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Antibacterial activity of some Holy Plants

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Abstract

Medicinal plants are being used from earliest without knowing about their active elements. Herbs are occupied the top source of novel drug with antimicrobial activity. With reference to these points, the ethanolic extract of three medicinal plants were subjected to *in vitro* antibacterial assay against human pathogenic bacteria Gram positive *Bacillus subtilis* and Gram negative *Pseudomonas putida* by employing disc diffusion method. Among the plants tested *Calotropis gigantean* was found to be the most effective against both bacterial strains followed by *Curcuma longa* and *Oscimum sanctum* found ineffective against both test organisms. *Curcuma longa* showed decreased zone of inhibition with increased concentration of sample, while *Calotropis gigantean* showed increased with concentration. Largest zone of inhibition (26 mm) was obtained with *Pseudomonas putida* and (27 mm) with *Bacillus subtilis* at 0.5mg/ml concentration when treated with *Curcuma longa*. In case of *Calotropis gigantean*, largest zone of inhibition (27 mm) was obtained with *Pseudomonas putida* and (29 mm) with *Bacillus subtilis* at 0.25 mg/ml concentration.

Keywords: Antibacterial, disc diffusion, zone of inhibition, *Curcuma longa*, *Calotropis gigantean.*

Introduction

The effectiveness of any antibiotic is limited and the increased resistance shown by microbes. This antibiotic resistance is the major concerns all around the world and this leads to the investigation of alternative sources such as phytochemicals (Akrayi and Abdulrahman, 2013). Due to this pharmaceutical properties and easily available nature, the plants are quite common in many developing countries. Plants are potent of phytomedicine and those can be derived from any part of plants i.e., from stem shoot to root tip (Gordon and David, 2001).

Turmeric is a wonderful compound, being used from ancient time in traditional system of medicine. Curcuminoids are the phytoconstituents of rhizome extracts of Curcumin (Jayaprakash *et al*., 2002) which are helpful as antimicrobial (Ravindran *et al*., 2007, Chang *et al*., 2008, Singh and Jain, 2011, and Hegde *et al*., 2012). *Curcuma longa* commonly known as turmeric (Luthra *et al*., 2001) contains various curcuminoids in rhizome (Srinivas *et al*., 1992) which are possess health promoting activities such as antibacterial agent (Ramprasad and Sirsi, 1956 and Shankarnarayanan and Jolly, 1993). *Ocimum sanctum* is incomparable one among all the herbs and has been used as antibacterial agent

(Geeta *et al*., 2001, Singh *et al*., 2005 Rahman *et al*., 2010 and Lalit Mohan *et al*., 2011). Due to higher content of linoleic acid in *Ocimum sanctum* possesses antibacterial activity. In Ayurveda *Ocimum sanctum* has been described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna) (Sirkar, 1989 and Shahedur Rahman *et al.*, 2011). *Calotropis gigantea* is a common wasteland weed and known for various medicinal properties such as toothache, earache, sprain, anxiety, pain, epilepsy, diarrhoea and mental disorders (Gaurav Kumar *et al.*, 2010a) and also reported for its anti-*Candida* activity, cytotoxic activity, antipyretic activity and wound healing activity (Chitme *et al*., 2005, Wang *et al.*, 2008, Saratha *et al*., 2009 and Gaurav Kumar *et al.*, 2010b).

This study was designed to explore the antibacterial efficacy of plant extracts of *Curcuma longa, Ocimum sanctum* and *Calotropis gigantea* on selected human pathogens *Pseudomonas putida* and *Bacillus subtilis*. The rhizome of *Curcuma longa*, leaves of *Oscimum sanctum* and shoot of *Calotropis gigantean* were used for the present study.

Materials and Methods

Collection of plant materials:

The rhizome of *Curcuma longa*, leaves of *Oscimum sanctum* and shoot of *Calotropis gigantean* were collected in and around Puthanampatti, Trichy District, Tamilnadu. Collected plant materials were dried in shade and ground into fine powder and stored in a closed container for further use.

Test organisms:

The Gram positive *Bacillus subtilis* and Gram negative *Pseudomonas putida* were obtained from clinical laboratory and used to evaluate antibacterial activity of test plant materials.

Ethanolic extraction of plant materials:

The each powdered sample with ethanol (100gm/100ml) used for extraction using Soxhlet apparatus at room temperature. The extracts from these solvent are soaked and evaporated.

Antibacterial Assay:

Petri plates containing 20ml of Nutrient agar medium were seeded with 24hr old culture of test organisms. The extracts were dissolved in Dimethyl Sulfoide (DMSO) separately. The assay was performed using Kirby - Bauer Disk Diffusion diffusion method (Hudzicki, 2009). Plant extract of 0.5mg/ml, 0.15 mg/ml and 0.25 mg/ml concentrations were impregnated into sterile 6mm diameter discs. Discs were dried and dispensed on the solidified Nutrient agar, inoculated with test human pathogens. Incubation was made at 37˚C for 24hrs in BOD incubation chamber. After incubation the plates were observed for zone formation. The length of the zone was measured.

Results and Discussion

Evidences for the use of plants as antibacterial agents are found in earliest records. By keeping this idea, the crude ethanolic extracts of 3 medicinal plants were subjected to *in vitro* antibacterial assay against human pathogens *Pseudomonas putida* and *Bacillus subtilis* by disc diffusion method and zone of inhibition were determined. In this present study the rhizome of *Curcuma longa*, leaves of *Oscimum sanctum* and shoot of *Calotropis gigantean*, traditionally used in India for various disorders and considered as holy and divine plants, were studied for their antibacterial activity.

The ethanolic extract of *Curcuma longa* showed significant effect on both test pathogens. In which the maximum inhibitory zone was observed at 0.5mg/ml concentration and zone on both pathogens showed decreasing the size of zone of inhibition when increasing the concentrations of sample. In case of *Pseudomonas putida* out of 0.25mg/ml all other concentrations of samples were sensitive to the pathogens. While *Bacillus subtilis* was the most sensitive to *Curcuma longa* at all test concentrations and this was resembled the article of Shagufta naz *et al*., 2010. The

methanol extract of turmeric revealed MIC values of 16 μg/mL against *Bacillus subtilis* (Ungphaiboon *et al*., 2005). Rachana and Venugopalan (2014) observed that hexane, dichloromethane and ethyl acetate extracts of Curcumin, showed maximum activity against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). In the study of Sara Albino Antunes *et al.*, (2012) *Curcuma longa* showed the highest antimicrobial activity against *Salmonella typhimurium* was 15.0 ± 1.41 mm at the concentration of 2.30 mg.mL–1 of essential oil and 2.0 mg.mL–1 of ascorbic acid and with regard to *Listeria monocytogenes*, the largest zone of inhibition (13.7 ± 0.58 mm) was obtained at the same concentrations.

The antibacterial test results of *Oscimum sanctum* revealed that both the pathogens were resistant and no zones were observed at any concentrations like the result of the study of Rashmi Chandra *et al*., 2011 and Ilhan Kaya *et al*., 2008 in case of *Pseudomonas putida* and *Bacillus subtilis* respectively.

The antibacterial activity of ethanolic extract of *Calotropis gigantean* against *Pseudomonas putida* and *Bacillus subtilis* showed maximum inhibitory zone at 0.25mg/ml concentration and the resulting zone on both pathogens increasing when increasing the concentrations of sample from 0.5mg/ml to 0.25mg/ml. Earlier studies on the antimicrobial activity of *C. gigantea* root bark extracts revealed its antibacterial potential against *B. subtilis* (Alam *et al.*, 2008) Table 1).

Table 1: Zone of inhibition (mm) of test plant extracts on test pathogens.

Test organism	Plant extracts	Zone of inhibition (mm) at different sample concentration (mg/ml)			Mean±SD	Significance
		0.5	0.15	0.25		
Pseudomonas putida	*Curcuma longa*	26	24	8	19.33±9.87	0.005
	Ocimum sanctum	-	-	-	0.00±0.00	
	Calotropis gigantean	23	24	27	24.67±2.082	
Bacillus subtilis	*Curcuma longa*	27	21	19	22.33±4.16	0.001
	Ocimum sanctum	-	-	-	0.00±0.00	
	Calotropis gigantean	19	29	29	25.67±5.77	

The mean and standard deviation of all the test plant extracts were given in the table 1. This ANOVA result revealed that the significance of variance, i.e., *p*-value, were 0.005 for *Pseudomonas putida* and 0.001 for *Bacillus subtilis.* Since, both the significance value were less than ($p<0.05$), the variance between different plant extracts is significant. Therefore, we conclude that the 3 plant extracts were differing from each other significantly.

Table 2: Tukey HSD Multiple Comparisons of plant extracts on *Pseudomonas putida.*

(I) Plant extracts	(J) Plant extracts	Sig.
Curcuma longa	*Ocimum sanctum*	.016
	Calotropis gigantean	.536
Ocimum sanctum	*Curcuma longa*	.016
	Calotropis gigantean	.005
Calotropis gigantean	*Curcuma longa*	.536
	Ocimum sanctum	.005

Turkey test selected in One-way ANOVA for Multiple Comparisons of plant extracts. In case of *Pseudomonas putida*, *Curcuma longa* significantly differ from *Ocimum sanctum* (*p-value*=0.016) but do not from *Calotropis gigantean* (*p-value*=0.536), *Ocimum sanctum* differ from rest of plants and *Calotropis gigantean* significantly differ from *Ocimum sanctum* (*p-value*=0.005) but do not from *Curcuma longa* (*p-value*=0.536) (Table 2).

Table 3: Tukey HSD Multiple Comparisons of plant extracts on *Bacillus subtilis*.

(I) Plant extracts	(J) Plant extracts	Sig.
Curcuma longa	*Ocimum sanctum*	.001
	Calotropis gigantean	.607
Ocimum sanctum	*Curcuma longa*	.001
	Calotropis gigantean	.001
Calotropis gigantean	*Curcuma longa*	.607
	Ocimum sanctum	.001

In case of *Bacillus subtilis*, *Curcuma longa* differ significantly from *Ocimum sanctum* (*p-value*=0.001) but do not differ from *Calotropis gigantean* (*p – value* = 0.607), *Ocimum sanctum* differ significantly from the both other plant extracts (*p-value*=0.001) and *Calotropis gigantean* differ significantly from *Ocimum sanctum* (*p-value*=0.001) but do not differ from *Curcuma longa* (*p-value*=0.607). When the result of *Ocimum sanctum* compared with other 2 plant extracts the values under the significance. So it differs significantly from others on both pathogens. The scientific inference is that the zone of inhibition of *Curcuma longa* was similar to *Calotropis gigantean* according to Turkey HSD multiple comparison analysis.

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