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Bio-synthesis and biopotential assessment of silver nanoparticles (AgNPs) by using leaf extract of *Ocimum sanctum*

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Abstract
Silver nanoparticles (AgNPs) have great potential for their potential role in biomedical researches. In the present study AgNPs have been synthesized utilizing *in vitro* grown leaf extract of *Ocimum sanctum* by the reduction of silver nitrate solution. The green synthesized AgNPs were partialy characterized by UV-visible spectroscopy with maximum absorbance (λ_{max}) at 427 nm. The Fourier transform infrared spectroscopy (FTIR) was carried out to identify the possible biomolecules responsible for efficient stabilization of AgNPs. Green synthesised AgNPs seemed to exhibit capacity to work as good antioxidant agent (a potent radical scavenger properties by 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay) and have antibacterial activity against *E. coli* bacterial strain in present study. Our results explain that *Ocimum sanctum* leaf extract have potential as bio-reducing agent for synthesis of AgNPs, which can be exploited as anti-oxidant, antimicrobial agent and depicting an effective way for utilizing bioactive resources in restoration of medicinal properties of this plant *Ocimum sanctum* with high efficacy.

Keywords: Antioxidants, antimicrobial activity, free radicals, nanotechnology, silver nanoparticles (AgNPs)

Introduction
Nanotechnology can be defined as a whole knowledge on fundamental properties of nano-size objects (Sergeev, 2003, Sergeev and Shabatina, 2008) [1, 2]. Nanotechnology has rapidly surged in prominence and now extends its influence across diverse domains, including health care, cosmetics, biomedicine, food production, gene delivery, environmental preservation, mechanics, optics, chemical industries, space exploration, energy science, nonlinear optical devices, and photo electrochemical applications (Ahmed *et al*., 2016, Tripathi *et al*., 2019, Sonker *et al*., 2022) [3-5]. Nanotechnologies have been used to develop nanoparticles (NPs)-based targeted drug carriers (Falanga *et al*., 2011) [6]. Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity (Sonker *et al*. 2017a, b) [7, 8]. At present time nano-chemistry becomes one of the main growing directions of nanoscience (Sergeev and Shabatina, 2008) [2]. Although chemical and physical methods may successfully produce pure, well-defined NPs but these methods were quite expensive and potentially dangerous to the environment. Normally, physical and chemical methods are found to be costly and dangerous. Biological methods are simple, single step method and exibhit high yield, high solubility as well as high stability and also doesn't need elevated temperature, pressure, force and deadly chemicals (Ge L *et al*., 2014) [9]. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner (Sastry *et al*., 2004, Bhattacharya and Rajinder, 2005, Mohanpuria *et al*., 2008) [10-12]. Plants are the richest bioresources of drugs in traditional and modern medicine (Prasad *et al*., 2011) [13]. Biomolecules are highly compatible with nanotechnology which makes unique assembly for development of metal NPs of biological molecules which are authentic and cost effective (Harekrishna *et al*., 2009) [14]. In recent years development of metallic NPs is an emerging field of research in material science. Metal NPs have a high specific surface area and a high fraction of surface atoms because of the unique physico-chemical characteristics of NPs (Crabtree *et al*., 2003) [15].

Different types of NPs like copper, zinc, titanium (Retchkiman-Schabes *et al*., 2006) [16], magnesium, gold (Gu *et al*., 2003) [17], alginate (Ahmad *et al*., 2005) [18] and silver have come up but silver nanoparticles (AgNPs) have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms (Gong *et al*., 2007, Tripathi *et al*., 2019) [19, 4]. AgNPs have wide range of applications such as catalysis (Tsang *et al*., 2004) [20], drug delivery (Baptista, 2009) [21], biosensing (Huang *et al*., 2007, Jain *et al*., 2006 [22, 23]) and optics (Murphy *et al*., 2005) [24]. Currently AgNPs are synthesized from natural herbs having medicinal potential such as synthesis of various metal NPs using fungi like *Aspergillus terreus*, *Paecilomyces lilacinus* and *Fusarium* (Devi and Joshi, 2012) [25]. *Penicillium* sp. (Hemanth *et al*., 2010) [26], *Fusarium oxysporum* (Nelson *et al*., 2005) [27] and *Euphorbia hirata*, green tea, neem, starch aloe vera, lemon etc. (Elumalai *et al*., 2010, Ankamwar *et al*., 2005, Kasthuri *et al*., 2008, Vijayaraghavan *et al*., 2012 [28-31]. In one of the studies, it was found that AgNPs had potent anti-biofilm action against bacterial species such as *P. aeruginosa*, *Escherichia coli*, and *S. aureus* (Mohanta *et al*., 2020) [32]. Antioxidants are known to safe guard cells from damage caused by free radicals, and biologically synthesized AgNPs have a high level of antioxidant activity as they possess bioactive molecules on their surface (Zehiroglu and OzturkSarikaya, 2019, Keshari *et al*., 2020) [33-34]. AgNPs have been most widely reported to show antidiabetic studies (Rajaram *et al*., 2015) [35]. Analytical techniques that are used for the analysis of this AgNPs are UV-Vis spectroscopy, X-ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FTIR), Dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Atomic force microscopy (AFM) etc. (Lin *et al*., 2016) [36]. The synthesis of metalic NPs have been the subject of substantial research in recent years (Li *et al*., 2001, Sharma *et al*., 2008, Iglesias-Silva *et al*., 2007, Huang and Yang, 2008, Pathak *et al*., 2019a, b, 2021, 2024) [37-44]. Demand of AgNPs is increasing rapidly in many of the streams like in medical, pharmaceutical companies, healthcare, food, consumer, cosmetics etc. Due to its uses, it has been used for its several applications like antibacterial properties, household, medical devices, and food industry, wound dressing, in diagnostic, orthopedics and an anticancer agent (Al-warthan *et al*., 2010) [45]. After considering the above facts and findings we worked on the biosynthesis, partial characterization and biopotential assessment of AgNPs by using leaf extract of *Ocimum sanctum.*

Materials and Methods

Collection of plant material (Leaf)

Ocimum sanctum, commonly known as holy basil or tulsi, is an aromatic perennial plant in the family Lamiaceae. Tulsi is a sacred plant for Hindus. It is worshipped as the avatar of Lakshmi, and is often planted in court yards of Hindu houses or temples to Hanuman. Leaves of *Ocimum sanctum* plants were collected from our college campus ground A.N.D Kisan P.G College, Babhnan, Gonda, U.P, India. Further the voucher specimen was preserved in the department as a record.

Preparation of leaf extract

Desirable weights of fresh leaves of selected plants were thoroughly washed using sterile distilled water and ground well using mortar and pestle. The well-grounded material was mixed with 100 mL of sterile distilled water and then transferred in 500 mL flask and the content was boiled for 3 minutes on hot plate. After boiling the content was filtered using cheese cloth / filter paper and the filtrate was stored in a sterile beaker at 4 °C for further use.

Biosynthesis of silver nanoparticles (AgNPs)

100 mL of aqueous silver nitrate, $AgNO_3$ (1mM) was added with 10 mL of the leaf extract. This mixture was kept in autoclave under 15 psi pressure at 121 °C for 5 minutes. A color change from green to yellowish brown, visually confirms the formation of AgNPs (Vigneshwaran *et al*., 2006) [46].

UV-Vis spectral analysis of AgNPs

The synthesised AgNPs solution was allowed for repeated centrifugation (Remi mini centrifuge RM-02) at 6000 rpm for 15 minutes. The supernatent was used for wavelength scan by using UV-Vis spectrophotometer (Shimadzu) with the resolution of 1nm at the wavelength range of 280-800nm, possessing a scanning speed of 300 nm/minutes.

FTIR spectral analysis

An FTIR spectrum was used to detect bio-active molecules which are responsible for the reduction of Ag^+ into Ag^0, during synthesis and stabilization of NPs. FTIR Spectrum for AgNPs was recorded using KBr pellets at a resolution of 4 cm^{-1} on a Perkin-Elmer Spectrophotometer instruments. This spectroscopic method was used to analyze the possible functional groups, which was involved in nanoparticle synthesis.

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay

DPPH (Sigma-Aldrich, M.W. 394.32) is a stable radical in solution and appears purple colour absorbing at 517 nm in methanol. This assay is based on the principle that DPPH on accepting a hydrogen (H) atom from the scavenger molecule i.e. antioxidant, resulting into reduction of $DPPH^+$ to $DPPH_2$, the purple colour changes to yellow with concomitant decrease in absorbance at 517nm. The colour change is monitored by spectrophotometrically and utilised for the determination of parameters for antioxidant properties. 0.5 ml of synthesised AgNPs sample was added to 3ml DPPH (1 mM DPPH) solution. The decrease in absorbance at 517nm was recorded at 3 min interval upto until the reaction reached a plateau. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al*., 2003) [47]. Ascorbic acid (Merck; M.W. 176.13) was used as standard. The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula:

$$\% \text{ DPPH radical scavenging} = [(Ab^c - Ab^t) / (Ab^c)] \times 100$$

Ab^c = Absorbance of control

Ab^t = Absorbance of test Sample

Anti-microbial activity of green synthesised AgNPs by disc diffusion method

Antibacterial activity of synthesized AgNPs were assessed by the disc diffusion method against gram negative (*Escherichia coli*) strains of bacteria. Microorganisms were grown in Muller Hinton Agar (MHA) medium at 37 °C. Freshly prepared inoculums were spread over solid medium plates and sterile discs (diameter 6 mm) (Hi-media) impregnated with equal concentrations of plant extract, $AgNO_3$ solution, synthesized AgNPs, water and streptomycin were placed on it and kept at 37 °C in incubator. After 24h incubation inhibition zone around discs were measured.

Results

A color change (due to reduction) from green to yellowish brown, visually confirms the formation of AgNPs. The outline of green synthesis, partial characterization and biopotential assesemnt of AgNPs from leaf extract of *Ocimum sanctum* is shown in Fig. 1. The bio-reduction of Ag^+ ions ($AgNO_3$ into Ag^0) in aqueous extract was monitored by UV-visible spectra.

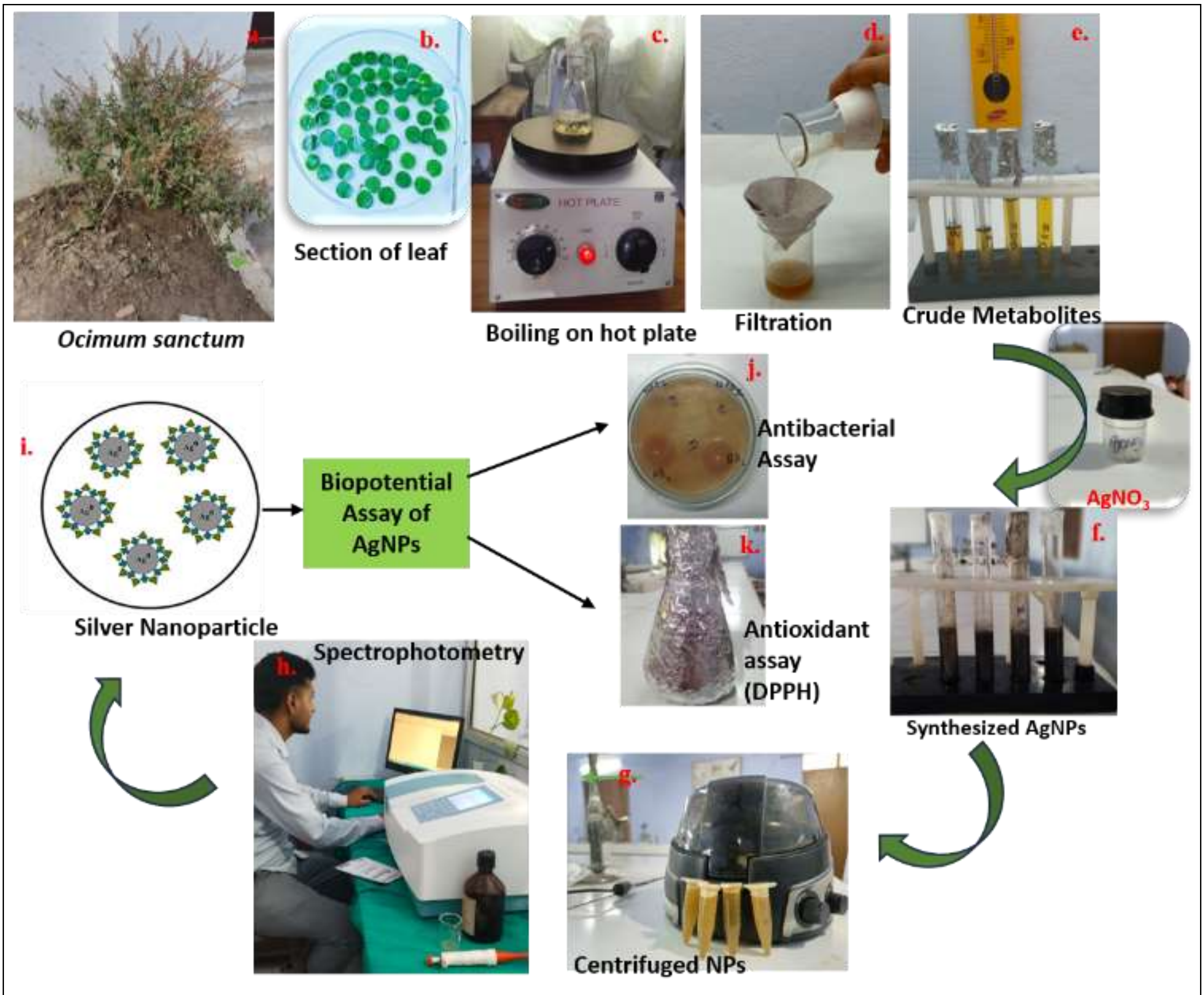

Fig 1: Outline of green synthesis, spectrophotometric characterization and biopotential assay of AgNPs from leaf extract of *Ocimum sanctum.*

UV-Vis spectrum

The absorption spectrum of the leaf extract and AgNPs are shown in Fig. 2a & b. The UV-visible spectral analysis of the extract with $AgNO_3$ solution showed maximum absorbance at 427nm (Fig. 2 a & b).

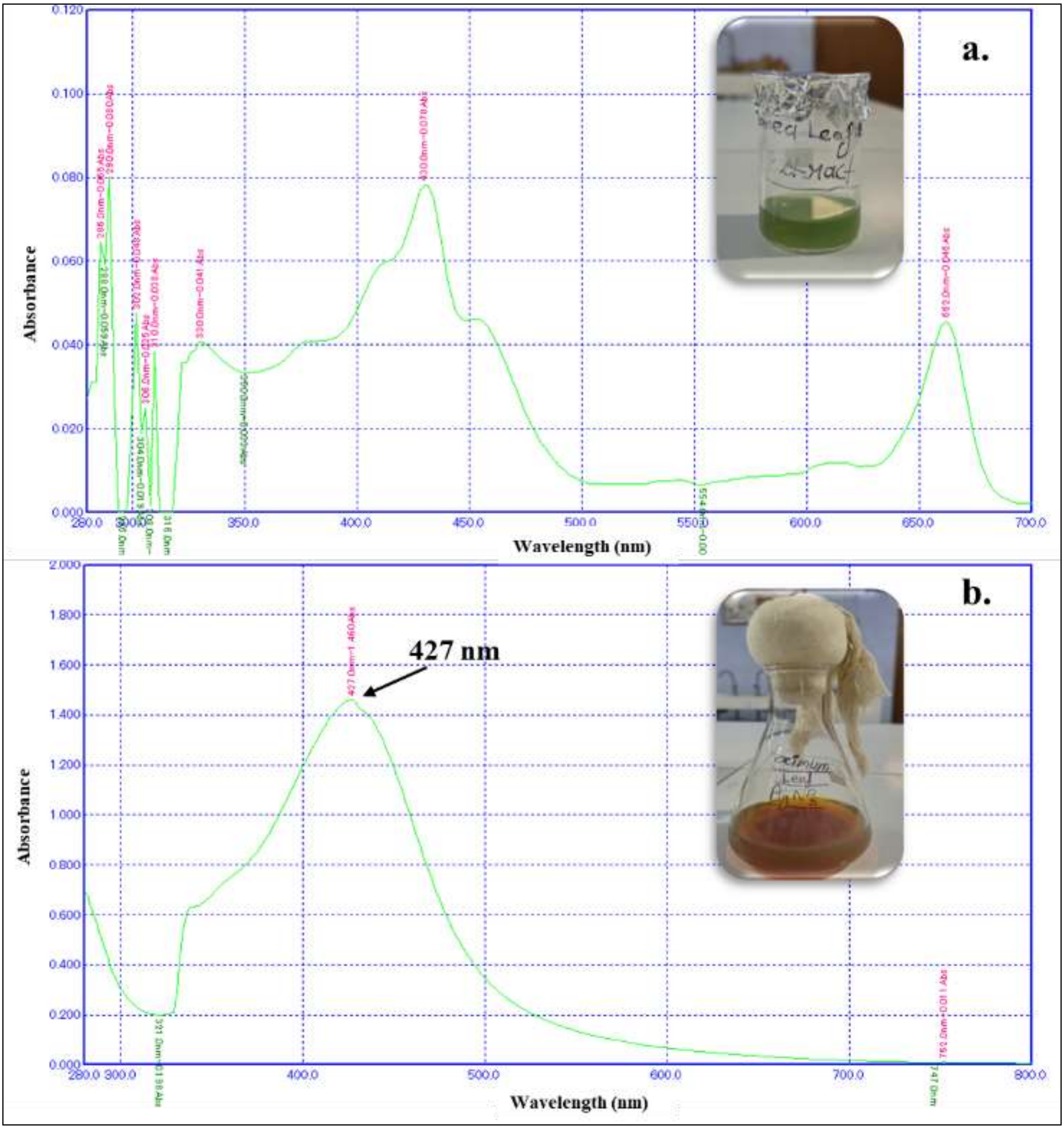

Fig 2: UV-Vis absorption spectra of leaf extract (a) and synthesized AgNPs (λ_{max} 427 nm) (b).

FTIR spectrum

The FTIR spectrum of plant extract showed distinct peaks at 2925, 2845, 1612, 1531, 1425, 985 and 615 cm^{-1} (Fig. 3). The earlier reports (Philip *et al.*, 2011) [48] show flavonoids and terpenoids were present in the *Ocimum* leaf.

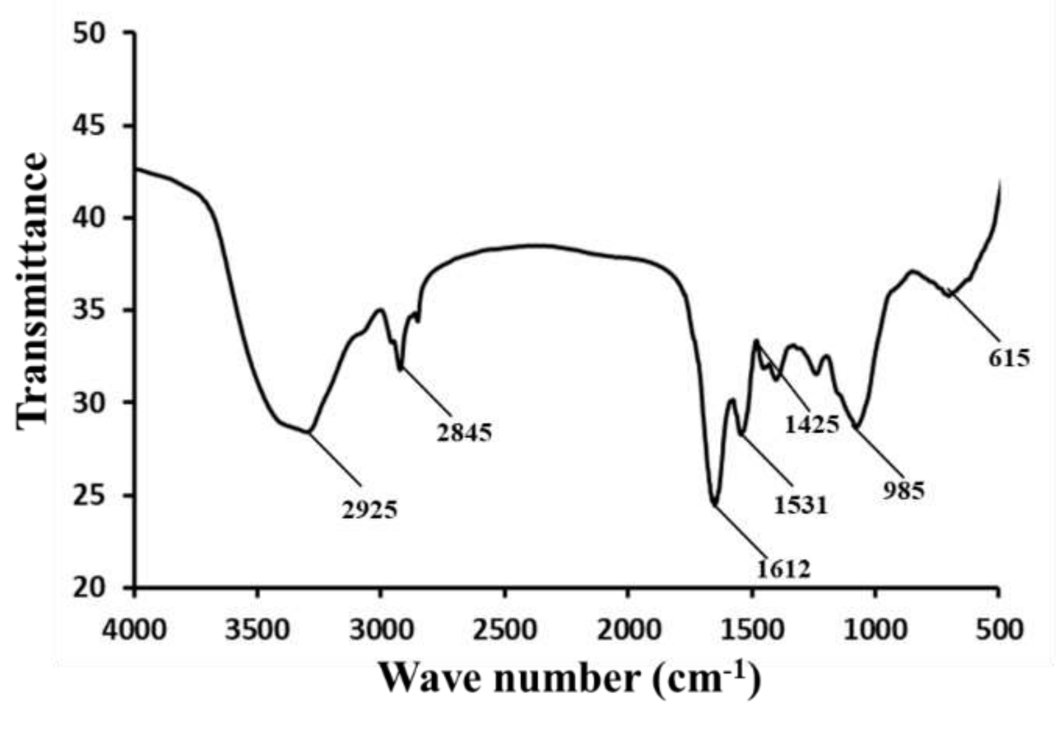

Fig 3: FTIR spectrum of synthesized AgNPs.

An intense band was found at 1612 cm^{-1} which reflects comparatively higher involvement of flavonoides in the reduction of Ag^+. The FTIR spectrum indicates the involvement of amides, flavonoides, phenols, amino acids during AgNPs synthesis.

Screening of antioxidant assay of synthesised AgNPs

The anti-oxidant activity of any compound is directly proportional to its reducing power. Antioxidant activity of AgNPs and Ascorbic acid (reference) were estimated by DPPH percent inhibition. DPPH is used as nitrogen centered stable free radical, it has capacity to accept electron and form stable diamagnetic molecule (Saha and Verma, 2018, Nimse and Pal, 2015) [49, 50]. The result obtained during DPPH assay showed the effective free radical scavenging activity in Ascorbic acid as well as in AgNPs samples (Fig. 4). Our results showed that green synthesised AgNPs have efficient antioxidative potential and therefore can be utilized as an antioxidant for natural health prevention.

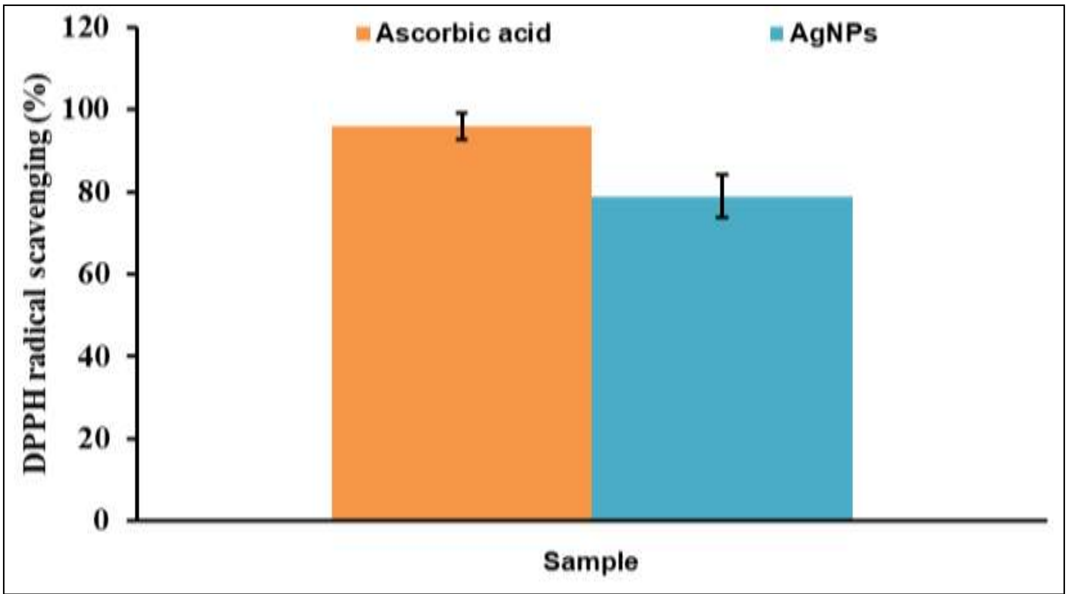

Fig 4: DPPH assay of Ascorbic acid and AgNPs.

Screening of antimicrobial assay of synthesized AgNPs

Antibacterial activity is well documented in metallic NPs like copper, nickel, gold including silver (Liu *et al*., 2009, Chatterjee *et al*., 2014, Piruthiviraj *et al*., 2015) [51-53], but among them AgNPs were highly used as antibacterial agent because silver has its own antibacterial properties. Khan *et al*., 2016 reported in his study that increased antibacterial efficiency of AgNPs than $AgNO_3$ was due to the presence of terpenoids (Khan *et al*., 2016) [54]. Similarly, in our results plant extract and silver nitrate indepedently showed slight low level of antibacterial activity against *E. coli*. Possibly, in our study there might be presence of various terpenoids responsible for antibacterial activity in synthesized AgNPs. Zone of inhibition of AgNPs revealed that *E. coli* were more susceptible to the streptomycin. Where as synthesised AgNPs showed low bacterial inhibitory effect comparable to that of streptomycin (Fig. 5).

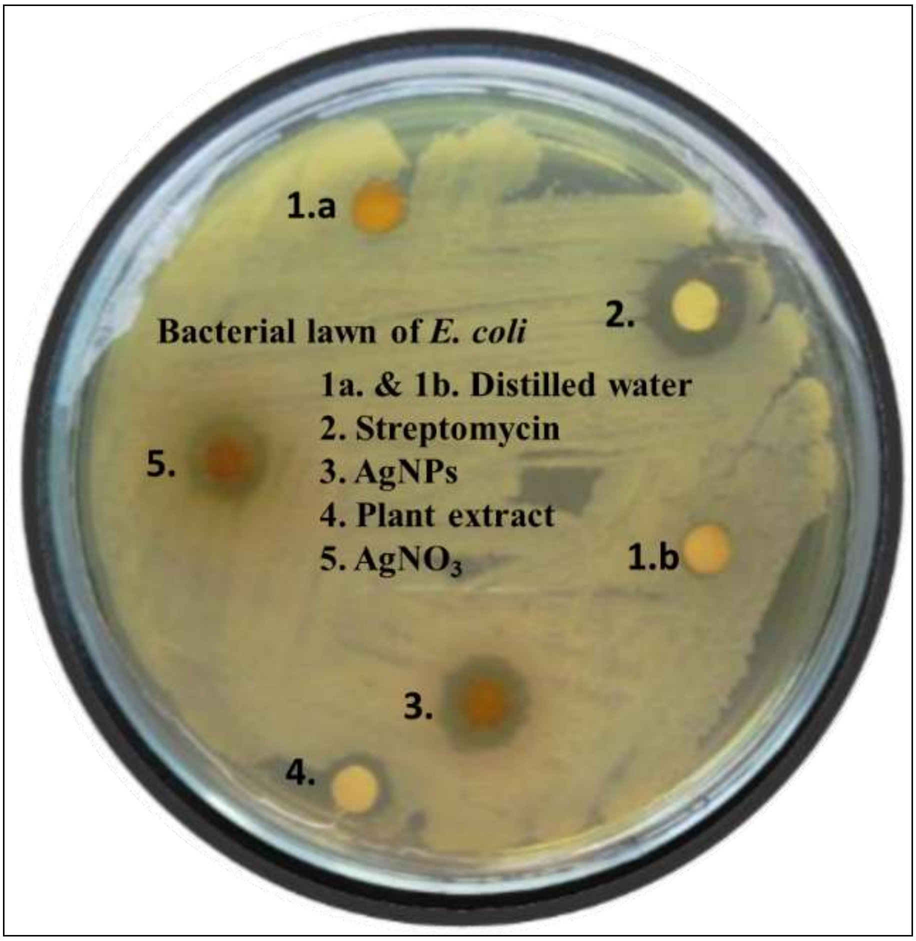

Fig 5. Antimicrobial activity assessment through disk diffusion method against *E. coli.*

Conclusion

In the present study, we have developed an eco-friendly, cost effective and convenient AgNPs from leaves of *Ocimum sanctum.* The extract having properties to reduce $AgNO_3$ solution and biosynthesized AgNPs. The present green synthesized AgNPs showed the capacity to work as good antioxidant agent and have antibacterial activity against *E. coli* bacterial strain. Thus, green synthesised AgNPs may be act as model for human pathogens as good therapeutic agent and also for the treatment of diseases caused by free radicals in the near future. From a technological point of view, these obtained AgNPs will have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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Conflicts of interest

There are no conflicts of interest between authors.

Author's contribution

Jainedra Pathak and Deepak Kumar Singh designed the experimental work. Experiments were performed by Khagesh Singh and Manoj Kuamr Shukla. Abha Pandey did FTIR analysis. Khagesh Singh and Abhinav Singh wrote the manuscript and Prof. Rajeshwar P. Sinha reviewed the manuscript.

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