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*** (L.) Gaertn. [Verbenaceae] against some of the human pathogenic bacteria. Five plant extracts (Petrol, Benzene, Methanol, Ethly acetate and Aqueous) under six different concentrations(500mg/ml, 1mg/ml, 2mg/ml, 5mg/ml,10mg/ml and 15mg/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 inerme*, sensitivity, inhibition.

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 (Chandel et al., 1997; Singh & Gautam, 1997; Satyavati et al.,1987; Pulliah, 2002; Jain, 1991; Khan et al.,2002 ; Kirtikar & Basu , 1935)

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 & Majumdar , 1976 ; Khan, 2002; Cox, 1994; Khan et al., 2002; Asolkar et al.,1992; Rastigi & Mehrotra, 1991,1993; Rastogi, 1998 ; Perry & Metzer, 1998 ; Fransworth , 1988).

An attempt was made to study the possible anti bacterial potential of the plant *Clerodendrum inerme* (L.) Gaertn. [Verbenaceae]. It is a straggling shrub, leaves obovate to elliptical oblong, and glabrous. Plant is commonly grown as hedged. Locally the plant is known as *Lanjai* , its leaves are used in chronic pyrexia (Khan , 2002).

Chemical constituents: 3- epicaryoptin, neolignan.

Pharmacology: Alcoholic extract of the plant proved to be hypotensive. While essential oil possess anti

fungal properties(Asolkar et al.,1992; Rastigi & Mehrotra, 1991,1993; 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 hours. Mother liquor (Crude MeOH extract) is filtered out and residual plant material is again refluxed with 95% methyl alcohol for 10 hours. 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 methanol extract is refluxed with light petrol (60-80°C) for five hours. After filtration, the residual methanol extract is again refluxed with petrol for five hours and filtered. This process is repeated five times. Petrol is evaporated under reduced pressure to obtain petrol soluble extract.

iv. Petrol insoluble fraction of methanol extract obtained in step (iii) is refluxed with benzene for five hours. 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 five hours. Thereafter, it is filtered and refluxed again with ethyl acetate for five hours 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 five hours, 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. The steps are graphically presented as a flow chart in Fig. 3B

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 hours at room temperature. The flask is then refluxed over hot water bath for 10 hours 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 1000 g dry material**: Petrol $\simeq$ 10.0 g, Benzene $\simeq$ 12.5 g, EtOAC $\simeq$ 8.0 g and MeOH $\simeq$ 13.0 g. aqueous extract material (500 g) (yield $\simeq$ 40.0 g).

Microorganisms

The leaf extracts were tested for possible antibacterial activity in the disk assay using eighteen (18) human pathogenic bacteria listed in table no. 1. The bacteria were obtained from the bacterial stock, Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh, India. The bacterial cultures were maintained at 4^0C on nutrient agar.

Anti microbial assay

The (Mueller& Hinton, 1941) agar plates were inoculated with inoculums 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 60^0 by each streak .The paper disk(what man filter paper no 1) with 500mg/ml, 1mg/ml, 2mg/ml, 5mg/ml,10mg/ml and 15mg/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 minutes. (Pre diffusion time). Inoculated petridishes were incubated at 37^0C over night and the inhibition zone were recorded.(Bauer et al., 1966, Cruickshank, 1968 ; Colle & Marr, 1989). The experiments were repeated thrice and the mean of the triplicate of the results is summarized in table no. 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 extract 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 Methanol 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 fifteen of the tested pathogens.(Table no. 1)

Table – 1

Antibacterial activity of *Clerodendrum inerme* (Whole plant)[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 mg	06	02	-	0	0	-	-	-	00	00	04	-	-	-	-	05	06	-
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 mg	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 mg	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 mg	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 mg	-	-	-	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 mg/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.

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 6mm/500mg/ml/disk). Followed by *Shigella dysenteriae* and *higella flexneri* (zone of inhibition 5mm/500mg/ml/disk). Benzene extract inhibited the growth of eleven tested bacteria and the maximum inhibition zone was recorded against *Staphylococcus albus*(zone of inhibition 6mm/500mg/ml/disk).Fifteen microorganisms were found sensitive to ethyl acetate fraction and the most affected bacteria were *Staphylococcus aureus, Klebsiella pneumoniae* and *Shigella boydii*(zone of inhibition 8mm/500mg/ml/disk/each).While methanolic fraction was found to most effective against *Streptococcus faecalis* and *Bacillus subtilis* gram positive and three of the gram negative bacteria(*Klebsiella pneumoniae, Proteus mirabilis and Shigella boydii)* (zone of inhibition 7mm/500mg/ml/disk/each).

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 was ethyl acetate for which maximum zone of inhibition was recorded. Followed by methanol fraction that also inhibited the growth of fifteen tested human pathogens. While petrol and benzene extracts as compared to the methanol 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 methanol, ethyl acetate and aqueous extracts showed antibacterial activity against both types of pathogens (Fig. 1). The above results revealed that plant extracts could be effective antibiotics. Both in controlling gram positive and gram-negative human pathogens. The results also confirm the utility of plant as a wound-healing agent.

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