BVG Trust

J. Sci. Trans. Environ. Technov. 2011, 5(2) :96-99

Scientific Transactions in Environment and Technovation

Bioassay - Fractionation Based antibacterial activity studies on the medicinal plants - *Ipomoea obscura, Clerodendrum inerme* and *Acalypha fructicosa*

Shubashini K. Sripathi*and Uma Sankari. U

Department of Chemisty, Avinashilingam University for Women, Coimbatore - 641 043, Tamil Nadu, India.

Abstract

The plants *Ipomoea obscura*, *Clerodendrum inerme*, *Acalypha fruticosa* were collected, authenticated and extracted with ethanol. The ethanolic extracts of these plants were subjected to bioassay-guided fractionation. The antibacterial activity was evaluated for the various extracted fractions of the plants against two gram positive and two gram negative bacteria using disc diffusion method and the zone of inhibition for various bacterial strains is reported.

Keywords: *Acalypha fruticosa,* antibacterial activity, bioassay-guided fractionation, *Clerodendrum inerme, Ipomoea obscura*

INTRODUCTION

A discussion of human life on this planet would not be complete without a look at the role of plants. Although we now live in a highly industrialized society, we have not lost this dependence on plants. Natural products, as the term implies, are those chemical compounds derived from living organisms, plants, animals, and insects. Natural products isolated from higher plants have been providing novel, clinically active drugs. Drugs derived from natural products are usually secondary metabolites and their derivatives.

A typical protocol to isolate a pure chemical agent from natural origin is bioassay-guided fractionation, meaning step-by-step separation. The key to the success of discovering naturally occurring therapeutic agents rests on bioassay-guided fractionation and purification procedures. Bioassay guided fractionation is an automated, high-throughput analytical tool for the unambiguous characterization of the active components of a combinatorially derived reaction mixture. The identification of the active component in a mixture is an essential step for subsequent synthesis or isolation of the active components or for removal of intractable wells from further consideration (Douglas *et al.,* 2002).The most promising initial plant compounds are fractionated to obtain pure samples in milligram amounts. These natural pure compounds are compared to the best available therapeutics by *in vitro* testing. If the bioassay is successful, the compound is structurally characterized and is subject to a confirmatory biological test (Veilleux and King, 1996).

The plants taken up for the present study are of immense pharmacological significance. Iridoid glycosides, phenylethanoid and neo-clerodane diterpenoids have been isolated from *Clerodendrum inerme* (Kanchanapoom *et al*., 2001). Indole alkaloids have been reported from *Ipomoea obscura* (Jenett-Siems *et al.,* 2003). There are reports on the use of *Acalypha fruticosa* in the treatment of dyspepsia, cholic, diarrhea and cholera, its ophthalmic use, antibacterial activity, use of its leaf juice to wash pustules and use of its root in gonorrhea etc. (Wealth of India, Raw materials, 1971)*Acalypha* ointment is used in the treatment of superficial fungal skin diseases (Oyelani *et al*., 2003).

MATERIALS AND METHODS

Collection and identification of plant materials

The plants were collected from the Lower Dam, Agastiar falls and Servalar regions of Agasthiyamalai, Tirunelveli district, Tamil Nadu, India during February 2009. The identity of each plant was confirmed at Botanical Survey of India, and IFGTB, Coimbatore, South India.

Preparation of the Extracts

Air-dried and cut pieces of *Ipomoea obscura* (115gm), *Clerodendrum inerme* (195gm) and *Acalypha fructicosa* (220gm) were extracted separately with ethanol for 6 hours under reflux temperature. The collected extracts were evaporated under vaccum to a volume of 100ml.

Fractionation Procedure

The concentrated ethanolic extract was divided into two portions. One portion was stored under refrigeration. The other portion was fractionated with (1:1) water and (1:2) chloroform till the chloroform layer remained colourless. The separated water (upper layer) and chloroform (lower layer) layers were collected separately. The aqueous extract was concentrated under vacuum and stored under refrigeration. The chloroform extract was concentrated to 25ml. One portion of this extract was concentrated and stored. The other portion

*Corresponding Author
email:*adusks@gmail.com*

Scientific name (plant) :*Clerodendrum inerme (L.) Gaertn*
Family : *Verbenaceae*
Common Name : *Sankankuppi*
Origin : India, Malaysia
Parts used : Stem, flowers, leafs

Scientific name (plant) : *Ipomoea obscura* (L.) Ker Gawl.,
Family : *Convolvulaceae*
Common Name : *Cirutali*
Origin : Tropical East Africa, Mascarene Islands, Tropical Asia, Malesia to northern Australia & Fiji
Parts used : Stem, flowers, fruits, leafs

Scientific name (plant) : *Acalypha fruticosa* Forssk
Family : *Euphorbiaceae*
Common Name : *Serucinni*
Origin : India, Kenya, Myanmar, Namibia, Sri Lanka, Sudan
Parts used : Stem, flowers, leafs

was fractionated with 90 percent ethanol and (1:1) petroleum ether. The two layers separated were collected separately. The layers were concentrated under vacuum pump and stored under refrigeration until activity studies wear done.

Antibacterial Screening of the Bioassay-fractions

The cultures to be used for antibiotic sensitivity assay were selected and labeled. Nutrient agar plates were prepared by suspending 38g of agar in distilled water which was heated to boiling till the medium was completely dissolved. The medium was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and mixed well. This was then poured into petri plates. A swab of the test culture was aseptically taken and inoculated on the surface of the nutrient plate completely so as to make a lawn. The antibiotic disc was carefully placed using the forceps over the agar plate 15mm from the edge of the plate. The disc was gently pressed to give a better contact with the agar. The plates were incubated in an inverted position for 24 hours at 37 °C. The zone of inhibition was observed around the antibiotic disc and measured.

RESULTS AND DISCUSSION

Among the bioassay-guided fractions of *Ipomoea obscura* the aqueous extract showed maximum zone of inhibition against the *Proteus sp* (Table 1). Ethanolic fraction of *Clerodendrum inerme* exhibited maximum zone of inhibition against *Staphylococci* (Table 2). The 90 percent ethanol fraction and aqueous extract of *Acalypha fruticosa* showed maximum zone of inhibition against *Escherichia coli* and *Proteus sp*. (Table 3). A comparison was done from the zone of inhibition values to check the efficiency of the extract from the three plants against each bacteria tested (Table 4)

Among the three chosen plants the ethanol extract of *Clerodendrum inerme* exhibited good antibacterial activity against *Streptococci*. Aqueous extract of *Acalypha fruticosa* exhibited good activity and the ethanol extract of *Clerodendrum inerme* showed good activity against *E.coli*. Aqueous extract of *Ipomoea obscura*, and aqueous extract of *Acalypha fruticosa* exhibited good activity against *Proteous sp*. The antibacterial activity results suggest that the bioassay fractions contain the effective active phytochemicals responsible for the elimination of microorganisms. Thus the plant extracts could be potential source of drugs to control both gram positive and gram negative bacteria.

ACKNOWLEDGEMENT

The authors thank the authorities of Avinashilingam University for Women,Coimbatore for providing the necessary facilities for carrying out the study.

Table 1. Antibacterial Activity of *Ipomoea obscura*

Extract	Zone of inhibition (mm)			
	Staphylococcus aureus	*Streptococci* sp	*Escherichia coli*	*Proteus* sp
Ethanol extract	8	10	8	10
Chloroform extract	10	0	8	10
Aqueous extract	10	0	10	20
Pet. ether extract	10	0	10	10
90% Ethanol extract	0	0	8	10
Control	20	25	20	0

Table 2. Antibacterial Activity of *Clerodendrum inerme*

Extract	Zone of inhibition (mm)			
	Staphylococcus aureus	*Streptococci* sp	*Escherichia coli*	*Proteus* sp
Ethanol extract	8	20	10	8
Chloroform extract	8	10	8	10
Aqueous extract	10	10	10	10
Pet. ether extract	8	0	8	8
90% Ethanol extract	8	0	15	0
Control	20	25	20	0

Table 3. Antibacterial Activity of *Acalypha fruticosa*

Extract	Zone of inhibition (mm)			
	Staphylococcus aureus	*Streptococci* sp	*Escherichia coli*	*Proteus* sp
Ethanolic extract	0	10	10	10
Chloroform extract	8	0	8	0
Aqueous extract	0	0	20	20
Pet. ether extract	10	0	0	10
90% Ethanol extract	10	0	10	20
Control	20	20	10	0

Table 4. Efficiency of the extracts from the three plants

Extracts	Zone of inhibition (mm) extracts against bacteria											
	Staphylococcus aureus			*Streptococci* sp			*E. coli*			*Proteus* sp		
	Ipomoea obscura	*Clerodendrum inerme*	*Acalypha fruticosa*	*Ipomoea obscura*	*Clerodendrum inerme*	*Acalypha fruticosa*	*Ipomoea obscura*	*Clerodendrum inerme*	*Acalypha fruticosa*	*Ipomoea obscura*	*Clerodendrum inerme*	*Acalypha fruticosa*
Ethanol	8	8	0	10	20	10	8	10	10	10	8	10
Chloroform	10	8	8	0	10	0	8	8	8	10	10	0
Aqueous	10	10	0	0	10	0	10	10	20	20	10	20
Pet. ether	10	8	10	0	0	0	10	8	0	10	8	10
90%Ethanol	0	8	10	0	0	0	8	15	10	10	0	20
Control	20	20	20	25	25	20	20	20	10	0	0	0

REFERENCES

Douglas W. Phillipson K. Eric Milgram, Alex Yanovsky I, Linda S. Rusnak, David A. Haggerty, William P. Farrell, Michael J. Greig, Xiaobing Xiong, and Mark L. Proefke. 2002. High-Throughput Bioassay-Guided Fractionation: A Technique for Rapidly Assigning Observed Activity to Individual Components of Combinatorial Libraries, Screened in HTS Bioassays, *J. Comb. Chem.* 4 : 591–599.

Kanchanapoom, T., Kasai, R., Chumsri, P., Hiraga, Y. and Yamasaki, K. 2001. Megastigmane and iridoid glucosides from *Clerodendrum inerme*, *J. Phytochem.*, 58: 333-3336.

Jenett-Siems, K., Robert Weigl, Macki Kaloga, Jutta Schulz and Eckart Eich. 2003. Ipobscurines C and D: macrolactam-type indole alkaloids from the seeds of *Ipomoea obscura*, *J. Phytochem.*, 62: 1257-1263.

Nan, Haihan, Wu, Jun, Zhang, Si. 2005, A new phenylethanoid glycoside from *Clerodendrum inerme*, *J. Pharm.*, 60: 798-799.

Oyelani O.A, Onayemi O, Oladimeji F.A, Ogundaini A.O, Olugbade T.A, Onawunni G.O. 2003. Clinical evaluation of *Acalypha* ointment in the treatment of superficial fungal skin diseases, *Phytother., Res.*, 17: 555-557.

Pandey, R., Ram K. Verma and Madan M. Gupta. 2004. Neo-clerodane diterpenoids from *Clerodendrum inerme*, *J. Phytochem.*, 66 (2005): 643–648.

Veilleux, C. and Steven R. King. 1996. An Introduction to Ethnobotany, Linda Morganstein, Edi. Shaman Pharmaceuticals, Inc. San Franciso.

Wealth of India. 1971. NISCAIR, New Delhi.