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In vitro antimicrobial activity of *Lantana camara* extracts against some selected microbial strains

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Abstract

Objective: The present study was aimed to ascertain the antimicrobial status of different solvent leaf extracts of *Lantana camara leaves* and its potential against the microbial strains by antimicrobial screening assay.

Methods: The four different solvent plant leaf extracts were screened against ten pathogenic microorganisms for possible antimicrobial activities was performed by agar disc diffusion method in Mueller Hinton agar plates.

Results: The results of this study revealed that, the most of the plant extracts exhibited antimicrobial properties. The highest potential was observed in the chloroform extract of *Lantana camara* against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. Whereas the petroleum ether and hydroalcoholic extracts show activity against *S. aureus*. Hydroalcoholic extract shows activity against *S. typhi*. Water extract does not show any activity against any bacterial strains. The hydroalcoholic extract also showed activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

Conclusion: The experiment confirmed the efficacy of some selected plant extracts as natural antimicrobials and suggested for the possibility of employing them in drugs for the treatment of infectious diseases caused by the test organisms.

Keywords: *Lantana camara*, antibacterial activity, agar disc diffusion method

Introduction

The herbal products which are obtained from plants are used for various therapeutic processes for long times^[1, 8]. Natural products are providing as a good source of remedies in order to cure many of diseases of mankind^[3, 6]. Medicinal plants are the valued source of nature for sustaining human healthiness. *Lantana camara* Linn. Generally famous as Lantana, wild sage, Surinam tea plant, West Indian lantana, red sage, white sage, tick berry and Spanish flag. It's an ornamental flowering plant belonging to the family Verbenaceae, often cultivated indoors, or in a conservatory^[5, 10, 11]. It has been initiated from its native central and South America to around 50 countries. In India, *L. camara* was distributed in the areas with moderate to high summer rainfall and found in well drained sloping sites. *L. camara* is a well-established medicinal plant in the traditional medicinal system and recent scientific studies have emphasized the possible use of *L. camara* in modern medicine^[21, 28]. Recently the public and medical health is facing a main risk on antimicrobial confrontation. It takes up a long-term investigation throughout for the prevention of globally elevated infectious disease caused by bacteria and fungi. With the persevering indication of restricting pathogenic microorganisms, there is a rising curiosity in the revelation of new antimicrobial specialists^[2, 4, 7]. The utilization of plant extract can be incredible importance in helpful medicines with known antimicrobial properties^[9, 15]. Over the most recent couple of years, various investigations have been going with in various nations to support such viability. Because of their antimicrobial characteristics a number of plants have been utilized that are because of secondary metabolism of the plant for compounds synthesis^[12, 13, 14].

Experimental

Materials and methods

Sample collection

The fresh leaves of plant, *Lantana camara* was collected from the local areas of Wayanad,

Kerala in the mid of February 2020 on the basis of its wide variety of uses in traditional medicinal history. The plant species was confirmed by a botanist Dr. Deena Meria Jose, Assistant professor & Head, Department of Botany, Providence Women's College, Kozhikode, Kerala (Accession No. 981). The plant species name, family, parts used, traditional uses, solvents used and extract yield was shown in table 1.

Preparation of extracts

The collected leaves of plant (*Lantana camara*) were firstly thoroughly sieved to remove the unwanted coarse particles washed with distilled water to remove dirt and it is air dried in a shade area in laboratory at room temperature for 2 weeks. The dried leaves were then crushed to coarse powder in a grinder [16, 22]. Then the fixed amount of powder is weighed (20g) and extracted by using various solvents such as Petroleum ether (PEE), Chloroform (CE), Hydroalcoholic (HAE) (7:3) [16, 29, 30]. The 20g of coarse powder was dissolved in 250ml of distilled water (WE) and kept in room temperature for three successive for maceration process [17, 23, 35]. The resulted extract was filtered through what man filter paper no. 4. All the filtrate was collected in a porcelain dish and condensed at 60 °C in a rotary evaporator, then dried under vacuum to yield the concentrated crude extract. The concentrated extracts were stored in a refrigerator at -4°C until use.

Percentage yield = Dry weight of plant extract / Dry weight of plant material X 100

The different solvent leaf extracts were subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, flavonoids, phenolic compounds, tannins, steroids, saponins, coumarins, phytosterols, alkaloids, carbohydrates and triterpenoids [18, 32, 33].

Phytochemical screening

Phytochemical analysis of the crude drug extracts was carried out to establish the presence of phytoconstituents [29, 31].

Screening of plant extracts for antibacterial activity

Bacterial strains

The antibacterial potency of each plant extract was evaluated by using five bacterial strains. Strains of Gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and three strains of Gram negative (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) bacteria. The bacterial strains were provided from the culture collection of Microbiology and Research Dept, Dr. Moopen's College of Pharmacy, Wayanad, Kerala, India.

Inoculum's preparation

Each bacterial strain was sub cultured overnight at 35°C in Mueller-Hilton agar slants. The bacterial growth was

harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 107CFU/ml using spectrophotometer [25, 27].

Antibacterial activity of plants extract

The disk diffusion method is used to evaluate antimicrobial activity of each plant extract. The plant extract residues (50 mg) were re-dissolved in 2.5 ml of ethanol, sterilized through Millipore filter (0.22mm) then loaded over sterile filter paper discs (8 mm in diameter) to obtain final concentration of 10 mg/disc [23, 26]. Ten ml of Mueller-Hilton agar medium was poured into sterile Petri dishes (As a basal layer) followed with 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 107CFU) to attain 105CFU/ml of medium. Sterile filter paper discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 5mg of Gentamycin was used as positive control. The plates were kept in the fridge at 5 °C for 2 h. to permit plant extracts diffusion then incubated at 35 °C for 24 hr. The presence of inhibition zones was measured by Vernier calliper, recorded and considered as indication for antibacterial activity [8, 10].

Determination of minimum inhibitory concentrations (MIC's) of the effective plants extract

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h. of incubation. The most effective plant extracts which exhibiting a strong antibacterial activity at 10 mg/ml was manipulated to determine their MIC using disk diffusion method and evaluate their efficiency in controlling bacterial strains causing food poisoning diseases. Different concentrations of the effective plant extract (1.25, 2.5, 5.0, 10.0, 12.5 and 15.0 mg/ml) were prepared separately by dissolving 50 mg in 2.5 ml of ethanol, sterilized through Millipore filter and loaded their requisite amount over sterilized filter paper discs (8 mm in diameter). Mueller-Hilton agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the pathogenic strains. The loaded filter paper discs with different concentrations of the effective plant extract were placed on the top of the Mueller-Hilton agar plates. The plates were kept in the fridge at 5 °C for 2 hr, then incubated at 35 °C for 24 hr. The inhibition zones were measured by Vernier calliper and recorded against the concentrations of the effective plant extracts [15, 18].

Results and Discussion

Plants extraction yield

The ethanobotanical data of the employed plants and their extract percentage yield are illustrated in Table 1. The highest plant extract of 50 g of dried plant materials with petroleum ether yielded plant extract residues ranged from 9.7%, hydroalcoholic extract 6.54%, water extract 5.26%, while chloroform plant gives the lowest extract yield respectively.

Table 1: The ethnobotanical data of employed plant species and their extract percentage yield

Plant species	Family	Plant part used	Traditional uses	Solvents used	Extract yield (100%)
<i>Lantana camara</i>	Verbenaceae	Leaves	Used for treating malaria, chickenpox, asthma, ulcer, swelling, eczema, tumor, high blood pressure, bilious fever, sores, measles, fevers, colds and high blood pressure	Petroleum ether	9.74%
				Chloroform	3.12%
				Hydroalcoholic	6.54%
				Water (Maceration)	5.26%

Antibacterial activity of plants extract

Evaluation of the antibacterial activity of four different plant extracts was determined initially by the disc diffusion method against different microorganisms. The study showed that all plant extracts used in the study exhibited a varying degree of antimicrobial activity against all microorganisms tested. It was observed that the chloroform extract of *Lantana camara* was the most effective among the four plant extracts tested. It showed a zone of inhibition against all gram-negative bacteria tested whereas there was no activity against gram positive bacteria. Hydroalcoholic extract was found to be effective against both gram positive and gram-negative bacteria. The petroleum ether extract

showed zone of inhibition against *S. aureus* as well as *S. Typhi*. The effectiveness of the extracts in tested bacteria strains was determined by measuring the minimum inhibitory concentration. MIC was performed for only those organisms which showed a zone of inhibition and were sensitive to the plant extracts in the antimicrobial assay by disc diffusion method. Chloroform extract of *Lantana camara* was found to be show strong antibacterial activity. In this case the ZOI of chloroform extract is 15 mm against *B. cereus* and 17mm against *E. coli*. Petroleum ether extract only effective against *S. aureus* with MIC value 12, 5 mg/ml. The MIC value of chloroform extract was 100 mg/ml against *S. typhi*.

Table 2: Diameter of ZOI of different plant extracts of *Lantana camara*

Test organism	Chloroform plant extract	Petroleum ether plant extract	Hydroalcoholic plant extract	Water extract
<i>Staphylococcus aureus</i>	-	10	10	-
<i>Bacillus cereus</i>	15	-	-	-
<i>Escherichia coli</i>	17	-	-	-
<i>Salmonella typhi</i>	13	-	13	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-

(-): No antibacterial activity

Table 3: MIC value of different plant extracts of *Lantana camara* against microorganism

Test organism	Chloroform plant extract	Petroleum ether plant extract	Hydroalcoholic plant extract	Water extract
<i>Staphylococcus aureus</i>	-	12.5	12.5	-
<i>Bacillus cereus</i>	20	-	-	-
<i>Escherichia coli</i>	25	-	-	-
<i>Salmonella typhi</i>	100	-	25	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-

(-): No antibacterial activity

Conclusion

In this study, antibacterial activities of four extracts of *Lantana camara* were assessed by Soxhlet solvent extraction method. The result showed potential antibacterial effects of chloroform plant extract against bacterial strains tested, such as *E. coli*, *B. cereus* and *S. typhi*. Whereas the petroleum ether and hydroalcoholic extracts show activity against *S. aureus*. Hydroalcoholic extract shows activity against *S. typhi*. Water extract does not show any activity against any bacterial strains. Here the work shows *in vitro* activity of certain plant extracts of *Lantana camara*. Further investigations are necessary to evaluate antimicrobial activity by using more bacterial strains. Moreover, other parts of the plants need to be studied to evaluate the studied plant extracts as a potential antimicrobial agent.

Conflict of Interest

The authors declare that there are no conflicts of interest

Authors Contribution

All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

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