ANTIFUNGAL ACTIVITY OF *CORALLOCARPUS EPIGAEUS* (HOOK. F.)

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ABSTRACT

In the present study, petroleum ether, hexane, chloroform, acetone and methanol extracts of leaf, stem and tuber of *C. epigaeus* were investigated for antifungal activity against *Candida albicans*, *C. tropicalis*, *Aspergillus niger*, *A. flavus* and *A. versicolor* by disc diffusion method. Methanol extract of *C. epigaeus* tuber exhibited maximum activity against most of the tested fungi. The petroleum ether and hexane extracts obtained from *C. epigaeus* stem was found to be active only against *A. niger*, *A. flavus* and *A. versicolor*. All the crude extracts exhibited activity against *A. niger* and *A. flavus*. The tuber extract of *C. epigaeus* showed higher inhibitory effect than leaf and stem. This kind of study could generate more such ideas for re-inventing and using herbs in combination to treat many more diseases.

Key words: Antifungal activity, *Corallocarpus epigaeus*, methanol extract, tuber.

INTRODUCTION

The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful (Sofowara, 1982). According to WHO reports, over 80% of the world population depends on traditional medicine for their primary healthcare needs (Duraipandiyan *et al.,* 2006). There is an alarming increase in the incidence of new and re-emerging infectious diseases (Chukwueneka *et al.,* 2011). Hence, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms, especially due to development of resistance to the antibiotics in current clinical use (Bauer *et al.,* 2003). Several plants used traditionally have potential antimicrobial and antiviral properties and this has raised the optimism of scientists about the future of phyto-antimicrobial agents (Zaika, 1988; Das *et al.,* 1999; Gandhiraja *et al.,* 2009). With this background, the present study was carried out to evaluate the antifungal activity of hexane, petroleum ether, chloroform, acetone and methanol extracts from leaf, stem and tuber of *Corallocarpus epigaeus. Corallocarpus epigaeus* (Cucurbitaceae) used in the treatment of chronic rheumatism, snake bite (Ganesan *et al.,* 2007; Kottaimuthu, 2008), asthma (Reddy *et al.,* 2006), dysentery and syphilitic disorders (Swarnkar and Katewa, 2008).

MATERIALS AND METHODS

Different parts of leaf, stem and tuber of *Corallocarpus epigaeus* were collected during Nov 2009-Feb 2010 from Maruthamalai Hills, Coimbatore, and Tamilnadu, India. The collected plant materials were identified and their authenticity was confirmed by Mathew (1981) and Gambel (1986) respectively. The voucher specimens were deposited in the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.

EXTRACTION OF PLANT MATERIAL

Various organic solvents were used for the extraction of bioactive compounds. The root and tuber powders (10g) of *Corallocarpus epigaeus* were first extracted with petroleum ether for defatting in a Soxhlet apparatus. The defatted powdered sample of *Corallocarpus epigaeus* were dried and successfully extracted with hexane, petroleum ether, chloroform, acetone and methanol in a Soxhlet apparatus. The extracts obtained were completely evaporated by using vacuum rotary evaporator. The concentrated extracts were used for antifungal activity.

Table 1: Antifungal activity of *Corallocarpus epigaeus*

Sample tested	Diameter zone of inhibition in mm														
	Aspergillus niger					*Aspergillus flavus*					*Aspergillus versicolor*				
Leaf	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**
Petroleum ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	1.33^{e}	2.00^{f}	3.25^{g}	4.87^{g}	6.56^{e}	1.00^{f}	1.53^{f}	3.00^{e}	4.20^{g}	5.97^{e}	2.50^{b}	4.93^{b}	5.33^{d}	0.00	0.00
Acetone	1.00^{e}	0.00	3.80^{f}	4.10^{h}	4.33^{h}	1.17^{f}	2.77^{e}	3.13^{e}	3.55^{h}	4.17^{f}	1.67^{d}	2.20^{f}	4.9^{e}	0.00	0.00
Methanol	3.03^{b}	5.80^{b}	6.97^{c}	7.83^{d}	3.87^{i}	1.53^{e}	1.43^{g}	3.17^{e}	4.93^{f}	6.80^{d}	3.00^{a}	4.00^{c}	6.07^{b}	1.03^{h}	2.17^{k}
Stem															
Petroleum ether	1.00^{e}	1.10^{g}	1.50^{h}	2.60^{j}	3.23^{j}	1.00^{f}	1.07^{g}	1.20^{g}	2.90^{i}	4.30^{f}	0.00	1.20^{g}	3.07^{g}	3.23^{f}	3.47^{g}
Hexane	0.00	0.00	0.00	0.00	1.34^{m}	1.00^{f}	1.03^{g}	2.27^{f}	3.60^{h}	3.80^{g}	0.00	0.00	1.27^{h}	2.80^{g}	3.17^{g}
Chloroform	2.00^{c}	3.33^{d}	5.33^{d}	7.17^{e}	8.00^{c}	2.00^{d}	2.70^{e}	8.13^{b}	9.93^{b}	12.00^{a}	1.23^{e}	4.43^{c}	5.00^{d}	7.80^{a}	8.57^{c}
Acetone	1.67^{d}	2.00^{f}	5.30^{d}	6.00^{f}	7.13^{d}	1.00^{f}	3.17^{d}	5.43^{d}	6.90^{d}	7.80^{c}	1.17^{e}	3.00^{d}	5.53^{c}	6.20^{c}	6.97^{d}
Methanol	2.05^{c}	3.57^{c}	4.67^{e}	8.67^{c}	10.67^{a}	2.67^{c}	5.77^{b}	7.10^{c}	9.50^{b}	12.20^{a}	3.13^{a}	5.50^{a}	6.47^{a}	7.00^{b}	9.36^{b}
Tuber															
Petroleum ether	1.67^{d}	1.00^{g}	1.10^{i}	1.43^{k}	1.77^{l}	1.50^{e}	0.00	1.07^{g}	2.63^{i}	3.17^{h}	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	3.07^{i}	2.20^{k}	0.00	0.00	0.00	0.00	2.27^{i}	0.00	0.00	0.00	0.00	0.00
Chloroform	2.02^{c}	2.80^{e}	8.00^{b}	9.33^{b}	10.00^{b}	3.67^{a}	4.00^{c}	5.20^{d}	7.03^{c}	11.83^{b}	3.00^{a}	2.67^{e}	3.72^{f}	4.20^{d}	6.00^{e}
Acetone	1.20^{e}	2.80^{e}	3.00^{g}	4.33^{h}	5.00^{g}	1.33^{f}	0.00	0.00	5.97^{e}	6.77^{d}	0.00	0.00	5.00^{d}	3.80^{e}	5.00^{f}
Methanol	3.67^{a}	7.47^{a}	10.67^{a}	13.67^{a}	6.00^{f}	3.17^{b}	6.03^{a}	9.07^{a}	10.67^{a}	12.00^{a}	2.43^{c}	3.17^{d}	5.80^{c}	7.17^{b}	10.10^{a}

TESTED MICROORGANISMS

Antifungal activity of crude extracts was tested against fungi *Candida albicans*, *C. tropicalis*, *Aspergillus niger*, *A. flavus* and *A. versicolor*. All the fungal cultures were procured from the Microbiology Laboratory, K.G Hospital, Coimbatore -641018. The stock cultures of fungi were maintained on nutrient agar slants and fungi on potato dextrose agar slants at 4º C.

ANTIFUNGAL ASSAY

Antifungal assay was performed using disc diffusion method (Bauer *et al.,* 1966).The extracts of the plant parts used were tested for their antifungal activity. The respective fungal spores were inoculated on the surface of the Potato Dextrose Agar plates and incubated at 25°C for 3 days. The different plant extracts were used to saturate the disc and placed on the seeded plates. Respective solvents act as a control. After incubation period, the antifungal activity was evaluated by measuring the zone of inhibition against test organisms.

STATISTICAL ANALYSIS

Statistical analysis was performed using statistical software package WINSAT 2007 in Microsoft Excel. The data were presented as Means ± S.E. Statistical analysis was performed using one way ANOVA, DMRT test was used for calculating for 5 % level of significance.

RESULTS AND DISCUSSION

The antifungal activity of petroleum ether, hexane, chloroform, acetone and methanol extracts obtained from *C. epigaeus* revealed that only the methanol extract showed a good antifungal activity against the fungal strains (Table 1 and 2). Next to methanol, chloroform exhibited moderate activity against *A. niger*, *A. flavus*, *A. versicolor*, *C. albicans* and *C. tropicalis*. No zone of inhibition was observed for acetone extracts against *C. albicans* and *C. tropicalis*.

Table 2: Antifungal activity of *Corallocarpus epigaeus*

Sample tested	**Diameter zone of inhibition in mm**									
	Candida albicans					***Candida tropicalis***				
Leaf	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**
Petroleum ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	3.23^{a}	1.00^{e}	2.93^{f}	4.43^{f}	6.03^{e}	2.67^{a}	4.47^{a}	4.67^{c}	5.09^{d}	2.00^{e}
Acetone	1.07^{d}	1.13^{e}	3.80^{e}	2.53^{h}	3.47^{h}	0.00	3.15^{b}	2.48^{g}	0.00	0.00
Methanol	1.80^{c}	3.17^{c}	4.30^{d}	5.13^{d}	7.43^{d}	1.34^{d}	1.00^{d}	2.50^{f}	3.00^{f}	5.33^{c}
Stem Petroleum ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	2.25^{b}	4.13^{a}	5.70^{a}	6.00^{b}	7.90^{c}	1.00^{d}	3.00^{b}	4.33^{d}	5.77^{c}	6.00^{b}
Acetone	1.10^{d}	3.00^{c}	4.00^{d}	4.77^{e}	5.20^{f}	1.00^{d}	0.00	2.37^{g}	2.80^{g}	3.17^{d}
Methanol	2.03^{b}	3.00^{c}	4.67^{c}	5.63^{c}	10.23^{b}	2.15^{b}	1.07^{d}	3.60^{e}	5.13^{d}	6.03^{b}
Tuber										
Petroleum ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	2.00^{b}	3.27^{c}	5.63^{a}	6.00^{b}	7.50^{c}	1.67^{c}	2.47^{c}	5.67^{a}	10.67^{a}	2.00^{e}
Acetone	1.17^{d}	1.53^{d}	2.30^{g}	3.10^{g}	3.77^{g}	2.67^{a}	3.47^{b}	3.67^{e}	4.07^{e}	6.00^{b}
Methanol	2.20^{b}	3.93^{b}	5.03^{b}	8.80^{a}	12.00^{a}	2.40^{b}	4.07^{a}	5.00^{b}	7.10^{b}	11.00^{a}

Values are expressed as Mean of 3 replicates. Means followed by a common letter aren't significantly different at the 5% level by DMRT.

The extracts obtained from *C. epigaeus* stem displayed mild to moderate activity against most of the fungi, but petroleum ether and hexane extract was found to be active only against *A. niger*, *A. flavus* and *A. versicolor*. Among the extracts, only methanol extract was found to be strongly active against all the fungal strains. The chloroform extract showed significant moderate activity against test fungal strains whereas acetone extract showed significantly less activity when compare to methanol and chloroform extract (Table 1 and 2).

C. epigaeus tuber extract showed mild to moderate fungal activities (Table 1 and 2). Methanol extract exhibited comparatively higher activity against most of the tested fungi than that of the other four extracts. All the crude extracts exhibited activity against *A. niger* and *A. flavus*.

This investigation revealed a pronounced antifungal activity. Similar results were reported in *Amarphophallus campanulatus* (Khan *et al.,* 2009) and *Gloriosa superba* (Hemaiswarya *et al.,* 2009). The tuber extract of *C. epigaeus* showed higher inhibitory effect than leaf and stem. It may be due to the reason that, the tubers have constant contact with soil, though; they may be infected with soil pathogen. As a result, they produce many antimicrobial substances in response to the infection (Kelamanson *et al.,* 2000). The broad spectrum antimicrobial activities of the plant extracts, possibly due to the identified phytocompounds. Bioactive substances from this plant can therefore be employed in the formulation of antifungal agents for the treatment of various fungal infections. Isolation, identification and purification of these phytoconstituents and determination of their respective antifungal potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agent should be the future direction for investigation.

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