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IN VITRO ANTIMICROBIAL ACTIVITY OF LANTANA CAMARA L. AND ITS PHYTOCHEMICAL SCREENING

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Abstract

In vitro antibacterial activity of aqueous extract from the leaves of Lantana camara L. was evaluated against four bacterial strains pure culture of gram positive Bacillus subtilis (MTCC-441) and Staphylococcus aureus (MTCC-96) and gram negative Escherichia Coli (MTCC-1687), Pseudomonas aeruginosa (MTCC-424) using disc diffusion method. Out of four bacterial strains tested maximum Zone of inhibition was observed against Escherichia Coli (ZO1, 16.3mm) followed by Pseudomonas aeruginosa (ZOI,15.3mm). Phytochemical studies indicated that the aqueous extract of leaves of Lantana camara possess alkaloids, tannins ,flavonoids, coumarins, emodins, saponins, protein, carbohydrate, glycosides, steroids and oil .

Keywords: Lantana Camera, Aqueous Extract, Antibacterial Activity, Phytochemicals, Zone Of Inhibition.

Introduction

Lantana camara L. is introduced in India as an ornamental and hedge plant, which is commonly known as wild or red sage belonging to Verbenaceae family**.** In Assam, it is popularly known as Gu-phul and grows wild on road side, boundaries of tea gardens and cultivated lands. It is a spiny and aromatic wild shrub. Leaves are ovate, acuminate and toothed. Flowers are white, pink or yellow and many flowered with peduncle head are ovoid. It is an important medicinal plant used in traditional system of medicine **[1]** . It has been found that different parts of the plant are rich source of bioactive principles [2] .Decoction of the aromatic leaves are widely used in folk medicine as a febrifuge and diaphoretic. It is also used to cure yellow fever due to the presence of quinine-like alkaloid, lantanine **[3]** . The fresh leaves are crushed and boiled in sufficient amount of water for tropical application in curing dermatitis, eczema, traumatic injury and wound bleeding for haemoptysis of pulmonary tuberculosis. It was found as natural potential source of remedy for the treatment of asthma, coughs, rheumatism, cold, dysentery, fever and other health problems such as lowering of blood pressure, acceleration of deep respiration and inhibition of uterine motility. Extracts from Lantana camara are known to exert antioviposition, antifeedant, phagostimulant, repellence and adult emergence reduction effects on insect pests **[4].** Pharmacological studies indicated that the extract of shoot of Lantana camara exhibited strong antioxidant activities **[5]**. The recent study on Lantana camara reported that the plant has been used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, catarrhal infections, rheumatism, malaria and abdominal viscera **[6]**. Different parts of Lantana camara are reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids, triterpens, sesquiterpenoides and tannin as major phytochemical groups **[7]** .The whole plant and its infusion are considered to be antipyretic, diaphoretic and anti-malarial [8] .Lantana oil contained sabinene, 1,8 –cineole, germacrene-D, β –elemene, γ -elemene, β- caryophyllene, bicyclogermacrene, α –humulene, α-

copaene and α-cadinene as major constituents. Lantana oil is used for the treatment of skin itches, antiseptic for wounds, leprosy and scabies [9] .

Lantana camara has been used as medicines over hundreds of years. Ethnomedical and scientific reports about the medicinal properties of L. camara represent it as a valuable plant and establishing it as a candidate for the future drug development. So it is required to determine whether their traditional uses are supported by actual pharmacological effect or merely based on folklore. Preliminary screening of photochemical is a valuable step, in the detection of bioactive compounds present in medicinal plants of Assam and it may lead to drug discovery and development. Therefore, the present investigation has been conducted on phytochemical screening of bioactive molecules from aqueous leaves extracts of Lantana camara and investigation of their in vitro antimicrobial activity.

Materials & Methods

Biological Materials

The plant materials were collected from the Dergaon area of Assam, India in the month of January, 2018. The taxonomic identification of plant material was confirmed at Department of Botany, D.K.D. College, Dergaon, Assam. A voucher specimen is deposited at the herbarium of the Department of Botany, D.K.D. College,Dergaon.

Preparation of Plant Extract

Collected leaves of the plant were washed under running tap water to remove dust. The plant samples were dried in shade for 2-3 days and grounded in a grinder and the powdered samples were stored in polythene bags for use.

The powdered plant material (50g) were soaked in 100 ml distilled water and shaken well. The solution then filtered using filter paper and filtered extract were taken for further phytochemical analysis.

Another set of extract was prepared by soaking 10 gm in 50 ml of ethanol, shaken well. After 48 hours the solution then filtered using filter paper and stored in a freezer until further analysis.

Nutrient Agar media were prepared , having P^H value 7.3 ±0.2 ,was autoclaved at 121 ^{0}C for 30 minutes 15 lb pressure and poured in to sterile petriplates (80mm) are allowed to solidify. The tested microorganisms were inoculated by streak plate methods separately. Under aseptic conditions sterile disc of 5mm diameter (Whatman filter no-1) were impregnated with 20 µl of essential oil and placed on the surface of petriplates with sterile forceps and gently pressed to ensure contact with inoculated agar surface. A standard disc containing tetracycline (30 µg/disc) was used as positive reference standard to determine the sensitivity of tested strains. The plates were inverted and placed in an incubator at $37^0 \pm 1^0$ C for 24 hours. After incubation periods each plate was examined for growth of inhibition. The zone of inhibition was measured for each test microorganisms. The above experiment done in triplicate and the mean value was calculated.

Phytochemical screening of Lantana camara was performed for the presence of Alkaloids, Tannins Flavonoids, Coumarins, Emodins, Anthocyanins Anthraquinones, Saponins, Protein, Carbohydrate, Glycosides, Steroids, Oil by using standard method.

Results & Discussion

The antibacterial activities of aqueous leaves extract and ethanol extract of Lantana camara are shown in the table:1 and table:2 respectively.

Table1- Effect of Antimicrobial Activity of Aqueous Extract of L. Camara on Disc Diffusion Method

Pathogen used	**Number of disc (D) & Zone of Inhibition in mm**			**Mean ±SD**	**Control (Tetracycline) in mm Mean ±SD**
	D_1	D_2	D_3		
Bacillus subtilis	12	13	14	13.0±0.58	20.0±0.58
Escherichia coli	15	17	17	16.3±0.67	17.0±0.58
Pseudomonas aeruginosa	17	14	15	15.3±0.88	14.7±0.67
Staphylococcus aureus	12	11	13	12.0±0.58	19.7±0.33

Table 2- Effect of antimicrobial activity of Ethanol extract of L. camara on disc diffusion method

Pathogen used	**Number of disc (D) & Zone of Inhibition in mm**			**Mean ±SD**	**Control (Tetracycline) in mm Mean ±SD**
	D_1	D_2	D_3		
Bacillus subtilis	12.3	13.6	10	11.86±0.97	19.3 ± 0.66
Escherichia coli	14.3	14.3	13.6	14.06±0.23	21 ± 0.57

The aqueous leaves extract of Lantana camara showed maximum Zone of Inhibition(ZOI) in Escherichia Coli (16.3 mm) followed by (15.3mm) against Pseudomonas aeruginosa. Inhibition zone of 13.0 mm and 12.0 mm were recorded against Bacillus subtilis, staphylococcus aureus respectively (Table:1). The ethanol extract of Lantana camara showed Zone of Inhibition(ZOI) in Escherichia Coli (14.06 mm) followed by 11.86mm in Bacillus subtilis (Table:2) .

The phytochemical analysis were carried out on Lantana camara and the study has revealed the presence of phytochemical constituents which are considered as active chemical compounds. Important medicinal phytochemicals such as Alkaloids, Tannins, Flavonoids, Coumarins, Emodins, Saponins, Protein, Carbohydrate, Glycosides, Steroids, Oil were present whereas, Anthocyanins and Anthraquinones were not found in the extract. The results were sum marized in Table: 3 & Table :4.

Table 3: Preliminary Phytochemical Tests for Plant Extracts (Aqueous)

Sl. No.	Phytoconstituents	Phytochemical Test	Observation
1	Alkaloids	2ml extract + acetic acid + Mayer's reagent	White Precipitate
2	Tannins	1ml extract + 2ml H_20+2-3 drops of $FeCl_3$(5%)	Green Precipitate
3	Flavonoids	1ml extract+ 1ml $Pb(OAc)_4$(10%)	Yellow Coloration
4	Coumarins	2ml extract+3ml NaOH(10%)	Yellow coloration
5	Emodins	2ml extract+2ml NH_4OH+3ml Benzene	Red coloration
6	Anthocyanins	2ml extract+ 2ml HCl (2N)+ NH_3	No Violet coloration
7	Anthraquinones	3ml extract+3ml Benzene+5ml NH_3(10%)	No Pink, violet or red coloration
8	Saponins	5ml extract+ Olive oil	Emulsion formed
9	Protein	2ml extract+Millon's reagent	White precipitate
10	Carbohydrate	2ml extract+10ml H_2O+2dropsethanolic α napthol(20%)+2ml H_2SO_4(conc.)	Reddish violet ring at the junction.
11	Glycosides	2ml extract+2ml$CHCl_3$+2ml CH_3COOH	Violet to Blue to Green coloration
12	Steroids	2ml extract+2ml $CHCl_3$+2ml H_2SO_4	Reddish brown ring at the junction
13	Oil	2ml extract+Sudan reagent	Oily layers turns red.

Table 4: Results of Phytochemical Analysis of the Aqueous Extract of Lantana Camara

Sl No.	Variable	Lantena camera (Aqueous extract)
1	Alkaloids	+
2	Tannis	+
3	Flavonoids	+
4	Coumarins	+
5	Emodins	+
6	Anthocyanins	-
7	Anthraquinones	-
8	Saponins	+
9	Protein	+
10	Carbohydrate	+
11	Glycosides	+
12	Steroids	+
13	Oil	+

The selected plant was the source of primary metabolites such as proteins, carbohydrates (reducing sugars). The secondary metabolites i.e alkaloids, tannins, flavonoids, coumarins, emodins, saponins, glycosides, steroids and oil plays an important role in proving the therapeutic uses of this medicinal plant in curing various diseases . The anti-bacterial and anti- fungal, anti-cancer, anti-malarial, anti-oviposition, anti-feedant activities of this medicinal plant may be due to the presence of the above mentioned secondary metabolites. Such type of phytochemical analysis of Lantana camara is also important and has commercial interest in both research institute and pharmaceutical companies for the manufacturing of the new drugs for treatment of various diseases. Therefore, the important phytochemical properties of Lantana camara of Assam identified by our study will be helpful in the copping different diseases of these particular regions.

Conclusion

Systemic analysis of medicinal plants provides a variety of bioactive compounds for the discovery of new pharmaceutical products. Antimicrobial compounds are a group of chemical compounds which either destroy or suppress the growth and metabolism of microorganisms which can cause various infectious diseases in plants, animals and human beings. Therefore, present work highlights the use of leaves extracts of L. camara containing a highly potential phytochemical which could be characterized and thus, find its way into the discovery of pharmaceutical drugs. Phytochemicals from L. camara have a broad antimicrobial spectrum and might be a novel source of antimicrobial drugs. It is expected that the important phytochemical properties recognized by this study in L. camara plant of Assam is very useful in the curing of various diseases of this regions.

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