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In vitro antimicrobial activity of silver nanoparticle synthesized using *Aster squamatus* flower extract against selected pathogenic microbial

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The aim of this study was to green synthesis and investigates the antimicrobial activities of *Aster squamatus* flower (ASF) extract and their silver nanoparticle (ASFAg) counter to numeral clinical pathogenic microbial. The biocompatible silver nanoparticle (ASFAg) was investigated by means of UV-Vis spectroscopy, X-ray diffraction and scanning electron microscopy (SEM) methods. The prepared ASFAg revealed a strong surface plasmon resonance (SPR) absorption band at 432 nm. In the results, all growth of the investigated pathogenic microbial strains had been ceased in varied range by the silver nanoparticles potent than the crude extract of *Aster squamatus* flower. ASF extract showed week antimicrobial activities against *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Proteus mirabilis* with inhibition zone between (10.33 mm to 18.33 mm) while no activities against *Klebsiella pneumoniae, Candida albicans* and *Pseudomonas aeruginosa*. *Klebsiella pneumonia* is the best prone microbes with inhibition zone (29.00 mm), while *Enterococcus faecalis* and *Shigella dysenteriae* establish as the lowermost prone microbes of inhibition zone between (12.67 mm to 13.33 mm) by ASFAg. As a result, ASFAg perhaps could be significant pharmacological drugs counter to specific microbial strains.

Keywords: Biogenic synthesis, silver nanoparticles, bioreduction, antimicrobial property.

Introduction

Environmental friendly and cost-effective procedures are likely essential for the synthesis of materials. Therefore, green synthesis methods that have been attracted significant attention worldwide to synthesize state-of-the-art materials because of low cost and easiness[1–3]. Over the past few years, the major concern in the area of science and technology can be improved by using nanomaterials as compared to bulk materials due to unique physical and chemical properties. The metal nanoparticles have attracted a significant attention in diverse area due to its notable optical, electronic, medicinal, catalytic and magnetic properties[4–6]. The metal nanoparticles become very distinctive because they show the surface plasmon resonance (SPR) spectra. Among metal nanoparticles, silver nanoparticles have attracted special attention as antimicrobial agents, electrical conductivity, photo electrochemical activity, sensor probes, textile industries, cosmetic, water treatment, disinfection and strong reduction power[7–10], etc. Many researchers are showing an interest to improve the natural properties of silver nanoparticles for research and development due to their enhanced antimicrobial activity and anti-infectious agent[11–13]. Various synthetic methods like physical, chemical and biological routes are already involved for the synthesis of silver nanoparticles with various morphologies and sizes. It is well known from the previous literature report that physical and chemical meth-

ods are expensive and associated with having various demerits like use of toxic chemicals, high energy and uneconomical decontamination which makes pollution and poisoned human health. Therefore, biological or green chemistry methods has been gaining much more impetus because of less cost, a facile approach and very eco-friendly and does not involve the use of high pressure, temperature, energy, and toxic materials[1–3]. Many reports are available on biological synthesis of AgNPs using various plant extracts like geranium leaves[14], fritillaria flower[15], neem leaves[16], aloe vera[17], fig leave[18], Opuntia Ficus fruit[19], Putranjiva Roxburghii leave[20], etc. Recent reports have been showed that beside leaves extract like stem, roots, seed, peel, bark etc. have been used for the synthesis of different shapes of AgNPs[21–25]. *Aster squamatus* is belonging to *Asteraceae* (Compositae) family. It is herbaceous, multi stemmed and soft wooded leaves. The flowers of *Aster squamatus* is white and usually grow in the month of September to November. The *Aster squamatus* plants have antidiarrhoeic effect because of presence of various bioactive and medicinal compounds. Herein, we report the facile one step and green synthesis of AgNPs using aqueous extract of *Aster squamatus* flower (ASF) which acts as a capping and reducing agent followed by antimicrobial activity.

Experimental

Materials:

Silver nitrate ($AgNO_3$) (BDH reagent, ≥99.0%) was purchased from Sigma-Aldrich Co. and utilized as obtained without purification. Double distilled water was used in all experiments. The microbial strains were obtained from the Microbiology Lab., Faculty of Medicine, King Khalid University, Saudi Arabia.

Preparation of A. squamatus flower extract:

Aster squamatus plant was collected from Abha, Assir region of Saudi Arabia. The *Aster squamatus* flower was washed several time with double distilled water and dried in dry place. The aqueous extract was prepared according to previous procedure[18].

In general, 5.0 g of sieved dry (*A. squamatus*) flowers were mixed with 250 ml distilled water and left to stand for 24 h at room temperature. Earlier to an experiment, aqueous flowers extract (bio-extract) was filtered using Whatman filter paper and finally centrifuged at 35000 rpm for 5 min to isolate any solid particles from it. The extract was stored and was used for the synthesis of AgNPs.

Synthesis of bio-capped AgNPs:

1 ml aqueous *Aster squamatus* flower extract was added drop wise to 10 ml of $AgNO_3$ solution (0.1 *M*) with constant stirring at ambient temperature and initially the color of mixture solution was colorless but after few minutes color slowly changed to brown (abbreviated as ASFAg). The color change represented the biomolecules present in aqueous *Aster squamatus* flower extract are responsible for the reduction of Ag^+ to Ag^0 nanoparticles (inset of Fig. 1). The prepared silver nanoparticles solution was stored at room temperature for further study. We also tried to prepare AgNPs with the aqueous extract of *Aster squamatus* stem powder but unfortunately the results was not suitable therefore we did not mentioned in the current report.

Pathogenic strains preparation:

Nine of human pathogenic isolates namely *K. pneumoniae, S. typhi, E. faecalis, C. albicans, P. aeruginosa, S. saprophyticus, S. aureus, S. dysenteriae* and *P. mirabilis* were first grown in liquid nutrient broth media (NBM) and incubated at 30°C for 24 h for all bacterial isolates but *C. albicans* incubated for two days[26–28]. All isolates pathogen were obtained from Microbiology Laboratory, Faculty of Medicine, King Khalid University, Saudi Arabia.

In vitro antimicrobial assay:

The agar well-diffusion method was applied to investigate the antimicrobial activity of both ASF and ASFAg[26–28]. ASFAg and ASF were tested for their antimicrobial activities against all the subculted microbes. First 20 ml from previously sterilized nutrient agar was dispensed into petri dishes, and then kept at 24°C till solidification and cooling the media. Sterilized petri dishes were inoculated with 0.1 ml of each microbial strain using a sterile loop. Three circled holes of 6 mm in diameter in each plate were made using sterile corkborer. Each well was filled by 100 μL from each extract either from ASFAg and ASF. All inoculated plates were then kept for about one hour at 24°C for well diffusion the extract into the hole. 100 μL (0.2%) of dimethyl sulfoxide as a negative control and synthetics Cefoxitin (30 μg disc) as positive controls has been applied also for each microbial strains. All plates have been incubated at the 29°C in an aerobic condition for 48 h and the inhibition activities formed were measured with a transparent ruler. Experiments were performed

in triplicate and the mean ± S.D. were calculated for each results.

Characterization:

The crystalline nature of prepared AgNPs were obtained using X-ray diffractometer (XRD) (Philips PW3040/60) with Cu-Kα radiation (λ = 1.5418 Å) in the range of 2θ from 30° to 80° at room temperature. The morphological studies were performed using scanning electron microscopy (SEM) Hitachi S4800. The UV-Vis spectra were obtained in the range of 200–700 nm using double-beam UV spectrophotometer (PG). Raw data of antimicrobial effect of ASF and ASFAg against tested pathogens were analyzed and tested by One-way ANOVA in SPSS Statistics.

Results and discussion

Bioreduction of silver ion and the formation of bio-capped silver nanoparticles in solution phase were confirmed by color change through naked eye as well as using UV-Vis spectroscopy. The characteristic peak of silver nanoparticles was obtained between 400–500 nm. The prepared silver nanoparticle using aqueous *Aster squamatus* flower extract exhibited a single sharp peak at 432 nm (Fig. 1), which ascribed to the surface plasmon resonance (SPR) and reduction of Ag^{+} to Ag^{0} by bioreductant present in the extract[29]. The shape of particles was almost spherical which were confirmed by single SPR peak and SEM analysis. We studied the effect of time during formation of silver nanoparticles under same condition. By increasing the reaction time until 30 min the peak shifted from 432 to 436 nm, which is clear indication of increasing size of AgNPs.

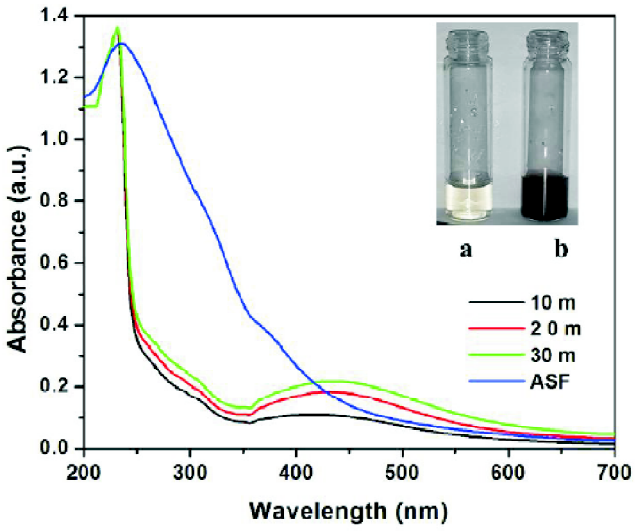

Fig. 1. UV-Vis spectra of extract and bio-capped AgNPs. Inset image shows the color change: (a) before and (b) after reaction.

The crystalline arrangement and phase of bio-capped AgNPs were studied by using XRD to confirm the presence of AgNPs. Fig. 2 showed the diffraction peaks corresponding to face centered cubic (fcc) phase of AgNPs were observed at 2θ = 38.8, 43.7, 64.2, and 77.2, respectively, which could be well indexed with (111), (200), (220) and (311) crystal planes (JCPDS file No. 89-3722). There are some unassigned and additional peaks besides AgNPs peaks which recommend the existence of capping agent on the surface of the prepared AgNPs and our results are similar to the results reported on green synthesis by Sathyavathi and Mehta[30,31].

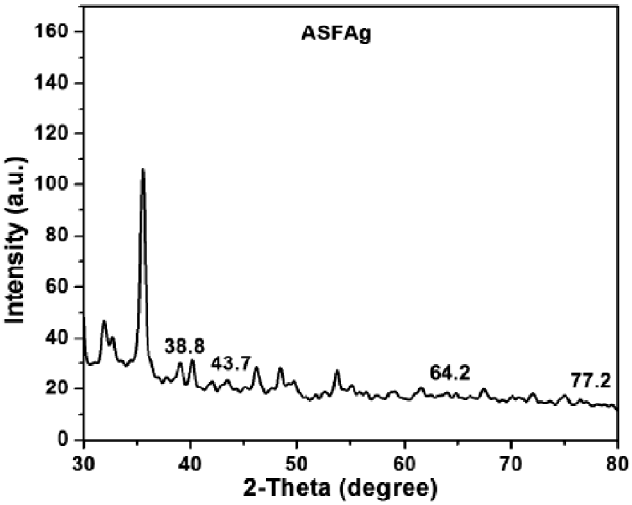

Fig. 2. XRD pattern of bio-capped AgNPs.

Fig. 3 shows the surface morphology of ASFAg film using scanning electron microscopy. SEM image of ASFAg nanoparticle is almost spherical, well dispersed and low agglomeration due to the effect of biomolecules present in the extract. The average diameter of ASFAg nanoparticle was obtained at 95±3.21 nm.

Antimicrobial activities of ASFAg and ASF against numbers of dangerous human pathogenic microbes are shown in Table 1. The results showed that ASF killing activities of the tested microbes decreases by 66.67%, which implies a

Table 1. Antimicrobial activities of ASFAg and ASF in Millimeter (mm)

	Strain								
Sample	*K. pneumonia*	*S. typhi*	*E. faecalis*	*C. albicans*	*P. aeruginosa*	*S. saprophyticus*	*S. aureus*	*S. dysenteriae*	*P. mirabilis*
ASFAg	29.00^{a}±4.58	14.33^{a}±1.15	12.67^{a}±2.89	14.33^{a}±3.51	12.00^{a}±0.10	20.00^{a}±0.05	18.0^{a}±0.00	13.33^{a}±0.58	16.00^{a}±1.00
ASF	NI	12.0^{b}±1.53	11.67^{a}±2.08	NI	NI	18.33^{b}±1.15	10.33^{b}±0.15	12.33^{a}±4.16	10.33^{b}±0.58
PC	36.33^{b}±2.08	21.0^{c}±1.00	23.67^{b}±1.53	20.0^{b}±1.00	24.5^{b}±0.50	22.9^{c}±0.85	22.83^{c}±0.76	23.0^{b}±0.00	24.67^{c}±0.58

NI, no inhibition activities; PC, Positive control, (Cefoxitin - 30 μg). Different superscript letters within columns show significant differences ($p \leq 0.05$) between treatments ± SD of the mean for $n = 3$.

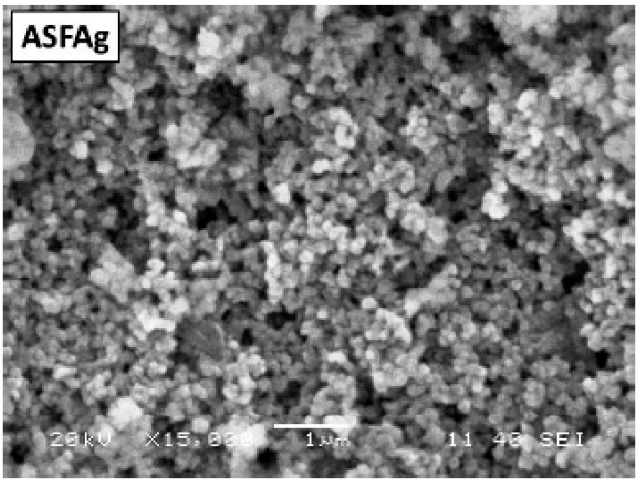

Fig. 3. SEM image of bio-capped AgNPs.

bioactivity function of the ASFAg (100% killing affect). In addition, it was apparent in this research that ASFAg more potent than ASF against all tested microbes in the form of zone of inhibition. *K. pneumoniae* and *S. saprophyticus* are the most susceptible microbes by ASFAg in the range between (29.00±4.58 mm to 20.00±0.05 mm) respectively followed by *S. aureus* (18.0±0.00 mm). While *S. typhi*, *E. faecalis*, *C. albicans*, *P. aeruginosa S. dysenteriae* and *P. mirabilis* had been inhibited in the range between (16.00±1.00 mm to12.00±0.10 mm). ASF shows the highest activity against *S. saprophyticus* (18.33±1.15 mm) while very low activity against *S. typhi*, *E. faecalis, S. aureus*, *S. dysenteriae* and *P. mirabilis* in the range between (12.33±4.16 mm to 10.33±0.15 mm). While *K. pneumoniae, C. albicans* and *P. aeruginosa* are not affected at all by ASF. Cefoxitin (30 μg) showed killing activity against all tested microbes in the range between (36.33±2.08 mm to 20.0±1.00 mm) while dimethyl sulfoxide (DMSO) did not exhibit any killing activities[32]. The outcomes of the results are in good covenant with previous reported studies against various pathogen[15,18,19,25].

Conclusions

The current investigations focus on an environmental friendly and low cost method for the preparation of silver nanoparticle using aqueous solution of *Aster squamatus* flower (ASF) extract at room temperature and studied their antimicrobial activity against various pathogens. The shape of prepared ASFAg nanoparticle is almost spherical and well dispersed with an average diameter of 95±3.21 nm. To sum up we obtained that our prepared nanoparticle exhibited significant antimicrobial activity counter to Gram-negative and Gram-positive bacteria and capability of bio-reducing agents present in *Aster squamatus* flower (ASF) extract act as novel and cheap. *Klebsiella pneumonia* is the best prone microbes with inhibition zone (29.00 mm), while *Enterococcus faecalis* and *Shigella dysenteriae* establish as the lowermost prone microbes of inhibition zone between (12.67 mm to 13.33 mm) by ASFAg. Therefore, ASFAg perhaps could be momentous pharmacological drugs counter to specific microbial strains.

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