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Rumax dentatus Synthesis of Silver Nanoparticles, Antimicrobial Activity and Characterization

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Abstract. AgNPs (Silver Nanoparticles) are frequently used in medicine due to their potent antimicrobial activity and AgNPs effectively produced by a variety of physico-chemical and photochemical reduction. Polygonaceae family comprises 200 species; *Rumax dentatus* are one of them present worldwide, used as food, exhibiting flavors, taste, and aroma and mild laxative properties, and utilized as medicine for a long time. In our current study, the chemical synthesis of silver nanoparticles (AgNPs) using the extract of plant species *Rumax dentatus* was reported. Extraction was performed through the soxhlet method. Additionally, the nanoparticles were screened against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacterial strains. The chemical-synthesized NPs (Nanoparticles) were characterized using UV–Visible spectroscopy, XRD (X-ray diffraction) and SEM (scanning electron microscopy) for structural morphology average size and confirmation of AgNPs. The shift of yellow to brown-red color indicated by the extract showed the chemical synthesis of AgNPs. The greater antimicrobial activity was found against *Escherichia coli* and closed to standard control. XRD and SEM morphological analysis showed morphological characteristics showed agglomerated polydispersed spherical shape of nanoparticles as and indicated high displayed inconsistent morphology which caused by aggregation or agglomeration of nanoparticles and is accountable of *Rumax dentatus* plant extract.

Keywords: *Extraction; Rumax dentatus; Silver Nanoparticles; XRD, SEM, UV–Visible, antibacterial activity.*

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Rumex dentatus синтез наночастиц серебра, антимикробная активность и характеристика

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Аннотация. AgNPs (наночастицы серебра) часто используются в медицине из-за их мощной антимикробной активности, а AgNPs эффективно образуются в результате различных физико-химических и фотохимических восстановлений. Семейство Polygonaceae насчитывает 200 видов; Rumax dentatus – один из них, присутствующий во всем мире, используемый в пищу, обладающий вкусом и ароматизатором и мягкими слабительными свойствами, а также долгое время применяемый в качестве лекарства. В нашем текущем исследовании сообщалось о химическом синтезе наночастиц серебра (AgNPs) с использованием экстракта вида растений Rumax dentatus. Экстракцию проводили по методу Сокслета. Кроме того, наночастицы были подвергнуты скринингу на грамположительный золотистый стафилококк и грамотрицательные штаммы бактерий Escherichia coli. Химически синтезированные NPS (наночастицы) были охарактеризованы с использованием УФ-видимой спектроскопии, XRD (дифракция рентгеновских лучей) и SEM (сканирующая электронная микроскопия) для определения структурной морфологии, среднего размера и подтверждения AgNPs. Изменение желтого цвета на коричнево-красный, отмеченное экстрактом, свидетельствовало о химическом синтезе AgNPs. Более высокая антимикробная активность была обнаружена в отношении Escherichia coli и не поддавалась стандартному контролю. Рентгеновский и СЭМ-морфологический анализы показали, что морфологические характеристики показали агломерированную полидисперсную сферическую форму наночастиц as и указали на высокую степень непоследовательности морфологии, которая вызвана агрегацией или агломерацией наночастиц и обусловлена растительным экстрактом Rumax dentatus.

Ключевые слова: экстракция, Rumex dentatus, наночастицы серебра, рентгеновская дифракция, SEM, УФ-видимость, антибактериальная активность.

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1. Introduction

Particulate materials with at least one dimension less than 100 nm are included in the broad group of substances known as nanoparticles (NPs). The AgNPs (Silver nanoparticles) are the noble MNPs (Metal nanoparticles) that have received the most attention due to their numerous uses in industries like lithography, photonics, microelectronics, photocatalysis, and medicine. AgNPs, for example, are frequently used in medicine due to their potent antimicrobial action against a variety of pathogenic microbes. Significant amounts of AgNPs are effectively produced using a variety of physical and chemical methods, including photochemical reduction, lithography, and laser ablation. However, these methods continue to be quite costly and may call for the use of some poisonous chemicals. Hence, it is crucial to create a green and efficient process for creating AgNPs [1]. In order to create nanoparticles, many metals including Zn, Pd, Au, Pt, Cu, and Ag, have been employed. Metal nanoparticles offer numerous useful features and extremely intriguing uses in a variety of industries, including agriculture, catalysis, food industry, and medicine. Silver nanoparticles (AgNPs), one of several noble metal nanoparticles, have drawn particular attention due to their special characteristics, including electrical conductivity, surface plasmon resonance properties, and good chemical stability. They are widely used in industrial, medical and pharmaceutical applications [2]. Various microorganisms and antibiotic resistance Infection has grown to be a significant barrier in the public healthcare system. Antibiotic resistance has evolved in almost every type of microorganism, primarily as a result of transfer of gene. Physical, chemical, and biological techniques can all be used to create nanoparticles. Low yields are produced by physical and chemical procedures, while contamination is brought on by the use of toxic solvents, precursor compounds, and hazardous byproducts in chemical methods [3, 4]. It is now known that the size, shape, composition, crystallinity, and structure of a noble metal NP dictate its intrinsic qualities (solid or hollow) [5]. Due to their distinctive physiochemical properties, such as their anti-inflammatory, antibacterial, antifungal, anti-angiogenesis, antiviral and antiplatelet actions, Ag NPs (silver nanoparticles) play a key role in the biomedicine field. Since silver compounds are widely known for their potent bacterial-killing and inhibitory properties, they have been utilized for many years to both prevent and cure a wide range of illnesses and infections [6]. Polygonaceae family comprises 200 species, *rumax dentatus* are one of them present worldwide. The leaves of

Rumex species, including *R. acetosa*, *R. acetosella*, *R. abyssinicus*, *R. tuberosus*, and *R. thyrsiflorus*, *R. crispus* and *R. sanguineus*, used as food in some regions, primarily in form of sour soups, salads, sauces, and often in milk [7]. In the instance *Rumex*, a species that are consumed as food exhibit the flavours, tastes, and aromas and mild laxative properties, many species' roots have been utilised in medicine for a long time. (For instance *R. crispus* and *R. obtusifolius*) [8]. Polygonaceae plants are well known to generate abundant amounts of biologically important secondary metabolites such as steroids, flavonoid glycosides, anthraquinones, leucoanthocyanidins stilbenoids, and phenolic acids [9]. Globally 200 kinds of *Rumax* species are present and mostly found in India. While specie *Rumax dentatus* is an annual herb and is commonly 30 to 50 cm taller than wheat, and in height of about 160 cm with numerous branches that is primary and secondary, climbing to particularly divaricate [10]. In *rumax* plant species main secondary metabolites found by researcher are Flavonoids, Quinone's and Anthroquinone and medicinally useful compounds while their roots contain bioactive substances such as myricetin, quercetin, chlorogenic acid, kaempferol, and vitamin C and possess antibacterial, anti-inflammatory antifungal, and antiviral activities [11]. This study evaluates *rumax dentatus* plant extraction performed through soxhlet as well as green synthesis of silver nanoparticles and characterization by XRD, SEM and UV-visible spectrometry and in vitro-antibacterial activity.

2. Material and Methods

2.1. Plant collection and identification

The important plant species *Rumax dentatus*, belongs to the family of Polygonaceae was identified and confirmed by botanist and also through comparison of various literature survey. The entire medicinal plant was chosen for the study/experiment. During the month of March and April, the entire fresh plant was picked from Katlang, Mardan.

2.2. Plant drying and grinding

The fresh leaf samples were cleaned properly with sterilized-distilled and tape water thrice. After cleaning, they were cut into little pieces using scissors and knives, and then placed for drying in a shaded area to protect them from environmental contamination and dust. The drying took place without any exposure to light in a room for 15 days. After the plants have dried fully, make sure the powder is consistent in size and that the surface area is increased for greater extraction.

2.3. Soxhlet extraction

The 25 gram of plant sample's finely ground, uniform-size powder is placed in a thimble, a porous bag made of cellulose strong filter (you will prepare the paper by hand), and the thimble is then placed into the Soxhlet thimble chamber. The bottom flask of the Soxhlet was used to hold 250 ml of ethanol for the extraction. By syphon mechanism the solvent was heated of around 45 °C (a moderate temperature) over mantox heater, and the solvent vaporized and went to the sample thimble chamber, condensed, and fell back when the liquid extract reached the syphon arm and was repeatedly emptied into a bottom flask of a Soxhlet. The upper part was fitted with a condenser by providing water inflow and outflow. 16 hours were spent carrying out the process until the solvent drop could not evaporate without leaving residues [12–14].

2.4. Synthesis of Nanoparticles

Silver nanoparticles (AgNPs) were synthesized by mixing 250 ml distilled water and silver nitrate to prepare 1 mM silver nitrate solution. In the next step take the plant extract, add it to burette, and then 50 ml silver nitate solution in a flask (with magnet for constant stirring) and add it on hot plate (providing 50–60 ^{O}C) then start the plant extract drop wise to flask until change in color (brown yellow to dark brown) appears that signaling the production of silver nanoparticles. The AgNPs separated from the dark brown solution by centrifugation (at 7000 rpm for minimum 15 minutes). Hereafter, the produced silver nanoparticles were washed thrice with n-hexane and distilled water to remove those substances which are soluble with water and then nanoparticles dry with the help of oven and were stored in clean bottles for further study.

2.5. Characterization of Synthesized Nanoparticles

The synthesized silver nanoparticles from plant were characterized with the help of advance and important techniques such as XRD (X-ray diffraction), SEM (Scanning electron microscopy) and by visual UV–Visible spectroscopy. The reduction of silver ions was seeing by the UV visible spectra of reaction medium 3 hours later after a small quantity of sample was diluted at double distilled water. This UV–Visible spectroscopy was performed under value λ=25. While the presence of silver nanoparticle (AgNPs) was confirmed through x-ray diffractometer in the angle range of 0 to 160 with a 2Ø (degree) values and CuKα (Wavelength = 1.5418 A) was used. SEM was used to take a picture of these AgNPs size distribution and morphological characterization (JEOL JSM-6490A). A drop of these AgNPs was applied to a copper grid that had been coated with carbon to make the sample for the SEM evaluation.

2.6. Antibacterial activity of Nanoparticles

2.6.1. Solution Preparation of Nanoparticles

The dried fraction of nanoparticles was dissolved to make 10mg/ml solution in DMSO (Dimethyl sulfoxide), then centrifuged for 30 minutes at a speed of 1300 rpm to ensure proper mixing the fraction with solvent. The Gram negative bacteria used was *Escherichia coli*, and the gram positive bacteria were *Staphylococcus aureus*.

2.6.2. Media Culture Preparation

The microorganism suspension was made in accordance with McFarland standards. For analysis of antibacterial sensitivity test, the MHA was used to prepare bacterial media. The culture media were prepared in 250 ml of distilled water by dissolving 9.5 g of MHA. The resulted amber color solution is thoroughly mixed and boiled with constant stirring to dissolve agar powder completely. A clear to slightly opaque gel is obtained. The media are then autoclaved for sterilization at 121 °C temperature and at 15 lbs pressure, for 20 minutes. Sterilized media allowed for cooling at room temperature in a laminar flow hood before being poured into Petri plates in 25 ml portions and let hardening for a short while. After the solidified culture-media a cotton swab was used for bacteria stains for media. The entire-media at 90 degree was covered with no gaps. In petri plate bores placed 2.5 cm apart, and 30μl of fraction, the other bore placed antibiotics, and solvent. Two Petri plates were used for both bacterial strain positive controls, and extra for negative control and sterility of the media, without any bacterial strain. All Petri plates kept in the BOD (biochemical oxygen demand) incubator for 24 hours at 37 °C.

The inhibited zone for each fraction and active drug evaluated and determined as mean standard deviation [15–17].

3. Results and Discussion

Shift of color in the solution initially the extract of *rumax dentatus* was greenish in color, after it adding $AgNO_3$ (silver nitrate) solution at room temperature with constant stirring, then the initial extract with silver nitrate solution turns into brown color. In other words the intensity of color increased as the incubation period went on, indicating the formation of silver nanoparticles and the formation of Ag ions Fig. 1. The most prominent and significant evidence for the formation of Ag nanoparticles is the shift of color in the solution caused by Ag nanoparticles surface plasmon excitation [18]. During UV–Vis analysis, a broad cone spectral curve was found. The plasmon band is made broad due to the addition of different metabolites from plant extracts to the solution since; they may also be detected in the range of this spectrophotometric. Silver's surface plasmon resonance arises at 450 nm. Fig. 2 shows the UV–Vis absorption spectra of Ag NPs. At 450 nm, silver experiences surface plasmon resonance (SPR). This peak grew over time until it reached 360 minutes. Mie theory states that spherical nanoparticles exhibit one silver surface plasmon resonance peak. The number of peaks rises as particle shape diversity increases. Thus, it can be assumed that silver NPs produced during biosynthesis are all proper shape. The XRD characterization technique was used for the confirmation of the crystal and structure of the silver nanoparticles (AgNPs). The silver nanoparticles modified XRD spectra showed in Fig. 3. At 2Ɵ values, the XRD pattern showed different peaks that can be referred to the crystalline planes of 311, 200, 220, and 111 silver-nanoparticles Fig. 3, these peaks are related to the face centered cubic (FCC) lattice. While the result is consistent with previous researchers' findings [19]. The particle approximate total size 25–40 nm based on the width of (111) Bragg's reflection. Additionally, other peaks of silver nanoparticles pattern at 2Ɵ values are due to the presence of organic compounds of the plant extracts. These peaks demonstrate the crystallization of a few plant metabolic components on the Ag NPs' surface.

The SEM (scanning electron microscopy) morphological characteristics of rumax dentatus AgNPs possess agglomerated poly-dispersed spherical shape of nanoparticles as shown in Fig. 4 which

Fig. 1. Shift of color change and confirmation of AgNPs

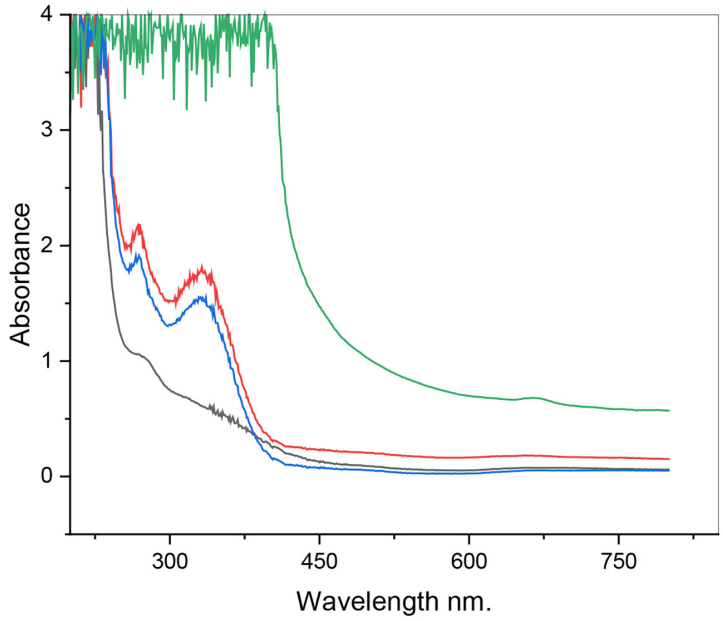

Fig. 2. UV-visible absorbance based on different concentrations

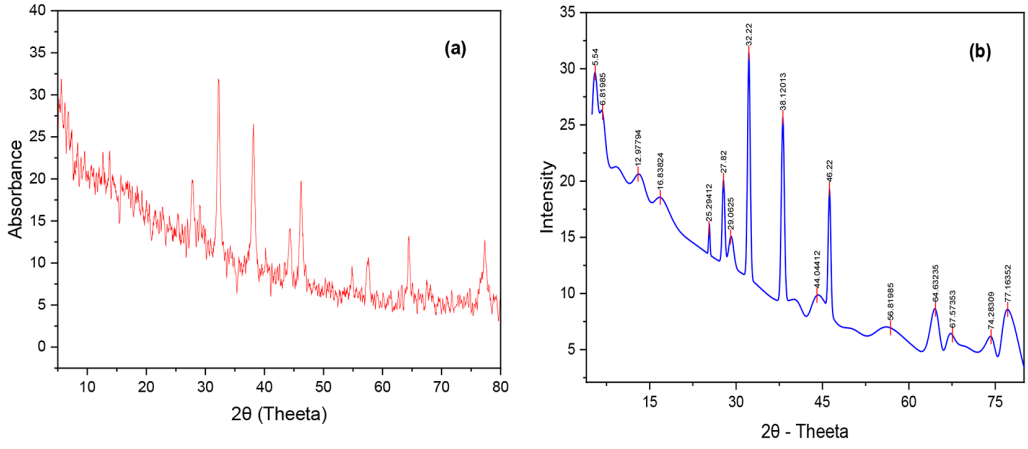

Fig. 3. XRD characterization of nanoparticles a) Initial peak b) cumulative peak

indicated high displayed inconsistent morphology which was caused by aggregation or agglomeration of nanoparticles and is accountable of *rumax dentatus* plant extract and is supported by previous literature studies.

The XRD graph of silver nanoparticles approved that it have face-centered crystalline structure and all the peaks were according to JCPDS No.00–004–0783, and were coincided with face-centered cubic symmetry (FCC) that shows the crystalline nature of nanoparticles [20]. The antibacterial activity of synthesized nanoparticles of *rumax dentatus* is shown in Table 1 and Fig. 5 the highest concentration of nanoparticles showed direct relationship with activity and greater inhibition found against *E. coli* bacterial strain and is closed in activity with standard control. Similarly NPs such as silver NPs

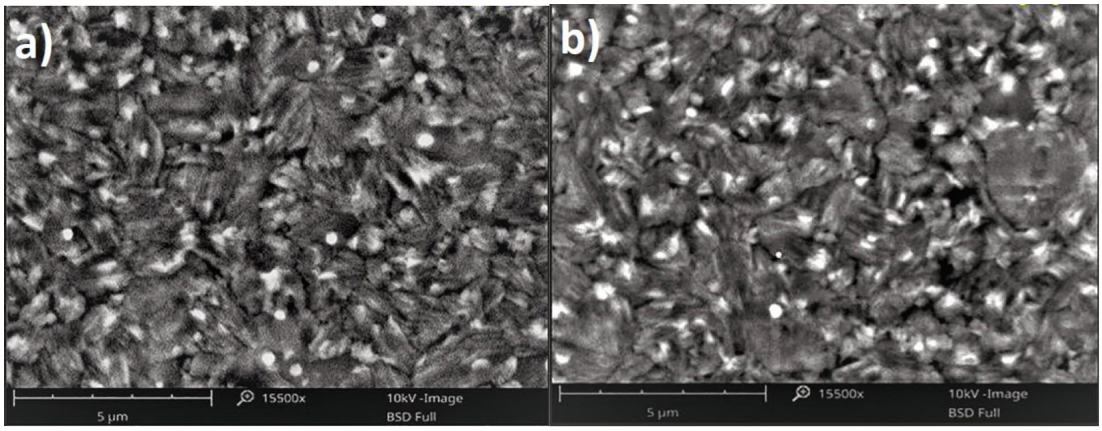

Fig. 4. SEM morphological characterization of AgNPs

Table 1. Antimicrobial activity of silver nanoparticles

AgNPs Concentration (μl)	Tested Microbes	
	Escherichia coli	*Staphylococcus aureus*
10μl	2.3±0.5 mm	1.90±0.1 mm
15μl	3.6 ±0.2 mm	2.2 ±0.05 mm
20μl	4.5 ±0.5 mm	2.9±0.05 mm

The control Ampicillin (3–5.2±0.5 mm) and Gentamicin 2.5–6±0.05 mm, closed in contrast in triplicates.

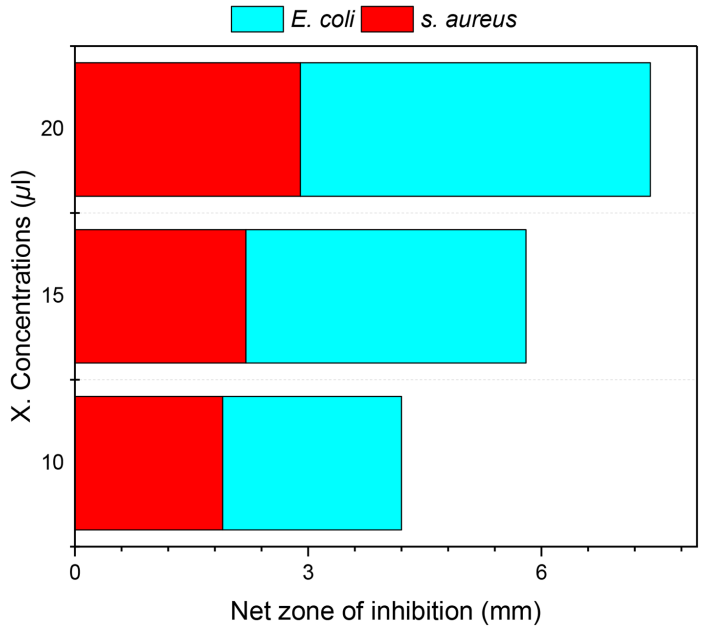

Fig. 5. Antibacterial activity comparison of AgNPs based on concentrations

have antibacterial properties which are also indicated by previous literatures [21, 22]. Generally, the antibacterial activity of silver NPs is mostly related to the release of cations from silver NPs, which serve as their reservoir. However, the possible process of antibacterial activity of silver NPs was discussed by some of the researches such as: i- Electrostatic attraction causes Ag NPs to connect to the cell wall of bacteria, as a result cell permeability and respiration was reduced due to the formation of reactive oxygen species. ii- Silver NPs connect to thiol groups in DNA and RNA, affecting bacterial protein synthesis [23]. iii- Silver nanoparticles cause cell death by producing pits on the cell surface and causing proton leakage. In terms of morphology the size and shape of nanoparticles affect antibacterial activity of silver NPs. Larger particles having lower surface area available for contact will have a lower bacterial affect as compared to smaller particles and will not easily penetrate into the cell. *Rumax dentatus* is medicinally essential plant that is found all over the world. As per phytochemical screening Rumax dentatus includes cardiac glycosides, alkaloids tannins, saponins, quinones, terpenoids and flavonoids. A total of 63 different compounds have been extracted and identified, among these compounds includes essential oils, chromones, quinones, stilbenes, flavonoids, naphthalene glucosides and c-glucosyl anthrones [24]. There is a wide range of activity and less evolving resistance. The antibacterial activity of SNPs is strictly influenced by their size. Flavonoids are essential for the production of nanoparticles. As a result, flavonoid content should be investigated as a primary factor of plant potential in nanosynthesis. The plant extract-to-metal precursor ratio is important for synthesizing silver nanoparticles with varied form, size, and stability [25]. We found that raising the Rumax dentatus specie extract ratio while keeping the silver nitrate amount fixed increases silver nanoparticle formation. The introduction of 1, 2, 3, and 4 gm of *Rumax dentatus* extract to 50 ml of 1 mM silver nitrate solution and noticed that increasing the amount of Rumax dentatus extract produced more nanoparticles, as shown by increased absorbance around the surface plasmon resonance peak in UV–Vis spectroscopy. Plant extracts from a variety of plant parts, including roots, fruits, flowers, bark, seeds and leaves have been used in the production of silver nanoparticles (Ag NPs) and may operate as stabilizing and reducing agents. It has been determined that the ethanolic extract of many plants contains a wide variety of primary and secondary metabolite compounds, varying from proteins to low molecular weight molecules like quinones, flavonoids, alkaloid, organic acids (malic, protocatechuic, ascorbic, tartaric, oxalic acid), amino acids, glutathiones, alcoholic compounds, polysaccharides, antioxidants, terpenoids, and phenolic acid. Commonly these metabolites are well known to be active in redox reaction processes. Secondary metabolites like terpenoids, sugars, polyphenols, phenolic acids, alkaloids, and proteins have also been related to the reduction of metal ions, which proceeds to the production of nanoparticles and sustains their stability. According to the relevant research, flavonoid ranked among the most usually reported or predicted compounds responsible for the ecofriendly formation of silver nanoparticles. Thus metabolites and plant species are responsible for synthesis of silver nanoparticles (Ag NPs). Hence, the size and rate of ion emission are defining toxicity of silver NPs. Further, the three-dimensional structure of the cell wall of gram-positive and gram-negative microbes is also recognized with the antibacterial effects of silver nanoparticles.

4. Conclusions

The current study report the extraction of plant *species rumax dentatus* and synthesis of their AgNPs which had a face-focused structure with average molecular size of 25–40 nm. The synthesized silver nanoparticles of *rumax dentatus* possess a viable antibacterial activity against gram negative

bacteria *Escherichia coli*, and gram positive bacteria *Staphylococcus aureus* and directly associated with concentration, and closed in contrast to standard control. The usage of plant extract for the combination of silver nanoparticles and our starter results might open a fascinating region for additional examinations, make changes in culture state of the plants utilizing various medicines and assessment their connection with the qualities of the synthesized nanoparticles. Positively, nanoparticle creation with wanted qualities can be a decent option in contrast to anti-toxins in different applications.

Data Availability

All data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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