Biomedical & Pharmacology Journal Vol. **4**(2), 297-299 (2011)

A Study on Antimicrobial Properties of *Phyllanthus niruri* and *Ocimum sanctum*

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(Received: August 27, 2011; Accepted: October 13, 2011)

Key words: Antimicrobial activity, *E.coli, Bacillus subtilis*, *Phyllanthus niruri and Ocimum sanctum.*

INTRODUCTION

Medicinal plants are considerably useful and economically essential. They contain active constituents which are used in the treatment of many human diseases. Since plants have been used to treat common infectious diseases, their healing potential has been utilized by Indian traditional medicines like Ayurveda and Unani (Jayshree & Manneemegalai, 2008). Medicinal plants such as *Phyllanthus niruri* is an annual herb of Euphorbiaceae family, have potential value in wide range of ailments like jaundice, gonorrhea, frequent menstruation and diabetes, as a poultice for skin ulcers, swellings and itching and in the treatment of chronic dysentery. *Ocimum sanctum* is used to manage immunological disorders. The essential oil extracted from this plant has insecticidal activity and anti-allergic properties. It is used in the curing of heart ailments, diabetes, cough, seasonal fever, malaria, bronchitis in children and ear infection. Test organism *Escherichia coli* is a classic example of enteric bacteria capable of producing diseases. Enterotoxigenic *E. coli* strains (ETEC) produce toxins that affect the small intestine. A single strain can produce one or both of these forms of toxins as determined genetically by the plasmids. It is a Gram negative rod shaped bacterium that is commonly found in the lower intestine of warm blooded organisms (endotherms) are easily manipulated because of their easy genetics. *Bacillus subtilis*, known as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium commonly found in soil. A member of the genus *Bacillus*. *B. subtilis* is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions.

MATERIAL AND METHODS

Healthy plants of *Phyllanthus niruri* and *Ocimum sanctum* were collected from the field, after proper identification. The plant material was immediately transferred to the laboratory and cleaned. Old and damaged leaves were discarded. Uniform sized leaves were separated from the stem, washed with water and blotted dry. Extraction from fresh leaves was performed on the same day. For extraction from dry leaves, the material was spread out on the laboratory tables and dried at room temperature for about 10 days followed by grinding into a fine powder using mortar and pestle. Fresh leaves of each plant under study were crushed separately with a small quantity of double distilled water. The respective pastes were transferred to conical flasks by adding more water. The flasks were plugged and left undisturbed for 24 hours. The contents were filtered using Whatmann filter paper and the filtrate evaporated in an evaporating dish over a water bath to obtain a concentrate. The concentrate was stored in refrigerator at 4 ºC for further use. Extraction was done in a similar manner using the remaining two solvents i.e. ethanol and

chloroform and their concentrates were also stored in the refrigerator. Extraction from dry leaves of each plant under study was crushed separately. For determination of antimicrobial activity, the lowest concentration of antimicrobial agent under the given experimental conditions was taken, Minimum Inhibitory Concentration (MIC) was observed, as indicated by a zone of inhibition. Stock cultures of *E. coli* and *B. subtili* were maintained at 4ºC on nutrient agar slants. Active cultures of the organisms under study were obtained by sub culturing on nutrient agar slants.The MIC of the various plant extracts against the test organisms was determined by agar disc diffusion method where a loopful of the respective active bacterial culture was suspended in 0.8% saline. 0.1ml of this suspension was spread plated on the surface of nutrient agar plates with the help of a glass spreader. Concentrates of the plants under study were redissolved in the respective solvents to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 mg/ml. Sterile filter paper discs (4mm diameter) were dipped in the various concentrations of the plant extracts and placed on the nutrient agar plates inoculated with the bacterial cultures. Positive control was maintained with antibiotics like tetracycline, penicillin, ampicillin and chloramphenicol while distilled water and the solvents used for extraction were used as negative control.The plates were incubated at 37ºC for 18-24 hours. The zones of inhibition (if any) formed around the discs were measured using mm scale.

RESULTS AND DISCUSSION

In the present study, all the test organisms demonstrated susceptibility to ethanol, aqueous and chloroform extracts of *Phyllanthus niruri,* and *Ocimum sanctum.* From the above study it was observed that ethanol and aqueous extracts are much more effective than chloroform extract. Fresh leaf extracts showed better activity against the microorganisms as compared to extracts from dried leaves. A comparison of the above results of the various plant extracts at a concentration of 20mg/ml on the two test organisms is depicted in Table 1. From the Table it is observed that fresh ethanol extracts *Phyllanthus niruri* showed the highest zones of inhibition (18mm) with *Ecoli*. Fresh aqueous and chloroform extracts of *Phyllanthus*

Table 1: Zones of inhibition (mm) of different extracts of *Phyllanthus niruri* and *Ocimum sanctum* at a concentration of 20mg/ml on test bacteria

Organisms	*Phyllanthus niruri*						*Ocimum sanctum*					
	Fresh			Dry			Fresh			Dry		
	EE	AE	CE	EE	AE	CE	EE	AE	CE	EE	AE	CE
E .coli	18	12	12	11	12	16	13	13	5	10	12	5
B. subtilis	14	25	17	12	12	12	12	12	3	13	12	3

EE- Ethanol extract, AE- Aqueous extract, CE- Chloroform extract.

Table 2: Minimum inhibitory concentration (MIC) of all the plant extracts of *Phyllanthus niruri* (fresh and dry) on the test organisms

Organisms	*Phyllanthus niruri*					
	Fresh			Dry		
	AE	DE	CE	AE	DE	CE
E. coli	8	8	8	6	8	10
B. subtilis	8	8	8	2	6	10

Table 3: Minimum inhibitory concentration (MIC) of all the plant extracts of *Ocimum sanctum* (fresh and dry) on the test organisms

Organisms	*Ocimum sanctum*					
	Fresh			Dry		
	AE	DE	CE	AE	DE	CE
E. coli	8	20	8	6	6	10
B. subtilis	8	6	10	4	8	12

niruri showed the highest inhibition zones of 25mm and 17mm respectively with *B. subtilis*. Aqueous extracts of dry *Phyllanthus niruri* showed highest inhibition zones of 16mm and chloroform extract of dry *Phyllanthus niruri* showed highest zone of inhibition of 12mm for *B. subtilis*. Ethanol, aqueous and chloroform extracts of fresh as well as dry *Oscimum sanctum* showed highest zones of inhibition with *E.coli*, but was less then *Phyllanthus niruri.*

Minimal inhibitory concentration (MIC) (Table 2 & 3) of plant extract gave different zones of inhibition based on the methods of extraction at different concentrations. All the extracts give inhibitory actions at various concentrations. Diameters of zones of inhibition recorded at various concentrations. The minimal inhibitory concentration ranged between 2-10 mg/ml depending on the microorganisms and various extracts. Zones of inhibition increased with concentrations of extracts that is, as concentrations of extracts were diluted, zones of inhibition were decreased. The result of Minimal inhibitory concentration (MIC) suggests that ethanol and aqueous extracts could possibly act as bactericidal agents to these microorganisms. The study has also shown that ethanol extracts and aqueous extracts are much more effective than chloroform extracts of fresh leaves and thus could possibly act as bacterial agents to the microorganisms.

The inhibitory effect of the plant extracts justifies the medicinal use of these plants and further study is required to find out the active compounds of medicinal value.

REFERENCES

1. Aboyade, O.M.; Yakubu, M.T. and Grierson, D.S., Studies on the toxicological effect of the aqueous extract of the fresh, dried and boiled berries of Solanum aculeastrum Dunal in male Wistar rats. *Human & Experimental Toxicology,* 28: 765-775 (2009).
2. Jayashree, A. and Maneemegalai, S., Studies on the antimicrobial activity of the extracts from Tridax procumbens and Ixora couinea. *Biomedical,* **28**: 190-194 (2008).
3. Kumar, V., Lipid lowering activity of Phyllanthus niruri in hyperlipemics rats. *Journal of Ethnopharmacol,* **28**: 908-915 (2007).
4. Naraynan, S. and Ramkumar, S.V., Antimicrobial and free radical scavenging activity of Corynebacterium L flowers. International Journal and Applied Sciences, **567**: 34-42 (2007).
5. Rojas, J.J.; Ochoa, V.J; Ocampo, S.A. and Munoz, J.F., Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. Universidad deAntequia, **67**: 1-23 (2005).