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Green Synthesis of Silver Nanoparticles using *Mentha asiatica* (Mint) Extract and Evaluation of their Antimicrobial Potential

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Abstract

The aim of this study was green synthesis of silver nanoparticles by using mint plant leaves extracts and to evaluate their antimicrobial activity. The fresh suspension of plant extracts was yellowish-green in colour. However, after addition of $AgNO_3$ within 20mins, the suspension showed change in colour and turned dark brown after 5 hrs of incubation at room temperature. Formation of silver nanoparticles was confirmed using UV-Vis spectral analysis and showed silver surface plasmon resonance band in the range of 200-600 nm. It is a well-known fact, that silver ions and nanoparticles are highly toxic and hazardous to microorganisms. It is found out that the silver nanoparticles have many inhibitory and bactericidal effects and so its application is extended as an antibacterial agent. The antibacterial activity of silver nanoparticles is estimated by the zone of inhibition. The silver nanoparticles biosynthesized by mint plant leaves extracts showed antibacterial activity against microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Baccillus subtilis* (Gram Negative) and *Staphylococcus aureus* (Gram Positive)**.** However, the antimicrobial effect varied with the variation in salt concentration. Additionally, the silver nanoparticles by mint plant leaves extracts showed good inhibition activity towards *Escherichia coli* and *Pseudomonas aeruginosa*. The use of silver nanoparticles in drug delivery systems might be the future thrust in the field of medicine.

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Introduction

Nanoparticles are those particles which have less than 100 nm in diameter and exhibits as new or which accelerate size dependent properties if compared with larger particles of the same material. Nanoparticles present widely in the nature for so many of year because of productive form of photochemical and volcanic activity, created by plants and algae, or as the product of combustion and food cooking. Recently it has found from vehicle exhausts. Nanoparticles are of interest because of their two main properties such as chemical reactivity and optical behavior. There are many ecofriendly protocols available for the synthesis of silver nanoparticles such as, reduction in solutions (Ahmed et al., 2015), chemical and photochemical reactions in reverse micelles (Bhakya et al., 2015), thermal decomposition of silver compounds (Chandran et al., 2006), radiation assisted (Deng et al., 2010), electrochemical (Dheeban Shankar et al., 2015), sonochemical, microwave assisted process (Ahmed et al., 2015; Deng et al., 2010) and recently from the green chemistry too (Ganaie et al., 2014).

Our environment possesses varieties of plants and microorganisms. Plant leaf extract, bacteria fungi and enzymes for the synthesis of silver nanoparticles are majorly used and they offer numerous benefits such as eco-friendliness and lots of compatibility for pharmaceutical and other biomedical applications as not been used any toxic chemicals for the synthesis of nanomaterials (Kanipandian et al., 2014). Nanocrystalline silver particles are found too much of applications in the field of detection of biomolecules and diagnostics, antimicrobials and therapeutics catalysis and micro-electronics.

In present study, a cost effective and eco-friendly technique for green synthesis of antibacterial silver nanoparticles from $AgNO_3$ solution with the aqueous leaf extracts of *Mentha asiatica* (mint) is reported. The experimental parameter that is the concentration of salt solution was varied, for optimizing the synthesis of the nanoparticles. Further these biologically synthesized nanoparticles were evaluated for their enhanced antimicrobial activities against pathogenic strains of *Escherichia coli*, S*taphylococcus aureus, Pseudomonas* and *Bacillus subtilis*.

Materials and methods

Preparation of plant leaf extracts

Twenty gram of fresh leaves of mint were taken and washed with distilled water separately. Leaves were cut into fine pieces and crushed with 100 ml sterile distilled water using motor and pastel. Contents were boiled with constant stirring for ten minutes. After cooling contents were filtered with Whatmann No.1 filter paper (pore size 25 µm). Dark yellow coloured extracts were obtained, which were used as reducing agent and stabilizer (Krishnaraj et al., 2010).

Synthesis of silver nanoparticles

Ten ml of mint leaves extract was added into 90 ml of aqueous solution of silver nitrate for reduction into Ag+ ions separately and kept at room temperature for 5 hrs. Different concentrations of silver nitrate were used to standardize the optimum concentration of silver nitrate for synthesis of silver nanoparticles. The concentrations ranged from 1mM, 2mM, 3mM and 4mM of silver nitrate. The colloidal suspension thus obtained was centrifuged at 3000 rpm for 20 min and the pellet obtained after discarding the supernatant was re-dispersed in sterile distilled water. The centrifugation process was repeated 2 to 3 times for the removal of any adsorbed substances on the surface of silver nanoparticles (AgNps). The synthesized nanoparticles were then used for the evaluation of their antimicrobial activity on selected human pathogens (Ahmed et al., 2015).

Characterization of the synthesized silver nanoparticles

The reduction of metallic Ag+ ions by plant extracts was monitored by measuring the UV- Vis spectrum after about 16 hours of reaction. A small aliquot was drawn from the reaction mixtures and diluted with sterile distilled water (1:5). Spectrum was then taken on a wavelength from 200nm to 800nm on UV-Vis spectrophotometer (Systronics Double beam spectrophotometer 2202) (Kumar et al., 2011).

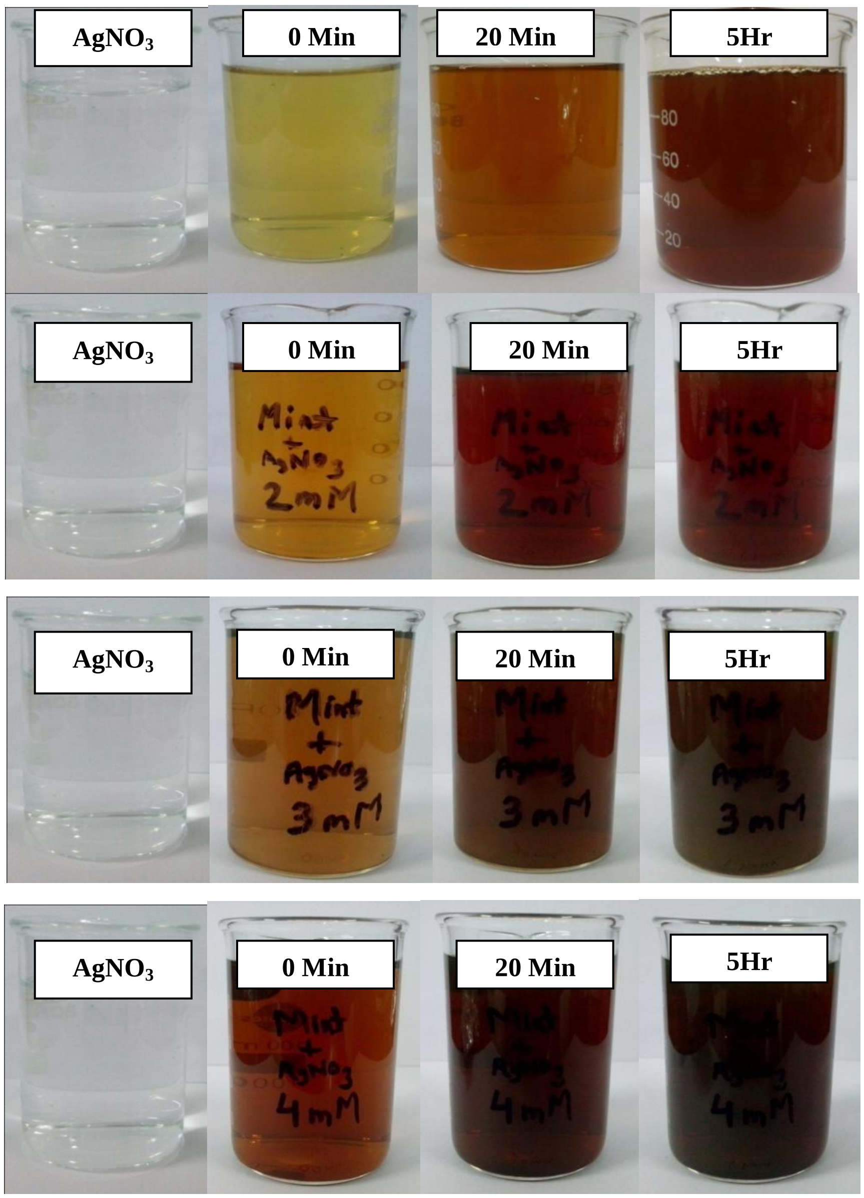
Antibacterial assay

The antibacterial activity of Mint aqueous extracts and the AgNPs developed at different AgNO3 concentrations (1, 2, 3, and 4mM) were evaluated against four types of bacteria. The antibacterial assessment was performed using agar well diffusion method against different pathogenic microorganisms *Escherichia coli*, *Pseudomonas aeruginosa, Baccillus subtilis* (Gram Negative) and *Staphylococcus aureus* (Gram Positive) and measuring the inhibition zones (mm).The pure cultures of bacteria were subcultured on Mueller-Hinton agar (MHA). Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 5 mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, 25 µL of nanoparticle solution was poured onto each well on all plates. After incubation at 37°C for 24 hrs, the diameter of zone of inhibition was measured in centimeter (Kumar et al., 2011; Lalitha et al., 2013).

Results

Mentha asiatica was used to make the aqueous extract. Leaves weighing 20g were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100 ml sterile distilled water and filtered through Whatmann No.1 filter paper (pore size 25 µm).

Fig. 1: Preparation of aqueous leaf extracts of *Mentha asiatica*.



a)

b)

c)

Fig. 2: Photograph showing color change of the Mint extract upon the formation of AgNPs from different concentrations of AgNO3: (a) 1 mM, (b) 2 mM, (c) 3 mM, and (d) 4 mM.

Synthesis of silver nanoparticles

The formation of silver nanoparticles is indicated by the change in colour of solution. This colour change was observed within 20min from addition of extract in the solution indicating the formation of silver nanoparticles. The colour of the blank (pure $AgNO_3$) remains practically unchanged during the entire period of incubation which can be easily seen from the photographs depicted in Fig. 2.

Characterization of the synthesized silver nanoparticles (AgNps)

The optical properties of AgNps were calculated by UV-Vis absorption spectroscopy, an important and most commonly used technique, to ascertain the formation stability of metal nanoparticles. Due to surface plasmon resonance (SPR), a strong absorption of electromagnetic waves is exhibited by metal nanoparticles in the visible range.

The SPR phenomenon arises when nanoparticles are irradiated with visible light, because of the collective oscillations of the conduction electrons. It is well known that AgNps exhibit a yellowish-brown color in aqueous solution due to the excitation in UV-visible spectrum depending upon the particle size (Lalitha et al., 2012 and Namratha et al., 2013).

The presence of nanoparticles was confirmed by obtaining a spectrum in visible range of 200nm to 600nm. The optimum concentration for synthesis of nanoparticles was standardized using different concentrations of silver nitrate (1, 2, 3, and 4mM). The optimum concentration suitable for nanoparticles synthesis by *Mentha asiatica* leaf extract was found to be 4mM (Fig. 2).

Antimicrobial assay

Biosynthesized silver nanoparticles by this method were studied for antimicrobial activity against pathogenic bacteria by well diffusion method; it was observed that silver nanoparticles have antibacterial activities at concentration of 25µl/well.

$AgNO_3$ was used as a control. The silver nanoparticles biosynthesized from plant extracts showed inhibition zone against microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Baccillus subtilis* (Gram negative) and *Staphylococcus aureus* (Gram positive) (Fig. 4). Maximum zone of inhibition (MZI) are listed in Table 1. From the table, it is evident that the nanoparticles synthesized are good candidates for their usage as antibacterial agent.

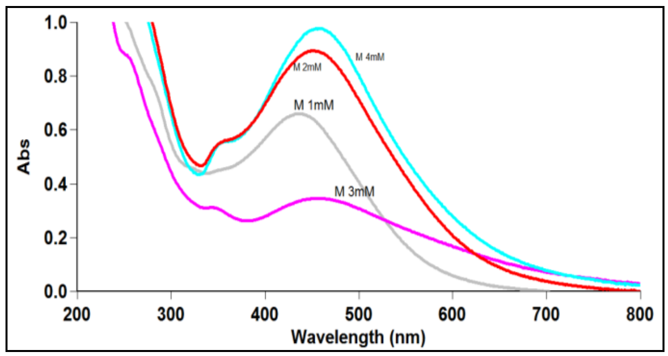
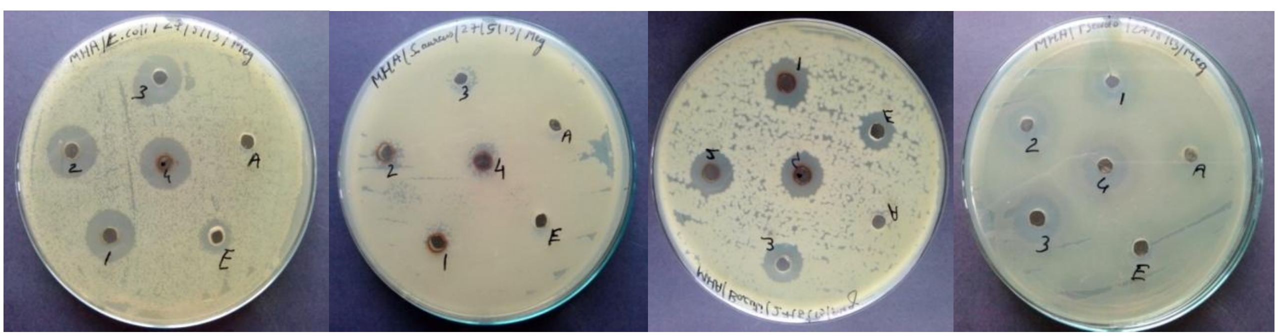


Fig. 3: UV-Vis spectra of the formed AgNPs by Mint extract recorded as a function of $AgNO_3$ concentration.



E. coli ***S. aureus*** ***B. subtilis*** ***P. aeruginosa***

Fig. 4: Antimicrobial activity of *Mentha asiatica* leaf extract formed AgNPs against selected pathogens: A-$AgNO_3$;E-Mint Extract; 1-1mM AgNP's; 2-2mM AgNP's; 3-3mM AgNP's and 4-4mM AgNP's.

Table 1. Maxmium zone of inhibition (MZI) by AgNPs formed by Mint leaves extract on selected pathogens.

Organism	$AgNO_3$ (cm)	Extract (cm)	AgNP's from different concentrations of $AgNO_3$ (cm)			
			1mM	2mM	3mM	4mM
Escherichia coli	-	0.8	1.4	1.4	1.4	1.5
Staphylococcus aureus	-	0.4	0.5	0.8	0.8	1
Bacillus subtilis	-	0.9	1.2	1.3	1.3	1.3
Pseudomonas aeruginosa	-	0.6	1	1.1	1.3	1.3

Discussion

Reduction of silver ion into silver nanoparticles when added to the plant extracts leads to color change. In the present study, silver nanoparticles exhibited dark brown color in aqueous solution. Excitation of the surface plasmon resonance in the metal nanoparticles might be the reason of characteristic color variations (Parashar et al., 2009; Parial et al., 2013). The frequency and width of the surface plasmon absorption depends on certain parameters such as the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium (Philip and Unni, 2011; Rajesh et al., 2009). It is generally recognized that UV–VIS spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions (Ramalingam et al., 2014). In this study the optimum concentration for synthesis of nanoparticles was standardized using different concentrations of silver nitrate (1, 2, 3, and 4mM). The presence of nanoparticles was confirmed by obtaining a spectrum in visible range of 200nm to 600nm. The optimum concentration suitable for nanoparticles synthesis by Mint extract was found to be 4mM (Fig. 2).

It is a reported known fact, that silver ions and nanoparticles are highly toxic and hazardous to microorganisms. It is proved that the silver nanoparticles have many inhibitory and bactericidal effects and so its application is exhibited as an antibacterial agent (Sable et al., 2012). In our study, the application of silver nanoparticles as an antimicrobial agent was investigated and exhibited better antimicrobial activity against some selected human pathogens. The antibacterial activity of silver nanoparticles is estimated by the zone of inhibition (Shivakumar et al., 2014). However, the antimicrobial effect was varied with the variation in salt concentration. Additionally, the silver nanoparticles by Mint plant extracts showed good inhibition activity towards *Escherichia coli* and *Pseudomonas aeruginosa.* Some researchers reported that the silver nanoparticles have relatively higher anti-bacterial activity against gram negative bacteria than gram positive bacteria, this is may be due to the thin peptidoglycan layer in cell wall and presence of beta barrel proteins called porins (Shukla and Vankar, 2012).

The mechanism of inhibitory action of silver nanoparticles on microorganisms, still not very clearly understood. Several possibilities could benanoparticle adhesion to the cell membrane and further penetration inside or by their interaction with phosphorus containing compounds like DNA and hampering the normal replication process, loss of cell viability and eventually resulting in cell death. It is also preferable fornanoparticlesto attack on the respiratory chain (Vanmathi Selvi and Sivakumar, 2012; Dheeban Shankar et al., 2015). It has also been suggested that a strong reaction takes place between the silver ions and thiol groups of vital enzymes ultimately inactivate them.

Nanoparticles have showed significance role in the field of biomedicine recently, due to its significant and distinguishable property of larger surface area to volume ratio (Parial et al., 2013; Lalitha et al., 2012). Smaller nanoparticles with a larger surface area to volume ratio demonstrated a more effective antibacterial activity even at a very lower concentration (Parial et al., 2012; Philip and Unni, 2011; Rajesh, 2009). Surface area contributes to the various properties such as the catalytic reactivity, antimicrobial activity etc. It is reported that when surface area of the nanoparticles gets increased, the energy remain in the surface will get increased and hence their biological effectiveness will also enhance.

Conclusion

In conclusion, the rapid biological synthesis of silver nanoparticles using *Mentha asiatica* (Mint) leaves extracts provides a stable, environmental friendly, simple and efficient route. For drug delivery systems in the field of medicine, in near future, the use of silver nanoparticles might be the thrust area.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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