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Antifungal Assay of Root Extract Fractionates of *Clerodendrum Inerme (Linn.)*

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Abstract: *Clerodendrum inerme* is a medicinal plant reported to be used for the treatment of skin diseases. Venereal infections, elephantiasis, asthma, topical burns and rheumatism. The plant possesses high medicinal potential. Fractionation of ethanol extract of roots of *Clerodendrum inerme* was done and antifungal activity of the fractions was assessed against microorganisms, *Monascus ruber, Aspergillus Niger, Aspergillus fumigatus* and *Candida albicans* by disc diffusion method. The ethanol extract showed antifungal activity against *Monoscus ruber* comparable to Standard clotrimazole.

Keywords: *Clerodendrum inerme,* Ethanolic Root extract, Antifungal activity.

INTRODUCTION

Clerodendrum inerme (Verbenaceae) is widely distributed throughout India and is a widespread evergreen commonly grown vanajai or garden Quine [1]. Leaves, stem and roots of this species are extensively used in the treatment of skin diseases, venereal infections, elephantiasis, asthma, topical burns and rheumatism [2]. In Indian tribal medicine, the leaves of *Clerodendrum inerme* are being used for curing umbilical cord infection and for cleaning the uterus [3, 4]. The plant has been studied with special

reference to its pharmacological activities [5]. The ethyl acetate and hexane extracts of the leaves have been screened for antifungal activity[6]. **Table-1** enlists the antifungal activity studies carried out on the stem and leaf parts of *Clerodendrum inerme*. The present study assesses the antifungal activity of various polar and non-polar solvent fractions of the roots of *C. inerme*.

Table-1: Reports on Antifungal Activity of *Clerodendrum inerme*.

Plant Part Analysed	Extract Analysed	Fungi Tested	Method Adopted	Reference
Leaves and root	1. Benzene 2. Methanol 3. Aqueous	*1.A. Niger* *2.A. Flavours* *3.C. albicans* *4.C. glabrata*	Disc diffusion method	Jasvindar Kaur *et al* [7].
Leaves	1.Petether 2. Chloroform 3. Ether 4. Ethanol	*A. Niger*	Disc diffusion method	Garima *et al* [8].
Leaves and Stem	1. Ethyl acetate 2. Hexane	*1.A. Niger* *2.A. Flavours* *3. Curvularialunata* *4. Botrytiscinerea* *5. Fusariumoxysporum*	Disc diffusion method	Rajasekaran *et al* [6].

MATERIALS AND METHODS

Collection of Plant Material: The plant material (roots) was collected during January – March 2011 from the local areas of Palani, Tamilnadu. India. The identity of the plant material was confirmed by Dr. P. Satyanarayana, Scientist D, Botanical Survey of India, Coimbatore. The stems were dried in shade, cut into small pieces and used for the study.

Sequential Extraction of Roots: Air dried pieces of roots of *Clerodendrum inerme* were sequentially extracted with petroleum ether (60–80°), ethyl acetate and ethanol for 6 hours at reflux temperature. The extracts were filtered; concentrates were labelled CIRP, CIREA, and CIRE respectively.

Fractionation by Column Chromatography: Column chromatographic analysis of the ethanol extract was carried out on a silica gel column. The column was eluted with pet-ether and a graded mixture of pet ether-ethyl acetate and ethyl acetate-ethanol.

Fractions of 200ml were collected and homogeneity assessed by thin layer chromatography. Similar fractioning were combined. The main fractions obtained are designated in **Table-2**. The compounds isolated from main fractions have been designated as IRC1, IRC2, IRC3, IRC4 and IRC5.

Antifungal Assay: The antifungal activity of the extract concentrates CIRP, CIREA and CIRE, the liquid –liquid fractions and the column fractions was assayed by disc diffusion method. The samples were dissolved in DMSO and stored as a stock solution.

Species cultures were grown on Sabouarud's dextrose agar (www.microbelibrary.org) at 28°C and the sample impregnated discs were placed on the agar and incubated at 28°C for 48h.The zone of inhibition was measured.

Table-2: Zone of Inhibition of the Sequential Extracts of Roots of *C. inerme.*

S. No	Plant Extract	Zone of inhibition (mm)			
		Mpnoscus rubber	*Aspergillus Niger*	*Aspergillus fumigates*	*Candida albicans*
1	Petether	22	17	15	17
2	Ethyl acetate	38	19	18	18
3	Ethanol	35	20	20	20
4	Std. Clotrimazole 10µg/disc	16	10	12	12

Table-3: Antifungal assay of Column Fractions and Compounds.

Fraction Code	Zone of inhibition (mm)				
	Eluant	*A. Niger*	*A. Fumigates*	*Candida albicans*	*Meniscus rubber*
IF1	98%pet ether and 2% ethyl acetate	10	7	8	6
IF2	95% pet ether and 5% ethyl acetate	8	8	10	9
IF3	95% pet ether and 5%ethyl acetate	8	6	17	9
IF4	95% pet ether and 5% ethyl acetate	6	8	6	8
IF5	95% ethyl acetate and 5% ethyl acetate	14	8	7	6
IF6	90% pet ether and 10% ethyl acetate	18	6	10	6
IRC1	90% pet ether and 10% ethyl acetate	7	6	7	7
IF8	80% pet ether and 20% ethyl acetate	9	9	9	7
IF9	80% pet ether and 20% ethyl acetate	7	9	10	8
IRC2	80% pet ether and 20% ethyl acetate	20	10	10	8
IF11	80% pet ether and 20% ethyl acetate	7	8	8	7
IF12	50% pet ether and 50% ethyl acetate	15	7	8	10
IF13	40% pet ether and 60% ethyl acetate	9	6	8	6
IF14	30% pet ether and 70% ethyl acetate	8	9	7	7
IRC3	30% pet ether and 70% ethyl acetate	7	6	7	6
IF15	20% pet ether and 80% ethyl acetate	9	9	8	8
IF16	10% pet ether and 90% ethyl acetate	7	7	8	8
IRC4	100% ethyl acetate	8	7	7	7
IF17	100% ethyl acetate	8	7	8	7
IF18	95% ethyl acetate 5%ethanol	20	8	7	7
IF19	80% ethyl acetate 20%ethanol	17	9	9	8
IRC5	80% ethyl acetate 20%ethanol	8	8	8	8
IF20	70% ethyl acetate 30%ethanol	10	6	6	7
IF21	60% ethyl acetate 40%ethanol	7	7	7	6
IF22	50% ethyl acetate 50%ethanol	7	8	8	7
IF23	40% ethyl acetate 60%ethanol	10	6	6	7
IF24	30% ethyl acetate 70%ethanol	16	10	7	7
IF25	20% ethyl acetate 80%ethanol	24	9	10	8
IF26	10% ethyl acetate 90%ethanol	10	8	6	7
IF27	100%ethanol	8	8	7	7
Std Clotrimazole 10µg/disc		7	15	16	14

RESULTS AND DISCUSSION

Antifungal screening results for the ethanol extract of root (CIRE) and for the various fractions are given in **Tables 2** and **3**. The root ethanol extract CIRE and the various column fractions showed varying degree of inhibition against all the fungal strains tested CIREA possessed antifungal activity *Monoscus ruber* comparable to standard *Clotrimazole*. Among the column fractions, the main factions IF5, IF6, IF10, IF12, IF18, IF19, IF24 and IF25 showed significant inhibition of *A. Niger*, All the isolated compounds showed moderate activity. From preliminary test compounds IRC1, IRC2 and IRC3 have been identified as steroidal in nature and compound IRC4 as a flavonoidal glycoside. The complete characterization of the isolated compounds will be dealt with in our next paper.

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