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Green Synthesis of Silver Nanoparticles from *Euphorbia heterophylla Linn*. Plant Latex and their Possible Applications as Antimicrobial Agent in the Agricultural Area

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A B S T R A C T

Presently, metallic nanoparticles have various uses for special scientific, pharmaceutical, and Agricultural applications. Nanobiotechnology, blended with green chemistry, has awesome capacity for the improvement of novel and important products that advantage human fitness, surroundings environment, and Industries. Green chemistry has a crucial position because of its contribution to the unconventional synthesis methods of silver nanoparticles from plant latex extracts from *Euphorbia heterophylla*, that have exhibited antimicrobial Capacity, among other top outstanding properties. The Fresh *E. heterophylla* latex was collected from Mandalavadi Village, Tirupattur district, Tamil Nadu, India. The confirmation of silver nanoparticles by UV-Vis Spectrophotometer, Fourier Transform Infrared Spectroscopy, and SEM-EDAX. The UV-Vis Spectral investigation of integrated AgNPs showed a peak. The FTIR characterization studies presence of biomolecules in the plant latex play a double part in synthesis and confirmation of silver nanoparticles. The Scanning Electron Microscope technique was studied to investigate the morphological characteristics. And EDAX spectra, plainly silver nanoparticles diminished by *E. heterophylla* have the weight percentage of Silver, Oxygen and Carbon. Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes. Various antimicrobial agents are used for this purpose. The synthesized silver nanoparticles are used in the agricultural field. *E. heterophylla* plant latex is naturally present in a small amount of antibacterial and antifungal activity.

***Key words*:** *Euphorbia heterophylla,* Plant latex, Green Synthesis, AgNps, UV-Vis spectrophotometer, FTIR, SEM-EDAX, Antimicrobial activity

Therapeutic plants assume a critical part in giving essential, medical care administrations to provincial individuals and are utilized by about 80% of the minor networks on the planet [1-3]. Therapeutic plants are vital to the wellbeing of people and networks as a rule. The therapeutic estimation of plants lies in some compound substances that produce a distinct physiological activity on the human body. Large numbers of the native therapeutic plants are utilized as flavors and food plants. They likewise once in a while added to food sources implied for pregnant ladies and nursing moms for therapeutic purposes [4]. Likewise, the utilization of homegrown medication for the treatment of sicknesses and diseases is just about as old as humanity. The World Health Organization upholds the utilization of customary medication gave they are demonstrated to be adequate and safe. In non-industrial nations, countless individuals live in outrageous neediness and some are languishing and biting the dust over the need for safe water and medication, they have no option for essential medical services [5].

The therapeutic handiness of the plant *Euphorbia heterophylla* has been the object of various synthetic and pharmacological investigations. *E. heterophylla* is a therapeutic plant with the normal name "spurge weed". It fills in semi-sticky places particularly in cassava, cowpea, and Soya bean manors. Report of past compound investigation on *E. heterophylla* is be that as it may, inadequate. *Euphorbia heterophylla L.* (wild poinsettia), is a tropical local plant. In currently subtropical America [6], is generally spread in a significant weed in any event 28 tropical nations and it is available in 37 more [7]. Wild poinsettia is a significant weed mainly in soybean fields in Brazil [8-12]. The year of 1970s in the United States it has been perceived as a significant weed in soybean in Louisiana since the last part. [13-17]. It has kept on expanding in dissemination and commonness during late years. Georgia and Florida in currently viewed as a weed of

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major financial significance in both nut and cotton. Nanotechnology is a field of science that manages the creation, control, and utilization of materials running in nanometers. With the progression of advances and improved logical information, a route for innovative work in the field of natural and therapeutic plant science towards the crossing point of nanotechnology has been noticed. One such impedance is applying plants source in the green amalgamation of nanoparticles. Nanoparticles can be effortlessly incorporated utilizing different strategies by different methodologies accessible for the blend of silver nanoparticles incorporate synthetic, electrochemical, radiation, photochemical strategies and Langmuir-Blodgett, and natural procedures. In any case, the greater part of the compound strategies utilized for the union of nanoparticles includes the utilization of harmful, dangerous synthetics that make organic dangers, and at some point, these substance measures are not eco-accommodating. This improves the developing need to grow harmless to the ecosystem measures through the green blend and other natural methodologies. Here and there the union of nanoparticles utilizing different plant materials and their concentrates can be gainful over other natural blend measures which include the intricate techniques of keeping up microbial societies. Silver Nanoparticles have been portrayed as a treatment agent for some infections and an effective antimicrobial agent.

In the course of recent many years, therapeutic plants have generally been read with the end goal of the alleviation and the treatment of different irresistible infections because microbial protections against customarily utilized manufactured antimicrobial specialists are expanding at a disturbing rate. Because of the nanotechnological blast, unordinary physical, synthetic, and organic strategies have been created for the combination and creation of metal NPs [18-23]. Accordingly, the curiosity of this paper lies in portraying the significant detailed green combination of AgNPs from plant separates their ability as antimicrobial specialists inside the agricultural field for battling against bacterial also, fungi microorganisms that can cause plant, waterborne, and foodborne sicknesses. Additionally, this work makes a concise audit of AgNPs commitment to water treatment and the improvement of "harmless to the ecosystem" Nano-fertilizers, Nano-pesticides, and Nano-herbicides, just as depicting the hurtful impacts of NPs collection in plants and soils.

MATERIALS AND METHODS

Collection of plant material

The *Euphorbia heterophylla* plant picture shows in (Fig 1. The Fresh *E. heterophylla* latex was collected from Mandalavadi Village, Tirupattur district, Tamil Nadu, in February 2021. The fresh plant latex was obtained from the stems and the leaves. The Plant latex was collected separately in sterile tubes in the early morning. The collected plant latex was lyophilized, weighed, and stored at 4°C until further use.

Green synthesis of silver nanoparticles

An aqueous solution of silver nitrate (1 mM) was prepared in double distilled water. Lyophilized latex ranging from 10 to 100 mg from plants was added in 25 ml of $AgNO_3$ (100 ppm) solution in separate vials and incubated at 37°C in dark with constant stirring for 30 min. During incubation, $AgNO_3$ solution showed a color change from milky white to reddish-brown.

Fig 1 *Euphorbia heterophylla* plant

Characterization of silver nanoparticles

The confirmation of silver nanoparticles by UV-Vis Spectrophotometer, Fourier Transform Infrared Spectroscopy, and SEM-EDAX.

UV-Vis spectrophotometer

The prepared AgNPs were perceived under UV-Vis spectrophotometer (Shimadzu-2700) detected by its maximum absorbance and wavelength. The stability of AgNPs from plant latex extract was recorded between 300-700 nm.

FTIR

Possible functional groups involved in the synthesis and stabilization of AgNPs were studied by Fourier Transform Infrared Spectroscopy.

SEM-EDAX

To determine the morphology of the synthesized silver nanoparticles using plant latex, the sample was analyses with Carl Zeiss Microscopy (SEM), integrated with Quorum Technologies (EDAX).

Isolation and identification of bacteria

Xanthomonas citri subsp. citri was isolated from citrus canker disease infected lemon leaf from Mandalavadi village, Tirupattur district, Tamil Nadu. The sample of a leaf of lime plants displaying Citrus canker symptoms (figure 2) was washed under running tap water. The lesions on each sample were cut into approximately 5 × 5 mm pieces with a sterilized scalpel and immersed in 0.6% NaOCl (Citric acid as an alternative to sodium hypochlorite) for 3 min followed by three rinses in sterile distilled water. The surface-sterilized samples and 2 ml of sterile 0.85% NaCl were combined and crushed in a sterilized mortar. The bacterial suspension was streaked in a sterilized loop on nutrient glucose agar (NGA) and then incubated at room temperature (28°C–32°C) for 2 to 3 days. Morphological characteristics regarding the shape and color of colonies, gram staining, cell shape, and cell arrangement were determined. The bacteria were streaked on NGA plates to confirm purity. The motility test to determine the motility of bacterium. The presence of bacteria was determined according to some biochemical tests described [24-25].

Fig 2 Citrus canker disease infected lemon leaf

Gram staining

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. Prepare thin smear of given bacteria on a clean glass slide. Allow the smear to air dry and fixed with heat. Place the slide on the slide rack for stating. Flood the smear with crystals violet and allow it for 30 seconds to one minute. Wash the smear with distilled water for few seconds using running water. Stain the smear with Iodine solution for one minute. Wash Iodine solution with 95% Ethyl alcohol. Add ethyl alcohol drop wise, until no more color flows from the smear. Wash the slide with distilled water and drained properly. Stain the smear finally with counter stain safranin for 30 seconds. Wash the slide with distilled water and dried properly. Observe the slide under the compound microscope.

Motility test

To determine the motility of bacterial cells. Place a small drop of culture in the center of a coverslip and place a little Vaseline in the cavity slide over the coverslip with the hanging drop suspended in the depression. Examine the preparation under microscope first under 4 X followed by 40 X and 100 X magnification.

Biochemical tests

Catalase test

Place a microscope slide inside a petri dish. Keep the petri dish cover available. Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18 – 24 hours colony and place it onto the microscope slide. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction. Using a dropper or Pasteur pipette, place 1 drop of 3% H_2O_2 onto the organism on the microscope slide. Do not mix. Immediately cover the petri dish with a lid to limit aerosols and observe for immediate bubble formation (O_2 + water = bubbles). Observing for the formation of bubbles against a dark background enhances readability [26].

Urease test

Urease test was used to determine the ability of an organism to split urea through the production of the enzyme urease [27]. Media were autoclaved at 121°C for 20 min. Then autoclaved medium was poured into two test tubes and kept in a rack in slant condition. The surface of a well-isolated colony and the tube was incubated at 37°C for 48 hours.

Isolation and identification of fungi

The infected citrus plants are raised spongy, pustules start a pinkish color (these pustules are slightly raised and pink to light brown in color) shows in (Fig 3a) and become grayish (Fig 3b-c). Citrus scab affects virtually all citrus types and also appears on leaves, stems, and twigs. *Elsinoe fawcetti* was isolated from citrus scab disease infected lemon leaf and fruit from Mandalavadi village, Tirupattur district, Tamil Nadu. The sample of leaf and fruit of lime plants displaying citrus scab symptoms (Fig 3a-c) were washed under running tap water. The lesions on each sample were cut into approximately 3 × 3 mm pieces with a sterile scalpel. These pieces were surface sterilized by sequential rinsing into 70% ethanol (C_2H_5OH) for 30 sec, 0.01% mercuric chloride ($HgCl_2$) for 5 mins. Then the pieces were surface sterilized with 1% of Sodium hypochlorite solution for 30 seconds and followed by three rinses in sterile distilled water. Then allowed to dry under sterile condition. Then the pieces were slightly crushed in a sterilized mortar. The fungi isolation by serial dilution method. The serial dilution method is one of the oldest and usable methods which is used for the isolation of fungi. In this method, we collected our desire samples (leaf and fruit skin) and make them in the test tubes (Master Test Tube). We inoculated the sample from the diluted test tubes in the prepared medium plates by using the pure plate method or spread method and then incubated the inoculated plates in incubated at 25-30°C for 2-3 days. The isolated fungi make a subculture from colonies of fungi. Then using further investigations.

Fig 3(a) Fig 3(b) Fig 3(c)

Fig 3(a), (b), and (c): Fungal infected citrus plant fruits and leaves

LPCB Staining

In a new report, better microscopic morphology of fungi was observed using Lactophenol Cotton Blue (LPCB) wet mount method. This LPCB staining helps to identify the fungal structures. The fungal spores and hyphae were appeared as pale to dark blue. Place a drop of 70% ethanol on a clean microscopic glass slide. Immerse the specimen in the drop of alcohol. Add one or at most two drops of the LPCB before the alcohol dries out. Holding the coverslip between the index finger and thumb, touch one edge of the drop of mountant with a coverslip edge and lower gently avoiding air bubbles. This preparation is now ready for examine. Make the initial examination using low power objective. Switch to higher power (40X) objective for more detailed examination of spores and other structures.

Antimicrobial activity

Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, and anti-fungal activity. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plant extracts [28-29].

Antibacterial activity

The antibacterial activity of the *Euphorbia heterophylla* plant latex and greenly synthesized AgNPs was investigated on bacteria strain namely *Xanthomonas citri subsp. citri.* The bacteria were isolated from Citrus canker disease infected lemon leaves.

Agar well diffusion method

Similar to the procedure used in the disk-diffusion method, the nutrient agar plate surface inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm has punched aseptically with a sterile cork borer or a tip, and a volume (50 µL) of the silver nanoparticles and plant latex extract solution at desired concentration is introduced into the well. And the positive control tetracycline disc kept in the agar plate surface. Then, agar plates are incubated at 37°C for 24 hours. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial stain tested.

Antifungal activity

The antifungal activity of the *Euphorbia heterophylla* plant latex and greenly synthesized AgNPs was investigated on a fungal strain namely *E. fawcettii.* The fungi were isolated from Citrus scab disease infected lemon leaf and fruits.

Agar well diffusion method

Similar to the procedure used in the disk-diffusion method, the Sabouraud dextrose agar (SDA media) plate surface inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm has punched aseptically with a sterile cork borer or a tip, and a volume (50 µL) of the silver nanoparticles and plant latex extract solution at desired concentration is introduced into the well. The standard positive control is used as a fluconazole drug. Then, agar plates are incubated at 25-30°C for 5-7 days. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial stain tested.

RESULTS AND DISCUSSION

Green synthesis and characterization of silver nanoparticles

The current assessment deals with the green synthesis of silver nanoparticles (AgNPs) utilizing *E. heterophylla* plant latex. Silver nitrate ($AgNO_3$) is used as reducing agent as a silver has specific properties like great conductivity, reactant and chemical stability. Figure 4 shows the picture of *E. heterophylla* plant latex preceding response with silver nitrate and color changes during the response of Ag^+ to Ag nanoparticles (Fig 5). Visual perception of the $AgNO_3$ solution (1 mM) tested with latex (10-100 mg) showed a color change from milky white to brown.

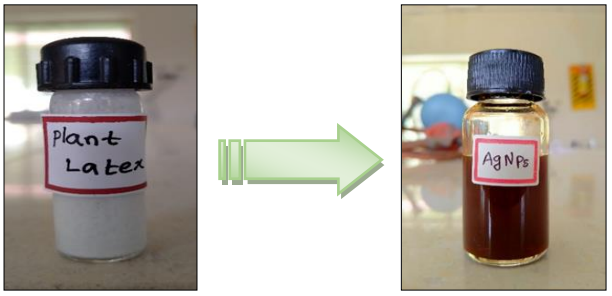

Fig 4 Plant latex sample Fig 5 AgNPs

UV-Vis spectroscopy

The presence of brown color in latex treated $AgNO_3$ solution was the primary sign of the presence of AgNPs. The first identification of synthesized silver nanoparticles by UV-vis spectroscopy has been demonstrated to be an exceptionally valuable technique for exploring nanoparticles. The UV-Vis Spectral investigation of integrated AgNPs showed a peak at 426 nm (Fig 6). A similar kind of result was observed in the synthesis of AgNPs from Pomelo (*Citrus Maxima*) [30]. An aqueous solution of AgNPs synthesized by latex from *E. heterophylla* was found to be a dark red-brown color.

FTIR

The FTIR examination was done to distinguish the potential biomolecules in *E. heterophylla* latex liable for lessening and capping the silver nanoparticles. The FTIR range showed the different peaks at 3390, 2925, 2853, 1735, 1636, 1415, 1364, 1244, 1025 cm^{-1}. The FTIR spectrum expressed that the absorption band at 3390 cm^{-1} are might be because of the presence of hydrogen bond N-H stretching, and characteristic for amino acids. The peak at 2925 cm^{-1}, relating to C-H stretching of the CH_2 groups, shows the presence of different amino acids, this peak may also be characteristic of the presence of aliphatic CH groups in these compounds. The peak at 2853 cm^{-1} alludes to the C-H balanced stretch vibration of alkanes. The peak at 1735 cm^{-1} showed carbonyl C=O stretch of esters likewise found in *P. nigrum* [31] leaves biosynthesized AgNPs. The peak at 1636 cm^{-1} corresponding to enol stretching frequency. The (Fig 7) shows an interesting peak at 1415 cm^{-1} for *E. heterophylla* latex. The peak is allotted to C=C ring stretching, CH_2 scissoring, lactone ring deformity, and OH out of plane deformity mode of vitamin C (ascorbic acid), respectively. Gum Arabic-capped silver nanoparticles showed disappearance of peak at 1364 cm^{-1}. The absorbance band at 1244 cm^{-1} was seen in (Fig 7). A sharp peak at 1025 cm^{-1} might be because of the - C-O stretching vibration from gelatin. In view of these FTIR Characterization studies

presence of biomolecules in the plant latex play a double part in synthesis and confirmation of silver nanoparticles.

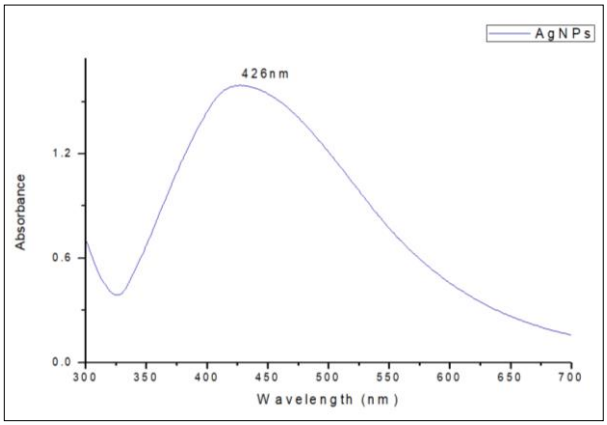

Fig 6 UV-Vis Spectra of AgNPs

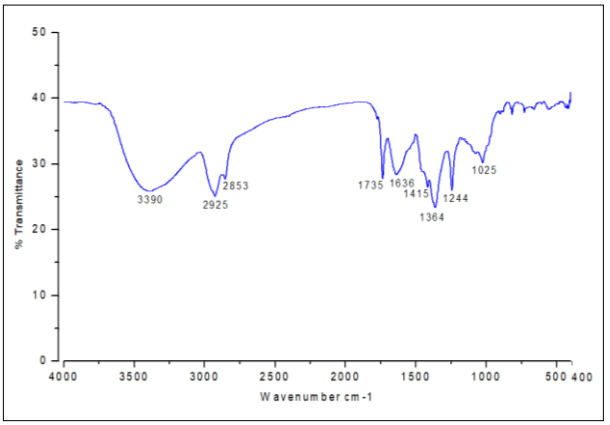

Fig 7 FTIR spectrum analysis of AgNPs

SEM-EDAX

The Scanning Electron Microscope technique was studied to investigate the morphological characteristics. The SEM image shows the AgNPs appearance in figure 8. The Scanning Electron Microscope (SEM) technique analyzes to show in most of the silver nanoparticles. The image shows the predominately spherical shape and well scattered with a close compact arrangement. The synthesized silver nanoparticles size was seen to be 20 to 70 nm. An EDAX spectrum recorded from the silver nanoparticles was shown in Figure 8. The EDAX range recorded showing a peak at 3KeV. From EDAX spectra, plainly silver nanoparticles diminished by *E. heterophylla* have the weight percentage of silver as 72.29%. Except for silver, we likewise show the presence of carbon and oxygen, the contents of which amounted to 17.53% and 72.29%, respectively.

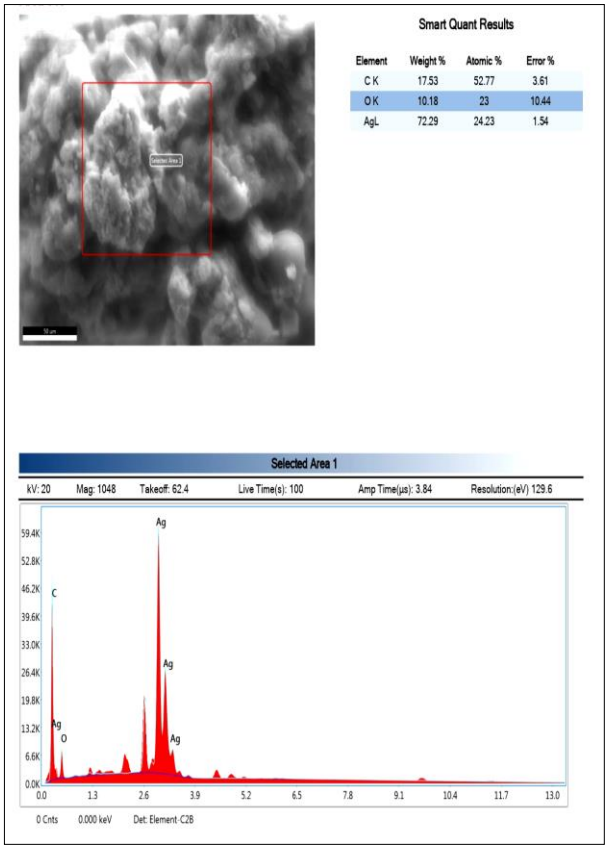

Fig 8 SEM-EDAX analysis of AgNPs

Fig 9 Bacterial colonies

Isolation and identification of bacteria

The Nutrient Agar medium used to isolation of bacteria from lemon disease affected leaf or fruit skin tissue segment. The streak plate technique was used to bacterial colonies purify on the Nutrient Agar medium. The individual bacterial colonies were seen observed as creamy yellow (Fig 9). The reason for yellow color colonies is Xanthomonadin. The Nutrient Agar medium was used to maintain the bacterial colonies for further investigations. An identification of presumptive *X. citri subsp. citri* colonies should be checked by a few methods to recognize the microorganisms. The techniques in addition to observing morphological characteristics, Motility testing, Biochemical tests, and Antibacterial Activity. The bacterial identification results show in (Table 1).

Isolation and Identification of fungi

Elsinoe fawcettii develop gradually, need broad airborne hyphae, and form fungal colonies with red or brown color (Fig 10). In a new report, better microscopic morphology of fungi was observed using Lactophenol Cotton Blue (LPCB) wet mount method. During microscopic visualization of this slide, it reveals a blue color-stained fungi spores, hyphae, and fruiting designs

against the light blue color background. This Lactophenol Cotton Blue (LPCB) staining helps to identify the fungal structures. The fungal spores and hyphae were appeared as pale to dark blue.

Table 1 Identification of bacteria

Characteristics	Bacteria isolated from citrus canker disease on lemon	*Xanthomonas citri subsp. citri* [32]
Gram staining		
(i) Cell shape	Rod shape	Rod shape
(ii) Color	Pink color cells were observed. The pink color cells are called Gram-negative bacteria	Pink color cells and the pink color cells are called Gram-negative bacteria
Motility test	+	+
Biochemical tests		
(i) Catalase test	+	+
(ii) Urease test	-	-
Antibacterial activity	Zone was formed	Zone was obtained

Fig 10 Fungal colonies

Antimicrobial activity

Antibacterial activity

Results got in the current investigation revealed that the plant latex and silver nanoparticles have possible antibacterial activity against *X. citri subsp. citri*. The silver nanoparticles antibacterial activity against isolated bacteria *X. citri subsp. citri* has a most maximum inhibition zone of 7 mm in figure 11 (50 µL of concentration). The plant latex antibacterial activity against isolated bacteria *X. citri subsp. citri* has an inhibition zone of 4 mm in (Fig 11) (50 µL of concentration). The positive control tetracycline showed an average inhibition zone of 8 mm in both concentrations.

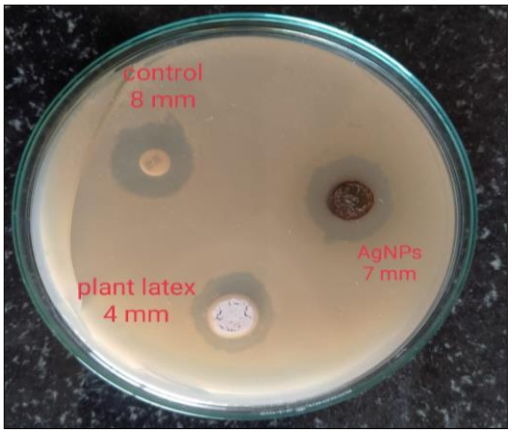

Fig 11 Antibacterial activity against *X. citri subsp. citri*

Antifungal activity

Results got in the current investigation revealed that the plant latex and silver nanoparticles have possible antifungal activity against *Elsinoe fawcettii*. The silver nanoparticles antifungal activity against isolated fungus *Elsinoe fawcettii* has a most maximum inhibition zone of 8 mm (50 µL of concentration). The plant latex antifungal activity against isolated fungus *Elsinoe fawcettii* has an inhibition zone of 1 mm (50 µL of concentration). The inhibition zone is shown in the (Fig 12). The standard positive control drug fluconazole showed an inhibition zone of 10 mm.

Fig 12 Antifungal activity against *Elsinoe fawcettii*

Agricultural applications

The synthesized silver nanoparticles are used in the agricultural field. *E. heterophylla* plant latex is naturally present in a small amount of antibacterial and antifungal activity. Using plant latex to synthesized silver nanoparticles which enhances the antibacterial and antifungal activity. So silver nanoparticles are used for bacterial disease like citrus canker and fungal disease like citrus scab disease against use to inhibit the growth of microorganism.

CONCLUSION

In the current investigation, we report an eco-friendly, non-harmful, practical strategy for the synthesis of silver nanoparticles was carried out utilizing a bio reducing agent obtained from *Euphorbia heterophylla* plant latex. The characterization results obtained from UV-Vis spectrometer, FTIR, and SEM-EDAX investigation revealed the presence of plant latex on the surface of the nanoparticles, showing that the plant latex was efficient in reducing the silver salt to silver nanoparticles. The UV-Vis Spectral investigation of integrated AgNPs showed a peak at 426 nm. The FTIR

peaks affirm the presence of biomolecules like secondary metabolites. SEM-EDAX investigation shown the affirmation of silver nanoparticles. The synthesized silver nanoparticles showed the spherical shape analyses by Scanning electron microscope. EDAX investigation completed affirming the presence of the silver element in green synthesized silver nanoparticles. The synthesized silver nanoparticles and plant latex showed antimicrobial activity. So synthesized silver nanoparticles utilized in an agricultural field in bacterial and fungal disease against.

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