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PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL SCREENING AND GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM LANTANA CAMARA FLOWER EXTRACT

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ABSTRACT In the present study, the ethanolic extract of the flowers of Lantana Camara plant was investigated for its phytochemical constituents. The extract was used to synthesize the silver nanoparticles and the antibacterial efficacy of extract as well as silver nanoparticles were evaluated against two micro-organisms Acinetobacter baumannii and Enterococcus faecalis. Results showed that the ethanolic extract of the flower contained alkaloids, carbohydrates, tannins, flavonoids, reducing sugars, protein, phytosterols. Comparative evaluation of the antibacterial efficacy revealed that the synthesized silver nanoparticles exhibited more efficacy with zones of inhibition ranging from 14 to 22 mm, were as the zones of inhibition of extracts was found to be in the range of 8 to 18 mm.

KEYWORDS : Anti-microbial, Lantana Camara, Silver Nanoparticles, Zones of inhibition.

INTRODUCTION

The use of herbal medicines and phyto nutrients continue to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings. This past decade has obviously witnessed a tremendous surge in acceptance and public interest in natural therapies both in developing and developed countries, with herbal remedies being available not only in drug stores, but now also in food stores and supermarkets[1].

Nanoparticles represents a particle with a nano meter size of 1-100nm. The nano scale materials have new, unique and superior physical and chemical properties when compared to its bulk structure due to an increase in the ratio of the surface area per volume of the particle. The most widely studied nanoparticles are metal nanoparticles because they are easier to synthesize. Moreover, these materials have a wide range of applications. They are used as detectors, catalysts, surface coating agents, and antibacterial/antimicrobial agents. Some of the most studied metallic nanoparticles include silver (Ag), gold (Au), platinum (Pt)], and palladium (Pd)[2].

Silver nanoparticles are an interesting metal to be studied, especially in the field of health and medicine. Silver is a strong antibacterial and also toxic to cells. Silver ions interact with the microbial cell and prevent protein synthesis, further decrease the membrane permeability, and eventually lead to cell death. The Silver nanoparticles are chemically more reactive than silver in their bulk. Therefore, silver nanoparticles are indicated to have stronger antibacterial capabilities[3].

Development of green synthesis of nanoparticles is environment friendly, cost effective and easy for large scale synthesis of nanoparticles, furthermore there is no need to use high temperature, pressure, energy and toxic chemicals for their synthesis hence the name green synthesis[4].

Phytochemicals like phenolics, flavonoids, lignans, sterols, vitamins play major role in preventing the development of chronic disease. They are an important source of antimicrobials, and many therapeutic agents[5].

Lantana camara Linn, (Verbenaceae), commonly known as wild or red sage, is the most widespread species around Asia, Africa and some parts of America. It is planted as an ornamental plant and is now a highly invasive weed in many parts of the world. Lantana camara is found at altitudes from sea level up to 2000 meters and can thrive very well under rainfall ranging from 750 to 5000 mm perannum[6].

Lantana camara has several medicinal uses- It is used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumours, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera[7].

The extracts from the Lantana camara leaves exhibited antimicrobial, insecticidal, nematicidal, antimicrobial, immunosuppressive and antitumor activities. Lantana oil is sometimes used for the treatment of skin itches, antiseptic for wounds, leprosy and scabies[8].

In the present study the ethanolic flower extract of Lantana camara was subjected to phytochemical screening. The silver nanoparticles of the extract were synthesized and comparative antibacterial activity of the extract and silvernanoparticles were evaluated.

MATERIALS AND METHODS

1. Collection of plant material

The flowers of Lantana camara were collected from the shrubs present in the region of Mysore, Karnataka, India and authenticated.

2. Extraction of the plant flowers

Fresh flowers of Lantana camara were collected and are separated followed by repeated washing several times with tap water to remove the dust particles.The extract was prepared by triturating the drug with solvent followed by maceration using 250ml of 60% ethanol.The extract was filtered for 2-3 times through nylon mesh and stored in the well closed container.

3. Phytochemical Analysis

Phytochemical screening of Lantana camara flower was performed as per the following procedure to confirm the presence and absence of various chemical constituents.

Test solution

0.5gm of the extract was dissolved in 10 ml of 60% ethanol and shaken well. Later the solution was used to perform the following phytochemical tests.

A) Test for Alkaloids
B) Test for Carbohydrates
C) Test for Tannins
D) Test for Flavonoids
E) Test for Reducing Sugar
F) Test for Proteins
G) Test for Anthraquinone glycosides
H) Test for Phytosterols
I) Test for Fats and Fixed oils

4. Green Synthesis of Silver Nanoparticles

0.01M aqueous solution of silver nitrate (AgNO3) was prepared and chemically reacted with the plant extracts at concentrations of 0.2, 0.4, 0.6 and 0.8 mg/ ml. Silver nano particles were synthesized and the synthesis confirmed. These nano particles were used for further comparative antimicrobial evaluation.

5. Antimicrobial screening

Antimicrobial screening of the extracts

All the extracts were prepared in the concentration of 0.2, 0.4, 0.6,0.8mg/ml and dissolved in DMSO and screened against the two microorganisms Acinetobacter baumanni and Enterococcus faecalis. Method used was agar well diffusion method. Ampicillin in the concentration of 0.1 mg/ ml was used as a standard.

Antimicrobial screening of Silver Nano Particles

The extract of flowers in a concentration of 0.2, 0.4, 0.6 and 0.8 mg/ ml was subjected to standardized antimicrobial screening procedure separately by agar diffusion method and comparative evaluation of the antimicrobial effects of individual extracts and nano particles was done.

RESULTS AND DISCUSSION

1. Collection of plant and extraction

The plant Lantana camara was collected from Mysore, Karnataka and authenticated. Extraction of flowers was carried out and the percentage extractive value was found to be 9.4%.

2. Phytochemical analysis of flower extracts

Phytochemical analysis of Lantana camara flower extract revealed the presence of constituents like alkaloids, carbohydrates, flavonoids, reducing sugars, tannins, proteins, and phytosterols. The results of phytochemical screening are shown in Table-01.

Table-01: Phytochemical analysis aqueous extract of Lantana camara flower

Phytoconstituents	Test	Result
Alkaloids	Dragendorff's Test	+
Carbohydrates	Molish Test	+
Tannins	Ferric Chloride test	+
Flavonoids test	Ferric Chloride	+
Reducing Sugars	Fehling's test	+
Anthraquinones	Hydroxy anthraquinone	_
Cardiac glycosides	Keller-killiani test	_
Fixed oil/ fat	Test for fats	_
Protein	Biuret Test	+
Phytosterols	Liebermann-Burchard's test	+
(+: Present, -: Absent)		

3. Synthesis of Silver Nanoparticles

The extract in the concentrations of 0.2, 0.4, 0.6 and 0.8 mg/ ml was used to carry out the green synthesis of silver nanoparticles using 0.01M aqueous silver nitrate solution. The formation of silver nanoparticles was confirmed by visual inspection against white background. There was difference in colour, the extract showing dark brown colour whereas the synthesized silver nanoparticles appeared light brown in colour. The image of extract and synthesized silver nano particles are shown in Figure-03.

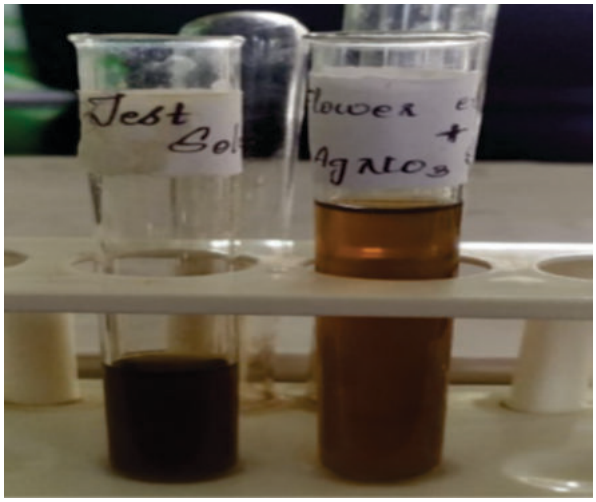


Figure-03: Plant extract along with synthesized silver nano particles

4. Antimicrobial Screening

Antimicrobial screening of the extracts

Results of antimicrobial screening of flower extracts of Lantana camara revealed that the extracts exhibited good antimicrobial activity. The zones of inhibition ranging from 8 mm to 18 mm were observed. Minimum zones of inhibition were observed by the extract at 0.2 mg/ml concentration and maximum zones of inhibition of 18 mm were exhibited at 0.8 mg/ml concentration against Acinetobacter baumanni. This was compared with the antimicrobial activity of the standard. Results are tabulated in Table-02, Graph-01 and Figure-04.

Antimicrobial screening of Silver Nano Particles

Results showed that the silver nano particles of extracts exhibited far better antimicrobial efficacy when compared to the plain extracts. Maximum zone of inhibition of 22.0 mm was observed by the nanoparticles at a concentration of 0.8 mg/ml whereas minimum zone of inhibition of 14.6 mm was observed by the nanoparticles at a concentration of 0.2 mg/ml. The standard drug showed zone of inhibition of 22.1 mm. Results are tabulated in Table-03, Graph-02 and Figure-05.

Table-02: Zones of inhibition of flower extracts at different concentrations against Acinetobacter baumanni and Enterococcus faecalis

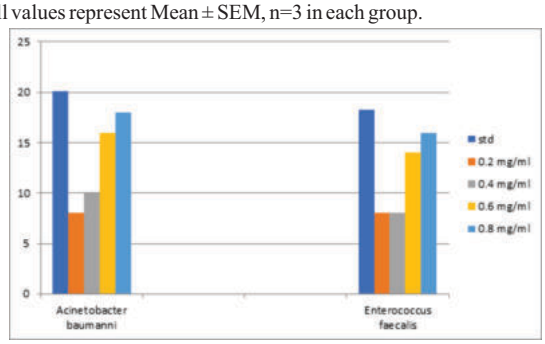
Sl No	Concentration of extracts (mg/ml)	Zones of Inhibition Acinetobacter baumanni	Zones of Inhibition Enterococcus faecalis
01	Std (0.1 mg/ml)	20.1 ±0.82	18.3±0.91
02	0.2	8.0 ±0.55	8.0 ±0.07
03	0.4	10 ±1.20	8.0 ±0.88
04	0.6	16.0 ±0.34	14 ±1.11
05	0.8	18.0 ±0.47	16.0 ±0.91

All values represent Mean ± SEM, n=3 in each group.

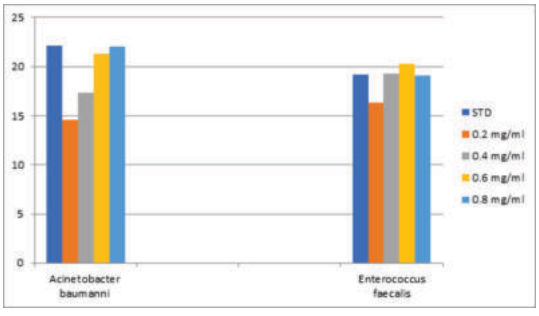
Table-03: Zones of inhibition of synthesized silver nanoparticles at different concentrations against Acinetobacter baumanni and Enterococcus faecalis

Sl No	Concentration of extracts (mg/ml)	Zones of Inhibition Acinetobacter baumanni	Zones of Inhibition Enterococcus faecalis
01	Std (0.1 mg/ml)	22.1 ±0.91	19.2 ±0.57
02	0.2	14.6 ±1.76	16.3 ±0.66
03	0.4	17.3 ±0.66	19.3 ±0.66
04	0.6	21.3 ±0.88	20.3 ±0.88
05	0.8	22.0 ±0.57	19.1 ±0.33

All values represent Mean ± SEM, n=3 in each group.



Graph-01: Zones of inhibition of flower extracts at different concentrations against Acinetobacter baumanni and Enterococcus faecalis



Graph-02: Zones of inhibition of synthesized silver nanoparticles

at different concentrations against Acinetobacter baumanni and Enterococcus faecalis

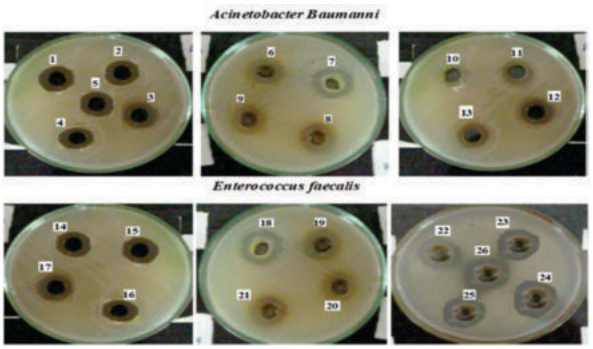


Figure-04: Zones of inhibition of flower extracts at different concentrations against Acinetobacter baumanni and Enterococcus faecalis

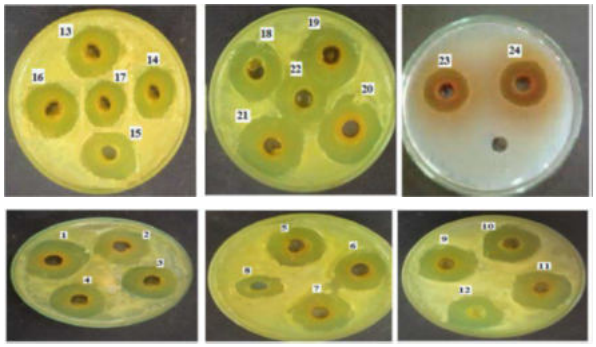


Figure-05: Zones of inhibition of synthesized silver nanoparticles at different concentrations against Acinetobacter baumanni and Enterococcus faecalis

CONCLUSION

In the present study it was concluded that the ethanolic flower extract of Lantana camara was found to contain alkaloids, carbohydrates, flavonoids, tannins, reducing sugars, protein, and phytosterols. The synthesized silver nanoparticles were far more active in inhibiting microbial growth when compared to the plant extract.

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