



Green Synthesis of Silver Nanoparticles using the Leaf Extract of *Filicium decipiens* and its Anti-Microbial Activity

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ABSTRACT

Silver nanoparticles has been used since ages; even now it is used in almost all areas like medicine, textiles, industries, cosmetics, purification and dyeing. There are many approaches to synthesize silver nanoparticles. However, these approaches are either harmful to the environment or highly expensive. An attempt has been made in this eco-friendly approach towards the green synthesis of silver nanoparticles (AgNPs) using the leaves of *Filicium decipiens*. Characterization of as-synthesized AgNPs were done using UV-Visible spectroscopy and Fluorescence spectroscopy which confirmed the formation of AgNPs. Scanning Electron Microscope (SEM) confirmed its rounded shape and X-ray diffraction (XRD) determined its crystalline nature as face centered cubic structure. Furthermore, Dynamic Light Scattering (DLS) was also done in order to know the average diameter and zeta potential of AgNPs. However, it did not show potential results due to the aggregates formed during the green synthesis of AgNPs. In addition to this, anti-microbial test against bacteria such as gram negative (*Escherichia Coli*) and gram positive (*Bacillus subtilis*) were done using well-diffusion method and also its application of anti-microbial activity was tested over fabric to understand its application in textile industries. In both the cases, AgNPs showed more efficiency in gram negative than gram-positive bacteria.

Keywords: Anti-Microbial; Characterization; Fabric; Green Synthesis; Silver Nanoparticles.

1. INTRODUCTION

Nanotechnology is one of the rapidly emerging field which has brought life-saving applications especially because of the fact that it is flexible with respect to its size, shape and other physical and chemical properties (Lee *et al.* 2019). According to European medical agency, nanomaterial is a material from the atomic level of 0.2 nm to 100 nm whereas, Organization for Economic Co-operation and Development (OECD) mentioned its definition as a material with 1-100 nm of size range. Actually, there is no particular size cut-off for the formation of nanomaterial and also 100 nm size limit does not have any clear evidence. However, for most of the materials, the change in its properties with respect to its size may happen within the boundary of 100 nm but again it is not evident that it cannot happen beyond 100 nm (Kreyling *et al.* 2010).

AgNPs in particular have gained much hype due to its extensive properties like electrical conductivity, chemical activity, anti-fungal and anti-bacterial properties (Benyettou *et al.* 2015; Kravets *et al.* 2016; Elahi *et al.* 2018; Liao *et al.* 2019). Many centuries ago, silver was used as anti-microbial and it was cheaper than

gold. However, silver is more toxic to prokaryotes like bacteria, fungi, viruses than to the eukaryotes such as human beings (Keshari *et al.* 2020). Since then it has been considered to be reliable and less harmful to human beings. In the present day, due to the rapid development in the field of nanotechnology, it has been possible to easily modify the physical and chemical properties of AgNPs which in turn made it possible for it to increase its anti-microbial efficiency (Kalmantaeva *et al.* 2020). In addition to this, AgNPs have been used for various other applications like biosensing (Lee *et al.* 2006), wound healing (Rajkumar *et al.* 2017), textiles (Xue *et al.* 2012), sensors for water pollutants (Proposito *et al.* 2020), cosmetics, toothbrushes (Hidayah N Ab Razak, Nik Ahmad Nizam, Juan Matmin, Wan Rosmiza Zana Wan Dagang, Nurliyana Ahmad Zawawi *et al.* 2021), dentistry (Fernandez *et al.* 2021) and agriculture (Singh *et al.* 2021).

Due to its larger surface to volume ratio, AgNPs has been extensively used as anti-microbial agent in various fields. Even though the mechanism of AgNPs as an anti-microbial is discussed broadly, its exact mechanism is still unknown (Yin *et al.* 2020). However, there are two well-known pathways to kill bacteria, viz.

ion-mediated killing and contact killing (Qing *et al.* 2018). In ion-mediated killing, Ag^+ ions released from AgNPs into the bacterial cells, interact with protein to cause protein deactivation. Besides this, it also uncouples the respiratory electron transport from oxidative phosphorylation and also can disrupt the cell division and reproduction in the bacterial cell (Hatchett *et al.* 1996; Monteiro *et al.* 2012); whereas in contact killing, AgNPs come in contact with the bacteria, infiltrate by causing damage to its membrane which lead to the leakage of cellular content and hence bacterial death (Seong *et al.* 2017; Khalandi *et al.* 2017).

AgNPs are notable nanomaterials that can be made through physical and chemical methods. However, they release toxic chemicals to the environment and have high temperature settings. Therefore, these methods fail to be eco-friendly and cost-effective. In order to overcome these drawbacks, green synthesis of AgNPs are largely employed using extracts of various plants, microorganisms, sugars and biodegradable polymers as reductant and capping agents (Ahmed *et al.* 2016b). These greener methods are toxic free, inexpensive, simple and convenient (Yousaf *et al.* 2020). Since synthesis through microorganisms is slower when compared to plant extracts, plant extracts are more preferred (Ahmed *et al.* 2016a). However, the capability of higher plants is less-explored in the green synthesis of AgNPs (Ahmed *et al.* 2016b). Considering this fact, in this study, a vascular plant known as *Filicium decipiens* has been used for the synthesis of AgNPs.

Filicium decipiens, which belongs to *sapindaceae* family, is a large, attractive and glossy fern-like foliage found mainly in Southern Africa (Lavaud *et al.* 1998), and Asian countries (Atiyaparveen *et al.* 2021). Studies have shown that it has high glycosides and saponin

content. This in itself is an anti-microbial, anti-oxidant and anti-inflammatory agent (Sharmila *et al.* 2017) which is mainly used for diabetics treatment in India (Atiyaparveen *et al.* 2021).

After the synthesis of AgNPs using the leaf extract of *Filicium decipiens*, they were characterized using UV-Vis., Fluorescence, SEM and XRD analyses. In addition, its anti-microbial activity was also evaluated to understand its efficacy against bacteria like *E. Coli* and *Bacillus subtilis*. Furthermore, its anti-microbial activity over fabric was analyzed for application in textile industries.

2. MATERIALS AND METHODS

2.1 Chemicals

AgNO_3 powder, nutrient agar and Nutrient broth were purchased from HiMedia Chemicals, India.

2.2 Preparation of Plant Extract

Leaves of *Filicium decipiens* were collected from the ground of D.G.S. Dhinakaran Auditorium, Coimbatore, Tamil Nadu, India and its identification was done by Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. After the collection of leaves, they were washed with tap water and the surface of each leaf was cleaned and then dried by using Hot Air Oven (Technico, Genuine). This was later powdered using a kitchen blender from which 20 g of powder was added in 200 ml of double distilled water. This mixture was then kept in water bath for 90-100 °C for two hours. Then the extract was filtered out by using Whatman Filter Paper in a separate conical flask.



Fig. 1: Trees, leaves and flowers of *Filicium decipiens* plant in D.G.S. Dhinakaran Auditorium, Coimbatore, Tamil Nadu, India

2.3 Green Synthesis of AgNPs

0.089 g of AgNO₃ was mixed with distilled water and made up to 10 ml by using Standard Measuring Flask (SMF), from which 2 mL AgNO₃ was mixed with 8 mL of plant extract. This was then kept on magnetic stirrer for 24 hrs. This AgNPs solution were then centrifuged at 6000 rpm for 10 min and then the supernatant was collected and dried. This dried supernatant was then used for the characterization. Bio-reduction of AgNPs were further studied using UV-Vis. spectroscopy at 330-770 nm (Double Beam Jasco V-630 Spectrophotometer) and fluorescence spectral readings with emission band width of 5 nm (Jasco FP-8300 Spectrofluorometer, with a 120 W Xenon lamp for excitation). Cuvettes of path length 1 cm were used for the above studies.

2.4 Characterization of AgNPs

In order to know the external morphology and shape, the as-synthesized AgNPs were powdered and analyzed through SEM analysis which was recorded using a JEOL, JSM-6390 instrument. In addition, to know the average particle size and its crystalline structure, XRD analysis was done by using SHIMADZU X600 and also DLS was conducted to know the size distribution by intensity and zeta-potential of AgNPs.

2.5 Anti-Microbial Activity studies

Well-diffusion method was performed for anti-microbial activity and *Bacillus subtilis* (gram positive) and *Escherichia Coli* (gram-negative) were used as test organisms. These bacteria were grown overnight by using HiMedia nutrient broth as the growth medium. On the next day, both the organisms were swabbed and three wells were made on each petri dish. Distilled water, 50 µL of silver nanoparticle (3 mg/ml) were filled in two wells respectively and streptomycin antibiotic disc was pressed over agar. Both the plates were incubated for 24 hrs. and zone of inhibition at various concentrations were measured and recorded.

2.6 Anti-Microbial Fabric studies

Anti-Microbial Fabric was done in order to know whether the as-synthesised silver nanoparticles are susceptible to the same organisms - *Bacillus Subtillus* (gram positive) and *Escherichia Coli* (gram-negative). These bacteria were swabbed all over the petri dish and then a strip of cotton gauge was dipped in 3 mg/ml of silver nanoparticle and another cotton gauge was dipped in raw *Filicium decipiens* plant extract. These plates were incubated at 32° C. On the next day the results were analyzed.

3. RESULTS AND DISCUSSION

3.1 UV-Vis and Fluorescence Spectral studies

The color change reaction from light yellow to dark brownish qualitatively confirmed the formation of AgNPs (Fig. 2). The reason behind this color change was due to an inherent property of metal nanoparticles known as surface plasmon resonance. However, its bio-reduction was further clarified by conducting UV-Vis and Fluorescence studies. Fig. 3 represents the formation of AgNPs since it gives its highest peak at 440 nm (Mosae Selvakumar *et al.* 2016). In addition, fluorescence studies revealed that when wavelength was measured with a peak ranging from 500 to 600 nm, the highest peak was formed in 560 nm, indicating the formation of AgNPs.

3.2 SEM analysis

Results shown in Fig. 4 reveal AgNPs to be round in shape and this has been evenly distributed showing a rough surface when taken in the lower resolution of 0.5 µm. This nanoparticle is poly-dispersed in nature and this shows that aggregation of particles might happen during the process of synthesis (Vijay Kumar *et al.* 2014).

3.3 X-Ray Diffraction

The crystalline structure of AgNPs were determined by XRD. The diffraction pattern of *Filicium decipiens* AgNPs is shown in Fig. 5 which depicts the highest diffraction peak as $2\theta = 32.32^\circ$, 38.25° and 44.50° and this dictate (100), (111) and (200) sets of lattice planes according to Bragg's Reflection. When compared with JCPDS No (89-3722), the pattern obtained for green synthesized AgNPs is face-centered cubic structure. The comparative results were shown by Mollick *et al.* (2012) and Jyoti *et al.* (2016). The crystallite size for AgNPs were calculated using Scherrer's formula:

$$d = 0.94\lambda/\beta \cos \theta$$

where, λ is the wavelength, β is the full width half maximum of corresponding peak. When the value of $2\theta = 38.25^\circ$ was taken, it gave average particle size as 16.15 nm which corresponds to following references (Anandalakshmi *et al.* 2016; Raj *et al.* 2018).

3.4 DLS Analysis

DLS analysis was done in order to know the size distribution of AgNPs. However, it showed a particle size of more than 100 nm. Moreover, study done by Béltéky *et al.* (2019) shows that the larger size of nanoparticles may be due to the large aggregates or organic matter present around the particle and this formed a larger hydration layer which led to larger average hydrodynamic diameter.

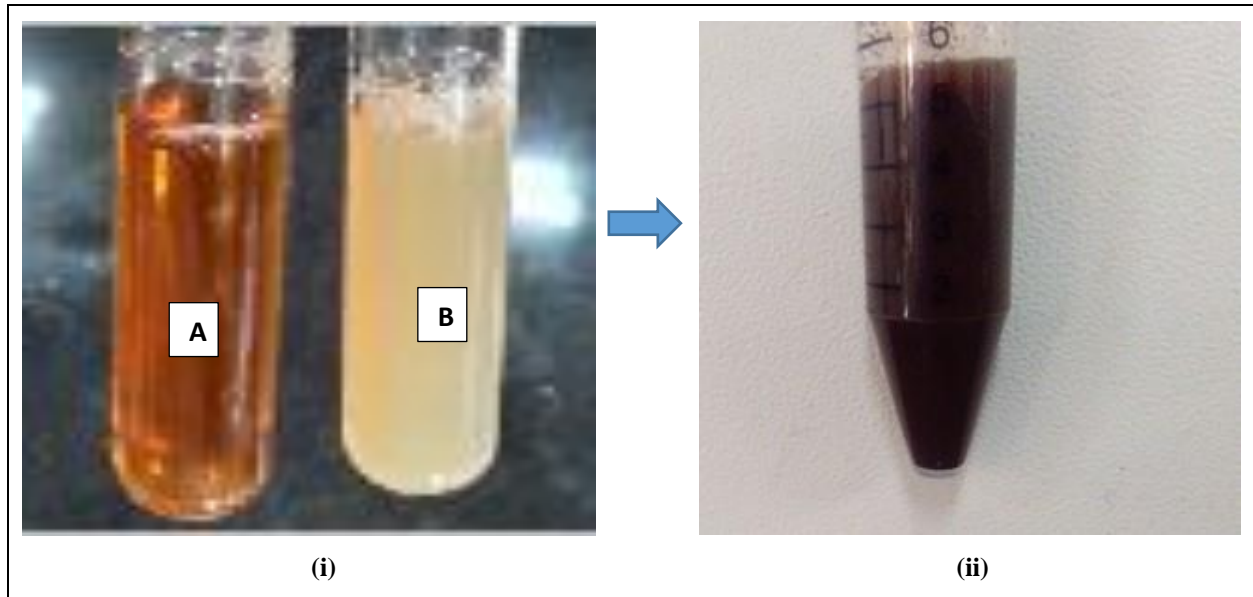


Fig. 2: Green synthesis of AgNPs: (i) (A) Plant extract (B) Plant extract after mixing with Silver nitrate (ii) After 24 hrs of magnetic stirring the mixture attains brownish color, indicating silver nanoparticle formation

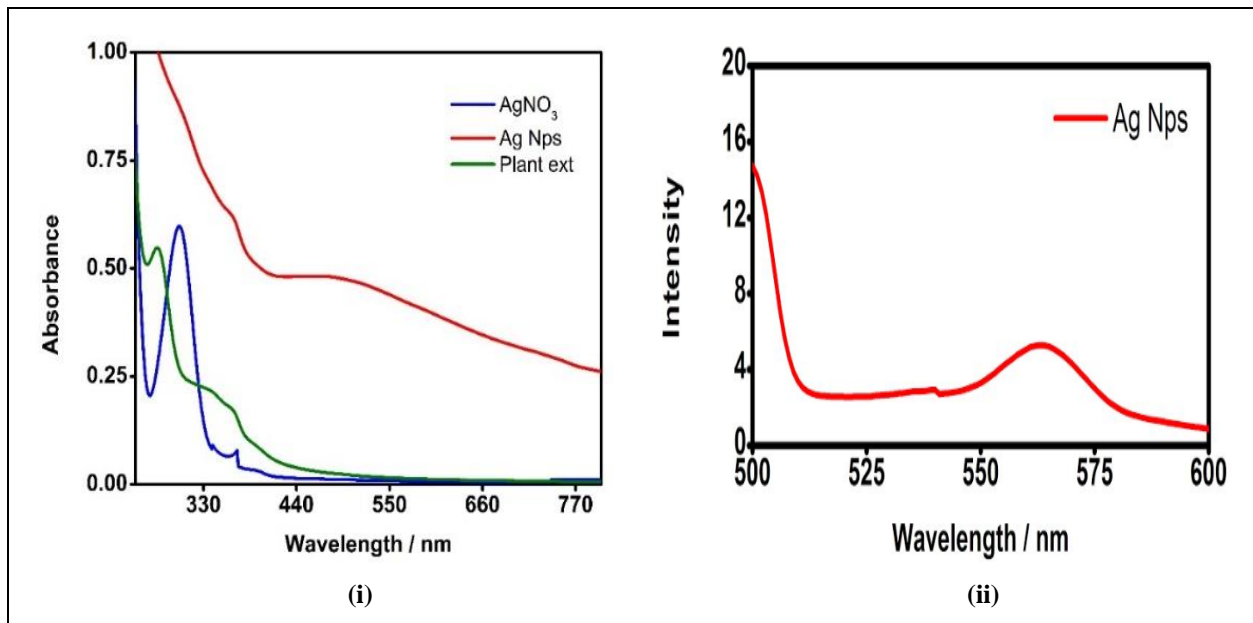


Fig. 3: (i) UV-Vis Spectra (ii) Fluorescence spectra of green synthesized AgNPs using *F. Decipiens* leaf extract at room temperature

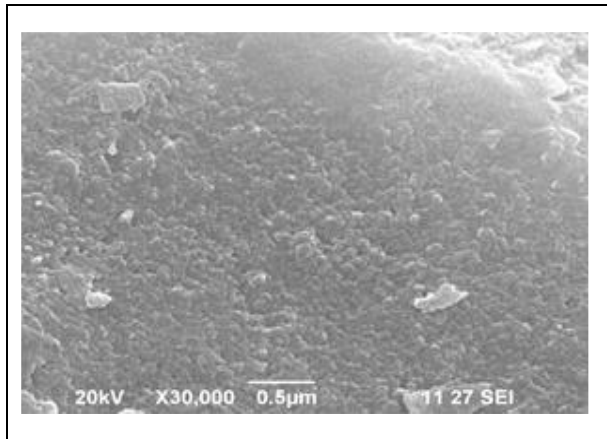


Fig. 4: SEM Analysis of *Filicium* based AgNPs

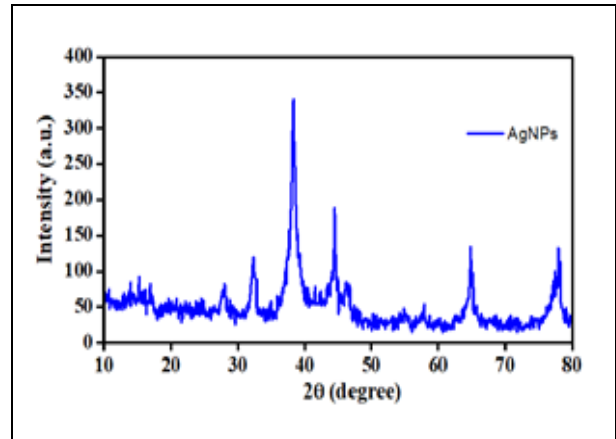


Fig. 5: XRD pattern for silver nanoparticles from *Filicium decipiens* extract

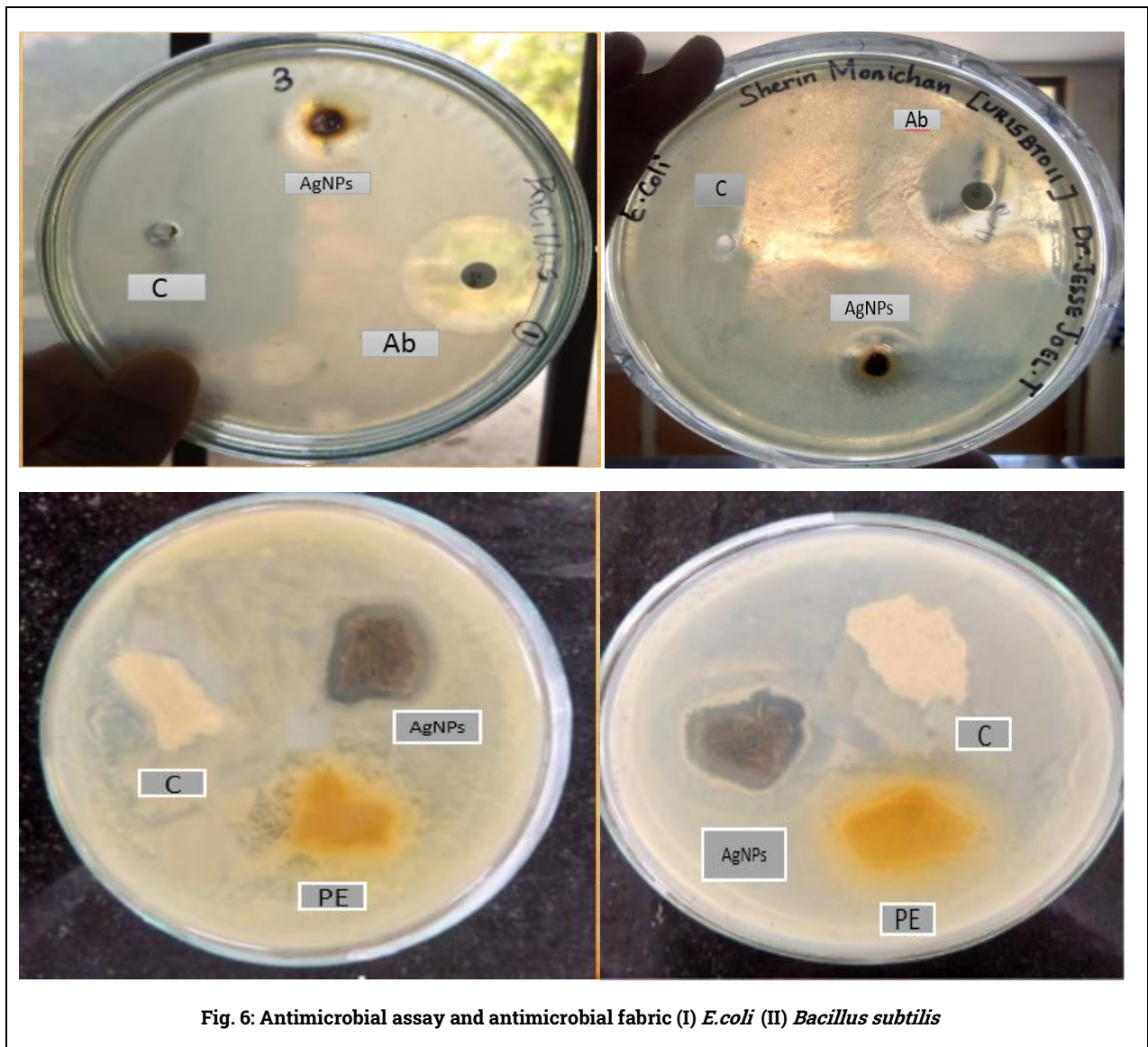


Fig. 6: Antimicrobial assay and antimicrobial fabric (I) *E.coli* (II) *Bacillus subtilis*

3.5 Antimicrobial Activity

Silver Nanoparticles have brought a major outcome as an anti-microbial in the field of pharmaceuticals and other drug industries mainly due to its size variation (Parthiban *et al.* 2019). However, its mechanism against a bacterium is not well-defined (Bragg *et al.* 1974). In this study, two bacterial strains were used each of Gram negative (*Escherichia. Coli*) and gram positive (*Bacillus subtilis*). These bacteria were swabbed around the plate and wells were made in which sample was added. Furthermore, it was then incubated for 24 hours and on the next day it showed a good zone of inhibition as shown in Fig. 6 and Table 1. This result interprets that gram negative bacteria shows a better result than gram positive bacteria and this is found to be more or less similar with the findings of Shrivastava *et al.* (2007).

3.6 Antimicrobial Fabric

AgNP is an excellent anti-microbial which made it capable to stand out in the arena of textile industry which includes towels, bandages, kitchen fabrics, furnitures, bed lines, re-usable surgical gloves, anti-bacterial dressings, suits against biohazards, ultra-hydrophobic fabrics, sports jerseys and also water repulsive materials (Rafique *et al.* 2017). The same study as an anti-microbial was done on cotton fabric which showed that after incubation of 24 hrs. AgNPs show a zone of inhibition against *E.coli* and *Bacillus subtilis*. However, this again indicated that the zone of inhibition was more prompt in gram negative bacteria than gram positive.

Table 1: Anti-Microbial Effect and anti-microbial fabric results of *Filicium Decipiens* AgNPs

Anti-Microbial Assay			
Zone of Inhibition in mm units in comparison with references			
Bacterial Strains	Control (Distilled Water)	3 mg/mL of silver nanoparticle	Values according to references
<i>Escherichia coli</i>	0	17	14 (Prema, 2009)
<i>Bacillus subtilis</i>	0	15	16 (Prema, 2009)
Anti-Microbial Fabric			
Zone of Inhibition in mm			
Bacterial Strains	Control (Cotton)	3 mg/mL of silver nanoparticle	Values according to references.
<i>Escherichia coli</i>	0	20	11 (Deena, 2015)
<i>Bacillus subtilis</i>	0	15	12 (Deena, 2015)

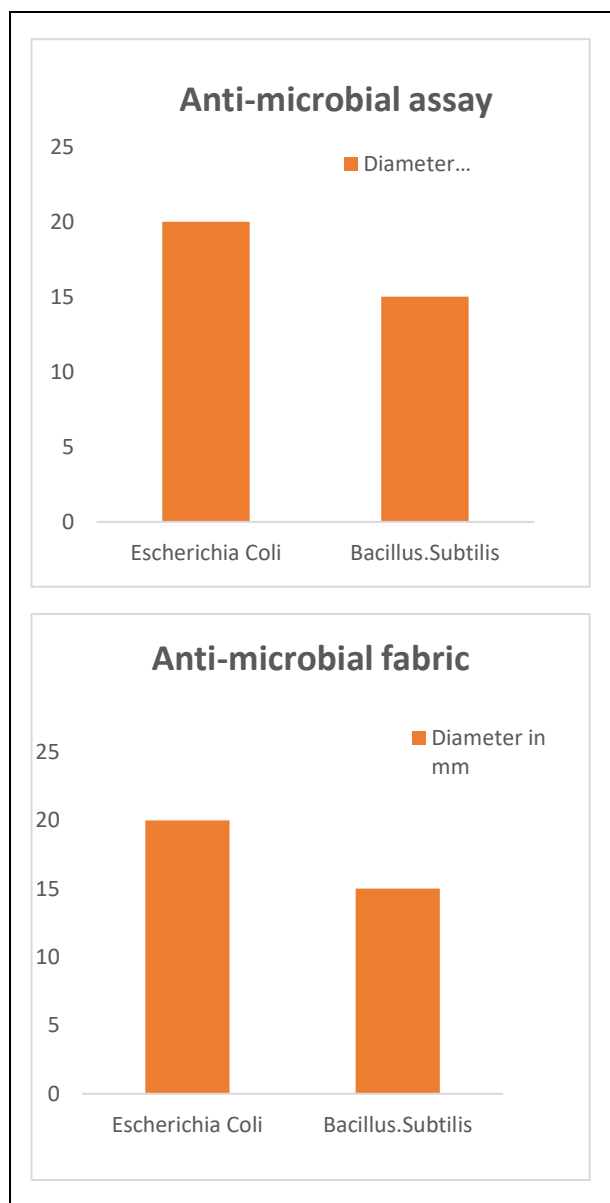


Fig. 7: Graph depicting E. Coli to be more susceptible to AgNPs than *Bacillus* (i) Anti-microbial assay and Anti-microbial Fabric

4. CONCLUSION

In this study, silver nanoparticles were synthesized using the leaves extract of *Filicium decipiens*. Furthermore, the formation of AgNPs were confirmed qualitatively through color change and quantitatively through UV-Vis and fluorescence spectroscopies. However, particle size and crystallinity were analyzed using SEM and XRD respectively which showed that the synthesized AgNPs are round, face-centered with average particle size as 16.15 nm. In addition to this, green synthesized AgNPs showed an excellent anti-bacterial efficacy against both gram negative (*Escherichia Coli*) and gram positive (*Bacillus Subtilis*). However, the results show clearly that gram

negative bacteria are more susceptible than gram positive bacteria to AgNPs. Moreover, this study reveals that *Filicium decipiens* in itself is an anti-microbial having the capability to enhance the properties of AgNPs and in addition to this the process is cost-effective, reliable and eco-friendly in nature, making it crystal clear that its application towards higher organisms is safer.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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