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Antibacterial potential of *Clerodendrum inerme*, crude extracts against some human pathogenic bacteria

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SUMMARY

This communication emphasized upon the sensitivity of the crude extracts of *clerodendrum inerme* against some of the human pathogenic bacteria. Five plant extracts (Petrol, Benzene, Methanol, Ethly acetate and Aqueous) under six different concentrations(500 μg/ml, 1 mg/ml, 2 mg/ml, 5 mg/ml, 10 mg/ml and 15 mg/ml) were tested by disk diffusion method. Methanol, Ethyl acetate and Aqueous extracts of the plant showed significant inhibition against fifteen of the eighteen bacteria tested. No earlier report on antibacterial activity of this taxon could be found in literature.

Key words: *Clerodendrum inerm*; Sensitivity; Inhibition

INTRODUCTION

Plants have been an integral part of human society since the start of civilization. India is rich in its plants diversity, a number of plants have been documented for their medicinal potential which are in use by the traditional healers, herbals folklorists and in Indian systems of medicine namely, Ayurveda, Unani, Siddha apart from a Homeopathy and Electropathy. These plant species play major role in the health care of the nations population.

Different national and international pharmaceutical companies are utilizing such plant based formulations in treatment of various diseases and disorders world around (Kirtikar and Basu, 1935; Satyavati *et al.*,1987; Jain, 1991; Chandel *et al.*, 1997; Singh and Gautam, 1997; Khan *et al.*, 2002; Pulliah, 2002).

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Many of the plant species have been documented pharmacologically and clinically which are endowed in phytochemicals with marked activity on human pathogenic bacteria. (Anonymous, 1976; Ray and Majumdar, 1976; Fransworth, 1988; Rastogi and Mehrotra, 1991, 1993; Asolkar *et al.*,1992; Cox, 1994; Perry and Metzer, 1998; Rastogi, 1998; Khan, 2002; Khan *et al.*, 2002). An attempt was made to study the possible anti bacterial potential of the plant *Clerodendrum inerme* (L.) Gaertn. [Verbenaceae]. Genus species: *Clerodendron inerme*, Family: Verbenaceae, English name: Glory Bower genus, Growth form: shrub, Growth location: terrestrial, Growth environment: cultivated garden, Growth zone: Tropical, Average height: Two to three meters, Stem: Woody, smooth, Leaf arrangement: Simple, opposite, Stipule: Absent, Petioles: 0.5 to 1.0 cm long, Leaf blade: Ovate to elliptical shape, 5 to 10 cm long, acute to acuminate tip, green, smooth slightly shiny upper surface, pinnate venation, margin is entire, Inflorescence: Cyme or umbel usually comprised of three flowers joined at a common base point, Floral bracts: None, Flower:

Rotationally symmetric, salver form, white– symmetry, sex, arrangement of male and female flowers if unisexual, Calyx: Green, fused and reduced to a cup-like structure surrounding the ovary, Corolla: Fused white corolla with five lobes, Stamens: Four reddish purple, upwardly curved stamens, Ovary: Ovary is inferior, Pistil: A single redish purple, upwardly curved style, style splits into a two forked stigma, Fruit: Not observed at collection time. Reported to be green to brown obovoid fruit, 1 to 1.5 cm long which splits into four one-seeded nut lets at maturity, Seed: Not observed, Anything else: No strong floral odor (Khan, 2002).

Medicinal uses

Locally the plant is known as *Lanjai*, its leaves are used in chronic pyrexia (Khan, 2002). Eye infection: Flower juice is squeezed in your eyes. Fever: Pound the leaves and boil the pounded leaves. Decoction is made which is used internally. Flu: Pound the leaves, squeeze the juice out, and drink it. Babies: Mix the juice with water so that it may not be bitter for the child. Adults: Do not mix with water but fill the juice in one cup and drink it. Flu with headache: Put leaves in pot of water at boiling temperature (removed from stove!) and then cover yourself with a sheet and breathe the steam. Skin rash: Pound the leaves and mix with coconut oil and use on your skin or just use the juice of the leaves on infected skin. Umbilical cord infection: Pound the leaves and put it in a can and burn it until it dries. When it cools, spread it on the baby's belly button. (Samoan Herbal Medicine, Whistler, Isle Botanica, 1996). Chemical constituents: 3- epicaryoptin, neolignan. Pharmacology: Alcoholic extract of the plant proved to be hypotensive. While essential oil possess anti fungal properties (Rastogi and Mehrotra, 1991,1993; Asolkar *et al.*, 1992; Rastogi, 1998).

MATERIALS AND METHODS

Plant material

Clerodendrum inerme (L.) Gaertn. [Verbenaceae], leaves of the plant were collected from the university campus, Aligarh Muslim University, Aligarh , India.

Preparation of extracts

Crude plant extracts; were prepared following Robinson (1963), the protocol is described below.

i. Freshly dried and healthy plant material is ground into fine powder in an electric grinder. Powder so obtained is stored in dessicator.

ii. Five hundred g plant powder is refluxed with 95% methyl alcohol (MeOH) in a round bottom flask on a water bath for 10 h. Mother liquor (Crude MeOH extract) is filtered out and residual plant material is again refluxed with 95% methyl alcohol for 10 h. The process is repeated four times to obtain maximum yield of MeOH extract. The extract is evaporated to dryness at 50°C under reduced pressure.

iii. Dried methanolic extract is refluxed with light petrol (60 - 80°C) for five hours. After filtration, the residual methanolic extract is again refluxed with petrol for 5 h and filtered. This process is repeated five times. Petrol is evaporated under reduced pressure to obtain petrol soluble extract.

iv. Petrol insoluble fraction of methanolic extract obtained in step (iii) is refluxed with benzene for 5 h. Thereafter, it was filtered and refluxed again with benzene for five hours and filtered. The process was repeated five times. Benzene is evaporated under reduced pressure to obtain benzene soluble extract.

v. Benzene insoluble fraction obtained in step (iv) is refluxed with ethyl acetate for 5 h. Thereafter, it is filtered and refluxed again with ethyl acetate for 5 h and filtered. The process is repeated five times. Ethyl acetate is evaporated under reduced pressure to obtain ethyl acetate soluble extract.

vi. Ethyl acetate insoluble fraction obtained in step (v) is refluxed with methyl alcohol (95%) for 5 h, filtered and is repeatedly refluxed for five times with methyl alcohol (Methanol). The methanolic soluble fraction is evaporated under reduced

pressure to obtain methanolic extract, while methanol insoluble residue is discarded.

Preparation of aqueous extract

Shade dried plant material (500 g) is ground to a fine powder, It is poured with distilled water, and left for 72 h at room temperature. The flask is then refluxed over hot water bath for 10 h and the mother liquor is filtered. The solute is again added with solvent (distilled water) that is again refluxed and filtered, this process is repeated for 4 times. The filtrate, thus obtained, is evaporated to complete dryness on a water bath. The residue thus obtained is aqueous plant extract. *Yields* per 1,000 g dry material: petrol ≃ 10.0 g, Benzene ≃ 12.5 g, EtOAC ≃ 8.0 g and MeOH ≃ 13.0 g. Aqueous extract material (500 g) (yield ≃ 40.0 g).

Microorganisms

The leaf extracts were tested for possible antibacterial activity in the disk assay using eighteen human pathogenic bacteria listed in Table 1. The bacteria were obtained from the bacterial stock, Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh, India. The bacterial cultures were maintained at 4°C on nutrient agar.

Anti microbial assay

The (Muellerand Hinton, 1941) agar plates were inoculated with inoculum of 10^6 size, a sterile swab is dipped into diluted culture inoculums, the agar surface of the plates is streaked in three directions turning the plates by 60° by each streak. The paper disk(whatman filter paper no. 1) with 500 µg/ml, 1 mg/ml, 2 mg/ml, 5 mg/ml,10 mg/ml and 15 mg/ml plant extracts were dried and placed at the agar surface with the help of a sterile forceps. Finally press the sensitivity disc with forceps to make complete contact with the surface of the medium. Allow the plates to stand at room temperature for 30 min(Pre diffusion time). Inoculated petridishes were incubated at 37°C over night and the inhibition zone were recorded(Bauer *et al.*, 1966; Cruickshank, 1968; Colle and Marr, 1989). The experiments were repeated thrice and the mean of the triplicate of the results is summarized in Table 1.

Studied activity

Antibacterial activity by disc diffusion method (Bauer *et al.*, 1966; Cruickshank, 1968). Diameters of petri dish and disc 9.0 cm and 0.5 cm respectively.

RESULTS

The petrol extracts of the plant was found to be effective against four of the gram positive and seven of the gram negative pathogenic bacteria. Benzene extract of the plant inhibited the growth of five gram positive and six gram negative bacteria. Ethyl acetate and methanolic fraction of the plant was found to be effective against all the tested gram positive bacteria while they were found inactive against three of the gram negative bacteria. (*Pseudomonas aeruginos, Salmonella typhi, Shigella dysenteriae*). The crude leaf extract (Aqueous extract) of the plant was effective against twelve of the tested pathogens (Table 1).

DISCUSSION

Very interesting facts were recorded during the sensitivity test performed. In case of petrol extract *Staphylococcus aureus* was the most affected bacteria (zone of inhibition 6 mm/500 µg/ml/disk). Followed by *Shigella dysenteriae* and *Shigella flexneri* (zone of inhibition 5 mm and 4 mm/500 µg/ml/disk). Benzene extract inhibited the growth of eleven tested bacteria and the maximum inhibition zone was recorded against *Staphylococcus albus* (zone of inhibition 6 mm/500 mg/ml/disk). Fifteen microorganisms were found sensitive to ethyl acetate fraction and the most affected bacteria was *Streptococcus haemoliticus Group-A* (zone of inhibition 5 mm/500 µg/ml/disk). Followed by three of gram negative bacteria *Edwardsiella tarda, Klebsiella pneumoniae and Proteus vulgaris* (zone of

Table 1. Antibacterial activity of *Clerodendrum inerme* (Leaf extract)[a] crude extracts Inhibition zone (mm)

	Gram Positive Bacteria							Gram Negative Bacteria									
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	9	10	11
Petrol																		
500 µg	06	02	-	0	0	-	-	-	00	00	04	-	-	-	-	05	05	-
1 mg	06	05	-	04	04	-	-	-	03	04	04	05	05	-	-	06	06	-
2 mg	11	08	-	09	08	-	-	-	08	09	09	08	08	-	-	09	09	-
5 mg	14	11	-	12	11	-	-	-	12	12	12	11	11	-	-	12	13	-
10 mg	18	16	-	17	16	-	-	-	17	17	17	16	16	-	-	18	17	-
15 mg	22	19	-	20	19	-	-	-	20	20	21	19	19	-	-	20	19	-
Benzene																		
500 µg	03	-	06	-	04	00	04	00	00	04	04	-	-	-	00	-	-	05
1 mg	03	-	07	-	05	04	05	05	02	05	06	-	-	-	03	-	-	06
2 mg	06	-	10	-	08	07	09	08	06	09	09	-	-	-	07	-	-	09
5 mg	12	-	14	-	11	10	12	11	09	13	12	-	-	-	12	-	-	11
10 mg	16	-	17	-	16	12	16	16	12	16	16	-	-	-	16	-	-	16
15 mg	19	-	20	-	19	16	21	19	16	19	19	-	-	-	19	-	-	19
Ethylacetate																		
500 µg	04	04	04	05	01	03	04	-	04	04	-	04	-	01	03	02	02	03
1 mg	04	05	05	05	02	04	05	-	04	05	-	04	-	02	05	03	03	04
2 mg	08	08	07	08	04	06	09	-	08	07	-	06	-	04	09	06	06	07
5 mg	14	14	12	14	07	10	15	-	10	10	-	09	-	06	14	09	09	10
10 mg	17	16	17	19	09	12	19	-	13	13	-	14	-	09	17	11	11	12
15 mg	21	20	21	21	12	18	21	-	17	18	-	19	-	11	21	13	15	17
Methanol																		
500 µg	03	04	04	-	-	02	04	05	-	-	03	-	03	-	-	02	02	04
1 mg	04	05	04	02	-	03	05	05	-	02	04	-	04	-	-	02	05	05
2 mg	08	09	08	06	04	06	08	08	-	06	08	-	06	-	04	05	06	07
5 mg	14	15	12	09	07	10	14	14	-	09	11	-	09	-	06	09	09	12
10 mg	17	17	17	13	09	12	17	17	-	12	13	-	12	-	09	11	11	14
15 mg	21	21	20	19	12	16	20	20	-	16	18	-	16	-	11	15	14	16
Aqueous																		
500 µg	-	-	-	02	-	-	-	-	-	01	-	01	-	-	-	-	-	-
1 mg	-	03	02	03	-	-	-	-	-	02	-	02	-	-	-	-	-	-
2 mg	05	06	05	06	-	05	03	-	02	05	-	05	-	-	04	-	05	-
5 mg	10	11	10	10	03	07	08	-	05	0810	-	07	-	-	06	-	07	-
10 mg	13	13	13	14	05	10	11	-	08	13	-	09	-	-	08	-	10	-
15 mg	16	17	15	16	08	12	15	-	10		-	11	-	-	11	-	12	-
Chloramphenicol																		
10 µg/disc	18	18	16	-	-	-	16	18	16	-	16	18	-	16	17	19	18	20

Gram positive bacteria: 1.Staphylococcus aureus 2. Staphylococcus aureus ATCC 25953 3. Staphylococcus albus 4. Streptococcus haemolyticus Group-A 5. Streptococcus haemolyticus Group-B 6. Streptococcus faecalis 7. Bacillus subtilis.

Gram negative bacteria: 1.Escherichia coli 2. Edwardsiella tarda 3. Klebsiella pneumoniae 4. Proteus mirabilis 5. Proteus vulgaris 6. Pseudomonas aeruginosa 7. Salmonella typhi 8. Shigella boydii 9. Shigella dysenteriae 10. Shigella flexneri 11. Plesiomonas shigelloides. [a]Values are the mean of replication of three; -, no inhibition.

inhibition 4 mm/500 μg/ml/disk/each). While methanolic fraction was found to effective against *Staphylococcus aureus ATCC 25953, Staphylococcus albus and Bacillus subtilis* (zone of inhibition 4 mm/500 mg/ml/disk/each), gram positive, maximum inhibition was recorded against the gram negative bacteria, *Escherichi coli* (zone of inhibition 5 mm/500 g/ml/disk/each). Aqueous extract showed moderate degree of sensitivity against tested pathogenic bacteria and was able to inhibit the growth of twelve of the tested pathogens.

From the results it is clear that leaves of *Clerodendrum inerme* are effective in controlling bacterial pathogens, particularly gram positive bacteria. In these investigations it becomes certain that most effective crude extract were ethyl acetate and methanolic fraction that inhibited the growth of fifteen tested human pathogens. While petrol and benzene extracts as compared to the methanolic showed weak anti microbial action. This action may be synergistic and not due to the efficacy of one single substance. It was also noticed that methanolic, ethyl acetate and aqueous extracts showed antibacterial activity against both types of pathogens (Table 1). Above results revealed that plant extracts can be effective antibiotics. Both in controlling gram positive and gram negative human pathogens. The results also confirm the utility of plant in many medicinal uses reported in the introduction.

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