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In vitro Antibacterial Activity of *Clausena dentata* (Willd.) M. Roem Leaf extracts Against Opportunistic Bacterial Pathogens of HIV/AIDS Patients

A. Freeda Rose[1], S. R. Senthil Kumar[2] and T. Francis Xavier[2]

[1]*Department of Botany, Holy Cross College, Tiruchirappalli – 620 002, Tamil Nadu, India*
[2]*Department of Botany, St. Joseph's College, Tiruchirappalli – 620 002, Tamil Nadu, India*

Corresponding author

Abstract

Human Immunodeficiency Virus (HIV) is one of the major threats to mankind. HIV infection is a global pandemic with numerous cases reported from many countries. The major threat to their life is not by the virus but due to these opportunistic pathogens and associated complications Infections induced by pathogenic bacteria are recognized as emerging threat to HIV/AIDS patients. *Clausena dentata* is a small tree belonging to the family Rutaceae with medicinal, nutritive value and volatile oils of four furanoid terpenic compounds, α-clausenan, rosefuran (γ-clausenan) and diclausenans A.In the present study, antibacterial activity of the ethanol, ethylacetate, chloroform, petroleum and acetone extracts of *Clausena dentata* leaf were tested against ten opportunistic bacteria isolated from the HIV/AIDS patients of Thuraiyur region of Tiruchirappalli District. Among the various solvent extracts tested, the ethanol extract showed significant antibacterial activity against most of the test bacteria. The zones of inhibition were higher in the case of *Staphylococcus hominis* and *Enterobacter faecalis Staphylococcus haemolyticus* and *Klebsiella pneumoniae*

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Introduction

Infections induced by pathogenic bacteria are recognized as emerging threat to global health and socio-economic problems (Walsh *et al.*, 1996; Iwu *et al.*, 1999).These are responsible for 14 million global deaths annually (Walsh *et al.*, 2003) and amongst them bacterial infections is a major threat to humans beings (Westh *et al.*,2004) Although there are several antibiotics for treating bacterial and fungal infections, they are not consistently effective against pathogenic organisms (Gearhart, 1994). Its use is limited by a number of factors such as low potency, poor solubility and drug toxicity (Fromtling and Rahway, 1987; Portillo *et al.*, 2001). Further, the development of resistance in pathogenic bacteria and fungi against most of the drugs have been reported (Cuenca-Estrella *et al.*, 2000).The increasing resistance of microorganisms to antimicrobial drugs in use has attracted the attention of the scientific community regarding the search for new cost-effective drugs of natural or synthetic origin (Pai *et al.*, 2004).Medicinal plants and corresponding preparations have been used for a wide range of purposes and for many centuries people have been trying to treat diseases as well as alleviate symptoms by using different plant extracts and formulations (Cowan, 1999).Ethnomedical literatures have also reported several medicinal plants which have been used by traditional people all over the world for

treating wounds, cuts, dysentery, diarrhea, cough, sore throats, fever, jaundice, skin ailments and venereal diseases (Tanira *et al.*, 1994; Buwa and Vanstaden, 2006).Ethnobotanical literature cleared that more than 180 different medicinal plant species used by the traditional medical practitioners are in use even today. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care (Penso, 1980; Alade and Irobi, 1993).

Clausena dentate (Willd.) M. Roem is a small belongs to Rutaceae family and widely distributed in India. (Agarwal, 1981).The vernacular name of this plant is called as Anai chedi. It is widely used by tribal people of Pachamalai Hills for its medicinal and nutritive value. The phytochemical studies of the plant have revealed the presence of volatile oils of four furanoid terpenic compounds, α-clausenan, rosefuran (γ-clausenan) and diclausenans A and B (Rao and Subramanian, 1934) and furanoterpenes (Subba Rao *et al.*, 1984). Hence, the present study was undertaken to screen the antibacterial potential against human pathogenic bacteria

Materials and Methods

Plant Collection and Storage

The plant materials (*Clausena dentata* leaves) were collected around Thalur village of Pachamalai Hills, part of Eastern ghats of Tamil Nadu located in Tiruchirappalli District and were authenticated by Dr.S.John Britto, Director, Rapinat Herbarium, St.Joseph's College, Trichy The leaves were separated from stems, washed in clean water, and dried at room temperature (Eloff, 1998).The dried plants were milled to a fine powder in an Electronic Blender and stored in the dark at room temperature in closed containers until required.

Solvent extracts

50 grams of the dried powdered plant materials (leaves) were soaked separately with 300 ml of each of the solvents *viz.* ethanol, ethyl acetate, chloroform, petroleum ether and acetone in a soxhlet apparatus for 48 hr at 310° C until complete extraction of the materials. At the end of 48 hr each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume. The paste like extracts were stored in pre-weighed screw scapped bottles and the yield of extracts has been weighed. These screw capped bottles were kept in refrigerator at 40° C. Each of the extract was individually reconstituted using minimal amounts of the extracting solvent prior to use.

Collection of clinical samples

Clinical samples *viz* blood, urine, sputum and oral swabs were collected from the suspected symptomatic HIV/AIDS patients of Government Hospital of Thuraiyur region in Tiruchirappalli District, under the watchful eye of experienced medical practitioner. All unsuitable specimens were discarded and a repeat specimen was collected.

Isolation and Identification of pathogens

Bacterial pathogens tested in this study were isolated from clinical samples of suspected symptomatic HIV patients. More than ten bacterial pathogens were isolated and confirmed by staining, morphological and biochemical characteristics. Among them, ten bacterial pathogens *viz Klebsiella pneumonia, Escherichia coli DH5α, Staphylococcus haemolyticus, Klebsiella, pneumoniae DSM, Staphylococcus hominis, Staphylococcus haemolyticus, Enterobacter faecalis, Staphylococcus saprophyticus, Enterobacter faecalis Pseudomonas lutea* were selected for antimicrobial screening test.

Maintenance of Bacterial Culture

The bacterial pathogens were inoculated on Nutrient agar slants and incubated overnight at 37^{o}C. These cultures were stored in a refrigerator at 4^{o}C. Fresh slant cultures were prepared every 2-3 weeks until tested for further antibacterial studies

Assay for antibacterial testing

Antibacterial activity of the above mentioned four different solvent and aqueous extracts were assayed separately using disc diffusion method (Bauer *et al.*, 1966). Petri plates containing 10 ml of Muller Hinton Agar medium were inoculated with 10^{8} CFC/ml of each test bacteria. Sterile filter paper discs (6 mm in diameter) were impregnated with 10µl of the 3 mg/ml plant extracts (30µg/disc) placed on the surface of the medium. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. A standard disc containing chloramphenicol antibiotic drug (30µg/disc) was used as a positive control and they were incubated for 24 h. The assessment of antibacterial activity was

based on the measurement of diameter of inhibition zone formed around the disc. Three independent trials were conducted.

Results and Discussion

Isolation and identification of opportunistic bacterial pathogens from HIV /AIDS patients

As per the guidelines and procedure for biochemical test referred from *Microbiology Laboratory Manual* (Sundarraj, 2010), 17 major bacteria were isolated from the clinical samples of suspected symptomatic HIV/AIDS patients. All the isolated bacterial species were confirmed through morphology, staining and biochemical tests and all the test results were compared with the standard test results for confirmation shown in Table 2. Isolated pathogens with their percentage of occurrence in respective clinical samples is mentioned in Table 1. Seventeen major bacterial pathogens were isolated from the samples; The predominant bacteria isolated in the study were *Escherichia coli* RV412 and *Klebsiella pnemoniae* (11.1%), *Escherichia coli* DH5 alpha, *Enterobacter cloacae* MB, *Klebsiella pneumonia* DSM and *Staphylococcus haemolyticus* CHB (7.4%), where as other organisms like *Enterobacter cloacae* DSM, *Citrobacter sedlakii*, *Staphylococcus haemolyticus DSM*, *Staphylococcus saprophyticus* DSM, *Pseudomonas lutea*, *Enterobacter faecalis* DSM, *Staphylococcus hominis* DSM, *Staphylococcus hominis* MB, *Corynebacterium aurimucosum*, *Lechlerchia adecarboxylate and Enterobacter faecalis* CHB were found to be very low such as 3.7%. Out of all pathogens isolated bacterial isolates constituted 92.6%. Among the isolated bacterial pathogens 52% of bacteria responsible for urinary tract infections and 24% cause respiratory infections and 24% causes blood borne infections. Among HIV positive patients, the most prevalent urinary tract pathogen is *Escherichia coli* (11.1%). The studies made by Ayyagari *et al.*, (1999) found such result in their study. The present study result is also supported by Aggarwal (2005) where they reported the similar kind of results whereas the other bacterial pathogens isolated in our study were varying from the study of other workers(Afessa and Grren,2000) who have reported *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Haemophilus influenzae* to be predominant isolates.

Table.1 Opportunistic bacterial pathogens isolated from HIV/AIDS patients

S. No	Name of the organism	Number of isolates	Percentage (%)
1	*Escherichia coli* RV_{412}	6	11.1
2.	*Escherichia coli* DH5 alpha	4	7.4
3.	*Enterobacter cloacae* DSM	2	3.7
4.	*Enterobacter cloacae* MB	4	7.4
5.	*Klebsiella pneumonia*	4	7.4
6.	*Citrobacter sedlakii*	2	3.7
7.	*Staphylococcus haemolyticus* DSM	2	3.7
8.	*Staphylococcus saprophyticus* DSM 20038	2	3.7
9.	*Pseudomonas lutea*	2	3.7
10.	*Lechlerchia adecarboxylata*	2	3.7
11.	*Enterobacter faecalis* DSM	2	3.7
12.	*Staphylococcus hominis* DSM	2	3.7
13.	*Staphylococcus hominis* MB	2	3.7
14.	*Corynebacterium aurimucosum*	2	3.7
15.	*Klebsiella pneumonia* CHB	6	11.1
16.	*Staphylococcus haemolyticus* CHB	4	7.4
17.	*Enterobacter faecalis* CHB	2	3.7

Table.2 Antibacterial activities of *Clausena dentata* leaf extracts on opportunistic bacterial pathogens

Inhibition zone diameter in mm (mean ± SD)											
Test bacteria	**Petroleum ether**		**Chloroform**		**Acetone**		**Ethyl acetate**		**Ethanol**		**Positive control**
	Experimental	**Negative control**	**Experi mental**	**Negative control**	**Experi mental**	**Negative control**	**Experimental**	**Negative control**	**Experimental**	**Negative control**	**Chloramphenicol (30 mg/disc0**
Klebsiella pneumoniae	9.3 ±0.94	-	-	-	1.4±0.9	-	8.0±1.414	-	**17.3±2.05**	-	16±0.0
Escherichia coliDH5α	10±0.00	-	-	-	1.0±0.4	-	10±1.414	-	12±0	-	-
Staphylococcus haemolyticus	8±4.14	-	-	-	17.0±0.53	-	9.6±1.247	-	18±2.16	-	16±0.0
Klebsiella pneumoniae DSM	4.6±3.29	-	-	-	15.6±0.94	-	11.3±1.69	-	13±2.94	-	18±0.0
Staphylococcus hominis	9±0.81	-	-	-	18.3±2.86	-	6.66±4.7	-	19.3±2.6	-	18±0.0
Staphylococcus haemolyticus	7.6±0.4	-	-	-	13.6±1.24	-	10.3±2.35	-	15.3±1.24	-	27±0.0
Enterobacter faecalis	7.3±1.24	-	-	-	11±2.16	-	13±2.160	-	13±2.82	-	18±0.0
Staphylococcus saprophyticus	8.6±0.47	-	-	-	15±4.08	-	8.66±1.24	-	11.6±3.09	-	15±0.0
Pseudomonas lutea	-	-	-	-	13±1.414	-	10.6±1.88	-	19.8±0.94	-	18±0.0
Enterobacter faecalis	11±1.414	-	-	-	16.3±0.94	-	11.3±2.6	-	11±2.16	-	21±0.0

Note: (-) Negative Controls; (+) Positive controls

Antibacterial activities

The evaluation of the antibacterial activity of the ethanol, ethylacetate, chloroform, petroleum and acetone extracts of *Clausena dentata* leaf by using the disc diffusion method is given in Table 2. The *in vitro* results were observed in terms of inhibition zone around each disc caused by diffusion of antibacterial properties from the plant extract impregnated disc into the surrounding medium. As can be seen from Table 2 among various solvent extracts tested ethanol leaf extracts exhibited high degree of inhibition followed by ethyl acetate and acetone solvent extracts. The petroleum ether extract showed moderate degree of inhibition against some bacteria and low degree of inhibition against other bacteria. The chloroform extract did not show any antibacterial activity. In addition, the inhibition zones formed by standard antibiotic disc (chloramphenicol 30 mcg/disc) and those filter paper discs injected with ethanol, ethylacetate, chloroform, petroleum ether and acetone (negative controls) are also depicted in Table 2. The diameter of inhibition zones for each of the samples were compared with standard antibiotics. It was noted that the inhibition zones of the samples to be either less than or greater than or equal to the inhibition zones of standard antibiotic. The ethanol extract showed significant antibacterial activity against most of the test bacteria. The zones of inhibition were higher in the case of *Staphylococcus hominis (*19.3±2.6) and *Enterobacter faecalis (*19.8±0.94), *Staphylococcus haemolyticus (*18±2.16) and *Klebsiella pneumoniae (*17.3±2.05). Similar results were drawn by Autade *et al.,* (2015) where they reported the acetone extract of Neem leaf exhibited significant antibacterial activities against the opportunistic bacterial pathogens of HIV/AIDS patients.

The present investigation revealed the antibacterial effectiveness of *Clausena dentata* leaf extract with ethanol as a solvent against the most common opportunistic infection associated with HIV/AIDS patients of Thuraiyur region of Tiruchirappalli District. Further study also is needed to determine the active principles that pose antibacterial l activities

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