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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *OCIMUM SANCTUM* L. AND *OCIMUM AMERICANUM* L. FOR THEIR ANTIBACTERIAL POTENTIAL

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ABSTRACT

Development of green nanotechnology is generating interest of researchers toward eco-friendly biosynthesis of nanoparticles. In this study, biosynthesis of stable silver nanoparticles was done using Tulsi (*Ocimum sanctum* L. and *Ocimum americanum* L.) leaf extract. These biosynthesized nanoparticles were characterized by different techniques including UV–Vis spectrophotometer, Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM). The surface plasmon resonance (SPR) absorption band obtained at 408 in *Ocimum americanum* L. and 427 nm in *Ocimum sanctum* L. reveals the reduction of silver metal ions into silver nanoparticles. FT-IR analysis was carried out to probe the possible functional group involved in the synthesis of AgNPs. SEM results indicated that AgNPs were predominantly spherical in shape with a particle size of 40-95 nm. Further biosynthesized AgNPs were evaluated for antibacterial activity by disc diffusion method against Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria. The leaf extract of *O. sanctum* L. showed height antibacterial activity (6.56±0.15) against*Escherichia coli*. This recent research study opens an innovative design to progress our understanding of how SNPs behave can be optimized to improve human antimicrobial activities.

KEYWORDS: ***Silver nanoparticles, FTIR, SEM, Antibacterial activity.***

INTRODUCTION

Nanotechnology is one of the most emerging fields of research in recent material science. They exhibit entirely enhanced properties based on specific features such as size, distribution and morphology. Nano Particles (NPs) of noble metals such as silver and gold exhibit drastically different physical, chemical and biological properties and are currently attracting increasing attention for broad range of applications in various industries. Silver nanoparticles (AgNPs) have been extensively used as antimicrobial agents, in high sensitivity biomolecular detection and diagnostics[1], therapeutics[2,3], micro-electronics[4] and in textile industry. They are also used in biomedicine, agriculture, forestry, environmental management, photo chemicals and food chemistry[5-9].Metal NPs can be prepared by different approaches including chemical, physical, and biological. Physical and chemical methods are extensively used in the synthesis of nanomaterials in huge amount in short duration of time with specific size and shape. But their main drawback is that they are expensive and have some toxicity which may pose potential environmental and biological threats[10].In these days, biological methods for the synthesis of AgNPs provide a feasible alternative, since they are eco-friendly, less expensive and can be carried out in a single step[11]. Green synthesis of NPs involves the reduction and stabilization of NPs by various biological materials such as microorganisms, whole plants, plant tissues and fruits, their extracts, marine algae and micro-fluids. Synthesis of NPs using bioactive products has several advantages over other methods, due to their broad range, least toxicity and easy availability[12].*Ocimumsanctum* L. and *Ocimum americanum* L. are an important medicinal plants, belonging to family Lamiaceae[13] a well known plants of Indian medicinal system, is gaining more attention for electing a wide spectrum of pharmacological activities. These leaves contain volatile oil eugenol, euginal and urosolic acid, carvacrol, linalool, limatrol, caryophyllene and estragol, methyl cinnamate, D-camphor, citral, ocimin, methylchavicol, linalook, nevadensin, beta-

sitoseterol, betulinic, ursolic, oleanolic acids, falvanoids and nevadensin[14]. They have been recommended for the treatment of several ailments[15] including skin diseases, chronic fever, bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, insect bite, arthritis, painful eye diseases, antifertility, anticancer, antifungal antidiabetic, antimicrobial, cardioprotective, antispasmodic, hepatoprotective, antiemetic, analgesic, adaptogenic and diaphoretic actions[16-21].Recent interest in *Ocimum* has resulted from its inhibitory activity against HIV-1 reverse transcriptase and platelet aggregation induced by collagen and ADP22 (adenosine 5-diphosphate).[22]Due to significant therapeutic ventures of medicinal plants the present study was designed for green synthesis of AgNPs as an alternative to conventional methods. Therefore leaf extracts of two species of *Ocimum*were used for bioconversion of silver ions to NPs. This plant is commonly available in India and each part of this plant has been used as a conventional source of natural drug against various human ailments against viral, bacterial and fungal infections. AgNPs can be produced at low concentration of leaf extract without using any additional harmful chemical methods. The method applied here is simple, cost effective, easy to perform and sustainable.

MATERIALS AND METHODS

Preparation of the extracts

Fresh and healthy leaves of two medicinal plants, *Ocimum sanctum* L. and *Ocimumamericanum* L. were collected from Rajasthan Agriculture Research Station, Durgapura, Jaipur. The leaves were thoroughly rinsed with tap water followed by distilled water to remove dust particles and were further dried. About 15 g of leaves were ground into fine powder and transferred to two beakers, each containing 100 ml distilled water. The broth was boiled for 15 min. The extracts were cooled to room temperature and filtered through Whatman filter paper No. 1 to remove particulate matter. The filtrate obtained was then stored in refrigerator at 4ºC.

Green synthesis of Silver nanoparticle (AgNPs)

Aqueous solution (0.01 M) of silver nitrate (AgNO3) was prepared in 250 mL Erlenmeyer flasks and extract was added for reduction into Ag+ ions. The composite mixture was then kept on hotplate for a period of 20 min at 60°C for complete bioreduction of silver nitrate. The change in colour from yellow to dark brown indicated the formation of AgNPs[23]. The reactions were carried out in darkness to avoid photoactivation of AgNO3. The dilute colloidal solution was then cooled to room temperature and was stored at 4°C for future use.

Characterization of AgNPs

UV-vis spectra analysis

The reduction of Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting the sample into double distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (SUV-S2100) at room temperature operated at a resolution of 1 nm between 200 and 700 nm ranges.

FTIR analysis

This technique gives information on the various biomolecules present in plant extract which are responsible for capping and reducing AgNPs which is validated by their functional groups and hence is an important technique for identification and characterization of a substance. FTIR analysis of the dried AgNPs was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and spectrum was recorded using the JASCO FTIR 680 plus (Japan).

SEM analysis

SEM analysis was done using cm30-Philphs SEM to obtain images of NPs. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film was allowed to dry by putting it under a mercury lamp for 5 min. The details regarding applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

Evaluation of Antibacterial activity

The antibacterial activities of AgNPs synthesized from leaf extracts were assessed against Gram (-)ve bacteria *Escherichia coli* (MTCC 443), *Pseudomonasaeruginosa* (MTCC 779) and *Klebsiella pneumoniae* (MTCC 530) by standard disc diffusion method[24]. Biosynthesized AgNPs were dispersed in autoclaved water in various concentrations (25, 50, 75 and 100 µg/mL). Stock culture of *E. coli*, *Pseudomonasaeruginosa* and *Klebsiella pneumonia* were grown separately in liquid nutrient broth medium for 24 h. Fresh bacterial cultures were spread on to Mueller-Hinton (MH) agar plates to cultivate bacteria. Sterile paper discs of 5mm diameter soaked in

double distilled water (negative control), soaked in solution containing AgNPs and antibiotic (Ampicillin) as positive control were placed in agar plate incubated at 37°C for 24 to 48 h. The maximum zone of inhibition was measured against each type of test bacteria.

STATISTICAL ANALYSIS

Analytical determinations were made in triplicate. Statistical analyses were carried out by using SPSS statistics 22 software. Statistical significance of the data was analyzed by one-way analysis of variance (ANOVA) at the 5 % level of significance ($P \leq 0.05$). All experimental data are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

UV-Vis spectral analysis

Ocimum sanctum L. and *Ocimum americanum* L. leaf extract were employed for eco friendly green synthesis of AgNPs. After the addition of the plant leaf extracts to aqueous silver nitrate solution, the colour of the solution changed from light yellow to reddish brown and finally to colloidal brown indicating reduction of silver thus leading to NP synthesis (Fig.1). AgNPs appear yellowish brown in colour in aqueous medium as a result of surface plasmon vibrations[25]. Similar changes in colour have also been observed in various researches[26, 27].The UV-Vis spectra recorded at different times of reactions is shown in Fig 2. AgNPs exhibited maximum absorption peak at 427 nm and 408 nm in *O. sanctum* L. and *O. americanum* L. respectively due to surface Plasmon resonance of AgNPs. The flat curves of the graph indicated the formation of polydisperse large NPs due to slow reduction rates[28].

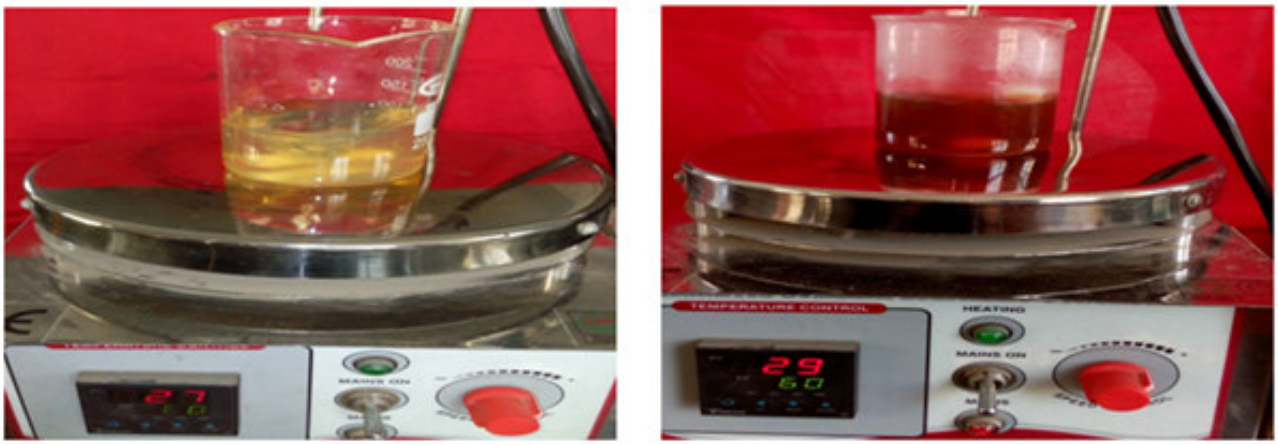


Figure 1

Colour change of AgNPs aqueous solution during NP synthesis

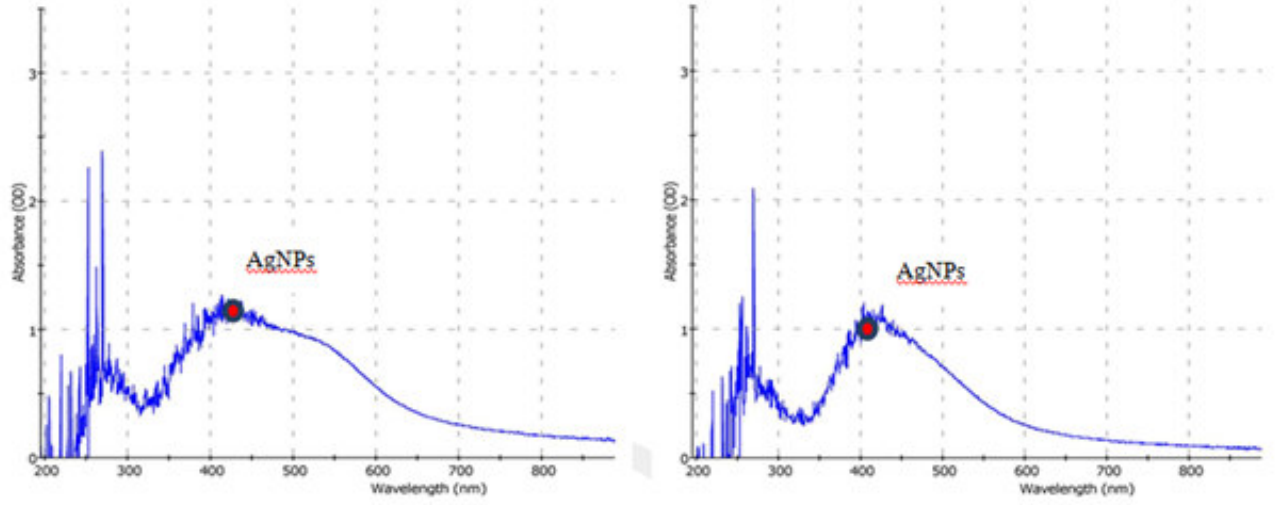
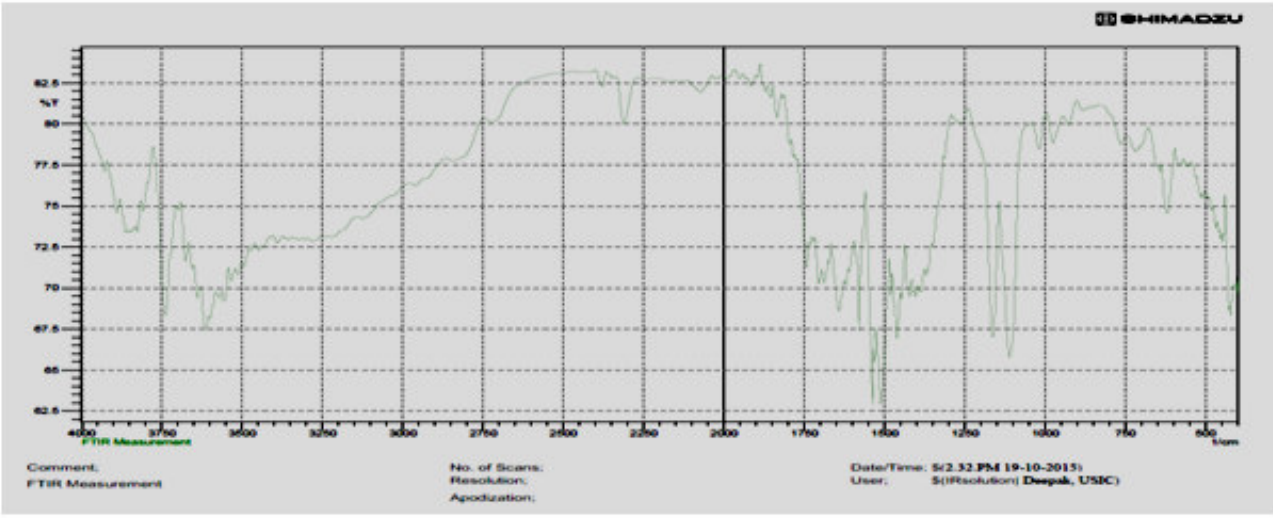


Figure 2

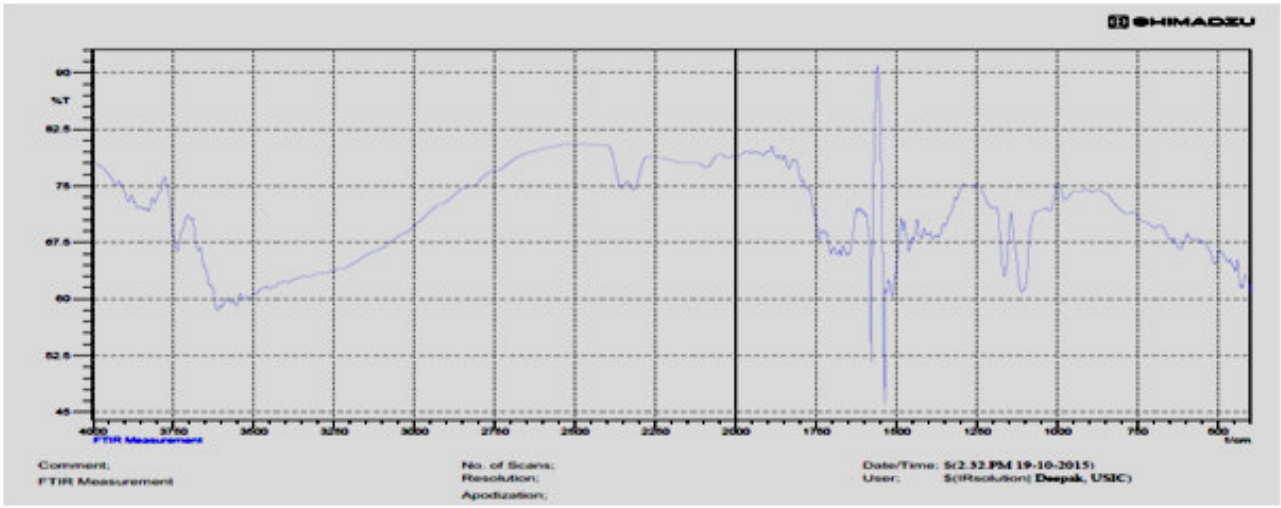
UV-Visible absorption spectrum of O. sanctum L. and O. americanum L.

FTIR analysis

FTIR spectroscopy was employed to decide the possible biomolecules and functional groups involved in reduction, capping and efficient stabilization of newly synthesized AgNPs. The band obtained in different regions of spectrum for AgNPs was analyzed and are shown in Fig. 3(a, b). The spectra showed absorption bands at 1850, 1650, 1615, 1495, 1635, 3650, 3250 cm^{-1}. Absorption peak at 1850 cm^{-1} may be assigned to C=O stretching in anhydride group and peak at 1650 cm-1 are assigned to C=O stretching in amide group which have strong binding ability with metal and acting as capping agent to prevent agglomeration and stabilizing agent for silver NPs[29]. Well-defined absorptions in the region 1615 and 1495 cm^{-1}were attributed to C=C stretching possibly due to the presence of an aromatic compound. The strong peak at 1635 cm^{-1} corresponded to N-H stretching due to primary amine[30] which suggest that amines are interacting with biosynthesized NPs and also their secondary structure were not affected after binding with AgNPs[31]. Absorptions in the region 3650 and 3250 are assigned to O–H stretching in hydroxy and amino group respectively, both giving very characteristic band profiles[32]. These results confirm that the *O. sanctum* L. and *O. americanum* L. leaf extract containing functional groups like- OH (hydroxyl), –C=O (Carbonyl), amino and N-H (amine) were involved in synthesis of silver NPs.



(a)



(b)

Figure 3
FTIR spectrum of green synthesized AgNPs formed by O. sanctum L. (a) and O. americanum L. (b).

SEM analysis

The size of AgNPs ranging between 85-95 nm for *O. sanctum* L. and 40-70 nm for *O. americanum* L. SEM image exhibited that the AgNPs synthesized were mostly spherical in shape. The SEM images also showed the formation of different structures of AgNPs like spherical shaped[23] and flake surfaced spherical and whiskers in both *O. sanctum* L. (Fig.4) and *O. americanum* L. (Fig.5). This may be due to the presence of various biomolecules as capping agents present in the different leaf extracts. This is also supported by the shifts and difference in areas of the peaks obtained in the FTIR analysis.

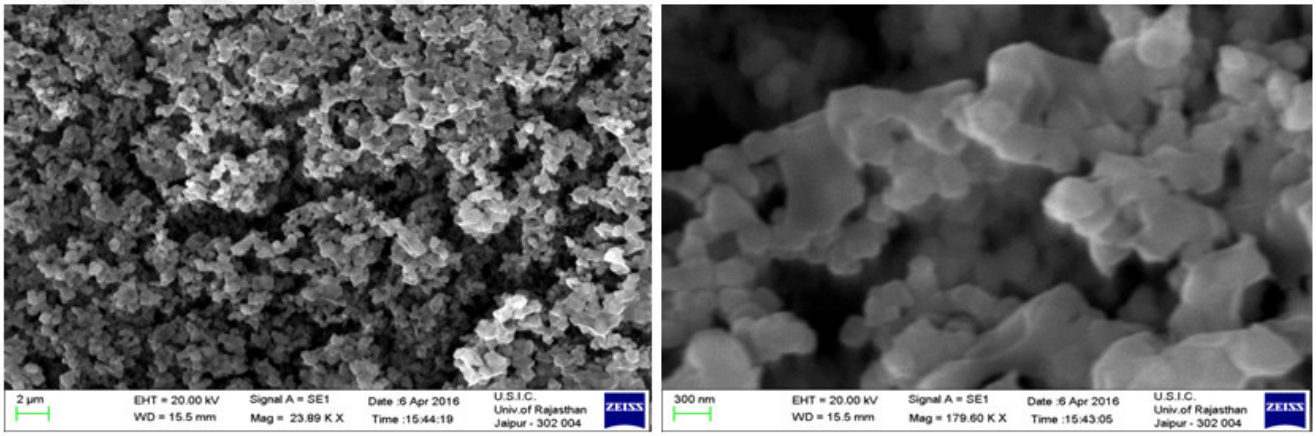


Figure 4

SEM image of AgNPs formed by O. sanctum L.

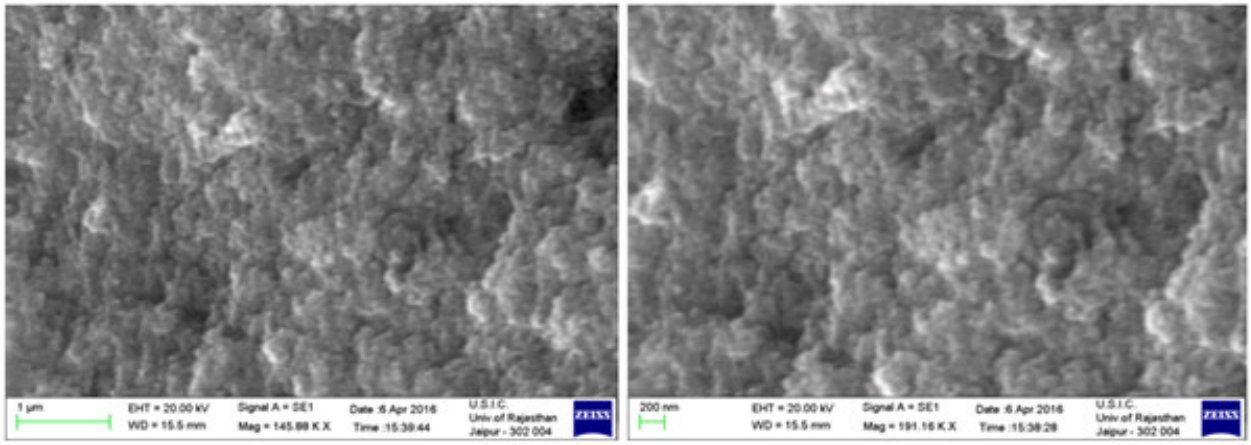


Figure 5

SEM image of AgNPs formed by O. americanum L.

Antibacterial activity

Antibacterial activity of AgNPs synthesized from *O. sanctum* L. and *O. americanum* L. leaf extract was investigated against Gram-negative (*Escherichia coli*, *Klebsiellapneumoniae* and *Pseudomonas aeruginosa*) bacteria using the agar disc diffusion assay, and the activity is shown in Table 1 (Fig.6 and Fig.7). The serial microdilution results were analysed using the Analysis of Variance (ANOVA) single factor statistical tool indicated that there is a significant difference in the sensitivity of the tested microorganisms to the both plants ($P \leq 0.05$).The microbial sensitivity to the different extracts represented by the mean MIC values ranged from 4.76 to 6.56 µg/ml.The AgNPs showed the maximum activity 6.56±0.15 (50µg/ml) for *E. Coli* and lowest by *Pseudomonas aeruginosa* 4.76±0.11 (100 µg/ml). AgNPs of *O. americanum* L. showed the maximum activity 5.93±0.05 (75µg/ml) for *E. Coli* and lowest by *Pseudomonas aeruginosa* 4.76±0.11 (100 µg/ml) against positive control (antibiotics) Ampicillin. The negative control (distilled water) did not show any zone of inhibition.The mechanism of prominent antibacterial activity by AgNPs against various pathogenic bacteria is still a point of research and requires further investigation[33]. Silver ions uncouple the respiratory chain from oxidative phosphorylation and lead to collapse of the proton motive force across the bacterial cytoplasmic membrane[34, 35]. AgNPs may attach to the surface of cell membrane of microorganisms, leading to the

disturbance of its functions like permeability and respiration. It is obvious, therefore, that the binding of particles to the microorganism depends on the surface area available for interaction. Small NPs have a larger surface area for interaction with bacteria, as compared to that of bigger particles, due to greater antibacterial activity[36,37].In our results, the leaf extract of *O. sanctum* L. showed maximum activity as compared to *O. americanum* L. This could be due to the size of the NPs.

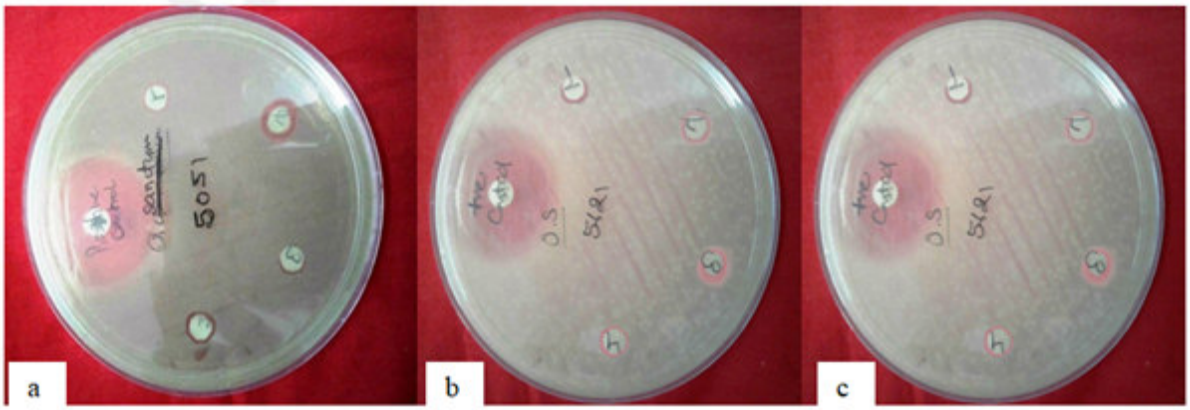


Figure 6
Antimicrobial activity of biosynthesized AgNPs from O. sanctum L. against Gram-negative bacteria (a)Escherichia coli, (b)Pseudomonas aeruginosaand (c)Klebsiellapneumoniae.

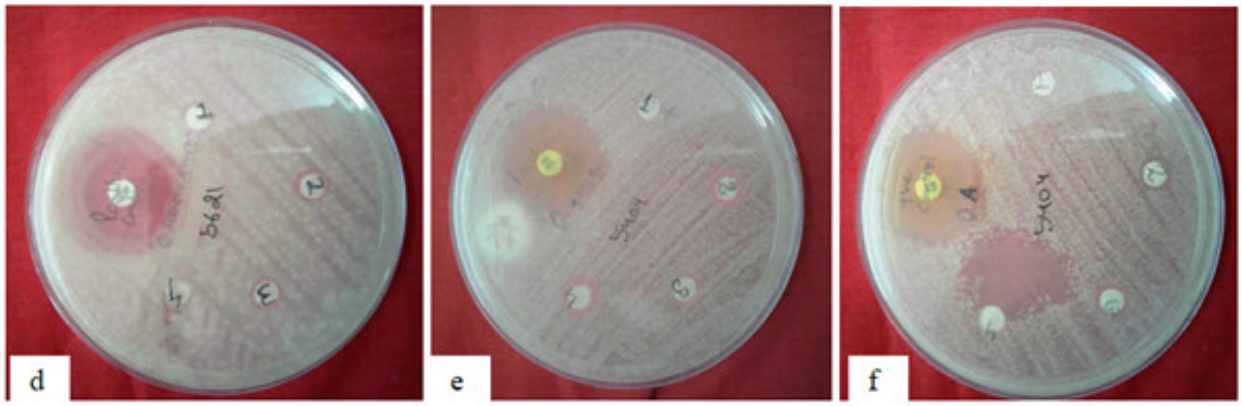


Figure 7
Antimicrobial activity of biosynthesized AgNPs from O. americanum L. against Gram-negative bacteria (d) Escherichia coli, (e) Pseudomonas aeruginosa and (f) Klebsiellapneumoniae.

Plant	Bacteria	Zone of inhibition (mm) (mean ± SD) against pathogenic bacteria			
		25µg/ml	50µg/ml	75µg/ml	100µg/ml
***O. sanctum* L.**	*Escherichia coli*	NA	6.56 ± 0.15	5.93 ± 0.15	5.63 ± 0.15
	Pseudomonas aeruginosa	NA	5.66 ± 0.15	5.63 ± 0.05	5.80 ± 0.10
	Klebsiella pneumoniae	NA	5.56 ± 0.11	6.03 ± 0.05	5.60 ± 0.20
	Average Zone of inhibition*	NA	5.92 ± 0.02^{a}	5.86 ± 0.05^{ab}	5.67 ± 0.05^{c}
***O. americanum* L.**	*Escherichia coli*	NA	5.86 ± 0.15	5.93 ± 0.05	5.13 ± 0.15
	Pseudomonas aeruginosa	NA	5.73 ± 0.11	5.56 ± 0.05	4.76 ± 0.11
	Klebsiella pneumonia	NA	5.76 ± 0.05	5.56 ± 0.15	5.10 ± 0.10
	Average Zone of inhibition*	NA	5.78 ± 0.05^{d}	5.68 ± 0.05^{e}	4.99 ± 0.02^{f}

NA- No activity
Values are mean ± SD of three samples; Significant at 5% Level (P ≤ 0.05)
****a-f: Mean values with different superscript are significantly different by One way ANOVA test***

Table 1
Antibacterial activity (mean ± SD) of AgNPs from O. sanctum L. and O. americanum L. leaf extract against tested bacteria.

CONCLUSIONS

The biosynthesis of AgNPs using leaf extract of *O. Sanctum* and *O. americanum* provides an eco friendly, simple and efficient method. These AgNPs were rich source of proteins and metabolites such as terpenoids, phenols etc. The AgNPs of *O. sanctum* L. were found to have wider antibacterial activity as compared to *O. americanum* L.Applications of such eco-friendly NPs in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other nanomaterials. Thus, we can be concluded that plant extract could be used in various fields for the biosynthesis of NPs as well as not require sophisticated equipments and toxic chemicals. Toxicity studies of AgNPs on human pathogen opens a door for a new range of antibacterial agents.

CONFLICT OF INTEREST

Conflict of interest

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