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SYNTHESIS, CHARACTERIZATION AND ANTIFUNGAL EFFECTS OF SILVER NANOPARTICLES OF *AGERATUM CONYZOIDES* L.

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Abstract

Synthesis and characterization of silver nanoparticles (AgNPs) produced from *Ageratum conyzoides* leaves extract, as well as their antifungal effectiveness against a variety of plant pathogens were investigated. Absorption peaks at 3674, 3264, 2980, 2940, 2096, 1739, 1545, 1370, 1080 and 700 cm^{-1} were recorded for the formed nanoparticles using FTIR analysis. According to SEM, XRD and UV spectroscopy results, nanoparticles have spherical and semi- spherical shape, size of 34 to 205 nm with a resonance wavelength at 460 nm. *Ageratum conyzoides*-AgNPs showed moderate antifungal activity against *Penicillium expansum*, *Macrophomina phaseolina*, *Alternaria alternata*, *Drechslera halodes* and *Fusarium oxysporum* f.sp. *lycopersici*. The fabricated NPs from leaves of *A. conyzoides* can be applied as antifungal agent.

Introduction

There has been an incredible increase in the use of nanoscience and nanotechnologies in recent years, due to advances in the manufacturing of nanomaterials. Because of their physical, chemical, and biological features in comparison to their macro-scaled counterparts, these developing nanoparticle items have piqued people's curiosity (Abou El-Nour *et al.* 2010). Silver (Ag) has shown promise in a variety of fields, including medicine, electronics, and household applications. AgNPs have been shown to have antimicrobial properties (Kashyap *et al.* 2013).

Ageratum conyzoides is a herb that is utilized for its hemostatic, anti-inflammatory, antispasmodic, and antiasthmatic characteristics and for the wounds treatment and bacterial infections (Kokwaro 1976, González *et al.* 1991). *Ageratum conyzoides*' *n*-hexane extract and methanolic extract showed antifungal activity against *Fusarium solani* (Javed and Bashir 2012). Root and leaf extracts showed antibacterial and antifungal properties (Omole *et al.* 2019).

The present study was aimed to synthesize AgNPs using *A. conyzoides* aqueous leaves extract. Antifungal effects of the prepared nanoparticles was tested against plant pathogenic fungi. The fabricated silver nanoparticles (AgNPs) were analyzed using a variety of analytical methodologies.

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Materials and Methods

Leaves of *A. conyzoides* were collected from road side area of Abha city, Asir region, Saudi Arabia. The leaves were washed using distilled water and air dried. Forty five grams of the fresh leaves were crushed into fine particles using mortar and pestle with the addition of 45 ml of deionized water. The aqueous extract was filtered and the filtrate was kept in -4°C for further use. $AgNO_3$ (Fisher Scientific, Leicestershire, UK) powder was dissolved in deionized water to prepare 60 mM $AgNO_3$ solution. Ninety ml of the prepared $AgNO_3$ solutions were mixed with 10 ml of the filtrate in a conical flask and heated at 70°C till the colour changed to be brown. The prepared solution was kept in a refrigerator (-4°C) for the antifungal activities test and to check the formation of NPs using X-ray diffractometry (XRD), at wavelength (x) = 1.5404 A^0. Fourier transform infrared spectroscopy (FTIR), ultraviolet visible spectroscopy (UV–vis spectroscopy) and scanning electron microscopy (SEM).

AgNPs obtained from *A. conyzoides* leaves aqueous extract were tested against *A. alternata*, *D. halodes*, *P. expansum*, *F. oxysporum* f. sp. *lycopersici* and *M. phaseolina*. Each fungus was cultured at 30°C on potato dextrose agar (PDA) for 14 days at dark in Petri dishes. Conidial suspension was prepared for each fungal strain using distilled water and Tween 80 and adjusted to 1.5×10^5 conidia/ ml.

Sterilized PDA (20 ml) was poured into a sterilized Petri dish and allowed to solidify. Five hundred microliters from each fungus was applied equally using a sterile cotton swab to the agar medium. Six mm diameter were punched in the solidified PDA media and then filled with 150 µl from the solution of AgNPs (Moustafa *et al.* 2021, Moustafa *et al.* 2013). Nystatin (10 µg disc) and 20% of Dimethyl sulfoxide (DMSO) were used as positive and negative control, respectively for each fungus. Three replicates from each sample were incubated at 37°C for 7 days and the clear zone diameter (mm) was measured.

UV-Vis spectral investigation analysis of *A. conyzoides*-AgNPs had been checked using (spectrophotometer HITACHI, Model U-2800) at of 300 - 600 nm wave lengths. Ag nanoparticles morphology were seen using scanning electron microscopy (SEM) (JSM-7500 F; JOEL-Japan) and SMILEVIEW software attached to the SEM device was applied. By using the FTIR diamond ATR platform spectrometer (Agilent, Cary 630, USA), the spectrum had been recorded. Grain size, phase identification and the crystalline nature had been determined using XRD (Shimadzu, 6000 Diffractometer, Japan) adjusted at 30 mA and 40 kV by using Cu Kα radiations with 1.54 A^0 and crystallite size corresponding to the recorded peaks was determined (Cullity 1978).

One way ANOVA and post hoc analysis using Tukey's test by using BM SPSS statistics software had been applied for analysis the results.

Results and Discussion

In vitro screening of AgNPs leaves aqueous extract showed moderate antifungal activity against test fungi (Table 1). Ag nanoparticles from leaves gave the highest activity against *P. expansum* (1.59 ± 0.12 cm) while the lowest against *M. phaseolina* (1.27 ± 0.08 cm). *Alternaria alternata* and *D. halodes* had a susceptibility from Ag nanoparticles with inhibition zone of 1.38 ± 0.29 cm and 1.43 ± 0.11 cm. Nystatin which had been applied as positive control had more antifungal activities against all the tested fungi (Table 1). Dimethyl sulfoxide (DMSO) showed no inhibition activities against the tested fungi (Shammout and Awwad 2021). Results of this study is corroborated with the fining of Ghojavand *et al.* (2020) and Anum *et al.* (2021). Acting nanoparticles with small dimensions enable them to enter fungal hyphae by engaging with cell membranes and then damaging fungal cell DNA and proteins and finally fungal cells death (Amini 2019, Nguyen *et al.* 2020).

Figure 1 showed analysis of the chemical bonding and composition of the synthesized NPs using FTIR. It was found that many peaks were in the spectrum, the weak band at 3674 and the broad band at 3264 cm^{-1} which could be assigned to O-H stretch. The bands at 2980 cm^{-1} and 2940 cm^{-1} refer to C–H stretching of alkyl chain indicating the presence of vibrations of methyl, methylene, or methoxy groups as capping agent with the AgCl particles (Aiad *et al.* 2014). The weak peak at 2096 cm^{-1} was due to the C = N stretching of R – N = C = S. The peak present at 1739 cm^{-1} was assigned to the C = O stretching mode in amine group which is commonly found in the protein, indicating the presence of proteins as capping agent for silver nanoparticles which increases the stability of the nanoparticles synthesized. The band at around 1545 cm^{-1} was ascribed to the OH group vibration. The strong band at 1370 cm^{-1} was attributed to the –C–N stretching vibrations of proteins. The peak located at 1080 cm^{-1} can be assigned as the absorption peak of C–O stretching. The peak observed below 700 cm^{-1} could be due to the AgCl stretching vibration (Daupor and Chenea 2017).

Table 1. Antifungal effect of AgNPs of *Ageratum conyzoides* leaves.

Test Fungi	Mean diameter of inhibition zone (cm)	
	NPs of *A. conyzoides*	Nystatin
Alternaria alternata	1.38 ± 0.29**	2.27 ± 0.09
Drechslera halodes	1.43 ± 0.11**	2.32 ± 0.15
Fusarium oxysporum f. sp. *lycopersici*	1.29 ± 0.08**	2.46 ± 0.13
Macrophomina phaseolina	1.27 ± 0.08**	2.51 ± 0.10
Penicillium expansum	1.59 ± 0.12**	2.33 ± 0.06

Values are Mean ± SEM; n=3, (*$p \leq 0.05$; **$p \leq 0.01$).

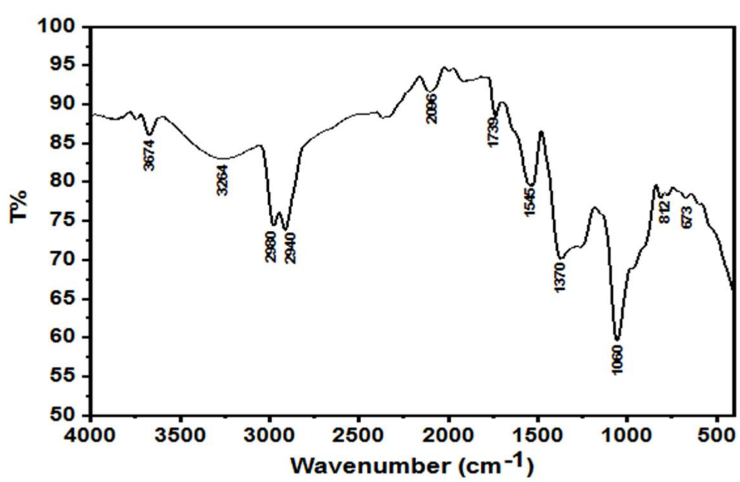

Fig. 1. Fourier-transform infrared (FTIR) spectrum of AgNPs of *Ageratum conyzoides* leaves.

Figure 2 showed the formation of the crystallite AgCl-NPs biosynthesized from the leaves of *A. conyzoides* aqueous extract using XRD, JCPDS No. 31- 1238, in the range of 2θ from 10 to 70 degrees. The XRD chart exposed the presence of six characteristic peaks at 2θ values of 27, 32, 46, 55, 57 and 67º indexed as 111, 200, 220, 311, 222, and 400, respectively were attributed to planes of the cubic crystalline phase of silver chloride. This interpretation of the AgCl-NPs diffraction profiles is consistent with the standards set by the Joint Committee on Powder Diffraction Standards (JCPDS No. 31- 1238, to silver chloride nanoparticles). There were no other peaks, in the sense that the high purity of AgCl-NPs and the highly crystalline structure was revealed from the strong and narrow diffraction peaks. The calculated average crystalline size of silver nanoparticles synthesized from *A. conyzoides* leaves aqueous extract was 34 nm (Table 2). The UV-Vis absorbance over the wavelength ranging from 200 to 800 nm, showed that the purified nanoparticles of *A. conyzoides* exhibited a surface plasmonic resonance (SPR) at 460 nm, confirming the formation of Ag-NPs (Fig. 3). SEM images of the synthesized NPs found to be spherical and semi- spherical in shape (Fig. 4). The SMILEVIEW software attached to the SEM system was used to obtain the diameter of AgCl NPs, ranging from 80 to 205 nm with a mean

Table 2. XRD data analysis of AgNPs of *Ageratum conyzoides* leaves.

2Theeta	FWHM(D)	hkl	d (A^o)	D (nm)
27.8833	0.39330	111	3.19715	20.81
32.3567	0.34000	200	2.76462	24.32
46.3333	0.34670	220	1.95802	24.92
54.8900	0.18000	311	1.67131	49.73
57.6000	0.20000	222	1.59895	45.32
67.5100	0.22000	400	1.38632	43.43
The average particles size				34.76

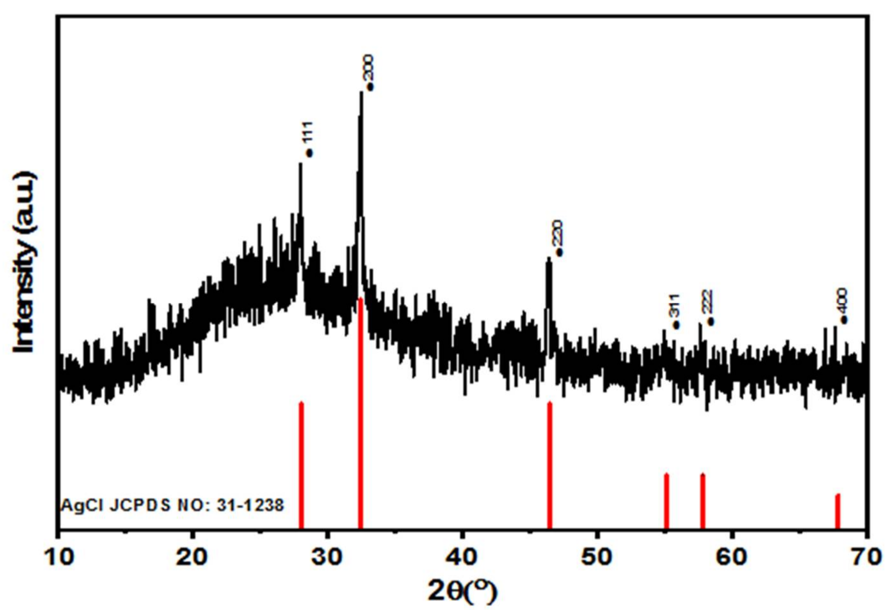

Fig. 2. XRD pattern of AgNPs of *Ageratum conyzoides* leaves.

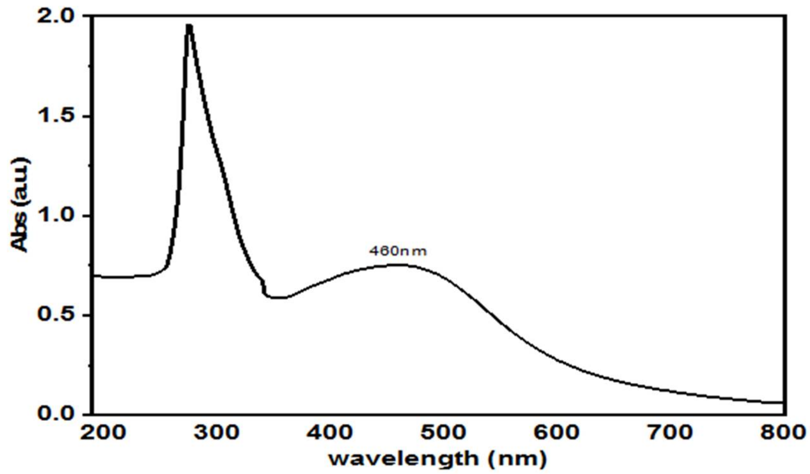

Fig. 3. Ultraviolet visible (UV-Vis) analysis of AgNPs of *Ageratum conyzoides* leaves.

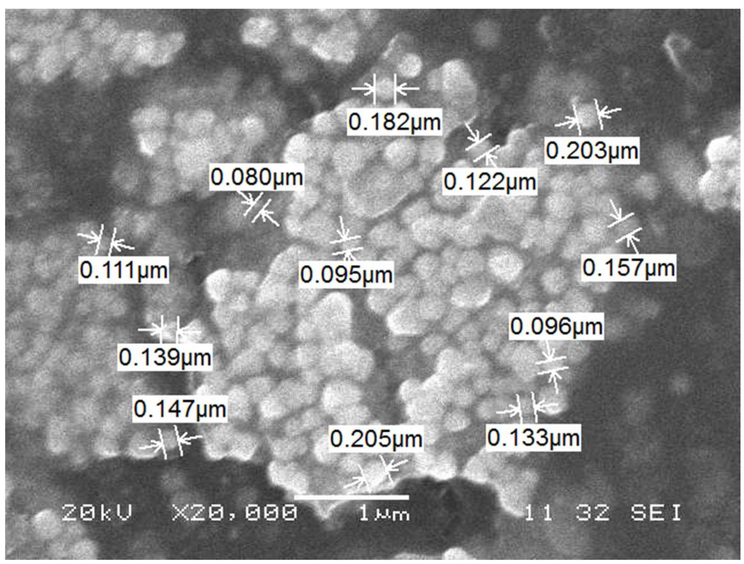

Fig. 4. SEM image of AgNPs of *Ageratum conyzoides* leaves.

value of 139 nm. The outcomes reveal that the plant extract is playing a key role in the synthesis fine AgNPs. These FTIR, XRD and SEM results illustrated that various functional groups, crystallite nature, surface plasmonic resonance wavelength and the shape of nanporticles of *A. conyzoides* leaves extract may play a role in the reduction and stabilization of generated AgNPs as well as antifungal activity (Wang *et al.* 2015, Szerencsés *et al.* 2020).

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