

ORIGINAL RESEARCH ARTICLE

**Antifungal Activity and Phytochemical Screening of *Anisochilus carnosus* (L) Wall and *Melaleuca alternifolia* (Maiden & Betche) against opportunistic pathogen *Candida albicans***

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**ABSTRACT**

This paper deals with the antifungal and phytochemical studies of *Anisochilus carnosus* (L) Wall and *Melaleuca alternifolia* (Maiden & Betche) against opportunistic pathogen *Candida albicans*. The medicinal plants selected for the study were collected from Navamalai, Pollachi, India. The plant extracts showed a wide range antifungal activity. The results were supported by phytochemical analysis.

**Key words:** *Candida albicans*, oral thrush, *Anisochilus carnosus* (L) Wall, *Melaleuca alternifolia* (Maiden & Betche).

**INTRODUCTION**

Mycotic infections are manifested unequivocally and ubiquitously all over the world with varied manifestations [1]. In tropical countries including India, fungal diseases are more prevalent, especially because of high temperature and socio economic conditions (poor hygiene).

Oral thrush occurs in debilitated or bottle fed infants. Creamy-white patches are found covering, red raw area of mucus membrane and tongue. It also occurs in adults as angular chelitis in sore mouth caused by ill fitting dentures and all too frequently after prolonged course of oral antibacterial therapy. It is also found in immuno compromised HIV patients.

The medicinal plants selected for the present investigation were *Anisochilus carnosus* (L) Wall and *Melaleuca alternifolia* (Maiden & Betche) collected from Navamalai, Pollachi, India. *Anisochilus carnosus* (L) Wall (Labiatae, Vernacular name- Karppuravalli) distributed throughout South India. Leaves are used for the medicinal purposes. *Melaleuca alternifolia* (Maiden & Betche) (Myrtaceae, Vernacular name- Tea tree oil) distributed native to Australia cultivated more in South Wales and Nilgiris. Oil is used for the medicinal purposes.

**MATERIALS AND METHODS**

The organism for the study *Candida albicans* 183 was obtained from M.T.C.C, Chandigarh, India. The medicinal plants selected for the study *A. carnosus* (L) Wall and *M. alternifolia* (Maiden &

Betche) were collected from Navamalai, Pollachi, India.

**Qualitative phytochemical studies:**

Hundred grams of shade dried leaves were powdered and extracted successively using petroleum ether, chloroform, ethyl acetate, ethanol and water using Soxhlet apparatus. The extracts were evaporated in a roto-evaporator under the reduced pressure. Preliminary phytochemical studies were conducted on all extracts following the prescribed procedures [2,3,4] and the results were presented in (Table 1).

**Table 1: Phytochemical screening:**

S.No	Phytochemical Components	Plant Extracts	
		<i>A.carnosus</i>	<i>M.alternifolia</i>
1	Alkaloids	-	-
2	Steroid and Sterols	-	-
3	Glycosides	-	-
4	Flavanoids	-	+
5	Saponins	-	-
6	Tannins and Phenolic compounds	-	-
7	Terpenoids	+	+
8	Carbohydrate	-	-

(+) Present; (-) Absent

**Anti fungal activity:**

The agar well diffusion method [5] was used to determine the growth inhibition. Sterile Muller Hinton agar plates were prepared. Three well of 6mm diameter were prepared with the help of a sterile well puncher. The 6 hour culture broth was taken and swabbed over the plate using sterile cotton swab to obtain a uniform lawn culture. The wells were filled with 10µl of the prepared

dilutions of the plant extract using dimethyl sulphoxide (DMSO). DMSO was used as the control. Then the plates were incubated at 37 °C for 24 hr. After incubation, diameters of the inhibition zones were measured and tabulated.

**Minimum inhibitory concentration:**

Minimum inhibitory concentration [6] of *A. carnosus* (L) Wall and *M. alternifolia* (Maiden & Betche) were found using broth dilution technique. Seven test tubes containing 1 ml of sterile Sabouraud’s Dextrose broth were prepared. For assaying plant extract, the starting concentration kept at 8mg/ml in the first tube containing 1 ml of sterile Sabouraud’s dextrose broth. The plant extract were serially diluted at the concentration 8, 4, 2, 1, 0.5, 0.25, 0.125 mg/ml. To each of this test tube, 0.1 ml of 6 hr culture of *Candida albicans* was added. The tubes were incubated at 30 °C for 24-48 hr. The test tubes were examined for visible turbidity. 1ml of the above mentioned tube was transferred to a microfuge and centrifuged at 5,000 rpm for 4 min. The supernatant was completely removed using micropipette and the pellet was suspended in 0.1 ml sterile distilled water. The resulting bacterial suspension was serially diluted and plated on Sabouraud’s dextrose agar plates. The end point of complete inhibition was defined as the minimum inhibition concentration of the test compound in the original tube which fails to yield discernible growth when sub cultured. The Nalidixic acid and Co-trimoxazole was used as the reference standard.

**Table 2: Antifungal activity of *A. carnosus* (L) Wall and *M. alternifolia* (Maiden & Betche) against *Candida albicans***

S.No	Extracts	Zone of Inhibition of <i>A. carnosus</i> (L) Wall (mm)			Zone of Inhibition of <i>M. alternifolia</i> (Maiden & Betche) (mm)		
		10mg/ml	5mg/ml	2.5mg/ml	10mg/ml	5mg/ml	2.5mg/ml
1	Petroleum Ether	11	9	6	11	11	7
2	Chloroform	12	10	8	16	11	8
3	Ethyl Acetate	15	15	10	15	11	10
4	Ethanol	13	11	9	12	10	9
5	Water	17	14	11	7	No Zone	No Zone

**Table 3: MIC of *A. carnosus* (L) Wall and *M. alternifolia* (Maiden & Betche) against *Candida albicans***

S.No	Extracts	MIC of <i>A. carnosus</i> (L) Wall (mg/ml)	MIC of <i>M. alternifolia</i> (Maiden & Betche) (mg/ml)	Reference Standards (mg/ml)	
				Na	Co
1	Petroleum ether	2	2	0.21	0.015
2	Water	2	4	0.21	0.015
3	Chloroform	4	2	0.21	0.015
4	Ethyl acetate	4	2	0.21	0.015
5	Ethanol	4	2	0.21	0.015

MIC- Minimum inhibitory concentration; Na- Nalidixic acid; Co- Co-trimoxazole

**CONCLUSION**

Antifungal screening was carried out against *C. albicans* by agar well diffusion method. Antifungal activities were indicated by clear zone of inhibition. Among all the extracts of *A.*

**RESULTS AND DISCUSSION**

The preliminary investigation of phytochemical studies and antifungal activity were reported from the leaves of *A. carnosus* (L) Wall and *M. alternifolia* (Maiden & Betche) for the first time. The antifungal activity of *A. carnosus* (L) Wall on *C. albicans* is presented in the (Table 2). It indicated that ethanol, ethyl acetate and water extracts showed high activity. Petroleum ether and chloroform extract extracts showed moderate activity. The antifungal activity of *M. alternifolia* (Maiden & Betche) on *C. albicans* is presented in the (Table 3). It indicated that ethanol, ethyl acetate and chloroform extracts showed high activity. Petroleum ether extract showed moderate activity. Water extract showed low activity.

The MIC concentration of *A. carnosus* (L) Wall (ethanol, ethyl acetate, water, petroleum ether and chloroform extracts) and *M. alternifolia* (Maiden & Betche) (ethanol, ethyl acetate, petroleum ether and chloroform extracts) on *C. albicans* is less than 8 mg/ml. Hence, the component present in the plant is active against *C. albicans*.

Similarly, the ethanolic extract of twelve plants selected through ethanomedical survey in Guinea Bissau were investigated for the *in-vitro* antifungal properties against *C. albicans* [7] and investigation for the anti fungal activity of organic and aqueous solvent extracts of *Micromeria nervosa* against *C. albicans* [8]. This confirms the present investigation provides an alternate search for ethno medicine against *C. albicans* for a novel drug formulation.

*A. carnosus* (L) Wall, ethanol, ethyl acetate and water extracts showed high activity. Petroleum ether and chloroform extract extracts showed moderate activity. Among all the extracts of *Melaleuca alternifolia* (Maiden & Betche),

ethanol, ethyl acetate and chloroform extracts showed high activity. Petroleum ether extract showed moderate activity. Water extract showed low activity. The phytochemical screenings of different plant extracts were studied to analyze the presence of various antifungal bio active substances. This study proves that *Anisochilus carnosus* (L) Wall and *Melaleuca alternifolia* (Maiden & Betche) can be used in the treatment of Candidiasis. The antifungal actions of these plants are due to the presence of antifungal agents in them. These results are valuable for discovering new drugs for various diseases.

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