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SYNTHESIS OF PERIWINKLE LEAF AND AMLA FRUIT SILVER NANOPARTICLES AND COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT AND BIOSYNTHESIZED SILVER NANOPARTICLES

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ABSTRACT: The study was performed with Methanolic Periwinkle leaf extract and aqueous Amla fruit extract. The activity was tested on *Staphylococcus aureus* and *Escherichia coli* by Agar Well Diffusion Method. Further, Silver Nanoparticles were synthesized from these Plant extract by Biological Method. Periwinkle leaf and Amla fruit extract showed antimicrobial activity against *E. coli* and *S. aureus* and also showed the synthesis of Silver Nanoparticles. The Biosynthesized SNPs were measured on a colorimeter. The λ_{max} for Periwinkle leaf was found to be 0.32 nm and that 0.65 nm for Amla fruit. This Biosynthesized SNPs of plant extracts was again tested for Antimicrobial Activity using Agar Well Diffusion Method and showed significant inhibition of S. for *S. aureus* and 24 mm for *E. coli*. The results of antimicrobial activity for plant extract of Periwinkle leaf and Amla fruit indicates that they inhibit the growth of *S. aureus* and *E. coli*. Antimicrobial activity of biosynthesized silver nanoparticles was tested against *E. coli* and *S. aureus*; the zone of inhibition obtained by biosynthesized silver nanoparticles of Periwinkle leaf was 37 mm for *S. aureus* and 42 mm for *E. coli*. There was no formation of silver nanoparticles of Amla fruit extract using biological method. The results of antimicrobial activity for the biosynthesized silver nanoparticles indicate that Periwinkle leaf inhibits the pathogenic organism *S. aureus* and *E. coli*. Hence, the comparative study shows inhibition of *S. aureus* and *E. coli* at greater extent.

INTRODUCTION: The use of plants and their extracts to treat different infections is an ancient practice in the traditional world. The practice of traditional medicinal use has been known for centuries worldwide. Numerous plants are used all over the world by many traditional medicine practitioners against infections.

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It is observed that the traditional practices that are to be used vary from country to country. Extract from roots, barks, leaves, and fruits of various plants are used in medicine production. It is well-established practice that different plant extracts can be given singly or as concoctions for various infectious diseases [19]

In 21st century, infectious diseases have the leading cause of death, which is classified by the World Health Organisation, where around 15 million people die every year from infectious diseases. To reduce the rate of death through infectious diseases, many medicines are to be manufactured from natural products.

There are no side effects in using natural products as medicine on the body or any particular organs; hence the use of natural products in medicine is efficient [19].

The study investigated here is antimicrobial activity of the products used are Periwinkle and Amla. Synthesis of silver nanoparticles of these products was performed, and their antimicrobial activity was also tested [19].

Periwinkle: *Catharanthus roseus* is a perennial plant commonly seen in tropical countries. Seven species of this genus are native to Madagascar, and one species is native to southern Asia. It is more commonly known as Madagascar periwinkle. The local name in Malaysia is Kemunting Cina. The national cancer council of Malaysia uses the periwinkle logo as it's a symbol of hope for cancer patients [16].

This plant produces beautiful flowers with a variety of colors such as purple, pink, and white and commonly planted for decorative purposes. Historically periwinkle has been used for various treatments, for example, diabetes mellitus, hypertension, and infections [16].

The stem of periwinkle produces a milky sap, which is the source of over 70 different indole alkaloids. Indole alkaloids contain a moiety of indole, which is related to neurotransmitter serotonin, which is widely implicated for brain function and cognition as the endogenous receptor agonist. An imbalance in serotonin levels may influence mood in a way that leads to depression. Hence the compounds contain indole moiety from plants that can serve as potent antidepressant [16].

Two of the common anticancer drugs which are derived from this plant are Vincristine and Vinblastine. Vincristine is used in the chemotherapeutic regime for Hodgkin's lymphoma, while Vinblastine is used for Childhood leukaemia [16].

Amla: Medicinal plants are a nature's gift to human beings to promote a disease-free healthy life. Many medicinal plants are present in a group of herbal preparations of the Indian traditional healthcare system named Rasayana proposed for their interesting antioxidant activities [18].

Phyllanthus emblica is commonly known as Indian gooseberry or Amla. It is an important herbal drug used in the ayurvedic system of medicine. The plant is used both as a medicine and as a tonic to build up lost vitality and vigour. Amla is highly nutritious and could be an important dietary source of vitamin c, amino acids, and minerals. The plant also contains phenolic compounds, tannins, *etc.* [18]

Various parts of the plant are used to treat a range of diseases, but the most important is the fruit. The fruit is used either alone or in combination with other plants to treat many ailments such as common cold and fever, as a diuretic, laxative, Liver Tonic, refrigerant, stomach ache, restorative, alternative, antipyretic, anti-inflammatory, hair tonic, To prevent peptic ulcer and as a digestive juice [18].

Preclinical studies have shown that Amla possesses antipyretic, analgesic, antihypercholesterolemia, wound healing, antidiarrheal, antiatherosclerotic, nephroprotective, and neuroprotective properties. Amla is also reported to possess Radiomodulatory, chemomodulatory, chemopreventive effects, free radical scavenging, antioxidant, antimutagenic and immunomodulatory activities [18].

Nanoparticles: Nanotechnology is the science that deals with matter and is also the study of manipulating matter at the atomic and molecular scale. In general, the size of nanoparticles spans to the range between 1 and 100 nm. Metallic nano-particles have different physical and chemical properties from bulk metals (*i.e.,* lower melting point, mechanical strength, *etc.*), properties that might prove attractive in various industrial applications [2]. The method for the synthesis of nano-particles can be grouped into physical methods, chemical methods, biological methods, and hybrid methods. The physical method includes a method based on physical grinding or a method based on the generation of materials that are then deposited in the form of nanoparticles. The chemical method is the simplest and includes a method based on reducing metals to form colloids, forming gels, films, and micelles. Biological methods use biological entities like microbes, plants, or animals or even pure bio-molecules such as DNA or proteins. The hybrid methods of synthesis combine two or more of the above methods to synthesize nanoparticles [2].

Most importantly, silver antimicrobial nature has been extensively explored due to its oligo-dynamic action, broad-spectrum killing, and lower possibility of microbial resistance development. Synthesis of silver nanoparticles using a biological system can be roughly divided into the following three steps:

1. Use of microbes like fungi, yeast or bacteria.
2. Use of plant extracts or enzymes.
3. Use of templates like DNA, membranes, viruses, and diatoms.

The overall work, i.e., to evaluate the antimicrobial activity of natural plant products and to synthesize silver nanoparticles from them and determine antimicrobial activity of biosynthesized silver nanoparticles solution of plant extract, is to be tested against the two gram-positive and gram-negative organisms *i.e., Staphylococcus aureus* and *Escherichia coli* [2].

MATERIALS AND METHODS: The study was conducted at the Department of Biotechnology, Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar, District Thane, Maharashtra, India.

Sample Extraction: The samples to be extracted are Periwinkle leaf and Amla fruit. Periwinkle leaves were washed in warm water and macerated in mortar and pestle using the solvent Methanol. The extract mixture is filtered through muslin cloth in the sterile test tube. The test tube containing filtered extract is then heated in a boiling water bath for 15 min; this is used as Periwinkle leaf extract [6, 8].

Amla fruit was cut into pieces and washed in warm water, and macerated in mortar and pestle using the solvent D/W. The extract mixture is filtered through muslin cloth in the sterile test tube. The test tube containing filtered extract is then heated in a boiling water bath for 15 min; this is used as Amla fruit extract [6, 8]. The extract of Periwinkle leaf and Amla fruit were tested for antimicrobial activity against the gram-positive and gram-negative organism *i.e., S. aureus* and *E. coli*. The antimicrobial activity was tested using the Agar Diffusion Method. After 24 h of incubation a clear zone of inhibition was found on Mueller-Hinton agar; both the extract shows inhibition against *E. coli* and *S. aureus* [6, 8]. Synthesis of silver nano-particles was done using Periwinkle leaf and Amla fruit extract. The synthesis was performed using the biological method with the help of silver nitrate. After 24 h of incubation, the diluted extract of Periwinkle leaf and Amla fruit shows a colour change to brown. Brown colour formation indicates the synthesis of silver nanoparticles [7]. The bio-synthesized extract of Periwinkle leaf and Amla fruit silver nanoparticles was again tested for antimicrobial activity against *S. aureus* and *E. coli* using the Agar Diffusion method. After 24 h of incubation, a clear zone of inhibition was found on Mueller-Hinton agar; both the extract shows inhibition against *E. coli* and *S. aureus* [7, 9].

Results:

Plant extract	*S .aureus* Zone of Inhibition	*E .coli* zone of inhibition
Periwinkle	23 mm	47 mm
Amla	32 mm	24 mm

Antimicrobial Activity of Periwinkle Leaf Extract:

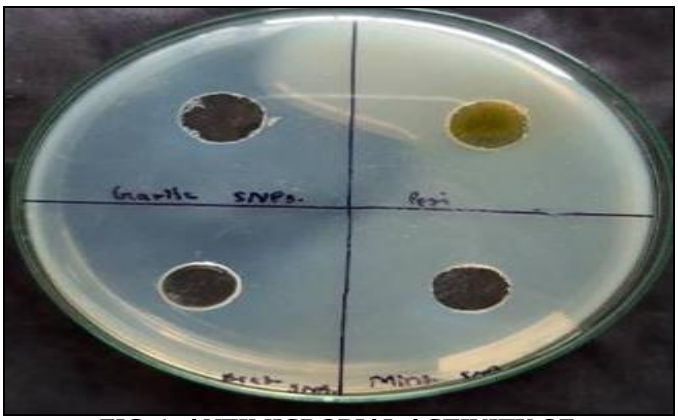


FIG. 1: ANTIMICROBIAL ACTIVITY OF PERIWINKLE LEAF EXTRACT WAS TESTED AGAINST *E. COLI*

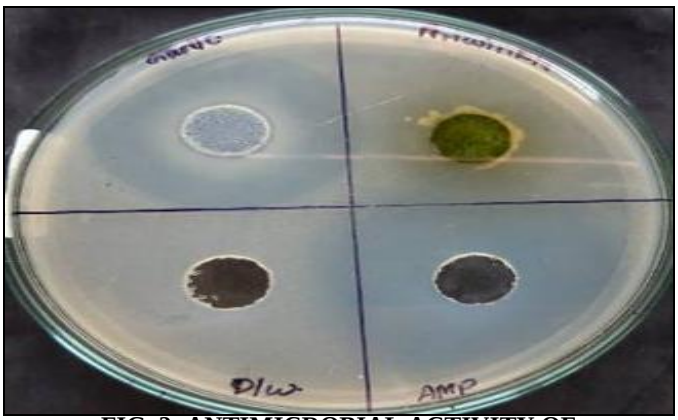


FIG. 2: ANTIMICROBIAL ACTIVITY OF PERIWINKLE LEAF EXTRACT WAS TESTED AGAINST *S. AUREUS*

Antimicrobial Activity of Amla Fruit Extracts:

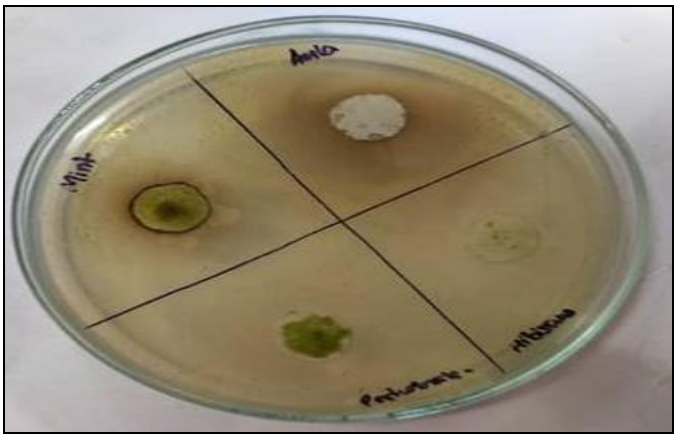


FIG. 3: ANTIMICROBIAL ACTIVITY OF AMLA FRUIT EXTRACT WAS TESTED AGAINST *E. COLI*

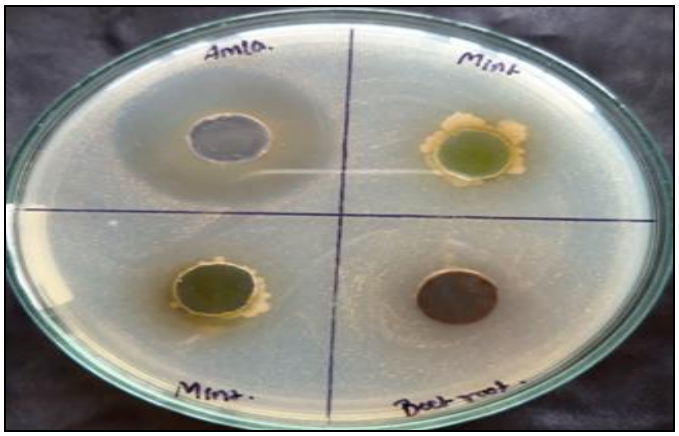


FIG. 4: ANTIMICROBIAL ACTIVITY OF AMLA FRUIT EXTRACT WAS TESTED AGAINST *S. AUREUS*

Synthesis of Silver Nanoparticles:

FIG. 5: SYNTHESIS OF SILVER NANOPARTICLES FROM PERIWINKLE. INITIAL COLOUR BEFORE INCUBATION

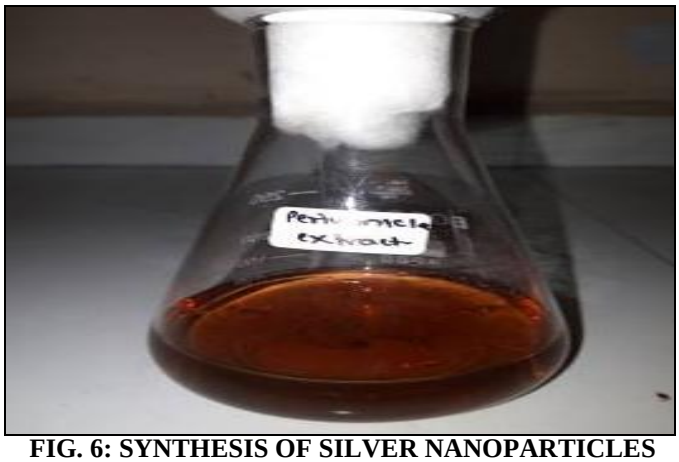
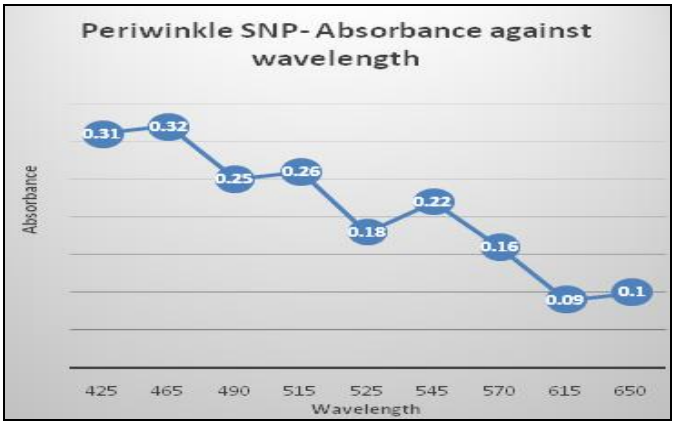


FIG. 6: SYNTHESIS OF SILVER NANOPARTICLES FROM PERIWINKLE. AFTER 24 HOURS OF INCUBATION



GRAPH 1: ABSORPTION SPECTRUM ANALYSIS OF PERIWINKLE

TABLE 1: ABSORBANCE OF PERIWINKLE

Wavelength (nm)	Absorbance
425	0.31
465	0.32
490	0.25
515	0.26
525	0.18
545	0.22
570	0.16
615	0.09
650	0.10

λ_{max}: 0.3

Antimicrobial Activity of Biosynthesized Silver Nanoparticles:

Biosynthesized SNPs of plant extract.	*S. aureus* Zone of Inhibition	*E. coli* Zone of Inhibition
Periwinkle SNPs	37 mm	42 mm

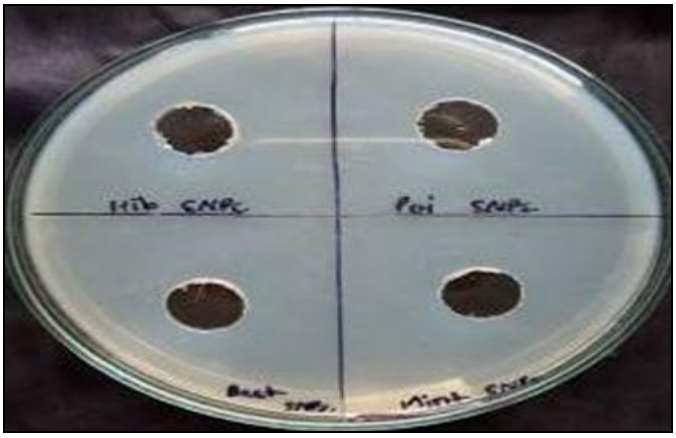


FIG. 7: BIOSYNTHESIZED SNPS OF PERIWINKLE AGAINST *E. COLI*

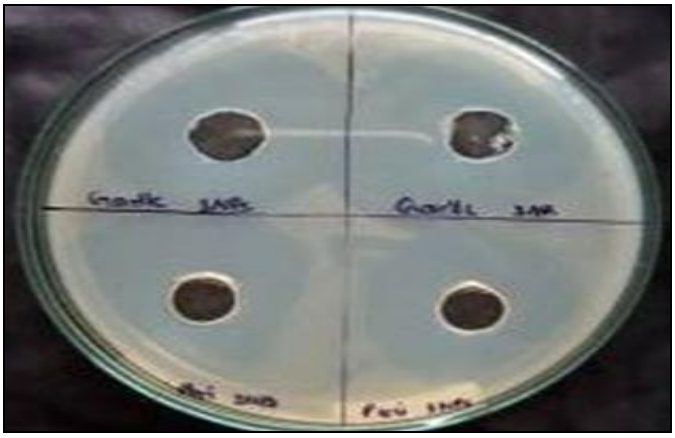


FIG. 8: BIOSYNTHESIZED SNPS OF PERIWINKLE AGAINST *S. AUREUS*

DISCUSSION: Two different plant samples were selected for testing antimicrobial activity, *i.e.*, Periwinkle and Amla. They were collected and extracted separately in a respective solvent, *i.e.*, Methanol and Distilled Water. The extract of Periwinkle leaves, and Amla fruit was extracted and tested for Antimicrobial Activity and shows positive result, *i.e.*, both the extract shows antimicrobial activity against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. A similar study was conducted by several other scientists, and similar results were found. Periwinkle leaf extract was prepared in methanol and was tested for antimicrobial activity against *E. coli* and *S. aureus* using agar well diffusion method. The results were observed after 24 hours of incubation at 37 ºC. The zone of inhibition was found around 47 mm for *E. coli* and 23 mm for *S. aureus*; similar results were found by Gloria Aderonke Otunola, Anthony Jide Afolayan, and Samule Wale Odeyemi [8]. Amla fruit extract was prepared in sterile distilled water and was tested for antimicrobial activity against *E. coli* and *S. aureus* using the agar well diffusion method. The results were observed after 24 h of incubation at 37 ºC. The zone of inhibition was found around 24 mm for *E. coli* and 32 mm for *S. aureus* after 24 hours of incubation at 37 ºC. Similar results were found by Nisrat Jahan and Salma Akter around 10 mm for *E. coli and* 20 mm for *S. aureus* using agar well diffusion method [4].

Synthesis of Silver Nanoparticles was performed for Periwinkle leaf and Amla fruit extract using a biological method, and both the plant extract forms silver nanoparticles with the help of silver nitrate used as a reducing agent. The formation of silver nanoparticles was checked using the colorimetric method. Amla fruit extract does not show the formation of Silver Nanoparticles; this was detected with no color change after 24 h of incubation. The absorbance was monitored on wavelength ranging from 400-700 nm. The λ_{max} was found at 465 nm for both plant extract. Graphical representation for wavelength against absorbance was done. A similar study was performed by Alexandra-Cristina Burdus, *el al.*, Oana Gherasim, Alexandru Mihai Grumezescu, Laurent, iu Mogoantă, Anton Ficai and Ecaterina Andronescu [1]; Md. Mahidul Islam Masum [1], Mst. Mahfuja Siddiqa , Khattak Arif Ali [1], Yang Zhang, Yasmine Abdallah [1], Ezzeldin Ibrahim [1] , Wen Qiu [1], Chenqi Yan and Bin Li [1, 7]; Henry F. Aritonang, Harry Koleangan and Audy D. Wuntu [9]; Ill-Min Chung, Inmyoung Park and Govindasamy Rajakuma [12]; Saba Pirtarighat, Maryam Ghannadnia, Saeid Baghshahi [13]; Mariana Guilger-Casagrande and Renata de Lima [14], Adnan Haider and Inn-Kyu Kang [15].

Antimicrobial activity of Biosynthesized silver nanoparticles was performed using Agar Diffusion Method against gram-positive (*S. aureus*) and gram-negative (*E. coli*) organisms. After 24 hours of incubation, the biosynthesized silver nanoparticles of Periwinkle was tested for antimicrobial activity on sterile Mueller-Hinton media. The media plates of biosynthesized silver nanoparticles were incubated at 37 ºC for 24 h. A clear zone of inhibition was found for biosynthesized silver nanoparticles periwinkle. The zone of inhibition for Periwinkle SNPs was found

to be 37 mm for *S. aureus* and 42 mm for *E. coli*. Periwinkle leaf extract and biosynthesized SNPs show inhibition against *Staphylococcus aureus* and *Escherichia coli*. A similar study was performed by Hoda Soliman, Ashraf Elsayed, Amira Dyaa [3]. Whereas Amla fruit extract shows inhibition against *Staphylococcus aureus* and *Escherichia coli* because it does not form silver nanoparticles; hence further study to check Antimicrobial activity of Biosynthesized Silver Nanoparticles was not performed [6, 11].

CONCLUSION: Two samples were processed to check for Antimicrobial Activity; they are Periwinkle leaf extract and Amla fruit extract. The organisms used were the pathogenic Gram Positive and Gram Negative bacteria *i.e., Staphylococcus aureus* and *Escherichia coli*. The crude extracts of Periwinkle leaf and Amla fruit were tested for Antimicrobial Activity using the Agar Diffusion Method. After 24 h of incubation at 37 ºC on Mueller Hinton Agar, a clear zone of inhibition was found by both organisms. Periwinkle leaf extract shows the zone of inhibition around 23 mm for *S. aureus* and 47 mm for *E. coli*, and that of Amla fruit extract shows the zone of inhibition around 32 mm for *S. aureus* and 24 mm for *E. coli*. This signifies that both Gram Positive (*S. aureus*) and Gram-Negative (*E. coli*) bacteria can be inhibited by Periwinkle leaf and Amla fruit, which are natural plant products. Hence it can be suggested that Periwinkle leaf and Amla fruit can be used against infection or diseases caused by *S. aureus* and *E. coli*. As these are natural plant products, they do not show any side effects. Periwinkle leaf and Amla fruit extract were also studied for the Synthesis of Silver Nanoparticles with the help of Silver Nitrate using the Biological Method. After 24 h of incubation, the formation of Silver Nanoparticles was detected by the brown color formation of periwinkle leaf extra. Amla fruit extract does not show any color change; hence no synthesis of Silver Nanoparticles was concluded.

The Biosynthesized Silver Nanoparticles of Periwinkle leaf were again tested for Antimicrobial Activity against the same organisms, *i.e., Staphylococcus aureus* and *Escherichia coli* using Agar Diffusion Method. After 24 h of incubation, Periwinkle leaf SNP shows a clear zone of inhibition. The zone of inhibition of Periwinkle leaf SNP was found around 37 mm for *S. aureus* and 42 mm for *E. col*i. The zone of inhibition of Periwinkle leaf SNP for *S. aureus* is more than Periwinkle leaf extract, and the zone of inhibition Periwinkle leaf extract for E. coli is more than Periwinkle leaf SNPs, but inhibition is at a greater extent. Hence Periwinkle leaf can be proved as a good medicinal remedy whether it is used directly or in the form of Silver Nanoparticles. Synthesis of Silver Nano-particles was not found from Amla fruit extract; hence further study to check Antimicrobial activity of Biosynthesized Silver Nanoparticles was not performed. Therefore the comparative study of Amla cannot be shown. Still, Amla fruit extract shows inhibition at a greater extent, hence proved to be a good medicinal remedy and can be used against infectious diseases caused by *S. aureus* and *E. coli*.

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CONFLICTS OF INTEREST: Nil

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