Comparative antimicrobial activity and phytochemical analysis of different extracts of potential medicinal plants of *Ocimum sanctum* Linn. and *Lantana camara* Linn.

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Abstract

Antimicrobial activity and phytochemical analysis of the hexane, ethyl acetate, acetone, chloroform and methanol stem and leaf extracts of *Ocimum sanctum, Lantana camara* were extracted, evaluated by agar well diffusion method against five selected bacterial (Gram +ve and Gram-ve) human pathogens and a fungus *C. krusei*. In antimicrobial activity, different organic solvent extracts (hexane, ethyl acetate, acetone, chloroform and methanol) were evaluated for antimicrobial activity against human pathogens *L. camara* ethyl acetate stem extract showed highest antibacterial activity against *S. typhi*, chloroform leaf extract showed activity against *C. krusei*, methanol stem, ethyl acetate leaf extract showed activity against *S. typhi*. *O. sanctum* chloroform stem extract showed activity against *B. subtilis* and *V. cholera.* Phytochemical analysis of leaf and stem powder of *L. camara* and *O. sanctum* showed the presence of alkaloids, carbohydrates, flavanoids, saponins, tannins and proteins. Concluded that ethyl acetate stem extract of *L. camara* showed potential antimicrobial activity compare to other extracts.

Key words: *L. camara, O. sanctum*, Verbenaceae, Lamiaceae, human pathogenic microbes, organic solvents.

Introduction

According to WHO approximately 80% of the people in developing countries chiefly rely on traditional medicines for their primary health care needs, which a major portion involves the use of plant extracts or their active principles. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[1]. Plant produces several secondary metabolic compounds namely alkaloids, cyanogenic glycosides, glucosionolates, flavonoids, saponins, steroids and terpenoids that protect the plants from the continuous attack of naturally occurring pathogens such as insects, fungi, bacteria, pests and environmental stresses[2].

The continued investigation into the secondary plant metabolites for anti-infective agents has gained importance, because of the alarming increase in the rate of resistance of pathogenic microorganism to existing antibiotics. Therefore the need to develop efficient, safe and inexpensive drugs from plant sources is of great importance in India, herbs have long been used for promotion of health, prevention and treatment of diseases[3].

Lantana camara is an aromatic plant belongs to family Verbinaceae, perennial flowering plants have about 150 species, heavily branched shrub which can grow in dense thick and compact clumps, stems are square in cross section, small and re-curved prickles, leaves are about 6 cm long and are covered with fine hair, bright green and grow opposite one another along the stem and when crush the leaves produce distinctive odour. Flowers are

cluster and are about 2.5 cm in diameter, colours vary from pale cream to yellow, white, pink, orange, purple and red, round, berry like fruit that turn from glossy green to purplish black when ripe. Extract from the leaves of *L. camara* possessed larvicidal activity[4] while extract from flowers of the plant showed repellent activity against mosquitoes[5]. The stalks are used as raw material for paper pulp, which is used for wrapping, writing and printing paper. Its bark is astringent and used as a lotion in leprous ulcers and other eruptions of the skin. Leaves are boiled and applied for swellings and pain of the body. Alkaloids from *Lantana* have been found to stimulate intestinal movements in experimental animals, lower blood pressure and accelerate deep respiration[6]. It is also used in treatment of the respiratory infection, skin itching and in gastric ulcer. Also plant extract are used for treatment of cancers, tumors, high blood pressure, asthma, malaria and chicken pox etc[7].

Ocimum sanctum, also known as Holy Basil, *tulsi*, or *tulasī*, is an aromatic plant belongs to family Lamiaceae[8]. It's an erect, much branched subshrub, 30–60 cm tall with hairy stems and simple, opposite, green leaves that are strongly scented, leaves have petioles and are ovate, up to 5 cm long, usually slightly toothed, flowers are purplish in elongate racemes in close whorls[9]. It is used in successful management of various disease conditions like bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, insect bite and in treatment of gastric, hepatic, cardiovascular and immunological disorders[10, 11]. *O. sanctum* has been reported to possess anti-carcinogenic, anthelmintic, anti-septic, anti-rheumatic, antistress and antibacterial properties[12]. The juice of the fresh leaves and slender roots is used as an antidote in snake bite and scorpion sting[13]. The seeds are mucilaginous and demulcent, and are given in disorders of the genitourinary system [14]. All the parts of the plant are being used without any side effects. The present study was carried out to test phytochemical analysis and antimicrobial efficacy of stem and leaf extracts of *L. camara, O. sanctum* against human pathogens.

Materials and methods

Chemicals:

Analytical grade chemicals from Hi-Media, Loba, Merck and Sigma were used throughout the study. Hexane, ethyl acetate, acetone, chloroform and methanol were of analytical grade. Mueller Hinton Agar (MHA) and Muller Hinton Broth (MHB) were purchased from Hi-Media was used for antimicrobial activity.

Collection of the plant material: Stem and leaves of *L. camara* and *O. sanctum* were collected from Thiruporur, Kanchipuram (DT) Chennai, Tamil Nadu, India. They were shade dried and dried parts were powdered using mechanical pulverizer and subjected for extraction.

Preparation of organic extracts:

Solvent and aqueous extracts: Air dried and powdered stem and leaves were cold macerated with different solvents (hexane, ethyl acetate, acetone, chloroform and methanol,) for 72 h in the ratio of 3:1. The extract was suction filtered using Whatmann filter paper (No. 1). This was repeated for 2 times and similar extracts were pooled together and concentrated at 40°C to 45°C The concentrated crude extracts were subjected to phytochemical analysis and antimicrobial studies.

Phytochemical analysis

The different phytochemical analysis was performed for establishing the profile of given extract for its chemical composition. Alkaloids, flavanoids, tannins, saponins and carbohydrates were determined according to the manual[15].

Antimicrobial activity

Microorganisms:

The test organisms used in this study were antimicrobial activity against human pathogenic gram positive and gram negative bacteria are *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-98), *Escherichia coli* (MTCC-1687), *Salmonella typhi* (MTCC-733) and *Vibro coloria* (MTCC-3906). The test organisms used in this study were one fungal strain namely *Candida krusei* (ATCC-24408) is budding yeast involved in chocolate

production. *C. krusei* is an emerging fungal nosocomial pathogen primarily found in the immuno compromised and those with hematological malignancies of different organic solvents (hexane, ethyl acetate, acetone, chloroform and methanol).

Preparation of Inoculum:

Fresh cultures were prepared by inoculating the organisms in Nutrient Broth (NB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi in incubating at 37°C for 24 h. Each organism was suspended in sterile broths and diluted.The culture thus obtained was the standardised bacterial and fungal suspension.

Antimicrobial activity by agar well diffusion method:

The *in vitro* antimicrobial activities of test compounds were determined by the well-diffusion method described by [16, 17]. Muller Hinton Agar (MHA) (Beef infusion, 300 g/L; Casein acid hydrolysate, 17.5 g/L; Starch, 1.5 g/L; Agar, 1.7 g/L; pH 7.3±0.1) for bacterial studies and Sabouraud Dextrose Agar (SDA) for fungi (Meat peptone, 5 g; casein peptone, 5 g; dextrose, 40 g; agar, 15 g, distilled water, 1000 mL) medium was used for the preparation of plates. The medium was poured onto sterile petridishes of 90 mm diameter. The agar was allowed to set at ambient temperature. Fresh bacterial cultures of *B. subtilis, S. aureus*, *E. coli*, *S. typhi*, were spread on the surface of the MHA; fresh fungal culture was spread on surface of the SDA plate with swabs. After incubation, using a sterile cork borer, wells were cut from the agar in the plate. The concentrated leaf and stem different solvent extracts of *L. camara* and *O. sanctum* were weighed and dissolved in dimethyl sulfoxide (DMSO) to prepare extract solution of 5 mg/mL of DMSO. To each well, concentration ranging from 25, 50, 75 µL of this extract solution was dispensed using a sterile micropipette. The inoculated plates were incubated within 15 min of inoculation at 37°C for 24 h. Turbidity was adjusted with sterile broth so as to correspond to 0.5 McFarland standards. The inoculated plates were incubated within 15 min of inoculation at 37°C for 24 h. Then the plates were examined for any zone of growth inhibition. Inhibition zones were as the diameter of growth free zones including the diameter of the well in mm at the end of incubation period.

$$\% \text{ of inhibition} = \frac{\text{I (Diameter of the inhibition zone) X 100}}{\text{90(Diameter of the petri plate in mm)}}$$

Statistical analysis

The data were subjected to One-way Analysis of Variance (ANOVA) to evaluate the significant of difference of means of various treatment groups using SPSS statistical software package (Version: 10). The values are presented as mean ± S.D and $P<0.05$.

Results and discussion

Phytochemical analysis, powder of leaf and stem of *L. camara* and *O. sanctum* showed the presence of alkaloids, carbohydrates, flavanoids, saponins, tannins and proteins, phytochemical analysis was given in Table 1. The herbal medicine has existed since the dawn of time, or knowledge of how plants actually affect human physiology remains largely unexplored. Numbers of plants are claiming various medicinal uses and many researches are going on in this view. Two such plants, which claims various medicinal properties is *L. camara* and *O. sanctum* in India [18].

The antimicrobial activity has been observed in leaf and stem extracts of *L. camara and O. sanctum* were evaluated at three different concentrations (25, 50 and 75 µg/mL, where only table given in 75 µg/mL) against six bacterial strains by well diffusion method and was compared with the activity of standard (Tetracycline) result was given only 75 µg/mL concentration. Hexane, chloroform, ethyl acetate, acetone and methanol extracts showed various levels of inhibitory effects against human pathogenic bacteria and fungi. The word herb refers to a plant used for medicinal purpose. Medicinal herbs and plants extracts are now generally considered as effective medicines to be respected, appreciated and they play a major role in modern pharmacy. There has been an explosion of scientific information concerning plants, crude plant extracts and various substances from plants as medical agents during last 20-30 year.

The *in vitro* antimicrobial activity analyzed by well diffusion method. It shows higher antimicrobial activity in higher concentration of extracts say it show good antimicrobial activity for the extract concentration of 25, 50 and 75 µg/mL, [19] has reported antibacterial activity of *L. camara* chloroform stem extract showed inhibitory effect against *S. saprophiticus* (9 mm). The maximum inhibitory zone was reported against *E. coli* (8 mm) in methanol extract. Gram positive bacteria and gram negative bacteria were found to be more susceptible to plant extracts than that of fungus. Similar results were observed by [20]. The susceptibility of gram positive bacteria may be due to the composition of cell wall. *L. camara* exhibited antibacterial activity against *P. aeruginosa* and *B. subtilis* [21]. The present results are slightly different from previous finding. The differences in the result may be due to the geographical origin, age of plant, or extraction procedure, antibacterial assays, and pH of media that were used for the testing antimicrobial activity, [22] have reported antibacterial activity of the hydro-ethanolic extract of white and pink flower extracts of *N. nucifera*. The maximum zone of inhibition against *E. coli* (16 mm and 14 mm), *B. Subtilis* (15 mm and 13 mm) and *S. aureus* (13 mm and 11 mm) was exhibited by the white and pink flower extracts in their study. In a similar earlier study by [23] indicated that the leaf extracts of *N. nucifera* exhibited antibacterial activity.

Table 1 Phytochemical analysis of *L. camara* and *O. sanctum*.

Phytochemical Test	***L. camara***		***O. sanctum***	
Types of Test	Leaf	Stem	Leaf	Stem
Detection of Alkaloids Mayer's Test	+++	+++	+++	+++
Detection of Carbohydrates Fehling's Test	+++	+++	+++	+++
Detection of Proteins Ninhydrin Test	+++	+++	+++	+++
Detection of Tannins Braemer's Test	+++	+++	+++	+++
Detection of Flavonoids Ferric Chrolide Test	+++	+++	+++	+++
Detection of Saponins	+++	+++	+++	+++

Table 2 Antimicrobial activity of *L. camara* leaf and stem different solvent extracts against human pathogens.

Microbes	Std	Zone of inhibition (mm)									
		H		EA		A		C		M	
		L	S	L	S	L	S	L	S	L	S
B. subtilis	25.00	20.00	-	20.00	-	17.77	-	-	-	18.88	18.88
S. aureus	23.32	16.66	14.44	21.11	-	15.55	-	-	16.66	17.77	20.00
E. coli	25.23	20.00	-	23.33	-	17.77	-	-	20.00	22.22	23.33
S. typhi	26.00	20.00	-	21.11	26.66	17.77	-	-	-	20.00	22.22
V. coloria	26.66	18.88	-	20.00	-	17.77	18.88	-	-	22.22	18.88
C. krusei	24.00	20.00	-	20.00	-	15.55	-	24.44	22.22	18.88	18.88

Table 3 Antimicrobial activity of *O. Sanctum* leaf and stem different solvent extracts against human pathogens.

Microbes	Zone of inhibition (mm)										
	Std	H		EA		A		C		M	
		L	S	L	S	L	S	L	S	L	S
B. subtilis	25.00	17.77	16.66	12.22	-	15.55	-	-	20.00	13.33	-
S. aureus	23.32	13.33	14.44	13.33	-	13.33	16.66	12.22	18.88	15.55	-
E. coli	25.23	13.33	-	-	-	-	15.55	-	17.77	14.44	-
S. typhi	26.00	13.33	15.55	-	-	-	-	12.22	18.88	14.44	-
V. coloria	26.66	-	-	13.33	-	14.44	16.66	14.44	20.00	15.55	-
C. krusei	24.00	14.44	-	14.44	-	-	-	15.55	15.55	13.33	-

H – Hexane, EA – Ethyl acetate, A – Acetone, C – Chloroform, M – Methanol, Std – Standard, L – Leaf, S – Stem.

Conclusion: Results concluded that ethyl acetate stem extract of *L. camara* showed potential antimicrobial activity compare to other extracts, due to crude extract synergism against various pathogens.

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