Ancient Science of Life, Vol. 29, No.2 (2009) Pages 28 - 31

Antibacterial activity of methanol extract of *Ruta chalapensis* (L), *Quercus infectoria* (Oliver) *and Canthium parviflorum* (Lam)

[1]P. Sathiya Priya
[2]J.M Sasikumar
[3]G.Gowsigan

Abstract

The present study aimed at evaluating the antibacterial activity of methanol extract of *Ruta chalapensis,* L., *(Rutaceae), Quercus infectoria* Oliver., *(Fagaceae)* and *Canthium parviflorum* Lam., *(Rubiaceae) against Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella oxytocoa, Klebsiella pneumoniae and Proteus mirabilis. The experiment was carried out using disc diffusion method. The results revealed that the methanol extract of aerial parts of Ruta chalepensis* (L) *presented the highest zone of inhibition against tested pathogens. Other plants showed significant zone of inhibition.*

Key words: *Ruta chalapensis,* (L) *Quercus infectoria* (Oliver)*, Canthium parviflorum,* (Lam) antibacterial activity and zone of inhibition.

Introduction

Medicinal plants have been a major source of cure for human diseases since time immemorial. Today one fourth of the world population depends on traditional medicines. For centuries plants have been used throughout the world as drugs and remedies for various diseases.[1] Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One such resource is folk medicines and systematic screening of these may result in the discovery of novel effective compounds. Making antibacterial drug therapy effective, safe and affordable has been the focus of interest during recent years[2].

Plants are the oldest source of pharmacologically active compounds and have provided mankind with many medically useful compounds for centuries. Screening of antimicrobial plants for new agents possesses an enormous challenge and is important especially with the emergence of drug resistant disease strains. It has only been in the past two decades or so that interest in higher plant antimicrobial agents has been reawakened worldwide and the literature in this area is becoming substantial[3]. Despite the introduction of antibiotics since the 1940's, even 80 percent of the population today relies on indigenous medicinal plants and the drugs[4].

Three potential traditional medicinal plants were selected for the present investigation is *Ruta chalepensis* (L)*, Quercus infectoria* (Oliver) *and Canthium parviflorum* (Lam).

R. chalapensis (L) belongs to Rutaceae is an aromatic perennial herb growing up to 75 cm in height. It is one of the important plant used in Ayurvedic medicine in vitiated conditions of *Kapha* and *Vata*. The oil acts as a stimulant for uterine and nervous systems. The juice obtained from fresh leaves of this plant is given to children for helminthic infections and is good for odontalgia and otalgia.[5] *Quercus infectoria*, Oliver (Fagaceae) is a large deciduous tree with a long main trunk, fan shaped crown and deeply furrowed, brownish grey bark. Ayurvedic practitioners use powdered galls in the form of infusion or decoction. Decoction is usually employed as an astringent, wash, gargle, enema or injection.[6] *C. parviflorum* (Lam) is a thorny subscandent shrub with spreading branches distributed throughout India, in scrub forest and dry plains. Traditionally the roots and leaves are used to cure vitiated conditions of *Kapha* in Ayurveda and the plant is used to cure diarrhea, fever and constipation.[7] The application of crude extracts from medicinal plants for the treatment of various ailments is one of the most intensive areas of medicinal plant research today. The significance of the study is to carry out preliminary antibacterial research with these medicinal plants. In this view the present study was carried out to evaluate the antibacterial activity of *R. chalepensis* (L)*, Q. infectoria* (Oliver) *and C. parviflorum* (Lam).

[1]Depart. of Biochemistry, Karpagam University; Coimbatore ,Tamil Nadu, India.
[2]Depart. of Industrial Biotechnology, Karpagam University, Cbe, Tamil Nadu, India.
[3]Depart. of Microbiology, Lister Metropolis laboratory, Cbe, Tamil Nadu, India.

MATERIALS AND METHODS

Collection of Medicinal Plants

The medicinal plants selected for this study is *R. chalepensis* (L), *Q. infectoria* (Oliver) *and C. parviflorum* (Lam). *The aerial parts of the R. chalepensis* (L) *were collected from* Ootacamund, *Nilgiris district,* Tamil Nadu, India. *Seeds of Q. infectoria* (Oliver) were obtained from herbal shop in Coimbatore, Tamil Nadu, India. *C. parviflorum* (Lam) leaves were collected from Pollachi region, Coimbatore district, Tamil Nadu, India. The plants *were identified by Dr. R. Gopalan, Professor & Botanist, in Karpagam University.*

Solvent Extraction

Aerial parts of R. chalepensis (L), seeds of Q. *infectoria* (Oliver) and leaves of *C. parviflorum* (Lam) were washed well with water. They were air dried at 25°C for 15 days in the absence of sunlight and powered well using a mixer. The powdered plant material was taken and subjected to solvent extraction using methanol.

Preparation of Methanol Extract

About 50 g of powered plant material was extracted with 250 ml methanol by using a separating funnel with occasional shaking for 16 hours and then the extract was concentrated by using rotary flask evaporator.

Test Microorganisms

Different Multi drug resistant strains of bacteria *Escherchia coli*, *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Klebsiella oxytocoa*, *Klebsiella pneumoniae* and *Proteus mirabilis*. The aforesaid microbes were procured from the patients of hospitals in Coimbatore region. The stock cultures were stored in Muller Hinton agar medium at 37^0C for future use.

Antibacterial activity

Antibacterial activity was done by disk diffusion method [8]. This is one of the widely used methodologies for rapid screening of antibacterial agents. All the Procedure was done in laminar airflow. Forceps, Petri dish, cotton swab, discs are sterilized and used for plating. Sterile condition was maintained by HEPA (High performance efficient particle air) in the laminar flow.

Inoculation of the test plates

A few colonies (3 - 5) of the organism are inoculated in 2 - 5 ml broth and grown for 2.5 hr. The cultures are diluted to a density equivalent to the 1 % barium sulfate standard. "(1 x1010 CFU/ml)" . Optimally within 15 minutes after adjusting the turbidity of the inoculum suspension to contain approximately (1-2 $x10^8$ CFU/ml), bacterial cultures were spread on agar surface by spread plating technique. After the inoculum has dried, impregnated discs are placed on the agar surface with flamed forceps and gently pressed down to ensure contact. The discs (5mm in diameters; Whatmann No.3 mm filter paper) are sterilized by autoclaving and subsequently dried at 80°C for at least 1hr. Sterile discs were dipped in methanol extract of concentration 1, 2, 5 mg/ml and placed on petriplates in circular manner. The impregnated discs are dried for 3 - 5 min and placed on pre - inoculated agar surface. The discs were gently pressed down to ensure complete contact of the disc with the agar surface. Ampicillin was used as positive control for antibacterial tests. The discs were spaced far enough to avoid both reflection waves from the edges of the Petri plates and overlapping rings of inhibition. Then the plates were incubated, (inverted position) at 37°C for 12 hours. The diameter of the zone of inhibition was measured.

RESULTS AND DISCUSSION

The results of antibacterial study of the methanol extract of *Ruta chalepensis* (L), *Quercus infectoria* (Oliver) *and Canthium parviflorum* (Lam)was showed in Table 1.

Three different concentrations of the plant extract (1, 2, 5 mg/ml) were used for the assay. All the organisms used were multidrug resistant strains from clinical samples. Methanol extract of *Ruta chalapensis* (L) at 5mg/ml concentration showed significant antibacterial potential against all tested organisms. *Escherchia coli, Klebsiella oxytocoa and Staphylococcus aureus showed* more sensitivity and had shown widest zone of inhibition. *Klebsiella pneumoniae, Pseudomonas aeruginosa* showed resistance, where all other organisms showed appreciable antibacterial activity at 2mg/ml concentration of the plant extract. The antibacterial activity may be due to the presence of essential oils like rutin. *Quercus infectoria* (Oliver) inhibited the growth of *Escherchia coli*, *Enterococcus faecalis*, *Klebsiella*

TABLE NO: 1

Antibacterial Activity of R. chalepensis (L)**, *Q. infectoria*** (Oliver) ***and C. parviflorum*** **(** Lam)

Sl.No	Organisms	Standard	Methanol extract								
			Zone of inhibition in mm								
			Ruta chalepensis			*Quercus infectoria*			*Canthium parviflorum*		
			*	**	***	*	**	***	*	**	***
1	*Escherchia coli*	23 mm	-	11	16	-	-	8	-	5	11
2	*Enterococcus faecalis*	17 mm	-	08	12	-	5	10	-	-	-
3	*Klebsiella pneumoniae*	13 mm	-	-	11	-	-	7	-	-	-
4	*Klebisella oxytocoa*	18 mm	-	13	17	-	-	-	-	-	-
5	*Proteus mirabilis*	20 mm	-	08	13	-	-	-	-	-	-
6	*Staphylococcus aureus*	18 mm	-	09	14	-	4	8	-	-	-
7	*Pseudomonas aeruginosa*	15 mm	-	-	11	-	-	-	-	-	-

*** Zone of inhibition in mm (tests were done in duplicate)**

'- ' Indicates no activity

Concentration of plant extract (* - 1 mg/ml, **- 2mg/ml, ***- 5 mg/ml)

Pneumoniae and *Staphylococcus aureus* at 5mg/ml concentration. *Klebsiella pneumoniae* and *Staphylococcus aureus* showed sensitivity at 2mg/ml concentration of plant extract, *Klebsiella oxytocoa, Pseudomonas aeruginosa and Proteus mirabilis* showed resistance at both these concentrations. This may be related to the presence of tannins, which have the ability to inactivate microbial pathogens. *Canthium parviflorum* (Lam) inhibited the growth of *E. coli* at 2 and 5 mg/ml concentration but all other organisms showed resistance at these concentrations. From this it was evident that the *Canthium parviflorum* (Lam) methanol extract is active in killing the pathogens at higher concentration. Tested organisms showed negligible antibacterial activity at 1mg/ml concentration of the methanol extract of all the medicinal plants. Activity indices of tested bacteria were different in value against ampicillin (1mg/ml). Above results suggested that among the three plants tested *R.uta chalepensis* (L) was found to have significant antibacterial activity against MDR strains. The broad spectrum activity exhibited by *Ruta chalapensis* (L) may be attributed to the various active constituents present in the crude extract.

Conclusion

From the present study it is proved that *Ruta chalepensis* (L) showed significant zone of inhibition against MDR strains. *Quercus infectoria* (Oliver) *and Canthium parviflorum* (Lam) was less active in inhibiting the growth of microorganisms, when compared to *Ruta chalepensis* (L). This study can be extended in future to isolate the chemical compounds responsible for antibacterial action and further extension of this research is possible in near future to find out the natural antibiotics from this plant origin.

Acknowlegement

The authors wish to thank Mr.G.Gowsigan, Microbiologist and Quality control manager, Department of Microbiology, Lister Metropolis Laboratory, Coimbatore for his help and support in providing cell cultures and technical assistance. We express our thanks to the management of Karpagam University for providing all facilities to carry out the research work.

REFERENCES

1. UNESCO: Culture and Health Orientation Texts World Decade for cultural Development 1988 -1997, Document CLT/DEC/PRO, Paris, France,`1996.
2. Janovska, D., Kubikov, K., and KoKosk, L., Czec, K., *J. Food. Sci.* **21**:107. (2003).
3. Cowan, M.M., Plant products as antimicrobial agents, *Clin. Micro. Rev.* **12 (4)**: 564 582. (1999).
4. Mehta, V., Indian herbal drug industry Future prospects http:// www. pharma biz.com/ article/ detnews. asp? (2004).
5. Narayan Das Prajapati,Purohit S.S, Sharma A.K and Kumar T, A Hand book of medicinal plants, a complete source book, Agrobios India Publishers, 652 (2003).
6. Singh M.P and Himadri Panda, Medicinal herbs with their formulations, Daya Publishing house 707 -708 (2005)
7. Kirtikar KR, Basu BD: *Indian Medicinal Plants with illustrations*. 2nd edition. Oriental Enterprises, Dehra Doon, India; 366 (2001)
8. Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M., Antibiotic susceptibility testing by a standard single disc method. *Am. J. Clin Pathol*, **36**: 493 - 496. (1966).