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Green Synthesis of Silver Nanoparticles (AgNPs) Using *Muntingia Calabura* and its Antibiogram Effects on Microorganisms Isolated in Drinking Water Samples Collected From Water Filters

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Abstract: ***Water is the mother liquor of all forms of life. The essentiality of water for living systems is very evident and without water there is no life. The water should be microbiologically safe for drinking. Hence it requires a thorough analysis before use. Green synthesis of nanoparticles is a promising area, because of its applications in the field of physics, chemistry, biology and medicine. The synthesized nanomaterials are used in medicinal and technological aspects. Biological synthesis also called green synthesis of nanoparticles where biological enzymes from plant extracts, fungi, bacteria are used. This method is eco-friendly and reduces toxicity and waste production.***

Keywords: Water, Nanoparticles, Green synthesis, Antibiogram effect

1. Introduction

Water is the mother liquor of all forms of life. The essentiality of water for living systems is very evident and without water there is no life. The water should be microbiologically safe for drinking. Hence it requires a thorough analysis before use. Water is very essential to life, but many people do not have access to safe drinking water., many die of water borne bacterial infections. Improving access to safe drinking water may results significant benefits to health. The major problem associated with ingestion of water contaminated with animal or human faeces. Safe water means water free from pathogenic organisms (George *et al*., 2002)

The emergence of nanotechnology has provided a vast research area in recent years by intersecting with various other branches of science and forming impact on all forms of life. Nanoparticles have expressed significant advances owing to wide range of applications in the field of bio-medical, sensors, antimicrobials, catalysts, electronics, agricultural, bio-labeling and in other areas. Nanoparticles are particles between 1 and100 nanometers (nm) in size with a surrounding interfacial layer. In nanotechnology a particle is defined as a small object that behaves as a whole unit with respect to its properties and transport. Green synthesis of nanoparticles is a promising area, because of its applications in the field of physics, chemistry, biology and medicine. The synthesized nanomaterials are used in medicinal and technological aspects.

There are several methods used for the synthesis of silver nanoparticles. It includes both physiochemical and biological methods. Examples, solution reduction methods (Goia *et al*., 1998), photo chemical reaction (Taleb *et al.*, 1997), thermal decomposition of silver compounds (Esumi et al., 1990), and biological methods or green synthesis include production of nanoparticle using bacteria (Saifuddin *et al*., 2009), fungi (Bhainsa *et al*., 2006), and using enzymes (Willner *et al*., 2007).

Biological synthesis also called green synthesis of nanoparticles where biological enzymes from plant extracts, fungi, bacteria are used. This method is eco-friendly and reduces toxicity and waste production. Silver nano particles synthesized by green chemistry methods offer a novel and potential alternative to chemically synthesized nano particles. The present study focus on the synthesis of silver nanoparticle by an aqueous leaf extract of *Muntingia calabura*. These silver nanoparticles were found to be extremely effective against different bacterial and fungal pathogens. The common name for *Muntingia calabura* is calabur tree, panama berry, ornamental cherry, jam fruit tree, Singapore cherry, West Indian cherry. It belongs to the family muntingiaceae. It is a shrub or tree up to 12 meter tall with spreading branches (Lim *et al*., 2012)., the leaves of the *Muntingia calabura* are alternate oblong or lanceolate, In the present scenario deals with the development of cheaper and effective plant based silver nanoparticles with better bioactive potential and less side effects.

2. Materials and Methods

Sample collection

100 ml of water sample was collected from 25 water filters after sterilizing the tap. Water is allowed to flow for few minutes and the sample was collected in a sterile conical flask and transported to the laboratory for further analysis.

Identification and Characterization

Isolated colonies were sub cultured on Mac Conkey agar. The medium was weighed as 5.3g and dissolved in 100ml of distilled water and add 1g of agar. After the sterilization the media was poured in to sterile petri plate and were allowed to solidify. The plates are incubated and the colony

morphology were observed by performing Gram staining and Motility test.

Biochemical characterization
Isolated bacteria were further identified by various biochemical testS.

Identification of fungal isolates
The isolated fungal cultures morphologically analyzed by lacto phenol cotton blue staining. A drop of lacto phenol cotton blue was placed on the center of a clean glass slide. A portion of culture from fungal colony was transferred into the drop of mounting fluid within the help of flamed and cooled needle. The culture was gently spread with the help of two needles. Coverslip was placed over the wet mount preparation without trapping air bubble. Excess stain was blotted with blotting paper and microscope first by low power objective and then high power objective.

Collection of leaf samples from *M. calabura*
Leaves that appeared healthy were collected from different branches of *Mutingia calabura* from Coimbatore, Tamil nadu. The plant leaves were collected and brought to the laboratory in a sterile container.

Preparation of plant extract
The leaves was taken and subjected to aqueous extract preparation, 25g of green tender leaves were thoroughly washed with tap water followed by double distilled water twice. The leaves are cut in to small pieces and were boiled in 100ml of distilled water. After 15 minutes the aqueous extract was filtered through What man No.1 filter paper. The aqueous extract of the plant used as reducing agent for the synthesis of silver nano particles.

Preparation of silver nitrate solution
A concentration of 1M $AgNO_3$ solution was prepared by dissolving 0.169 $AgNO_3$ in 1000ml of double distilled water and used for the green synthesis of silver nano particles (AgNPs).

Green synthesis of AgNPs
The filtered aqueous extract of *M. calabura* leaves was added individually to 90 ml of 1M $AgNO_3$ in a 250 ml Erlenmeyer flask. Then kept in room temperature for 48hrs at dark. The process was continued till the change of colour occurred from yellow to dark brown indicating the completion of silver nanoparticle synthesis.

Antibacterial activity
Antibacterial activity of sample was determined by well diffusion method on Muller Hinton Agar (MHA) medium. The Muller Hinton Agar medium was weighed as 3.8 gms and dissolved in 100 ml of distilled water and add 1 gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify. After the medium was solidified, the inoculums were swabbed on the MHA plates with sterile swab moistened with the bacterial suspension. Wells were made by using cork borer. Different concentration of samples (20µl, 40µl and 60µl) and positive control (streptomycin 1mg/ml-10µl) were loaded in respective wells. These plates were incubated for 24 hours at 37^0C. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

Antifungal activit

Preparation of inoculam
Stock cultures were maintained at 4^0C on slant of potato dextrose agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of potato dextrose broth for fungi that were incubated at 4 days at room temperature. The assay was performed by agar diffusion method. Antifungal activity of sample was determined by well diffusion method on Potato Dextrose Agar (PDA) medium.

The Potato Dextrose Agar medium was weighed as 4.4gms and dissolved in 100 ml of distilled water and add 1 gm. of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify for 1 hr. After the medium was solidified, the inoculums were swabbed on the PDA plates with sterile swab moistened with the fungal suspension. Wells were made by using cork borer. Different concentration of samples (20µl, 40µl and 60µl) and positive control (Griseofulvin 1mg/ml-10µl) were loaded in respective wells. These plates were incubated for 48-96 hours at 28^0C. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

3. Result and Discussion

Microbiological analysis
Out of 25 samples 5 samples showed the presence of bacterial isolates. Out of 5 two were aerobic spore formers and remaining 3 were identified as *Escherichia coli*, *Staphylococcus* sp., and *Pseudomonas* sp. Two fungal isolates namely*Aspergillus*sp and *Candida albicans* were also isolates (Table 1)

Table 1: Microbiological analysis

Serial No.	Sample Results
1	No growth
2	No growth
3	No growth
4	No growth
5	No growth
6	Bacillus sp.
7	No growth
8	No growth
9	Bacillus sp.
10	No growth
11	No growth
12	*E. coli*
13	No growth
14	No growth
15	No growth
16	*Staphylococcus* sp.
17	No growth
18	No growth
19	*Pseudomonas* sp.
20	No growth
21	*Aspergillus* sp.
22	No growth
23	No growth

24	*Candida albicans*
25	No growth

Fungal identification

The isolated fungal cultures were identified as *Aspergillus* sp and *Candida albicans* based on the morphological characters under the light microscope and colony morphology on the growth medium employed. *Aspergillus* sp easily grow on PDA at room temperature. Colonies starts white to pale yellow but quickly form jet black conidia. The conidia are spherical and roughen with maturity. *Candida albicans* are small. Oval shaped colonies with 2-4µm in diameter.

Biological Synthesis Of Silver Nanoparticles

Silver nano particles were syntheised by using aqueous extract of *Muntingia calabura* leaves. The plant extract were pale yellow in colour before the addition of silver nitrate solution. The synthesis of silver nano particles exhibited as the colour change from yellow to brown. The samples were observed periodically for the change of colour from pale yellow to different shades of brown.

Antimicrobial assay

Antimicrobial assays are important tools to test and screen the inhibitory effect of compounds against microorganisms before establishing their inhibitory spectra. Determine the antibacterial activity; and determine the reduction of microorganisms within a specified time period upon exposure to an antimicrobial item or substances.

Antibacterial activity

The inhibitory effect of silver nanoparticles synthesized from the aqueous leaf extract of *M. calabura* were tested by using well diffusion method on MHA (Muller Hinton Agar) plates against 3 human pathogens such as *Staphylococcus* sp., *Escherichia coli*., and *Pseudomonas* sp. and the results obtained are given in Table 1.

On the basis of antimicrobial activities of silver nano particle synthesized from the leafs of *Muntingia calabura*, it was evident that they shows significant antibacterial activity against Pseudomonas sp., but it does not showed any activity against *Staphylococcus* sp. and *Escherichia coli.*

Antifungal activity

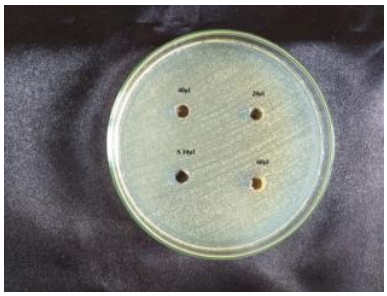
The inhibitory effect of silver nano particles synthesized from the aqueous leaf extract of *M. calabura* were tested by using well diffusion on PDA (Potato Dextrose Agar) plates against 2 fungus such as *Aspergillus* sp. and *Candida albicans* and the results obtained are given in Table 2.

On the basis of antimicrobial activities of silver nanoparticles synthesized from the leaves of *M. calabura*, it was evident that they does not showed any antifungal activity against *Aspergillus* sp. and *Candida albicans.*

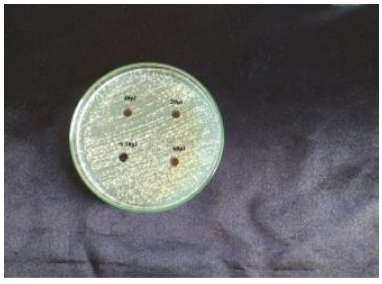
***Muntingia calabura* Plant Showing Leaves and Inflorescence**

Leaf extract: Before and after the formation of silver nanoparticle

Antifungal Activity of Silver Nanoparticles from the leaves of *Muntingia Calabura* against Fungal Pathogen

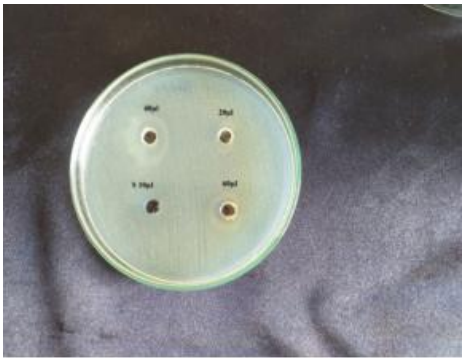


***Aspergillus* sp.**



Candida albicans

Antibacterial Activity of Silver Nano Particles from the leaves of *Muntingia calabura* against Bacterial Pathogens



***Pseudomonas* sp.**

Escherichia coli

***Staphylococcus* sp.**

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