартным раствором аскорбиновой кислоты. Синтезированные наночастицы могут быть использованы в различных областях благодаря своей экологичности, нетоксичности и совместимости с фармацевтическими и другими приложениями.

Ключевые слова: антиоксидант, наночастицы, Rumex nervosus.

Финансирование. Работа не выполнялась в рамках фондов.

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1. Introduction. Nanothecnology is the fastest growing area of frantic search for new nanomaterials and methods to make them [1]. Lately, widely nanobiotechnology have been utilize to enhance and improve avails of nanoscale materials for a broad range of applications in advanced biotechnology [2]–[3]. Nanoparticles have small sizes and large surface area making it possesses unique physical, chemical and biological properties [4]–[6]. Silver nanoparticles have been used in various applications due to their individual characteristic properties such as size depending optical, shape and electrical [7]. Furthermore, there are many applications of silver nanoparticles such as antimicrobial, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components [8]–[10]. Rumex nervosus is a medicinal plants that have been used by indigenous people for many diseases [11]–[12]. It has been reported as influenza virus A and antioxidant, anticancer agent and urease inhibitor [13]-[14]. The current investigation aim is to synthesize Ag nanoparticles using methanol extracts of R. nervosus (roots) evaluated its antioxidants activity.

2. Materials and methods.

2.1. Plant collection and extraction. Rumex nervosus (roots) was collected from Taiz city (Yemen) and was identified by Ghulam Jelani, Department of Botany University of Peshawar (Pakistan). A voucher specimen (UPESH-Bot: 20040O(pup)) was deposited in the herbarium of the said department [17]. Roots of R. nervosus was dried under shade at room temperature and grinded by using local grinder, then extracted by hot methanol. After collecting extract, the methanol was removed under reduced pursuer, using vacuum rotary evaporator.

2.2. Phytochemical Screening. The chemical test was performed on the crude extract of R. nervosus using standard procedure to recognize the bio active secondary metabolite by known preliminary phytochemical screening following the methodology of Harborne [18] and Kokate [19].

2.2.1. Test for alkaloids. About 0.2 g of methanolic extract were warm with 2 % H_2SO_4 solution for two minutes. The solution was filter and added a few drops of Dragendroff's reagent to each filtrate. The presence of alkaloid was detected by the formation of an orange-red precipitate.

2.2.2. Test for anthraquinones. About 0.5 g of methanol extract was boiled with 10 % HCl solution for few minutes on water bath. After it was filter and allows to cool. The $CHCl_3$ was added after cooling to each filtrate. Few drops of 10 % ammonia solution were added to each mixture and heated. Rose pink color formation indicates the presence of anthraquinone.

2.2.3. Test for phlobatanins. About 0.5 g of methanol extract was dissolved in distilled water and filtered. The filtrate was boiled with 2 % HCl solution. Red precipitate shows the presence of phlobatanins.

2.2.4. Test for terpenoids. About 0.2g of methanol extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3ml) solution was carefully added to form a layer. Positive results for the presence of terpenoids are indicated by the development of reddish-brown coloration.

2.2.5. Test for coumarines. Exact 3 ml of 10 % NaOH was added to 2 ml of crude methanol extract, the formation of yellow color indicates the presence of coumarines.

2.2.6. Test for emodins. Exact 2 ml of NH_4OH and 3 ml of benzene was added to methanol extract. The presence of emodins is indicated by the appearance of red color.

2.2.7. Test for saponins. 0.2 g of methanol extract was shaken with 5 ml of distilled water and heated to boiling. The presence of saponins is indicated by frothy appearance, which resembles a creamy mixture with small bubbles.

2.2.8. Test for tannins. A small quantity of methanol extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added to each filtrate. A dark green solution indicates the presence of tannins.

2.2.9. Test for cardiac glycosides. To 2ml of plant extract was added 1 ml of glacial acetic acid and 5 % ferric chloride. The few drops of concentrated H2SO4 solution were added after. The presence of greenish blue color indicates the presence of cardiac glycosides.

2.2.10. Test for soluble starch. Few quantities of each portion was boiled with 1 ml of 5 % KOH solution, cooled down and acidified with H_2SO_4. A yellow coloration was taken as the presence of soluble starch.

2.2.11. Shinoda's Test for flavonoids. About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of concentrated HCl solution. The presence of flavonoids is indicted by a color change from pink-orange or red to purple.

2.2.12. Test for anthocyanin and betacyanins. To 2 ml of methanol extract was added 1 ml of 2 N NaOH solution and heated for 5 minutes at 100 °C. Formation bluish green color indicates the presence of anthocyanins and formation of yellow color indicates the presence of betacyanins

2.3. Green synthesis of nanoparticles.

2.3.1. Preparation of stock and salt solution. 0.5mg of crude methanol extract was dissolved in 100ml of water to prepare 1mM stock solution of crude extract of R. nervosus. 1mM solution of silver was prepared by dissolve 20.27 mg of silver sulfate (Ag_2SO_4) with 65 ml of distilled water. The solution has been placed in the refrigerator at 4 °C.

2.3.2. Green synthesis of nanoparticales (NPs). Nanoparticles were synthesized by simple procedure and without the addition of any reducing or capping agents [15]. 1 mM of crude methanol extract solution was mixed with the silver salt solution in a small round bottom flask with ratio 1:2 and the reactions were performed under stirring at temperature 30-40 °C, the colors of mixtures change after one hour, what indicated the formation of AgNPs successively.

2.3.3. UV-visible analysis. The optical properties of AgNPs were determined by UV-visible spectrophotometer. The range of UV-spectrum was from 395-750 nm in wave length. The spectra were taken for the stock solution as well as when color change (reduction due to the addition of metallic salt). The spectra were compared for the confirmation of nanoparticles synthesis.

2.3.4. FT-IR analysis (Fourier-transform infrared spectroscopy). FT-IR instrument (IR Prestige – 21, Shimadzu 400-4000 per cm) was used to find the chemical composition of the plant crude extract and synthesized nanoparticles. Both were analyzed in the range from 400 to 4000 cm. The spectra were compared for the conformation of nanoparticles synthesis.

2.3.5. SEM analysis. Scanning electron microscopy (SEM) instrument was used for characterization of synthesized silver nanoparticales.

2.4. Antioxidant activity. The antioxidant activity was done by DPPH radical scavenging activity according to standard protocol and previous literature reported [15]. The electron donation capabilities of the corresponding crude extracts, fractions, nanoparticles and standards were measured from the changing of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Various concentrations (10-50, 100, 200, 400 µg/ml) of AgNPs were prepared and mixed with 1 ml of the of DPPH solution in methanol. The mixed solutions were stand for 30 minutes in dark then absorbance was measured at exact 517 nm. The ascorbic acid was used as a standard solution. Antioxidant activity by DPPH as percent radical scavenging

activities (% RSA) was calculated as follows:

$$\%DPPH = \frac{ODcontrol - ODsample}{ODcontrol} * 100\%$$

where, OD control – is the absorbance of the blank sample, and OD sample – is the absorbance of samples.

3. Results and Discussion.

3.1. Phytochemical screening. Phytochemical test of roots of R. nervosus was carried out and the result are present in the Table 1. The results showed that in root present of scenery metabolic compounds including anthraquinones, terpenoids, coumarines, emodins, tannins and Shinoda's. While alkaloids, phlobataninns, saponins, cardiac glycosides, soluble starch, anthocyanins and betacyanins are absent in the crude extract.

Table 1. Phytochemical Screening of crude extract of R. nervosus

Test	**Results**
Alkaloids	-
Anthraquinones	+
Phlobatanins	-
Terpenoids	+
Coumarines	+
Emodins	+
Saponins	-
Tannins	+
Cardiac glycosides	-
Soluble starch	-
Shinoda's test	+
Anthocyanins and betacyanins	-

(+) = positive, (-) = negative

3.2. Synthesis of silver nanoparticles. Synthesis of silver nanoparticles from crude methanol extract of R. nervosus was performed successfully. The color change after stirring the solution of plant extract and silver salt (1:2) at one hour. Change the color due to reduction of silver salt. The change in color indicates the presence of silver nanoparticles.

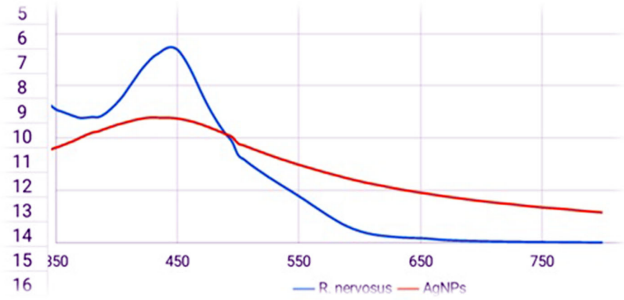

Fig. 1. UV-visible spectra of silver nanoparticles In the case of crude extract, while the AgNPs shows an absorption band at wavelength from 350-500 nm in UV-visible spectral analysis indicates the formation of silver nanoparticles.

3.3. Characterization of synthesis of silver nanoparticle.

3.3.1. UV-visible analysis. Silver salt solution were added to methanol extract of R. nervosus in flask, the change of color of reaction mixture take place after one hour of heating. The change in color shows the convertion of silver ion to silver nanoparticles. Surface Plasmon Resonance (SPR) process take place due to which the color of the reaction mixture was observed to be changed. The SPR absorption band was due to the free electrons of metallic nanoparticles. The highest absorption of silver nanoparticles was noticed at a wavelength of 455 nm. In Figure 1 shows the graph for plant crude extract and AgNPs (wavelength against the absorption, band was absorbed at a wavelength from 370-450 nm).

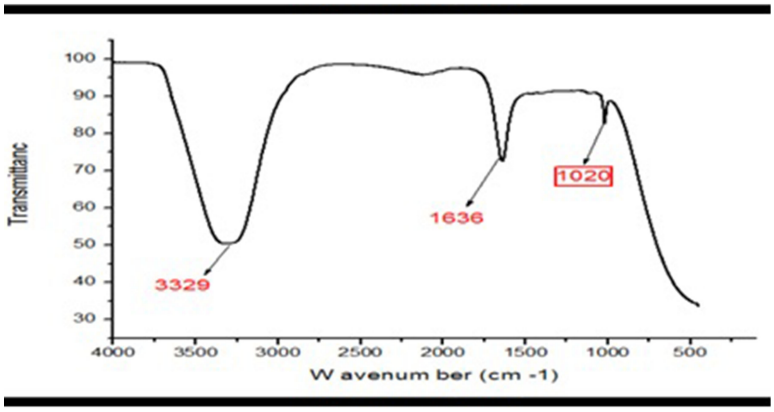

Fig. 2. FT-IR spectra of crude extract of R. nervosus.

3.3.2. FT-IR analysis. FT-IR spectrum of crude metabolic extract of R. nervosus is given in the Figure 2. The peak appeared in the region from 3329 cm^{-1} shows the stretching of aromatic and aliphatic hydroxyl group (OH-) and the peak at 1664cm^{-1} attributed for C=O, while the peak in 1013cm^{-1} attributed to C-N(amines). The intensity of the silver nanoparticles in the FT-IR spectrum was 3315cm^{-1}. Peak was reduced, what indicated the weaking of hydrogen bonding as showed in Figure 3. Three new peaks were appearing at 2952cm^{-1}, 2829 cm^{-1} and 1446cm^{-1} additionally to the peak at 634 cm^{-1} in the broad band range, which confirmed the presence of AgNPs.

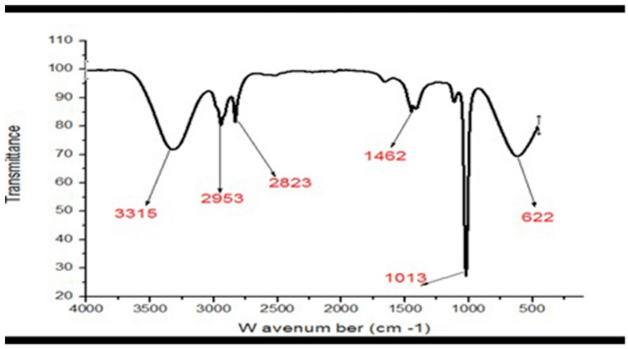

Fig. 3. FT-IR spectra of AgNPs.

3.3.3. Result of SEM analysis. The microscopic structures of AgNPs was investigated by using scanning electron microscopy (SEM) (Figure 4). SEM images confirm the formation of silver nanoparticles. The shape of silver nanoparticles was spherical with diameter range size average 75nm.

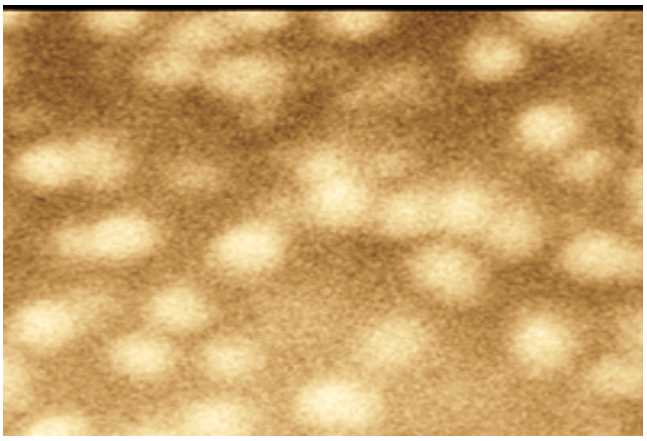

Fig. 4. SEM Image of AgNPs.

3.4. Result of Antioxidant activity. Antioxidant activity of AgNPs from roots of R. nervosus was evaluated by DPPH free radical scavenging assay. The ascorbic acid was used as a standard (positive control). Table 2 summarize the result obtained from the antioxidant assay which show effective free radical scavenging by AgNPs. Various concentration including (10, 50, 200 and 400) µg/ml were examined in this investigation and noted that the activity increased with increasing concentration of AgNPs (fig. 5, table 2). The highest free radical scavenging was recorded as 88.46% at concentration 400µg/ml followed by 69.0059 %, 67.8 % and 59.09 % at concentration 200 µg/ml,100 µg/ml and 50 µg/ml respectively. The lower free radical scavenging capacity was 44.68 % at 10 µg/ml the significant of AgNPs to scavenging free radical of DPPH attributed to AgNPs consider as act as electron donors and reacting with free radicals.

Table 2. Antioxidant activity of AgNPs

Concentration µg/ml	**100 DPPH %**	
	AgNPs	**Acorbic acid**
10	44.68	30.5
50	59.09	95.58
100	67.8	96.37
200	69.0059	96.41
400	88.46	96.73

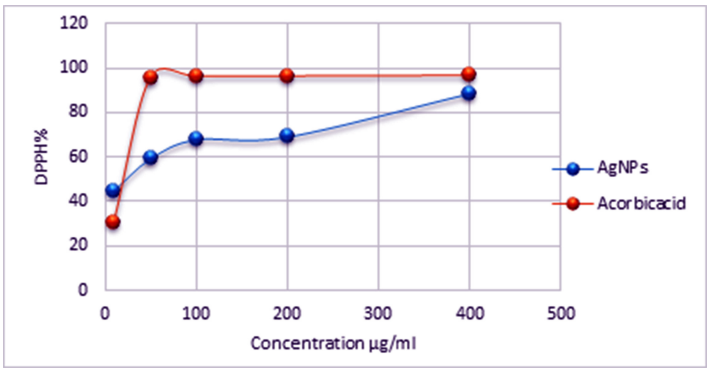

Fig. 5. Antioxidant activity of AgNPs.

4. Conclusion. In this study we have synthesized the silver nanoparticles from the R. nervosus root extract using silver sulfate salt. The confirmation of the Ag nanoparticles was done by the help of UV-vis and FT-IR analysis. The SEM analysis of the samples results extra small size of Ag nanoparticles with an average size of 75 nm, which makes this green method as useful technique to develop small particles for multiple use with no toxic or damaging effects to the environment. AgNPs containing root extract showed the high antioxidant activity. It could be concluded that R. nervosus root extract can be used effectively in the production of potential antioxidant AgNPs for commercial application.

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