Green synthesis of silver nanoparticles using leaf extracts of *Mentha arvensis* Linn. and demonstration of their *in vitro* antibacterial activities

Santhanamari Thiyagarajan[1*], Subramanian Kanchana[2]

[1]*Department of Medical Laboratory, Technology, College of Applied Medical Sciences, Northern Border University, Arar, Kingdom of Saudi Arabia,* [2]*Department of Chemistry, Quaid-E-Millath Government College for Women (Aut.), Chennai, India*

A study was carried out to synthesize silver nanoparticles (Ag-NPs) using plant extracts and to explore their pharmaceutical application as antibacterial agents. Dried leaves of *Mentha arvensis Linn.* were powdered and extracts were prepared using three different organic solvents. Preliminary screening on antibacterial activity by agar well diffusion method indicated that the methanolic extract has superior potential than the other two. Phytochemical analysis of the leaf extract revealed that it possesses tannins, steroids, terpenoids and flavonoids. Ag-NPs prepared using this extract were of spherical to cuboidal shape ranging in size from 40 to 70 nm. It retained essential chemical groups and had the required surface plasmon resonance. Further to the antimicrobial assay, the Ag-NPs, in contrast to the whole methanolic leaf extract, showed better (100% vs. 80%) bactericidal activity against the indicator organisms. The zones of growth inhibition for gram positive bacteria ranged between 14±0.6 and 23±0.3, while it was between 12±0.6 and 22±0.2 for gram negative bacteria. The Ag-NPs presented the minimum inhibitory concentration values of 250 μg/mL for gram positive bacteria and 250 – 500 μg/mL for gram negative bacteria. These findings suggest that the natural compounds present in *M. arvensis* L. possess the potency to facilitate the synthesis and antibacterial action of Ag-NPs.

Keywords: *Mentha arvensis*. Leaf extract. Silver nanoparticles. Characterization. Antibacterial.

INTRODUCTION

Herbalism or botanical medicine, studied under the broad category of Pharmacognosy, is a traditional or folk medicine practice based on the use of plants and plant extracts. Many of the herbs and spices used by humans to season the food encompass useful medicinal compounds. *Mentha arvensis* Linn. is a small perennial herb with a branched stem bearing trifling blue flower clusters on a spike of scented green leaves and growing up to 75 cm (Nair, Chanda, 2007). It belongs to the family Lamiaceae which includes roughly of 200 genera and more than 4000 species (Snoussi *et al.*, 2015). It is believed that it was originated in Eurasia and now available in different habitats around the world. It is commonly known as field mint, corn mint, podina and so on. It has long been known as a herb of choice for food seasoning and food decoration purposes.

Wild mint is often used for domestic herbal remedy as it offers numerous health benefits. Studies have reported that this plant owns a variety of chemicals like menthol, menthofuran, isomnethone, α pinene, carvone, piperitenone oxide, linalyl acetate, tannin, phenols, flavonoids, terpenes, alkaloids, eugenol, steroids, α-tocopherol, glycosides, sugars and so on. It also possesses the flavonoids such as menthoside, quercetin and isorhoifolin. The oil yield is 5% by distillation of leaves, which contain 40-50% menthol (Santos *et al.*, 2010; Pandey, Dubey, Saini, 2010; Biswas, Saha, Ali, 2014). The leaves, in particular, have substantial pharmacological properties. Studies in recent years have demonstrated the antiviral, antibacterial (Gardiner, 2000; Coutinho *et al.*, 2009), antifungal (Pramila *et al.*, 2012) and anti-helminthic (Sugandhi, Bai, 2011) properties of *M. arvensis*. The antimicrobial property of this plant is

*Correspondence: S. Thiyagarajan. Assistant Professor of Microbiology & Immunology. College of Applied Medical Sciences. Northern Border University. Arar, P.O. Box: 01321. Zip code: 91431, Kingdom of Saudi Arabia. Phone: +966 - 559354527. E-mail: drsthiyagarajan@live.com, Thiyagarajan.S@nbu.edu.sa. ORCID number: https://orcid.org/0000-0001-7943-8707. Scopus Author ID: 57191414106

attributable to its possession of essential oil (Snoussi *et al.*, 2015). Some researchers have established that the secondary metabolites such as tannins prevent the growth of microorganisms by way of inhibiting the enzymes required for protein synthesis and oxidative phosphorylation (Satya Prasad *et al.*, 2015; Sosnowska *et al.*, 2017).

In recent years, the effectiveness of antimicrobial chemotherapy is being questioned due to increasing incidences of complications, development of resistance by bacterial pathogens and deposition of drug residues in feed and environment (Mehdi *et al.*, 2018; Hembram *et al.*, 2018). As the plant based natural products are endowed with antimicrobial properties, there has been growing interest in carrying out research in this area. Interestingly, these phytochemicals, besides exerting direct lethality, have the potency to modify the natural antibiotic resistance of pathogenic microorganisms through facilitating the elimination of resistance coding plasmids and inhibition of drug transport via plasma membrane (Coutinho *et al.*, 2009).

Nanoscience and nanotechnology, dealing with the materials of 1-100 nm size, are the fast developing sciences of 21st century. Owing to the morphology, size and distribution characteristics the nanoparticles (NPs) exhibit different physical and chemical properties. In recent years, the NPs have become essential objects in various emerging fields dealing with optoelectronics, biosensors, nanocatalysts and so on (Parashar *et al.*, 2009; Masurkar *et al.*, 2011). Since the introduction of nanotechnology phenomenal modernizations have been taking place in the field of medical sciences (Banerjee *et al.*, 2017). Compared to bulk metals, the metal NPs display extraordinary catalytic properties by virtue of their higher surface area-to-volume ratio (MubarakAli *et al.*, 2011). As the usual physical and chemical methods of synthesis of metal NPs are encountered with higher production cost and environmental pollution, synthesis using plants and microbes is gaining vested interest in recent years. In green synthesis, the phytochemicals present in the plants parts such as leaves, fruit peels, bark, callus and root are explored as bio-reductants as well as capping agents for the synthesis of NPs, thus becoming advantageous (Hembram *et al.*, 2018).

Silver has long been utilized since ancient times as an important agent in biological sciences, particularly in medicine, for various purposes. It is considered as a safe antimicrobial agent as it causes high lethality to bacteria while exerting minimal toxicity to animal cells (Hembram *et al.*, 2018; Banerjee *et al.*, 2017; Ali *et al.*, 2015). Studies have reported that owing to their extreme stability and unique catalytic and biological properties, silver nanoparticles (Ag-NPs) display considerable antibacterial and antioxidant activities (Hembram *et al.*, 2018; Abdel-Aziz *et* al., 2014). Manjari *et al.* (2018) suggested that by virtue of these properties, the Ag-NPs could serve as potential candidates for catalytic and recycling applications. Researchers in recent years have explored the extracts of different plants for the synthesis of Ag-NPs. However, common edible plants are often much sought after for significant research studies owing to their commonness and easy availability (Saikia *et al.*, 2015).

Emergence and eventual complications caused by antibiotic resistant bacteria, such as vancomycin resistant *S. aureus*, extended spectrum beta-lactamase producing *Escherichia coli* and *Pseudomonas aeruginosa* in recent decades is considered as a global threat to health care system from public health view point. In order to combat such precarious pathogens, safe drugs of natural origin that are prepared using environment friendly and cost effective methods should be the high priority need in the present scenario. Therefore, the present study was carried out to synthesize Ag-NPs using leaf extract of *M. arvensis* L. and characterize their properties and also to evaluate their antibacterial potential.

MATERIAL AND METHODS

Material

Mint plant (*Mentha arvensis* Linn.) was collected from a local vegetable market in Egmore area, Chennai, India. Plant material was identified and authenticated by the experts in the Department of Botany, Quaid-E-Millath Govt. College for Women (Aut.), Chennai. Leaves of the plant were removed and cleaned twice in sterile water. The wet leaves were spread over a tissue paper sheet and left for drying under shade for about 2-3 weeks. Then,

the dry leaves were ground using a mechanical blender and the fine powder was collected in a sterile container.

Solvents such as methanol, petroleum ether and chloroform (each of >95% purity) were procured from Sisco Research Laboratories (India). Silver nitrate (AgNO$_3$) of 99.5% purity was purchased from Sigma-Aldrich (USA). Mueller Hinton broth and agar media required respectively for maintaining bacterial cultures and antibacterial assay were received from HiMedia Ltd. (India). Dimethyl sulfoxide (DMSO) and other analytical grade chemicals used for phytochemical analysis were obtained from Merck (India).

Preparation of leaf extracts

Extracts of the leaves of *M. arvensis* L. were prepared using three different solvents namely, methanol, petroleum ether and chloroform. Twenty grams of dry leaf powder was mixed with 100 mL of solvent in a conical flask and kept in rotary shaker at 150 rpm for 24 h. Then, it was filtered in a glass beaker and evaporated to make a final volume of one-fourth of the original volume. It was stored at 4°C until further studies.

Screening of antibacterial activity of leaf extracts

The extracts of *M. arvensis* L. leaves were tested for antibacterial activity against five indicator bacteria including one gram positive (*Staphylococcus aureus*) and four gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Peudomonas aeruginosa* and *Proteus mirabilis*). These bacteria were obtained from the laboratory of Department of Microbiology, Institute of Basic Medical Sciences, University of Madras, Chennai, where they were isolated from clinical specimens of wound, stool, sputum and urine.

In order to screen the antibacterial activity of different leaf extracts of *M. arvensis* L. the method of agar well diffusion (Sugandhi, Bai, 2011; Singh, Shushni, Belkheir, 2015) was adopted with slight modifications. Briefly, five plates of sterile Mueller Hinton Agar (MHA) were prepared and each plate was labelled for testing the drug (leaf extract) against an indicator bacterium. In each plate five wells each measuring 5 mm diameter were cut using a sterile cork borer. These plates were seeded respectively with fresh cultures (18-24 h) of indicator bacteria (0.5 McFarland's turbidity standard) by swab inoculation. Stock solution (1 mg/mL) of the drug was prepared using DMSO and four different volumes viz., 25 μL, 50 μL, 75 μL and 100 μL (corresponding to the concentrations of 250, 500, 750 and 1000 μg/mL) of this solution were loaded into four wells of MHA plate. Fifth well was loaded with 100 μL of DMSO, which served as the control. Plates were incubated at 37°C for 24 h and diameter of zone of growth inhibition around each well was measured using calibrated scale. Zone diameter of >10 mm was considered positive for antibacterial activity.

Analysis of phytochemicals of leaf extract

Crude solvent extract of *M. arvensis* L. leaves was tested by standard procedures to analyze and determine the presence of various phytochemical constituents (Pramila *et al.*, 2012; Mojab *et* al., 2003; Ghani, 2003) as discussed below:

Tannins: Fifty milligram of the extract was mixed with 5 mL of distilled water, heated in water bath and filtered. Ferric chloride was added to this filtrate slowly until it turned dark green indicating the presence of tannins.

Saponins: The plant extract of about 0.2 g was mixed with 5 mL of distilled water and boiled. Presence of saponins was confirmed by persistent frothing.

Steroids: To 20 mg of the extract, 1 mL of methanol was added and filtrated. Subsequent addition of 1 mL conc. H$_2$SO$_4$ resulting in yellow green fluorescence indicated the presence of steroids.

Terpenoids: The extract measuring 0.5 g was mixed in 2 mL of chloroform. Conc. H$_2$SO$_4$ was carefully added over to this mixture to form a layer. Reddish brown coloration of interface confirmed the existence of terpenoids.

Flavonoids: The plant extract (0.2 g) was dissolved in diluted NaOH. A slow addition of HCl to this solution

caused change of color from yellow to colorless, thus indicating the presence of flavonoids.

Anthraquinone: To 5 mL of chloroform 0.5 g of the extract was added and shaken for 5 min. and filtered. The filtrate was mixed with an equal volume of 10% ammonia solution and shaken until the ammonia layer turned pink violet or red showing positive result.

Synthesis and characterization of silver nanoparticles (Ag-NPs)

In order to synthesis the silver nanoparticles the cold extraction method (Kelkawi, Kajani, Bordbar, 2017) was followed. First of all, crude leaf extract (0.5 g) of *M. arvensis* L. was added to 100 mL of de-ionized water and kept in vigorous stirring for 1 h. Then, 100 mL of $AgNO_3$ (1×10^{-3} M) was added to this and incubated at room temperature (25°C) in dark for 48 h. Formation of Ag-NPs was confirmed by observing the change of color of solution into brown. The mixture thus obtained in this process was lyophilized to obtain the powder of Ag-NPs. Characterization of Ag-NPs was carried out using UV-visible Spectrophotometry, SEM-EDX analysis and FT-IR analysis and by carefully following the protocols adopted by Hembram *et al.* (2018), Banerjee *et al.* (2017), Ali *et al.* (2015), Elumalai *et al.* (2010), and Gabriela *et al.* (2017).

UV-visible Spectrophotometry: The optical properties i.e., surface plasmon resonance (SPR) of Ag-NPs and the bioreduction of Ag+ in aqueous solution were determined using double beam UV-Visible spectrophotometer (Thermo Spector_IC ~ Visio_pro Software V1.06, USA). The reaction time was calculated through periodical recording of absorbance values of whole extract and reduced Ag-NPs at a resolution of 1.5 nm between the band width ranges of 400 and 800 nm.

SEM-EDX analysis: Scanning Electron Microscopy for analysis of physical properties (size, shape and surface morphology) of Ag-NPs was performed using Hitachi S-4500 SEM machine (Japan). Parallel identification of elements and quantitative information on chemical composition was achieved through Energy Dispersive X-ray analysis.

FT-IR analysis: Fourier transform infrared spectroscopy (Perkin Elmer system, USA) was adopted for detection of reducing agents from the plant extract and also for determining the size distribution, identification of chemical bonds and functional groups of Ag-NPs. Spectroscopic grade potassium bromide (KBr) was dissolved (1:100) in extract and the FTIR spectra of Ag-NPs were recorded at the diffuse mode of reflectance before and after the addition of $AgNO_3$.

Assay of antibacterial property of Ag-NPs

Toxicity of Ag-NPs on bacterial growth was tested by following agar well diffusion method (Singh, Shushni, Belkheir, 2015) as described above. Results were expressed in terms of minimal inhibitory concentration (MIC), i.e., the lowest concentration of drug which can cause visible inhibition of bacterial growth.

Statistical analysis

Experiments were carried out in triplicate and the results of analyses were presented as mean values ± standard deviations (SD; $n = 3$). The data were subjected to one-way analysis (ANOVA) and compared using student's t-test. Differences in values at $P \leq 0.05$ were considered statistically significant.

RESULTS

Antibacterial activity and phytochemical analysis of leaf extract

The antibacterial efficacy of the solvent extracts was determined in relation to their ability of inhibiting the number of indicator bacteria. Among the three solvent extracts of leaves of *M. arvensis* L. tested against five indicator bacteria, the chloroform leaf extract (CLE) did not show any inhibitory effect (0%). While the petroleum ether leaf extract (PELE) could inhibit only *E. coli* (20%), the methanolic leaf extract (MLE) exhibited lethality

against all the bacteria except *P. aeruginosa* (80%) (Table I). The results of the analysis for phytochemical constituents of the leaf extract are presented in Table II. The analysis showed the abundant presence of flavonoids and a moderate presence of tannins, steroids and terpenoids.

TABLE I - Results of assay of antibacterial activity of Leaf extracts of *M. arvensis* Linn. against indicator bacteria

S. no	Indicator bacteria	Leaf Extract	Antibacterial activity of leaf extract (zone dia. in mm)*			
			250 μg/mL	500 μg/mL	750 μg/mL	1000 μg/mL
1.	*Staphylococcus aureus*	CLE	3 ± 0.9	4 ± 0.4	6 ± 0.3	9 ± 0.7
		PELE	6 ± 0.3	7 ± 0.2	7 ± 0.6	8 ± 0.2
		MLE	11 ± 0.4	12 ± 0.2	16 ± 0.5	18 ± 0.1
2.	*Escherichia coli*	CLE	5 ± 0.9	6 ± 0.8	6 ± 0.7	8 ± 0.2
		PELE	5 ± 0.3	7 ± 0.7	10 ± 0.2	12 ± 0.4
		MLE	9 ± 0.3	13 ± 0.6	15 ± 0.4	16 ± 0.1
3.	*Klebsiella pneumoniae*	CLE	3 ± 0.4	4 ± 0.5	6 ± 0.1	7 ± 0.6
		PELE	2 ± 0.9	5 ± 0.4	6 ± 0.8	7 ± 0.8
		MLE	4 ± 0.6	8 ± 0.2	12 ± 0.6	13 ± 0.5
4.	*Pseudomonas aeruginosa*	CLE	5 ± 0.4	5 ± 0.9	7 ± 0.4	8 ± 0.2
		PELE	4 ± 0.3	6 ± 0.6	7 ± 0.2	9 ± 0.1
		MLE	4 ± 0.5	6 ± 0.4	7 ± 0.9	9 ± 0.3
5.	*Proteus mirabilis*	CLE	4 ± 0.1	6 ± 0.3	6 ± 0.8	7 ± 0.5
		PELE	5 ± 0.7	7 ± 0.2	7 ± 0.6	8 ± 0.4
		MLE	7 ± 0.7	11 ± 0.5	13 ± 0.3	14 ± 0.8

CLE, Chloroform leaf extract; PELE, Petroleum Ether leaf extract; MLE, Methanol leaf extract. *Mean value of triplicate tests; Standard deviation ±0.953 mm

**TABLE II -** Phytochemical constituents of methanol extracts of *M. arvensis* Linn

S. no.	Phytochemicals tested	Results*
1.	Tannins	++
2.	Saponins	-
3.	Steroids	++
4.	Anthraquinone	-
5.	Terpenoids	++
6.	Flavonoids	+++

*-, negative / absent; ++, moderate presence; +++, abundant presence

Synthesis and characterization of Ag-NPs

Synthesis of Ag-NPs from the MLE was recognized by the change of color of reaction mixture into dark brown and development of dense precipitate at the bottom of container after the 35th hour of incubation. The UV-Vis spectroscopy enabled the detection of silver SPR bands at 430 nm. Formation of Ag-NPs was recorded through development of absorption peaks between the ranges 330 and 500 nm (Figure 1).
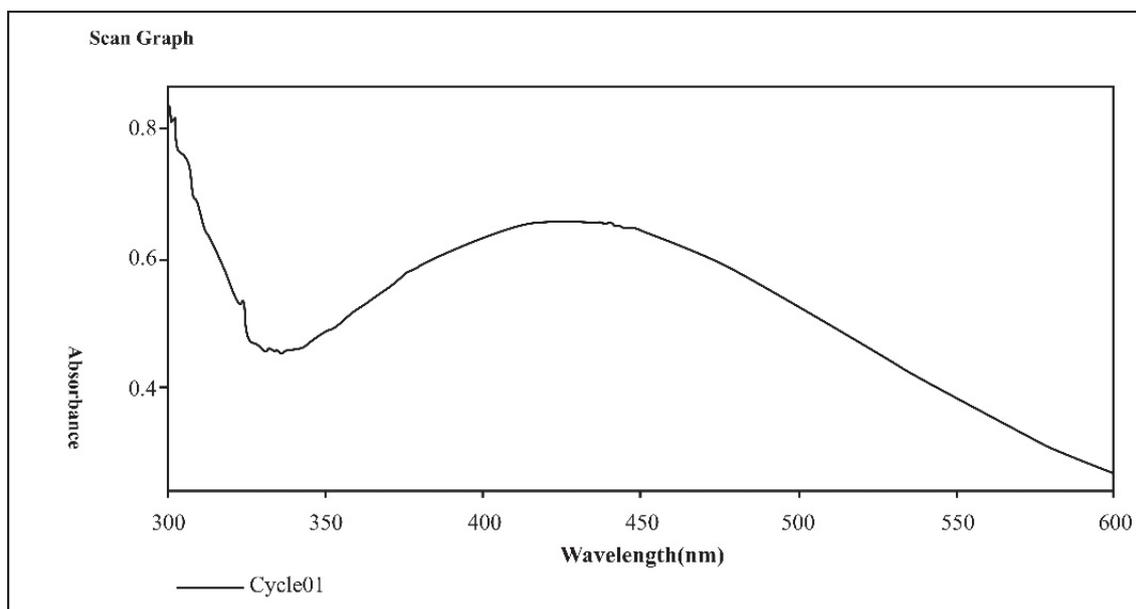

FIGURE 1 - UV-Visible Spectroscopy of *M. arvensis* Linn. mediated silver nanoparticles.

[Absorbance recorded between 300 and 600 nm; Appearance of SPR band at 430 nm]

SEM analysis indicated that the Ag-NPs were small, spherical to square shaped and found in agglomerated form (Figure 2). Analysis using dynamic light scattering indicated that these particles were of the sizes ranging from 40-70 nm. While the particles with the sizes of 56 and 61 nm were abundantly present, the mean size of the particles determined was 60 ± 23 nm endowed with the zeta potential values ranging between -18 and -22 mV. The EDX spectrum deciphered the signals corresponding to elemental silver (2.5-5.4%) and other associated compounds such as Carbon, Nitrogen, Oxygen, Sodium, Chlorine, Potassium, etc. producing signals with less volume (Figure 3).

The FTIR analysis of Ag-NPs revealed that the particles were potentially active encompassing functional groups ranging from 720 to 3413 cm^{-1} (Figure 4). Subsequent comparison with standard interferrogram showed the occurrence of functional groups such as O-H (Alcohols, Phenols), C-H (Alkenes), C-H (Phenyl ring), NO_2 (Nitro compound), N-H and C-N (Amines). While the broad peak at 3003 cm^{-1} corresponded to the N-H group of the peptide linkage occurring in the extract, there was a preponderance of alcohol and amine groups.
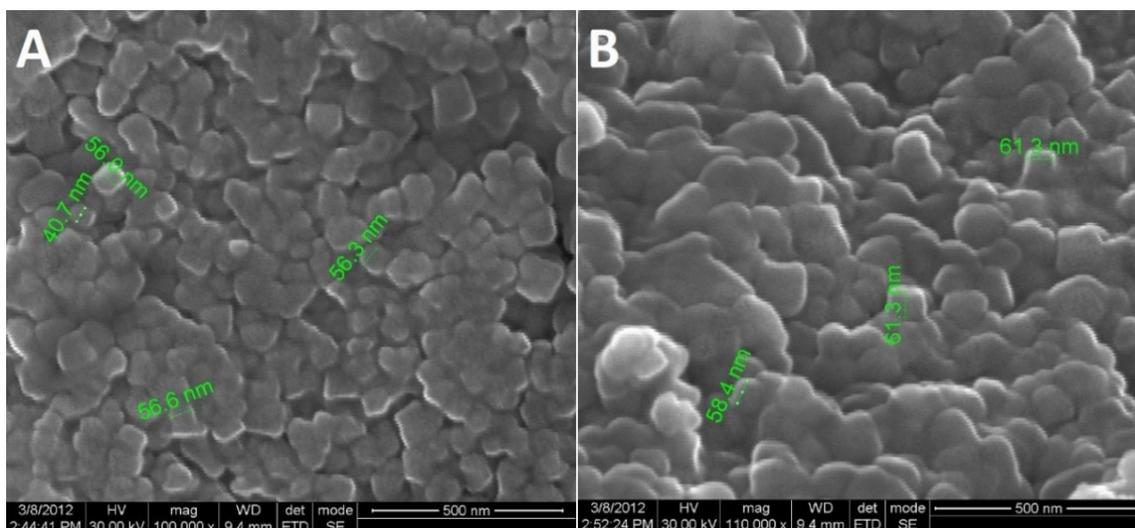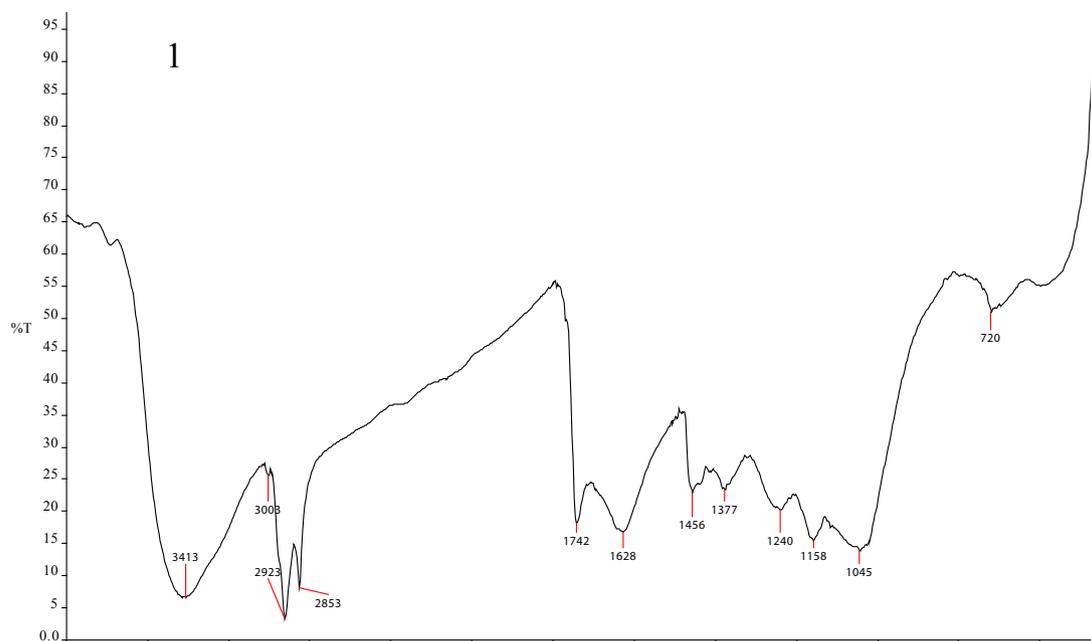
FIGURE 2 - Scanning Electron Micrograph of *M. arvensis* Linn. mediated silver nanoparticles. A, Spherical nanoparticles; B, cuboidal nanoparticles

[Particles with sizes ranging 40-70 nm]
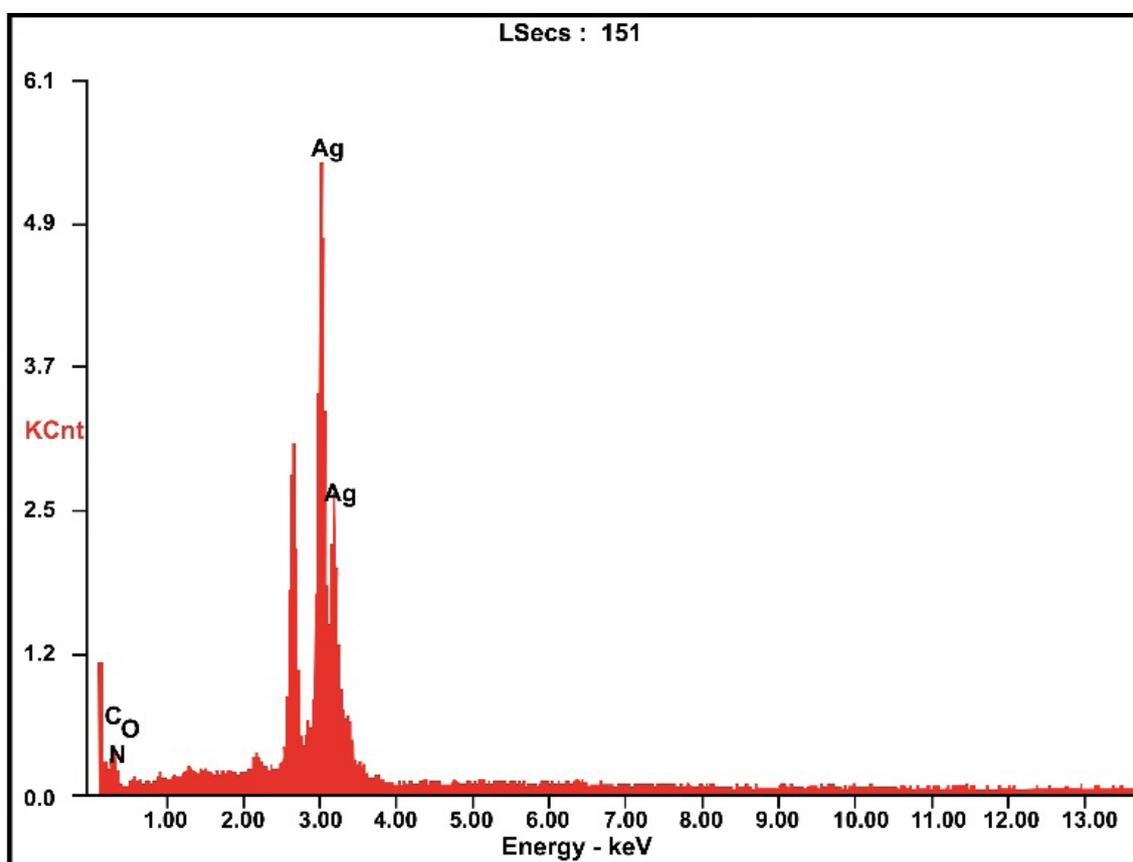
FIGURE 3 - EDAX spectrum of *M. arvensis* Linn. mediated silver nanoparticles.

[Spectrum showing peaks corresponding to C, O, N and Ag compounds]

FIGURE 4 - FTIR Spectrum of *M. arvensis* Linn. mediated silver nanoparticles.

[Spectrum showing peaks corresponding to different functional chemical groups of Ag-nps]
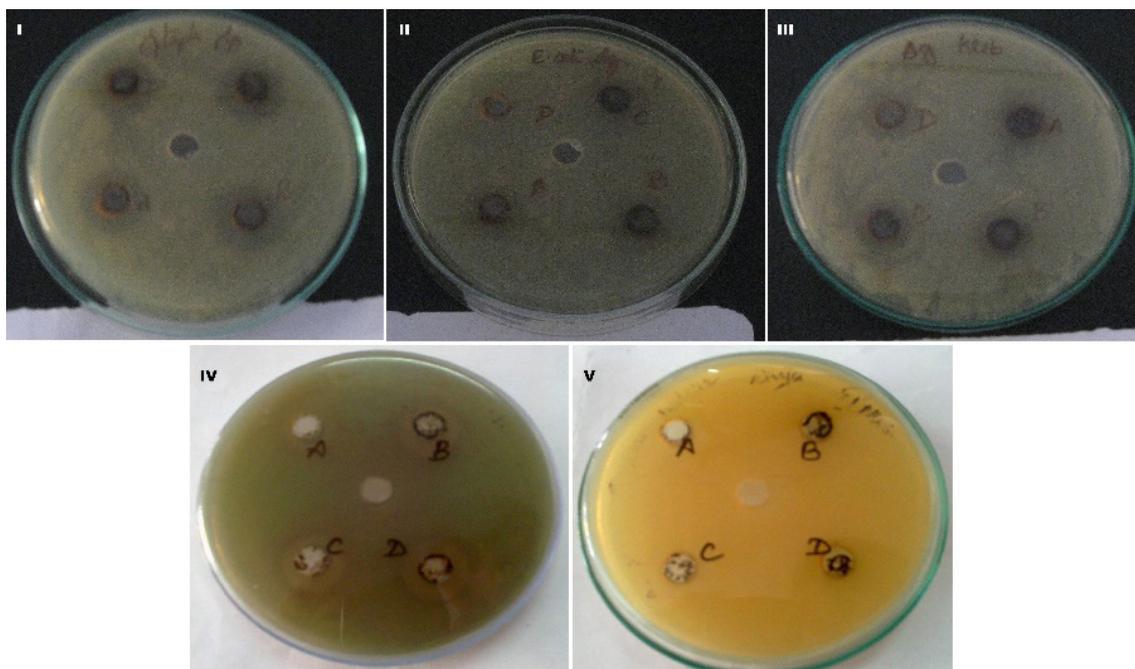
Antibacterial activity of Ag-NPs

The Ag-NPs of MLE of *M. arvensis* L. exhibited substantial antibacterial activity against all the five indicator organisms. Bacteria such as *E. coli*, *S. aureus*, *K. pneumonia*e and *P. aeruginosa* were inhibited even at the lowest concentration (250 µg/mL) of the drug. The diameters of zones of growth inhibition proportionate with the ascending concentrations of drug for the bacteria *E. coli* and *K. pneumoniae* were 12+0.6 to 19+0.8 and 14+0.1 to 22+0.7 respectively. A comparatively high MIC (500µg/mL) was required for the bacteria *P. mirabilis* (Table III; Figure 5) with zone diameters ranging 5+0.8 to 14+0.7. However, higher susceptibility (14+0.6 to 23+0.3) was observed with the gram positive bacteria *S. aureus*.

TABLE III - Results of assay of antibacterial activity of silver nanoparticles

S. no	Indicator bacteria	Antibacterial activity of Ag-nps (zone dia. in mm)*			
		250 µg/mL	500 µg/mL	750 µg/mL	1000 µg/mL
1.	*Staphylococcus aureus*	14 ± 0.6	18 ± 0.2	20 ± 0.9	23 ± 0.3
2.	*Escherichia coli*	12 ± 0.6	15 ± 0.6	16 ± 0.4	19 ± 0.8
3.	*Klebsiella pneumoniae*	14 ± 0.1	16 ± 0.5	19± 0.4	22 ± 0.7
4.	*Pseudomonas aeruginosa*	13 ± 0.3	17 ± 0.7	18± 0.6	20 ± 0.4
5.	*Proteus mirabilis*	5 ± 0.8	10 ± 0.4	13 ± 0.5	14 ± 0.7

*Mean value of triplicate tests; Standard deviation +0.953 mm

FIGURE 5 – MHA plates showing antibacterial activity of *M. arvensis* Linn. mediated silver nanoparticles against indicator bacteria.

I, *S. aureus*; II, *E. coli*; III, *K. pneumoniae*; IV, *P. aeruginosa*; V, *P. mirabilis*
Drug concentrations (µg/mL) A, 250; B, 500; C, 750; D, 1000

DISCUSSION

Screening of leaf extracts for antibacterial efficacy

For the purpose of preparing leaf extract of *M. arvensis* L. three solvents namely, chloroform, methanol, and petroleum ether were employed in the present study. Pramila *et al.* (2012), Singh, Shushni and Belkheir (2015) and Bupesh *et al.* (2007) in their individual studies with another plant *Mentha piperita* used other solvents such as ethyl acetate, butanol, ethanol petroleum ether, chloroform and so on for extraction of leaf contents. However, the methanolic extract has been advocated as a superior solvent especially in studies pertaining to antimicrobial assays. This is in agreement with the findings of the present study as the methanolic leaf extract (MLE) of *M. arvensis* L. showed comparatively better activity by inhibiting 80% of indicator organisms (Table I) including gram positive and gram negative bacteria except *P. aeruginosa*. Concomitant findings were obtained by Sugandhi and Bai (2011), Farah, Zabta and Imran (2012) and Saxena, Patil and Khan (2011), who independently demonstrated the antibacterial activity of MLE of *M. arvensis* L. against both gram positive and gram negative bacteria.

A comparative study of antibacterial activities of *M. arvensis* revealed that the MLE caused more lethality on gram positive bacteria compared to gram negative bacteria. While this extract required an MIC of 500 µg/mL for most of the gram negative bacteria, it inhibited the gram positive bacteria *S. aureus* even at a lower concentration (250 µg/mL). The relative resistance of gram negative bacteria to the antibacterial activity of MLE could be due to the presence of lipopolysaccharides on the cell wall (Singh, Shushni, Belkheir, 2015). Interestingly, the MLE of our study showed superior inhibitory activity with a zone diameter of 18 ± 0.1 and MIC of 250 µg/mL (Table I) than that of Farah, Zabta and Imran (2012) (15.66 ± 0.351; 2.5 mg/mL). The differences in antibacterial activities between these two studies may be attributed to the environmental and soil conditions of the plant's habitat, cultivar and age of the plant, method and extraction of constituents of the plant material.

Phytochemistry of methanol leaf extract

Phytochemical analysis of the MLE prepared for the present study revealed that it possessed constituents such as tannins, steroids, terpenoids and flavonoids (Table II). Similar report has been made from the studies of Sugandhi and Bai (2011) and Do Nascimento *et al.*, (2009). It is noteworthy that the biological and therapeutic properties of any plant are influenced by its phytochemical components. Possession of these compounds in the leaves of *M. arvensis* L. could be attributed to its various pharmacological properties. Pramila *et al.* (2012) proposed that the antibacterial and antifungal activity of methanolic leaf extract of *M. piperita* is attributable to the presence of tannins and flavanoids. Besides, the terpenoids, owing to their inherent affinity towards the cell membrane, could cause the bacteriolysis through lipolytic activity (Snoussi *et al.*, 2015). Menthol, principal component of mentha plants, is added now-a-days in many commercial tooth pastes to offer protection against oral microbial infections (Biswas, Saha, Ali, 2014).

Green synthesis of silver nanoparticles

In recent years, researchers in the field of nanotechnology are showing exhilarated interest in alternative methods of synthesis of metal nanoparticles (m-NPs) using natural plants as an eco-friendly approach. As the plant components possess non-toxic phytochemicals, the green synthesis of m-NPs intends to employ them as sources of reducing and stabilizing agents (Sosnowska *et al.*, 2017; Ali *et al.*, 2015). As the plants belonging to the genus Mentha have been reported to contain various phytoconstituents, the present study chose leaf extract of *M. arvensis* as a natural reducing agent for synthesizing Ag-NPs. MubarakAli *et al.* (2011) in their studies on the synthesis of silver and gold NPs using the leaf extract of *M. piperita* suggested that phytochemicals such as steroids, flavones, alkaloids, polysaccharides, oximes amino acids, etc. contribute significantly in the reduction of Au+ into Au0. Particularly, the menthol, a secondary alcohol present abundantly in these plants, could play a pivotal role in reducing $AgNO_3$ in the process of production of Ag-NPs. During the reaction with Ag^+, the menthol gets converted into menthone with the release of Ag0 (Sosnowska *et al.*, 2017; Kamatou *et al.*, 2013).

Physico-chemical characterization of Ag-NPs

The UV-Vis spectrophotometry confirmed the presence of silver nanoparticles in the MLE of *M. arvensis L.* through recording the change in color of the reaction mixture followed by raise in OD value (> 0.7). The absorption spectra depicted the appearance of surface plasmon band at 430 nm, which is the characteristic of Ag-NPs (Figure 1). Similar result has been obtained by Tambe and Tumane (2017), who recorded characteristic absorption band for Ag-NPs at 439 nm. As the SPR is dependent on the morphology and size of nanoparticles (Saikia *et al.*, 2015), red shifting of its band indicated the formation of larger Ag-NPs during the reduction reaction caused by the phytochemicals of the extract. As the phenolics present in the extract are considered to possess strong antioxidant property (Hembram *et al.*, 2018; Abdel-Aziz *et al.*, 2014), by way of donating electrons they could have caused the reduction of Ag+ ions to Ag-NPs.

Occurrence in the medium of dense, aggregated Ag-NPs with a mean size of 60 ± 23 nm was recorded using SEM (Figure 2). Some independent studies which synthesized the Ag-NPs from the leaves of another plant of the genus, *M. piperita* reported that the particles were spherical and of varying sizes viz., 5-50 nm (Sosnowska *et al.*, 2017), 12 nm (Manjari *et al.*, 2018), 15-65 nm (Parashar *et al.*, 2009) and 90 nm (MubarakAli *et al.*, 2011) respectively. In contrast to these reports, Ag-NPs of much smaller sizes viz., 1.9-4.3 nm and 5-30 nm have been synthesized respectively from the leaves of *Eucalyptus globus* (Ali *et al.*, 2015) and *M. piperita* (Reddy *et* al., 2015) in other studies. Occurrence of relatively larger particles may be attributed to the influence of lower pH and temperature which tend to cause lesser nucleation during the reduction reaction (Ali *et al.*, 2015). The EDX spectrum showing high peaks of Ag ions in the synthesis medium (Figure 3) further confirmed the efficacy of MLE in the

reduction of AgNO$_3$. Manifestation of large numbers of feeble signals revealed the possibility of adherence of other chemical elements on the surface of Ag-NPs. Occurrences in the plant extract of phytochemicals could contribute to the stability of nanoparticles thereby preventing their aggregation (Ali *et al.*, 2015). The existence of discrete Ag-NPs, as witnessed in the SEM of our study, could be due to the capping effect and steric hindrance caused by the compounds such as tannins, terpenoids, flavonoids, carbohydrates, proteins and other metabolites in MLE.

The FTIR analysis revealed the chemical complexity of Ag-NPs owing to the possession of various functional groups (Figure 4). The stability of Ag-NPs in reaction milieu depends on its stereochemistry which in turn rests on presence of chemical groups. The phytochemicals in green synthesis could act as reducing agents and contribute to the steric conformation of Ag-NPs. Tambe and Tumane (2017) have suggested that while O-H groups are offered by the flavonoids, C-H, N and C-O groups are donated by alkaloids, amines and alcohols of the plant extract respectively.

Evaluation of antibacterial activities of Ag-NPs

The main focus of this study, i.e., evaluation of antibacterial activity of green synthesized Ag-NPs revealed that their activities were much superior than that of whole leaf extract. While the whole leaf extract inhibited the growth of four out of five indicator bacteria (80%), the Ag-NPs exhibited toxicity against all of them even at lower concentrations (100%). This finding inferred that the utilization of Ag-NPs in antimicrobial therapy has substantial advantage over the use of whole or purified herbal extract. This is in agreement with the reports of Ali *et al.* (2015), who demonstrated that while the mixture of ethanolic extract of *E. globulus* leaves and Ag-NPs inhibited the bacterial growth at a concentration of 25 µL (zone dia. 19-21 mm), the lone extract could cause only insignificant cytotoxic effects (zone dia. 8-10 mm) even at a concentration of 100 µL.

Studies conducted in recent years suggest that efficacy of Ag-NPs in exhibiting antibacterial activity is significantly influenced by the plant extract utilized for their synthesis

(Sosnowska *et al.*, 2017; Saikia *et al.*, 2015). Gabriela *et al.* (2017) in their studies on *M. piperita* demonstrated the antibacterial activity of leaf extract against *S. aureus* and *E. coli* with the respective MIC values of 51.5 and 2.49 µg/mL, which are much lesser than that of our study. While the better activity of Ag-NPs in their study could be attributed to smaller size of particles (mean, 50 nm), the reduced performance of Ag-NPs in our study may be due to their comparatively larger (mean, 60 ± 23 nm) size. Smaller the size of Ag-NPs, the easier would be the transportation of particles across the bacterial cell wall, thereby manifesting better lethal effects inside the cell.

The overall analysis of antibacterial activity of Ag-NPs in our study revealed that they are equally toxic to both gram positive and gram negative bacteria. However, in terms of producing sizes of inhibitory zone, a sort of higher lethality was observed with gram positive bacteria than its counterpart. It can be noted from table III that the diameters of growth inhibition caused by Ag-NPs against the gram positive bacteria *S. aureus* vs. across the gram negative bacteria (maximum zone for each dilution) were 14 ± 0.6 vs. 14 ± 0.1, 18 ± 0.2 vs. 17 ± 0.7, 20 ± 0.9 vs. 19± 0.4 and 23 ± 0.3 vs. 22 ± 0.7 at concentrations respectively for 250, 500, 750 and 1000 µg/mL (Table III). Higher susceptibility of gram positive bacteria could be due to the presence of only the peptidoglycan layer which is not a strong barrier to the permeability of Ag-NPs in their cell wall. The cell wall of gram negative bacteria has more complex structure by virtue of a rigid phospholipid membrane along with lipopolysaccharide compounds, which makes it less susceptible to antibacterial agents (Farah, Zabta, Imran, 2012).

Although the exact mechanism of antibacterial action of Ag-NPs is not understood yet, some studies have proposed possible ways by which they cause lethality. The silver present in the forms of Ag0 and Ag+ interact negatively with the cytoplasmic contents and DNA thus preventing the replication and division of bacterial cell (SivaKumar *et al.*, 2015). Studies have established that the bacteria are incompetent in developing resistance against silver as they do with antibiotics. So, nanoparticles made out of this metal could be best employed for controlling antibiotic resistant bacteria. Contemporary studies have demonstrated the efficacy of Ag-NPs against drug

resisting bacteria such as MRSA (Methicillin-resistant *S. aureus*) (Chung *et al.*, 2016), ESBL (extended spectrum beta lactamase) producing *P. aeruginosa* and *E. coli* [18] (Ali *et al.*, 2015) and multidrug resisting (MDR) *Salmonella enterica* (Das *et al.*, 2017).

In recent years, there has been increasing demand for ecofriendly and cost effective synthesis of metal nanoparticles. Our study suggests that proper standardization of synthesis of silver nanoparticles using leaf extract of *M. arvensis* L. could help in developing a promising drug to overcome the infections caused by antibiotic resistant bacteria.

CONCLUSION

Mentha arvensis L. plant, as a whole, is regarded as a source of multiple nutraceutical and pharmaceutical chemicals which offer numerous health benefits. The methanolic extract of leaves of this plant is

comparatively a better source for exploring its antimicrobial properties than other extracts. The phytochemical constituents of leaves, especially menthol, tannins, flavonoids, terpenoids and steroids could act as natural reductants in the synthesis of silver nanoparticles. Besides, these compounds contribute to the stability and stearic conformation of synthesized nanoparticles by way of donating essential chemical groups. Antibacterial activity of nanoparticles is plant extract dependent and is significantly influenced by its size. Smaller silver nanoparticles exhibit better antibacterial activity as they can be easily transported across the cell membrane. Owing to its higher permeability characteristic, the gram bacteria are more susceptible to silver nanoparticles than gram negative bacteria. Since plant based natural compounds serve as non-toxic reducing and stabilizing agents, green synthesis of silver nanoparticles could be considered as a safe and most advantageous advantageous method.

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