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The Effect of Various Concentrations of AgNO3 Aqueous Solutions on Silver Nanoparticles Biosynthesis Using Tarchonanthus Camphoratus Leaf Extract and Their Antibacterial Activity

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Abstract: Because of the enormous range of biomedical uses and scientific interest in nanotechnology, the production of metallic nanoparticles using plant extracts is being examined. To investigate the antibacterial activity, silver nanoparticles (SNPs) were produced from aqueous silver nitrate by a simple and environmentally friendly approach employing leaf extract of *Tarchonanthus Camphoratus* (TC) as a reductant and stabilizer. A constant volume of plant extract (1mL) with various concentrations (1, 2, 4, 6, 8, 10 mM) of the aqueous silver nitrate ($AgNO_3$) was mixed separately to estimate their effects on the synthesized SNPs. The optical, surface morphological features, composition, and antibacterial activity of the SNPs were studied. The optical characteristics were evaluated at 429-446 nm using a UV spectrophotometer. Scanning Electron Microscopy (SEM) was used to examine the morphology. The analyzed SNPs were spherical in shape and well-distributed, with sizes ranging (21- 36 nm). Energy-dispersive X-ray spectroscopy (EDX) was used to estimate metal ion concentrations in the prepared SNPs. Using the cup-plate agar diffusion approach, the produced SNPs showed an inhibition zone that confirmed antibacterial effects against both *Staphylococcus aureus* and *Escherichia coli* bacteria. The maximum inhibition zones (13 mm) for *S. aureus* and (13 mm) for *E.coli* bacteria were observed. Finally, this study demonstrated that biosynthesized SNPs production using the described methodology was successful, as evidenced by several analysis methods, and demonstrated a new application of SNPs syntheses against the two types of bacteria growth.

Keywords: Silver nanoparticles, Biosynthesis, Leaf extract, *Tarchonanthus Camphoraus*, Antibacterial activity.

1. Introduction

Many researchers are interested in silver nanoparticles (SNPs) because of their numerous and exciting uses in diverse sectors. SNPs are one of the most appealing nanomaterials for expanding the

market for silver-based industrial applications. SNPs are used in a variety of fields, including engineering, biomedicine, and agriculture (Otsuka *et al.*, 2003; Sajjad *et al.*, 2018; Abasi *et al.*, 2022). Physical (Vasileva *et al.*, 2011), chemical (Sikkander *et al.*, 2013; Elnashaie *et al.*, 2015; Hassan *et al.*, 2022), photochemical (Obaid *et al.*, 2016), and biological methods and procedures for the production of SNPs have now been established (Bahwirth and Bamsaoud, 2020; Bamsaoud and Bahwirth, 2017; Tenzin *et al.*, 2022). As common difficulties, these approaches have advantages and disadvantages in terms of cost, scalability, particle size and size distribution, and so on (Dimitrieska *et al.*, 2018; Tenzin and Kaur, 2022). Furthermore, harmful chemicals are used in physical and chemical procedures, limiting their application (Dimitrieska *et al.*, 2018). Because of the cheaper cost, restricted availability of the main material, and its stable the green way to synthesize SNPs is chosen and may be suited for the large-scale synthesis of nanoparticles (Mahmud and Sarbast 2017;Bamsaoud *et al.*, 2021) . Most plant extracts can be employed in this manner since they contain the bulk of antioxidants that can act as reducing agents (Sajjad *et al.*, 2018), (Sardjono *et al.*, 2022). As a result, much studies must be done in synthesizing SNPs utilizing various plant leaves, and their appropriateness in various applications must be determined. In this method, most plant extracts can be used because of their antioxidants and potential redactors for the synthesis (Sardjono *et al.*, 2022), and nowadays, researchers are working to synthases SNPs using the different leaves of plants, and their suitability in some applications is required to be discovered.

The plant extract in this research is *Tarchonanthus Camphoratus (TC)*. *TC* is a small tree that has attractive grey leaves and is particularly suited to tough conditions. The plant belongs to the family of Asteraceae (Herman *et al.*, 2002) and it is known as "*Qummer*" in Hadramout, Yemen, and grows on the highlands (Nasser *et al*., 2013). It is modest, with a maximum height of 2-9m, and develops in large, homogeneous groupings (**Photo 1**). It has pale brown bark on its stem as well. The leaves are grey-green on top and pale grey and felted on the bottom, with pronounced venation. The leaves have whole or slightly serrated edges and are thin. Their leaves extract can be used to cure a variety of illnesses, including high fever and human body ailments (Nasr *et al.*, 2020). There have been numerous studies on the essential oil of *Tarchonanthus Camphoratus* and a wide variety of biological properties (e.g., antimicrobial, antifungal, antioxidant, antidiabetic, insecticidal, insect repellant, analgesic, and antipyretic) have been reported (Dimitrieska *et al.*, 2018; Bamsaoud *et al.*, 2021; Sardjono *et al.*, 2022).

Photo 1: *Tarchonanthus Camphoratus*

The antibacterial activity of SNPs against Staphylococcus *aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) was demonstrated in this study. Bacteria of this kind are extensively employed in research. This bacteria is one of the most common gram-positive and gram-negative bacteria on the planet and several materials have been employed as antimicrobials against *S. aureus* and *E. coli* (Breijyeh *et al.*, 2020). SNPs have significant cytotoxicity for microorganisms and are commonly employed as antibacterial agents (Bamsaoud *et al.*, 2021). Recently, the antibacterial action of SNPs has been widely utilized in antibiotics around the world, with superior results to previous traditional medicines (Mousavi *et al.*, 2018). Researchers recently observed that using natural plants in the SNP green synthesis process results in considerable antibacterial action (Sajjad *et al.*, 2018).

The purpose of this study is to look into the usage of *TC* leaf extract in SNP synthesis and to test the antibacterial activity of the SNPs that have been synthesized against *S. aureus* and *E. coli*.

2. Materials and methods

2.1. Materials

The *TC* leaves were collected from *Ra's Huwirah* market, Hadhramout, Yemen in the autumn season of 2021. Analytical-grade silver nitrate ($AgNO_3$) was purchased from *Fisher Scientific*, UK. There was no further purification of the chemicals and they were used as produced. For cleaning and other aqueous solutions preparation, deionized (DI) water was used.

2.1 Crude Extracts Preparation

The *TC* leaves were washed several times with DI water after being refluxed into running tap water. After the debris and any other contaminating organic materials were removed, 10g of the leaves were placed in a 250 mL beaker with 100 mL DI water and boiled for 30 minutes at 60°C. When the color of the combination turned yellowish, it was allowed to cool to room temperature before being filtered through Whatman No. 1 filter paper (pore size 25 µm) to modify the result. Before further investigation, the filtered TC leaf extract was kept at 4°C.

2.2 Green synthesis of SNPs.

1 mL of *TC* leaf extract was mixed separately in 50 mL of silver nitrate solution with various concentrations in a typical reaction (1, 2, 4, 6, 8, and 10 mM). All of the mixtures' reactions were completed at room temperature. *TC* extract, as well as silver nitrate solution (1 and 10 mM), were used as a control for antibacterial activity

2.3 Characterization techniques

The prepared SNPs' size, morphology, and composition were analyzed using various characterization techniques. Using a quartz cuvette with an optical path of 1 cm, the UV–vis absorption spectra of SNPs dispersions were confirmed on a spectrophotometer within the resolution of 1 nm and range between 200 and 800 nm. The scale and structure of the SNPs were investigated using high-resolution scanning electron microscopy transmission running at a 10 kV accelerating voltage. The metal ions concentrations were determined by using energy-dispersive X-ray spectroscopy (EDX) spectroscopy.

2.5 Antibacterial Activity

The cup-plate agar diffusion method was used to analyze the antibacterial activity of the produced silver nanoparticles with a small modification to examine the antibacterial properties of the biologically generated SNPs. 250 mL of sterile nutritional agar medium was well mixed with 1 mL of standardized *S. aureus and E.coli* bacterial stock suspensions (10^8–10^9 CFU/ml). In sterile Petri dishes, 20 mL of the inoculated nutrient agar medium was dispersed. The agar was allowed to be set before being cut in each plate using a sterile cork borer from 4 to 6 cups, 10 mm in diameter, and the agar disks were removed. Each cup was filled with 80 µl of samples and left to diffuse for 2 hours at room temperature (Balouiri *et al*., 2013). The plates were then incubated for 24 hours in an upright position at 37°C. Measurement of the inhibition zone was used to investigate the antibacterial activity of all samples. The inhibitory zone's diameter was averaged and the mean values were obtained (Vijayakumar *et al*., 2013).

3. Results and Discussion

3.1 Variation of $AgNO_3$ concentrations

The SNPs which prepared from a mixture of $AgNO_3$ as a metal source and *TC* leaf extract as a reducing and capping agent, shows a gradually change in mixture color. The mixture began with faint light yellow, and in the second stage, the color changed to a dark brownish which probably indicate to the reduction of Ag^+ to Ag^0 and due to excitation of electrons and changes in electronic energy levels (Elemike *et al*., 2014). Before completing 1hr and after the stirring process, the solution color stabilized to its final color which is dark brown (**Figure 2b**). It can be noted that the morphology of the nanoparticles is greatly influenced by the surface plasmon resonance (SPR), since it is the basis for measuring adsorption of material onto the surface of metal nanoparticles (Elemike e*t al*., 2014). Moreover, no observable change was noticed in the mixture color during the investigation period of several weeks. Typically, silver metal has free electrons on the surface of the particle, those electrons well affected with the incident light from the UV-vis spectroscopy, which create simultaneous vibration of these electrons with this incident light to make SPR absorption band (Bamsaoud *et al*., 2021). The SNPs formation was tracked using absorption data recorded by UV-vis spectroscopy. **Figure 2a** shows the UV-vis absorption spectra of SNPs prepared by different concentrations of $AgNO_3$ (1, 2, 4, 6, 8, and 10 mM).

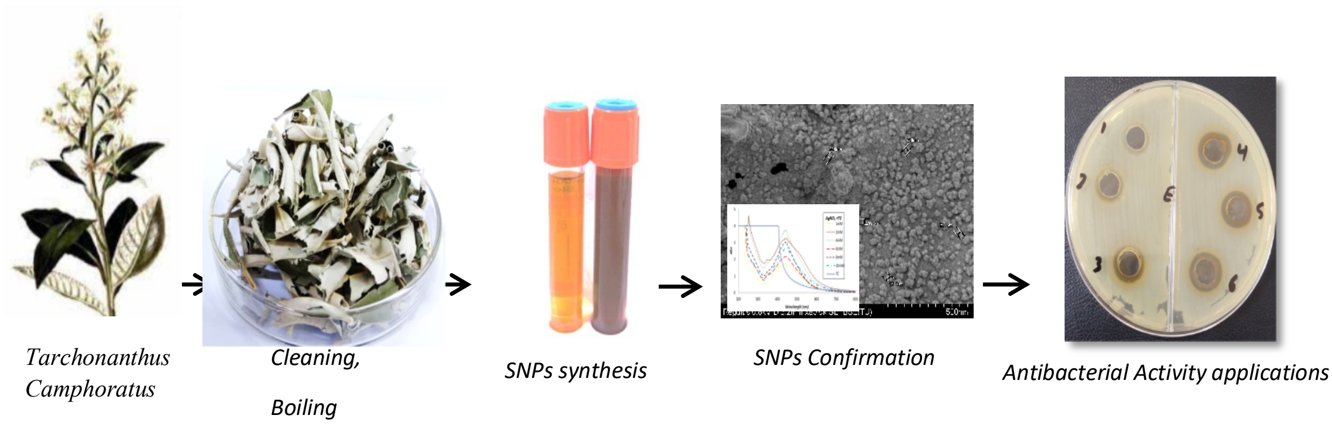

Figure 1. Schematic presentation for biosynthesis, characterization and antibacterial applications of synthesized silver nanoparticles using *TC* leaf extract.

At all the concentrations, there are mostly a single and narrow absorption peak except for pure *TC* leaf extract where no peak is observed. The SPR band was found to be between 440-448 nm for each sample. The similarity in SPR bands for all samples may indicate that all the samples have almost the same average particle size (Bamsaoud *et al*, 2021). In addition, the full width at half maximum (FWHM) of SPR is thought to be a reliable measure of particle size uniformity (size distribution) (**Figure 2c**). The narrow peaks in **Figure 2c** indicate that they have similar particle sizes. If the UV-vis intensity is a function of the Ag^0, a remarkable change was observed in the UV-vis intensity which indicates that a higher number of silver ions were converted to SNPs such as in 4mM while in 1mM indicates that the higher concentration of the protein in *TC* extract (**Figure 2a**).

In the chemical structure, *Tarchonanthus Camphoratus* (*TC*) leaves contain several compounds, such as oxygenated monoterpenes and oxygenated sesquiterpenes which are common constituents of many essential oils as the major groups also endo-fenchol, trans-pinene hydrate, caryophyllene oxide, α-terpineol, τ-cadinol, and α-cadinol (Herman *et al.*, 2002; Nasser *et al.*, 2013; Breijyeh *et al.*, 2020). Using the fact of *TC* leaf extract contains some deferent phenols in the essential oils react with silver nitrate by converting Ag^+ to Ag^0 resulting in silver nanoparticles (Nasser *et al.*, 2013), (Nasr *et al.*, 2020). The phenols work as a capping agent preventing more silver atoms to combine in order to form bigger silver nanoparticle sizes(Lignin *et al.*, 2021). In the present case, a fixed volume of *TC* extract with various concentration of $AgNO_3$ produces SNPs in average between (20 -60 nm) (**Figure 4a,b**).

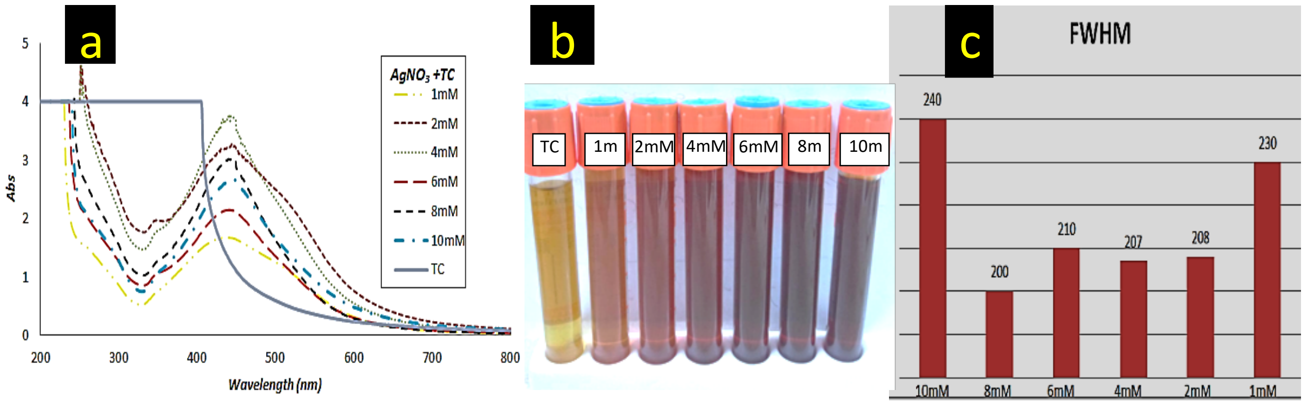

Figure 2. (a) UV-vis absorption spectra for SNPs prepared by different concentrations of *$AgNO_3$*, **(b)***TC* and SNPs color intensity,**(c)** FWHM of SPR

Silver nanoparticles are more widely used for medical purposes because of their antimicrobial characteristics(Bamsaoud *et al*, 2021). The results of the antibacterial activity effect of *TC* extract were simply determined by the cup plate method. The diameter (in mm) of the inhibition zone was determined after 24 h incubation at 37°C. **Figure 3** demonstrates the antibacterial test results of the SNPs synthesized from various $AgNO_3$ aquatic solutions. The SNPs (prepared from 1-10 mM and 1 mL of *TC* leaf extract) create an inhibition zone for both gram-negative and gram-positive bacterial.

3.2 Anti-bacterial analysis

Figure 4 shows the effect of produced SNPs on the target bacteria activity in this research. The test was carried out with SNPs and plant extract alone, as well as 1.10mM of $AgNO_3$. In the **Figure 4-a**, the activity of the SNPs was tested against *S.aureus* bacteria, the results were that there was no effect

at concentrations of 1.2mM but when the concentration was increased to 4mM, the SNPs began to resist the activity of bacteria, and the highest inhibition zone of *S.aureus* bacteria was recorded at concentrations 6-10mM (13mm). **Figure 4-b** shown that there is a relatively noticeable effect on the *E.coli* bacteria at the concentrations 1,2mM and an inhibition zone was shown at the concentration 4mM (11mm), while the best stabilized inhibition zone of *E.coli* bacteria at the concentrations 6-10mMM (13mm). **Figures 4-c** and 4-d show the effect of the extract only and silver nitrate only on the *S.aureus*, the results were that there was a small inhibition zone (11mm) for $AgNO_3$ and the *TC* plant extract only, and this indicates that the prepared concentrations of SNPs are better than the separate SNPs components itself.

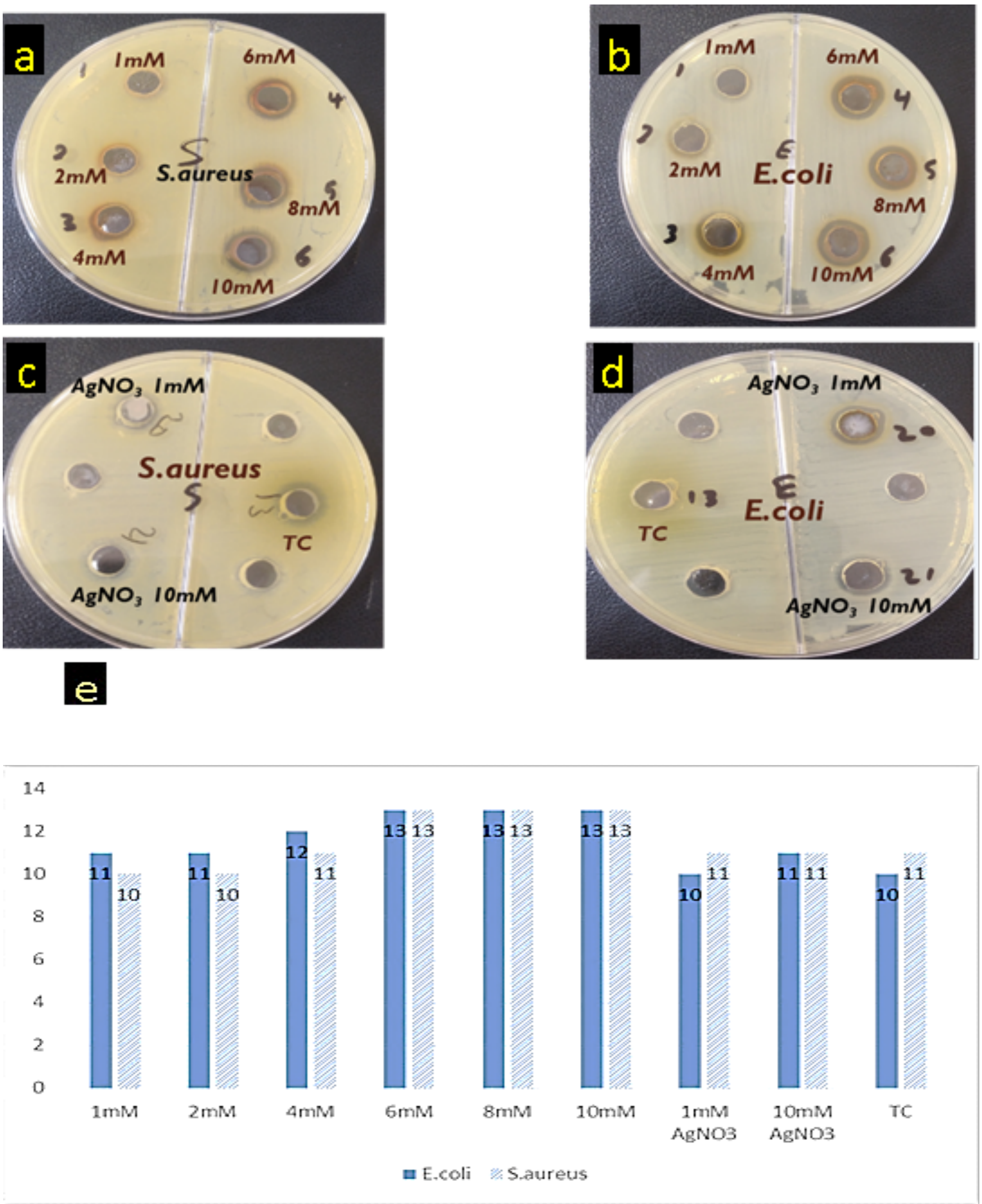

Figure 3. Antibacterial activities of SNPs against (**(a)** *S.aureus* and **(b)** *E.coli*) both with (1-10mM) SNPs and (**(c)** *S. aureus*, and **(d)** *E.coli*) both with 1mM, 10mM & *TC* all separtily alone, **(e)** shown the inhibition zone diameter (mm) of prodused SNPs against *S.aureus* and *E.coli* in.

These aforementioned results give indications that the new preparation SNPs have the ability to inhibit the activity of the gram-positive (*S.aureus*) and gram-negative bacteria (*E.coli*) at certain concentrations. The use of silver nanoparticles (SNPs) has become common recently because of their antimicrobial effects in pharmaceutics, cosmetic and textile applications. Antimicrobial effects of SNP are due to structural changes in the bacterial cell membrane, loss of DNA replication, dissipation of the proton motive force, and finally cell death (Sondi and Salopek, 2004; Maneerung *et al.*, 2007; Navarro *et al.*, 2008) .

3.3 SEM analysis of silver nanoparticles

SEM micro-graphs show aggregates of silver nanoparticles and the particles are in the range of 21–36 nm and there are not in direct contact even within the aggregates indicating the stabilization of nanoparticles by capping agents (**Figure 4(a) and (b)**). All the SNPs samples show that the particles are nearly spherical with smooth surface morphology and the particles were monodisperse, with only a few particles of different size.

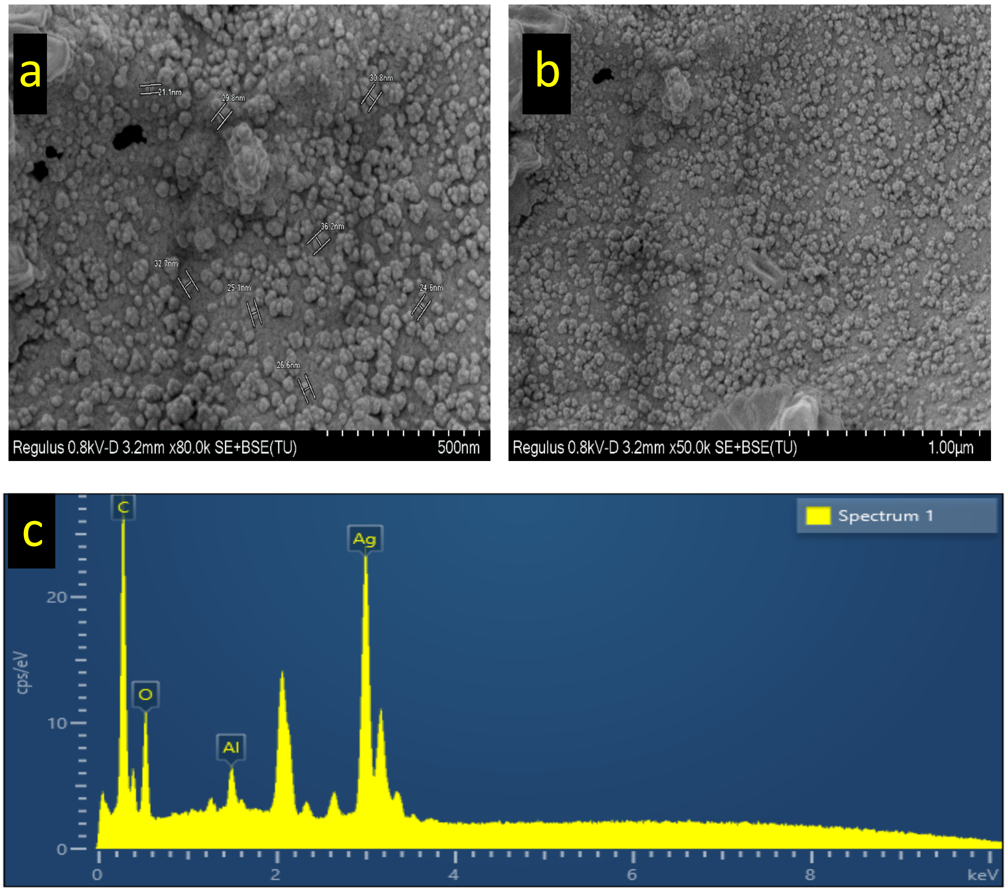

Figure 4. SEM (**a** and **b**) and (**c**) EDX spectrum of Ag NPs.

3.4 EDX analysis of silver nanoparticles

The EDX analysis of Ag nanoparticles showed strong signals for silver particles (**Figure 4-c**). The EDX pattern shows that the Ag nanoparticles are crystalline in nature, which is caused by the reduction of silver ions (**Table 1**). The EDX analysis confirmed the presence of silver nanoparticles and mostly showed strong signal energy peaks for silver particles in the range of 2.5–3.5 keV. Recently, (Vijayakumar *et al.*, 2013) obtained the formation of individual spherical-shaped AgNPs in the range

2–4 keV in Artemisia Nilagirica. Previously, Gardea-Torresdey et al. (2003) obtained individual spherical-shaped AgNPs in the range 2.5–4 keV by using Alfalfa. The minor amounts of C and O observed here are ascribed to the plant biomass attached to the SNPs. The high surface area to volume ratio causes high biological efficiency (antimicrobial, antioxidant, and anticancer activity) of metal nanoparticles (Yallappa *et al*., 2015; Sardjono *et al*., 2022). The encouraging results of AgNPs activity growing with time, their association with the dialdehyde cellulose (DAC) exhibits strong antibacterial activity against Gram-negative (E. coli) and Gram-positive (S. aureus) (Bruna *et al*., 2021; Akshaya *et al*., 2022, Du *et al*., 2022).

Table 1. EDX characterization confirm the presence of silver, carbon, oxygen and aluminum substances in the tested sample:

Spectrum 1	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma
C	K series	13.34	0.13343	20.34	0.26
O	K series	6.48	0.02181	12.97	0.27
Al	K series	1.47	0.01055	1.70	0.08
Ag	L series	49.15	0.49152	64.98	0.34
Total				100.00	

Conclusion

The current study shows that SNPs are easily, economically, and greenly synthesized with aqueous *Tarchonanthus Camphoratus* leaf excerpts. SNPs biosynthesis formation was confirmed by different characterizations. Different concentrations of $AgNO_3$ with a fixed volume of *TC* leaf extract give a change in particle size of SNPs. The biosynthesized SNPs with *TC* leaf extract aqueous showed different anti-bacterial activity against the gram-positive (*S.aureus*) and gram-negative *(E.coli*) bacteria. The Variation of SNPs concentrations showed various anti-bacterial activity but the 6mM concentration had been shown best activity against both bacteria types. The best anti-bacterial activity result was at the particle size of SNPs (21-36nm) and was conformed and had been the success in the formation. The SNPs with TC leaf extract aqueous showed much greater antibacterial activity than the *TC* leaf extract and $AgNO_3$ only

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