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SILVER NANOPARTICLES SYNTHESIZED USING *LANTANA CAMARA* FLOWER EXTRACT BY REFLUX, MICROWAVE AND ULTRASOUND METHODS

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Abstract. Green synthesis of silver nanoparticles (AgNPs) using *Lantana camara* yellow flower extract *via* microwave irradiation and ultrasound methods was accomplished. The research was aimed at evaluating the effect of the synthesis method and of the treatment time during synthesis on the particle size and antioxidant and antibacterial activities of the AgNPs. Analysis of the nanoparticles was performed using UV-Vis spectroscopy, transmission electron microscope (TEM), particle size analyzer, and Fourier transform infrared spectroscopy (FTIR). The antioxidant activity of the nanoparticles was determined using the radical scavenging (DPPH) assay, while the antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus*. The nanoparticles enhanced antioxidant and antibacterial properties compared to the plant extract. The present results support the advantages of the green method for the production of nanoparticles for further potential applications.

Keywords: silver nanoparticle, green synthesis, *Lantana camara*, microwave, ultrasound.

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Introduction

Nanotechnology has recently received popular interest and considerable attention in science and technology. The discovery of specific physicochemical features of materials on the nanometer scale resulted in the achievement of significant advances and those features proved useful for a wide range of applications such as bio-medical, sensors, antimicrobials, catalysts, electronics, optical fibers, agriculture, waste treatment, *etc*. For medical applications, silver nanoparticles (AgNPs) are by far the most popular ones [1-4]. AgNPs are mainly used as antibacterial and antioxidant agents, and recently they have been successfully applied in cancer identification and therapy. Due to these aspects, studies on techniques of nanomaterials synthesis, including the synthesis of AgNPs, are gaining momentum. The bottom-up synthesis method involving the use of a silver precursor solution employ conventional reducing agents such as sodium tetraborohydrate ($NaBH_4$), hydrazine, and dimethyl formamide (DMF), which are known to be carcinogenic and environmentally unsafe chemicals. In the green chemistry approach, many investigations have reported the synthesis of AgNPs by using plant extracts as alternatives for the above mentioned reducing agents. Some studies used plant extracts of *Bergenia ciliata*, *Zizyphus xylopyrus*, *Malus domestica*, *Clitoria ternatea* and *Solanum ningrum*, *Nictantes abror*, *Zelanicum* bark, *Sargassum angostifolium* and apple extract for similar types of syntheses [5-12].

Lantana camara is a shrub plant growing in tropical areas. *Lantana camara* plant contains a variety of chemical compounds such as mono- and sesquiterpenes (bisabolene, *β*-curcumene, safrole), triterpenes (lantanolic and lantic acids, ursolic acid, lupine), alkaloids, flavonoids (hispidulin, glycoside camaraside, trimethoxy quercetin derivatives), proteins, phenolic compounds and essential oil [13]. Studies related to the biosynthesis of AgNP using *Lantana camara* have been reported, where some of these used leaf and flower extracts [14-17]. Although finding a potentially useful plant for the synthesis is vital, choosing a proper and effective synthesis technique is also of importance. Green synthesis of AgNPs has been previously reported using methods such as hydrothermal, microwave-assisted, and sonication, and some of these are listed in Table 1. All these methods showed that the reaction time significantly affects the particle size of nanoparticles.

Table 1

Some methods for the green synthesis of AgNPs using plant extracts.

Plant extract	*Method*	*Results*	*Lit. ref.*
Red cabbage	Hydrothermal method	Monodispersed spherical nanoparticles exhibiting antibacterial activity towards *Staphylococcus aureus* (Gram+), *Escherichia coli* (Gram−) and *Candida albicans*.	[18]
Orange peel	Microwave	AgNPs with mean size of 7-17.31±0.84 nm.	[19]
Zizyphus xylopyrus bark	Sonication	Nanoparticles ranging from 60 to 70 nm.	[6]
Pigment *Streptomyces coelicolor* klmp33	Microwave	AgNPs were synthesized in only 90 seconds, in size of 50 nm.	[20]
Camellia japonica leaf	Aging at room temperature	AgNPs exhibiting photocatalytic activity in the degradation of EY dye and nitrobenzene under UV-Vis irradiation.	[21]
Citrus peel	Microwave	Nanoparticles of 7.36±8.06 nm synthesized in 15 min.	[22]
Ocimum basilicum leaf extract	Sonication and aging	Both methods and variation of the ratio of plant extract: $AgNO_3$ solution influence the synthesis.	[23]
Portulacaoleracea	Sonication	AgNPs less than 60 nm in size.	[24]
Phoenix Dactylifera L. (date palm) leaf	Microwave	The synthesis is influenced by: pH, Ag^+ concentration and exposure time to microwaves.	[25]
Pisonia grandis (R. Br)	Sonication	Spherically shaped AgNPs of 20-150 nm	[26]
Pleurotus florida mushroom	Photo-irradiation	AgNPs of 20±5 nm were obtained.	[27]

In view of the effective results of sonication and microwave methods, the study of both methods for AgNPs synthesis using *Lantana camara* flower extract is an interesting topic. Taking into account published reports showing that the synthesis parameters affect the morphology and activity of the nanoparticles, we investigated the effects of the synthesis method and also of the treatment time during synthesis on the particle size and antioxidant and antibacterial activities of AgNPs. Analysis of the nanoparticles was performed using UV-Vis spectroscopy, transmission electron microscope (TEM), particle size analyzer, and Fourier transform infrared spectroscopy (FTIR).

Experimental

Plant material collection and chemicals

Yellow flowers of *Lantana camara* were collected from the area of Universitas Islam Indonesia campus (Sleman District, Yogyakarta, Indonesia). Silver nitrate, dimethyl sulfoxide (DMSO), ethanol, and methanol were purchased from Merck, Germany. 1,1-Diphenyl-2-picrylhydrazyl (DPPH, >99.5%) was purchased from Sigma-Aldrich, USA. Bacteria strains of *Escherichia coli* and *Staphylococcus aureus* were purchased from ATCC Company, USA, and stored at the Microbiology Laboratory, Department of Pharmacy, Universitas Islam Indonesia. Deionized water was used throughout.

Preparation of extracts

Fresh yellow flowers of *Lantana camara* were collected and dried under sunlight for 24 h to reduce the water content. The flower extract was obtained by boiling 3 g of dried flower for 15 min, followed by cooling and filtration on Whatman 41 filter paper. The freshly prepared extract was used for further experiments and was referred to as *Lantana camara* extract (LCE).

Synthesis of silver nanoparticles

Mixtures of 10^{-3} M silver nitrate solution and LCE in a volume ratio of 9:1 were prepared and further reacted by various methods: reflux, ultrasound-assisted synthesis and microwave-assisted synthesis. In the reflux method, the mixture was refluxed for 1 h to complete the bio-reduction, while in the microwave-assisted and ultrasound-assisted syntheses, the mixture was treated for 10, 20 and 30 min. A commercial microwave oven was used for the microwave-assisted synthesis, and a Hielscher ultrasonic batch mixer was employed in the ultrasound-assisted synthesis.

Characterization of the synthesized AgNPs

The resulting solutions were characterized using UV-Vis spectroscopy (Tokyo, Japan), X-ray diffraction analysis (XRD) (Shimadzu X6000, Tokyo, Japan), particle size analyzer (HORIBA Scientific, Kyoto, Japan), transmission electron microscopy (TEM) (JEOL-JEM 1400, Freising, Germany) and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) (Perkin Elmmer, New York, USA).

Antioxidant activity assay

The antioxidant activity of AgNPs was measured by using the DPPH method. For each analysis, 0.2 mL of AgNPs was mixed with 2 mL of DPPH and 2 mL of methanol. The mixture was incubated and allowed to stand at room temperature for 30 min, afterwards the absorbance of the solution was measured at 517 nm. The

antioxidant activity percentage was calculated using Eq.(1):

$$Scavenging\ (\%) = \left\{1 - \frac{[Abs]_{30}}{[Abs]_0}\right\} \cdot 100 \qquad (1)$$

where, $[Abs]_{30}$ and $[Abs]_0$ are the absorbance values of solution at 30 min and at 0 min, respectively.

Antibacterial assay

The antibacterial test of the AgNPs was carried out using two different pathogens: gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*. The antibacterial activity test was performed using the disk diffusion method, by placing different types of disks after immersion in the AgNPs solution. Chloramphenicol and double-distilled water were used as positive and negative controls. The disks in agar plates were incubated for 24 h at 37°C, and then the diameter of the zone of inhibition was measured with a meter ruler. The mean value of inhibition around each disc was calculated and expressed in millimeters.

Results and discussion

The UV-Vis spectra of *Lantana camara* extract and synthesized AgNPs are shown in Figure 1. The LCE shows a shoulder peak in the region 300-400 nm, and the maximum registered wavelength is at 220 nm. The formation of AgNPs after the reaction with $AgNO_3$ solution was identified from the peak at around 420-450 nm due to the plasmon resonance formation in all methods [28]. The UV-Vis spectra of AgNPs synthesized using microwave-assisted and ultrasound-assisted syntheses are depicted in Figures 2 and 3. The spectra allow us to conclude that longer treatment time gives higher absorbance, which indicates a higher concentration of AgNPs in the solution.

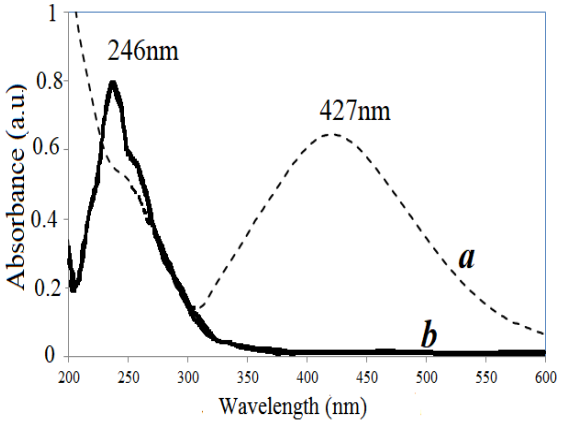


Figure 1. UV-Vis spectra of LCE and AgNPs obtained by reflux method.

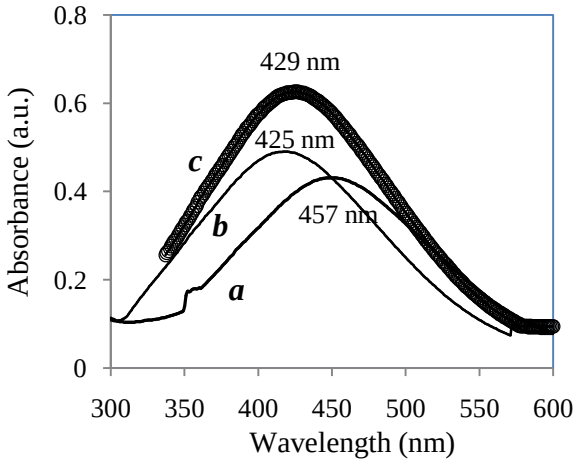


Figure 2. Comparison of UV-Vis spectra of AgNPs obtained by microwave-assisted synthesis, exposure time of *(a)* 10, *(b)* 20 and *(c)* 30 min.

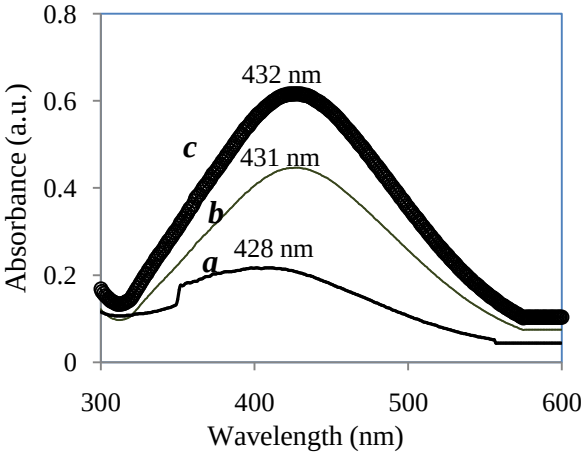


Figure 3. Comparison of UV-Vis spectra of AgNPs obtained by ultrasound-assisted synthesis, exposure time of *(a)* 10, *(b)* 20 and *(c)* 30 min.

An increased intensity indicates a faster rate of bio-reduction [6]. However, there is no linear correlation of the maximum wavelength with the reaction time in both methods. Visually, in the synthesis of AgNPs, there was a change in color from yellowish to reddish-brown.

Particle size distribution of AgNPs

A particle size analyzer provides information about the distribution form and particle size distribution of the formed AgNPs. Figure 4 shows the effect of treatment time on the particle size distribution of AgNPs, and the parameters are listed in Table 2. In general, smaller particle sizes of AgNPs were achieved by both treatments for 30 min. For the ultrasound-assisted treatment, longer treatment time produces a smaller particle sizes, which is in agreement with a previously reported investigation [29]. In contrast, the microwave treatment shows a deviation at 20 min of irradiation, which gives the highest particle size. This phenomenon may be

related to agglomeration during the reaction process, which causes destabilization of the particles. This assumption is also strengthened by the higher polydispersity index of the AgNPs compared to the result obtained at 10 min of irradiation.

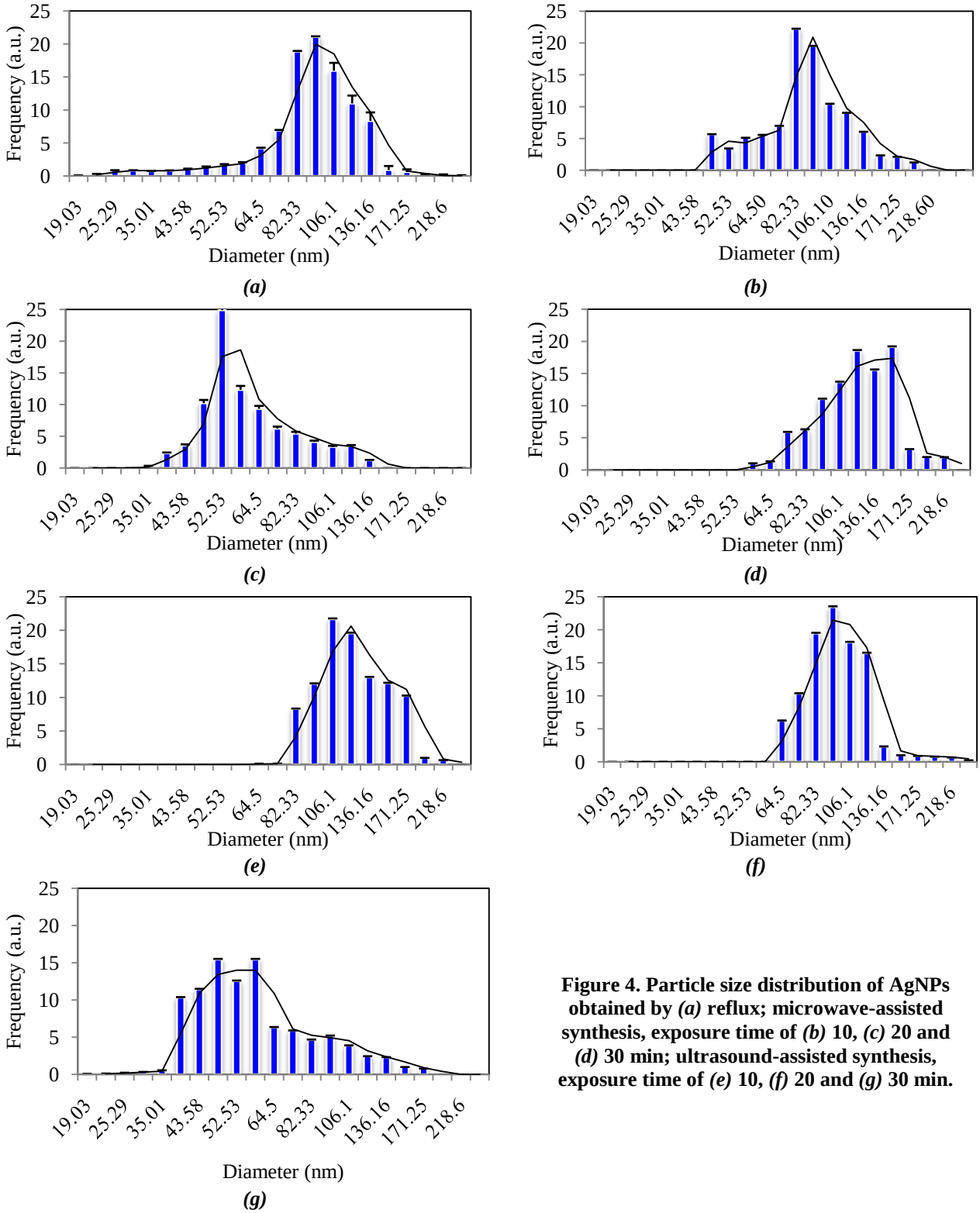


Figure 4. Particle size distribution of AgNPs obtained by *(a)* reflux; microwave-assisted synthesis, exposure time of *(b)* 10, *(c)* 20 and *(d)* 30 min; ultrasound-assisted synthesis, exposure time of *(e)* 10, *(f)* 20 and *(g)* 30 min.

Table 2

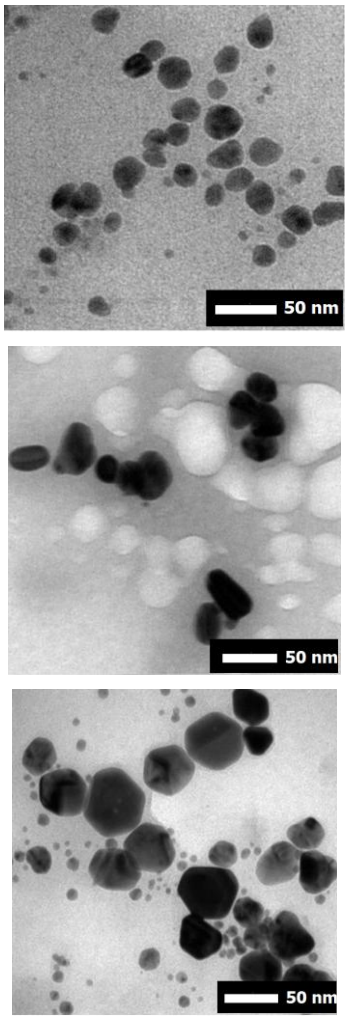
Results from the particle size analyzer.

Method	*Reflux*	*Microwave-assisted synthesis*			*Ultrasound-assisted synthesis*		
Time (min)	60	10	20	30	10	20	30
Mean particle size (nm)	62.8	51.5	124.3	45.32	126.3	64.9	47.1
Polydispersity index (PI)*	0.275	0.229	0.441	0.324	0.569	0.443	0.35

***PI indicates distribution of mean particle size, the higher PI means the wide range of particle size.*

TEM analysis

Figure 5 presents the morphology and size of AgNPs confirmed by TEM analysis. All synthesis methods produce nanoparticle sizes distributed at around 50-100 nm, and a significant difference in the shape of AgNPs is observed depending on the synthesis method. The reflux and ultrasound methods tend to produce AgNPs with irregular spherical forms, while microwave irradiation gives hexagonal-like forms. AgNPs obtained after 30 min in the ultrasound method demonstrate capping formation, which indicates the presence of organic functional groups in the solution [20].



(a)

(b)

(c)

Figure 4. TEM profile of AgNPs obtained by *(a)* reflux, *(b)* microwave-assisted synthesis, *(b)* 30 min exposure, *(c)* ultrasound-assisted synthesis, 30 min exposure.

FTIR analysis

FTIR analysis is helpful for analyzing the possible interaction between AgNPs with different functional groups as a result of reduction mechanism.

Figure 6 presents the obtained FTIR spectra. It is noted that all samples show a sharp band at 3306-3308 cm^{-1}, which is attributed to the hydroxyl functional groups in the phenolic compounds in the extract and also from water. The band at around 1632 cm^{-1} in the LCE is attributed to C=O, and this band is shifted to a higher wave number (around 1634 cm^{-1}) due to the formation of metal particles. Another shift identified after the AgNPs formation is the shift of the O-H vibration from 3293.6 cm^{-1} in LCE to higher wavenumbers in AgNPs samples. This shift is also related to the identification of phenolic interaction in the reduction reaction, which is determined from the bands at around 2118 cm^{-1} and the disappearance of the 1023 cm^{-1} bands attributed to the presence of $–CH_2$- and C-N, respectively. During the performed investigations, AgNPs were not separated from the LCE extract so the data of FTIR analysis also contain the functional groups from the extract. FTIR analyses results suggest on the reduction mechanism in the synthesis and there are functional groups related to the LCE maintained in the AgNPs solution as capping agent [30,31].

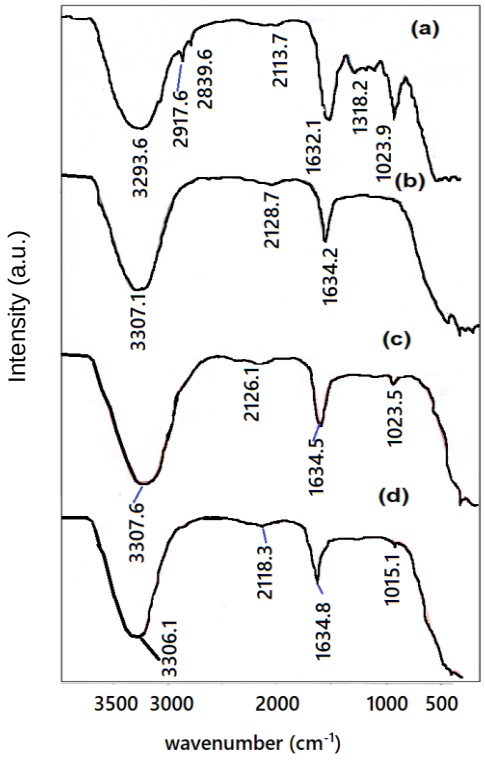


Figure 6. FTIR spectra of *(a)* LCE; *(b)* AgNPs obtained by reflux; *(c)* AgNPs obtained by microwave-assisted synthesis, 30 min exposure; *(d)* AgNPs obtained by ultrasound-assisted synthesis, 30 min exposure.

Antioxidant activity

Table 3 lists the antioxidant activity of the AgNPs compared to the LCE, represented by % of scavenging. These data show that AgNPs have higher antioxidant activities as compared to LCE.

In the ultrasound method, a longer ultrasound treatment gives a higher % of scavenging, but this trend is not observed in the microwave method. The highest % of scavenging was registered for the sample obtained by microwave-assisted synthesis, 30 min exposure, and had a value of 89.76%. These data suggest that the antioxidant activity is not only related to AgNPs size but also to some bioactive compounds from the LCE contained in the solution.

Table 3

Antioxidant activity of AgNPs compared to LCE.

Tested sample	*Scavenging (%)*
LCE	20.80
AgNPs (Reflux)	56.18
AgNPs (Microwave 10 min)	45.58
AgNPs (Microwave 20 min)	35.89
AgNPs (Microwave 30 min)	89.76
AgNPs (Ultrasound 10 min)	35.68
AgNPs (Ultrasound 20 min)	54.83
AgNPs (Ultrasound 30 min)	66.08

Antibacterial activity

Although the antimicrobial effect of silver is widely known, the mechanism of the antimicrobial action is not well explored.

Bactericidal activity is presumably due to the possible interaction between AgNPs with sulphur- and phosphorus-rich biomaterials, which include intracellular components, such as proteins or DNA, and extracellular components such as membrane proteins. These components influence the respiration, division, and ultimately survival of cells.

Table 4 shows the antibacterial activity of AgNPs [32]. These data show that LCE does not have any antibacterial activity towards any of the two bacteria, while the AgNPs, on the contrary, show antibacterial activity, as expressed by the inhibition zone measurements. Data analysis demonstrates a similar pattern as in the case of the antioxidant activity with the microwave and ultrasound treatment time. Thus, in the ultrasound-assisted method, a longer treatment time gives a higher antibacterial activity, but this is in a random order for microwave-assisted synthesis, *i.e.*, microwave 30 min> microwave 10 min> microwave 20 min. The main reason for this is also related to the content in the solution of the functional groups responsible for both the antibacterial and antioxidative activities.

Table 4

Antibacterial activity data of synthesized AgNPs.

Tested sample	*Inhibition zone after 24 h of incubation*			
	Escherichia coli		*Staphylococcus aureus*	
	Measurement 1	*Measurement 2*	*Measurement 1*	*Measurement 2*
Chloramphenicol	22.3 mm	22.1 mm	14.3 mm	14.8 mm
DMSO 10%	-	-	-	-
LCE	-	-	-	-
AgNPs (Reflux)	9.2	8.9	7.1	7
AgNPs (Microwave 10 min)	11.8	11.4	9.0	9.4
AgNPs (Microwave 20 min)	8.8	8.7	8.0	8.1
AgNPs (Microwave 30 min)	11.5	11.9	10.1	10.2
AgNPs (Ultrasound10 min)	7.9	8.1	8.2	8.1
AgNPs (Ultrasound20 min)	9.8	9.7	10.4	10.5
AgNPs (Ultrasound 30 min)	10.9	10.8	17.1	17.0

Conclusions

Green and fast synthesis of AgNPs using *Lantana camara* flower extract *via* microwave irradiation and ultrasound methods was successfully conducted. It was found that AgNPs synthesized by the ultrasound method led to nanoparticles with hexagonal forms, while in the microwave and reflux methods, the nanoparticles were spherical. Furthermore, the time of treatment for both methods significantly affected the particle size and the antioxidant and antibacterial activity of the AgNPs. The AgNPs showed antioxidative and antibacterial activities and it was found that there was no linear correlation between the particle size and both activities.

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