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Research Article **Botany**

Studies on the antifungal activities the leaves of *Achyranthes aspera.* L against oral *Candidiasis*

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ABSTRACT

In the present work has been designed and planned to evolve strategy for the identification of bioactive compounds from the medicinal plants against *C. albicans* causing oral candidiasis in human beings. Thirteen compounds were obtained in *Achyranthes aspera* and characterized as 1-tetradecene, phenol, 3,5–bis (1,1–dimethylethyl), 2-tetradecene, benzene, 1,1'–(1,2-cyclobutanediyl) bis-trans, 1–octadecene, 1H–indole–3–carboxylic acid, nonadecanol–1, methyl stearate, 1–docosene($_{C22H44}$), 1-heptacosanol, 1 – benzylindole, 1H–indole-2–methyl–3– phenyl and 5–methyl–2–phenylindolizine. The individual fractions (ethanol, methanol) of *A. aspera* leaves extracts showed moderate antifungal activity. From the above results it is concluded that *A.aspera* methanolic leaves extracts have great potential to use a new phytomedicine against multiple resistant *Candida albicans*.

Keywords: *C. albicans, Achyranthes aspera*, GC MS,

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INTRODUCTION

Candidiasis describes a number of different disease syndromes that often differ in their causes. Majority of patients, notably immunosuppressed individuals with human immunodeficiency virus (HIV) infection, experience some form of superficial mucosal candidiasis, most commonly thrush, suffer from recurrent infections. *Candida* infections of the later category are also referred to as candidemia and are usually confined to severely immune compromised persons, such as cancer, transplant, and AIDS patients as well as non-trauma emergency surgery patients (Moran *et al*. 2002**).** The occurrence rate of *Candida* has also been reported to be increased in patients with diabetic mellitus. Among the various types of candidiasis, oral candidiasis is a common opportunistic fungal infection of the oral cavity caused by *C. albicans*. Oral candidiasis is a source of oral discomfort, pain, loss of taste, and aversion to food in dental patients. It encompasses infections that range from superficial to systemic and potentially life-threatening diseases.

At the most serious level, mortality rates of candidiasis are high. By keeping all these in mind, the present work has been designed and planned to evolve strategy for the identification of bioactive compounds from the medicinal plants against *C. albicans* causing oral candidiasis in human beings.

MATERIALS AND METHODS

Phenotypic analysis of *C. albicans*

Collection of plant

Healthy and young leaves (3-6) months old of *A.aspera* were selected.They were collected from in and Thanjavur,Tamil Nadu,India and identified with help of the standard mannuals such as "The flora of the presidency Madras"Gamble,1967" and Indian Medicinal plants(Kiritikar and Basu,1988).The leaves were separated from leaves,washed in clean water,and dried at room temperature. The shaded dried leaves were weighted and ground in the sterile morter.

Sample collection and clinical examination

Oral samples were collected from patients of different age groups who were admitted to various clinics in and around Thanjavur Tamil Nadu, India. Oral sample collection involved oral swab and mouth rinsing with 10 ml of sterile phosphate-buffered saline (PBS; 0.1 M; pH 7.2) and it was collected in sterile container. Each rinse was centrifuged at 2,000 ×g (10 min), the supernatant was removed, and pellet was dissolved in 1 ml of PBS IT WAS USED STORED.

Preparation of Leaf power.

The leaves of the plants were collected, washed, cut into small pieces and dried at room temperature (39 degree c) for two weeks and made into power for the further analysis.

Preparation of leaf Extract;

Extraction is a process,to the separate or isolate the secondary matabolites from the plant material.it is basically two types i.e. heat cold extraction has some advantage over cold extraction like time consistency and also no contamination by microbes.an apparatus called soxhlet is used for heat extraction.10 of the plant leaf power were packed in to the thimble of the soxhlet apparatus.the ratio of the plant power and solvents were maintained at 1:4ratio

Ethanol extract:

The plant extraction was dried and extracted with 100ml of ethanol (59.5-61.5degree c) by continuous hot percolation,until extraction was completed.after the completion of extraction.the extract was filtered and the solvent was removed by distillation under reduced pressure.A dark green colored residue was obtained.

PHYTOCHEMICAL ANAYSIS

Qualitative Analysis

was then filtered and kept in hot air oven at 40°C for 24 hrs to evaporate the methanol from it. Phytochemical analysis of the plant extracts was undertaken using standard qualitative methods as described by various authors (Kapoor *et al*., 1969; Odebiyi and Sofowora, 1990). The plant extracts were screened for the presence of biologically active compounds such as alkaloids, flavonoids, carbohydrates, phytosterols, proteins, phenolics, tannins and saponins

Gas Chromatography – Mass Spectroscopy (GC-MS) analysis (Ivanova *et al.*, 2002)

The sample was dissolved in 75 ml of methanol for 24 h. Then the filtrates was collected by evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 x m df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS.

The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Anti-*Candida*l activity

Disc-Diffusion Method

Various solvent fractions of the leaves of *Achyranthes aspera* were checked for anti-candidal activity using disc-diffusion method. *C. albicans* (C2 isolate) was swabbed on the surface of the sabouraud agar plates .The disc (Whatman No.1 filter paper with 9 mm diameter) was impregnated with the 50 µl of each test plant sample and it was placed on the surface of sabouraud agar plates .

To compare the antifungal activities, nystatin (20 µg/disc) used as standard antibiotic and blank disc impregnated with water act as negative control. The plates (triplicates) were incubated 28°C for 72 h. The

antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

RESULTS

Morphological study of *C. albicans*

Methylene blue stained oral swab smear showed abundant branched pseudohyphae and true hyphae with blastoconidia which was observed under compound microscope. The blast conidia appeared as grape-like clusters along the length of the hyphae. Chlamydoconidia were observed in the terminal part of hypha(Figure 6).Further the germ tube germination was observed in some isolates of *C. albicans*

Determination of minimum fungicidal concentration

The antifungal activities of Leaves of *A.aspera* against *C. albicans* were evaluated by minimal fungicidal concentration (MFC). The MFC of *A.aspera* effectively inhibited the growth of *C. albicans* at concentration of 9.65 mg/ml (Table 5), which was higher than others. Figure4 (Table5)

Gas Chromatography – Mass Spectroscopy (GC-MS) analysis

The phytochemical compounds present in the leaves of *A. aspera* was identified by GC-MS analysis.GC-MS analysis of methanolic extract of *A. aspera* displayed Thirteen peaks with the retention times ranging from 11.341 to 27.127 min (Figure 2). The positive electron impact mass spectrum of Thirteen compounds were obtained and characterized as 1-tetradecene ($C_{14}H_{28}$),phenol, 3,5–bis(1,1–dimethylethyl) ($C_{14}H_{22}O$), 2-tetradecene(C14H28), benzene, 1,1'–(1,2-cyclobutanediyl) bis-trans(C16H16), 1–octadecene(C18H36), 1H–indole–3–carboxylic acid ($C_{10}H_9NO_2$), nonadecanol–1 ($C_{19}H_{40}O$), methyl stearate($C_{19}H_{38}O_2$), 1–docosene($_{C22H44}$), 1-heptacosanol ($C_{27}H_{56}O$), 1 – benzylindole ($C_{15}H_{13}N$), 1H–indole-2–methyl–3– phenyl(C15H13N), 5–methyl–2–phenylindolizine($C_{15}H_{13}N$).Figure3,(Table 2)

Table :1. Qualitative analysis of The leaves of *Achyranthes aspera*

PHYTOCHEMICALS	*Achyranthes aspera*
Alkaloids	+
Flavonoids	+
Carbohydrates	+
Protein	+
Phenols	+
Saponins	+
Tannins	+
Phytosterols	+
Terpenoids	+
Phlobatannins	-

Table :2. Gas Chromatography –Mass Spectrometry analysis of the leaves of *A. aspera*

S. No	RT	Compound	Molecular Formula	Relative content (%)
1	11.341	1-Tetradecene	$C_{14}H_{28}$	2.79%
2	12.912	Phenol, 3,5–bis (1,1–dimethylethyl)	$C_{14}H_{22}O$	0.09%
3	13.845	2-tetradecene	$C_{14}H_{28}$	0.31%
4	15.766	Benzene, 1,1'–(1,2-cyclobutanediyl) bis-trans	$C_{16}H_{16}$	0.33%
5	16.074	1–Octadecene	$C_{18}H_{36}$	0.57%
6	16.938	1H–indole–3–carboxylicacid	$C_{10}H_9NO_2$	5.15%
7	17.440	Nonadecanol–1	$C_{19}H_{40}O$	1.27%
8	17.790	Methyl stearate	$C_{19}H_{38}O_2$	0.39%
9	18.087	1–Docosene	$C_{22}H_{44}$	93.44%
10	19.343	1-Heptacosanol	$C_{27}H_{56}O$	0.19%
11	21.620	1 – Benzylindole	$C_{15}H_{13}N$	0.10%
12	23.062	1H–Indole-2–methyl–3– phenyl	$C_{15}H_{13}N$	0.38%
13	27.122	5–methyl–2–phenylindolizine	$C_{15}H_{13}N$	2.55%

Anti-Candidal activity of *Achyranthesaspera*

Disc diffusion method

In the present study from the *A. aspera* showed antifungal activity against *Candida albicans*. The individual fractions (ethanol, methanol) of *A. aspera* leaves extracts showed moderate antifungal activity. The average zone of inhibition of 3, 8, 4, 2, 1 and 1 mm wereobserved.Amongall the fractions, only ethanol and methanol showed maximum activity against *C. albicans.* Hence these two fractions were once again treated with multiple resistant *C.albicans*strain at various combinations, which showed effective results (Table3-5) Figure.5.

Table 3: Antifungal activity of *A. aspera* tested against *C. albicans* by disk diffusion method.

Plant sample / Solvent	Zone of inhibition (mm)	
A. aspera	**Ethanol**	**Methanol**
	8	4

Table 4: Antifungal activity of Ethanol /Methanol fraction of *A. aspera* tested against *C. albicans* by disk diffusion method.

S.No	Plant sample	Diameter of zone of inhibition (in mm)
		Candida albicans
1	***A. aspera* (mg/ml)**	9.65
4	**Nystatin(mg/ml)**	14

Table 5: Determination of minimum fungal concentration of the leaves against *C. albicans*.

Plant sample / Solvent	Zone of inhibition (mm)								
	18:2 (E/M)	**16:4 (E/M)**	**14:6 (E/M)**	**12:8 (E/M)**	**10:10 (E/M)**	**8:12 (E/M)**	**6:14 (E/M)**	**4:16 (E/M)**	**2:18 (E/M)**
A. aspera	1	2	2	1	8	2	0.5	-	9

Fig-1.*Achyranthes aspera* .*L*

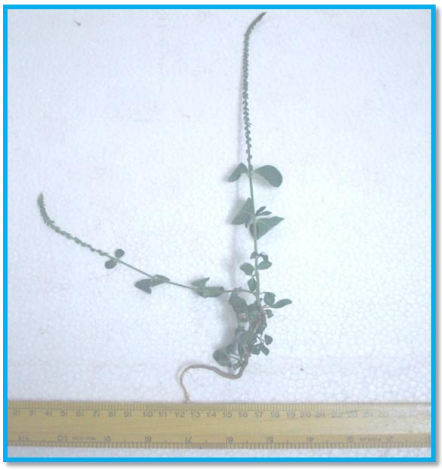


Fig-2.Phytochemical Analysis of leaves of the A.aspera

Fig-3.GC- MS analysis of the leaves of *A.aspera*

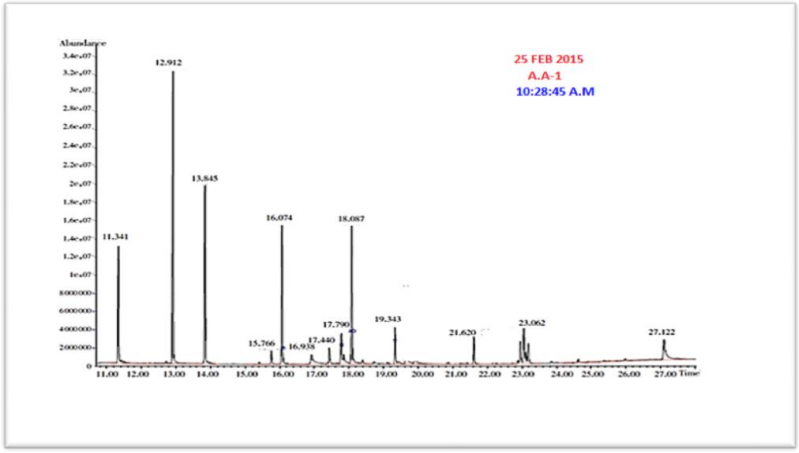
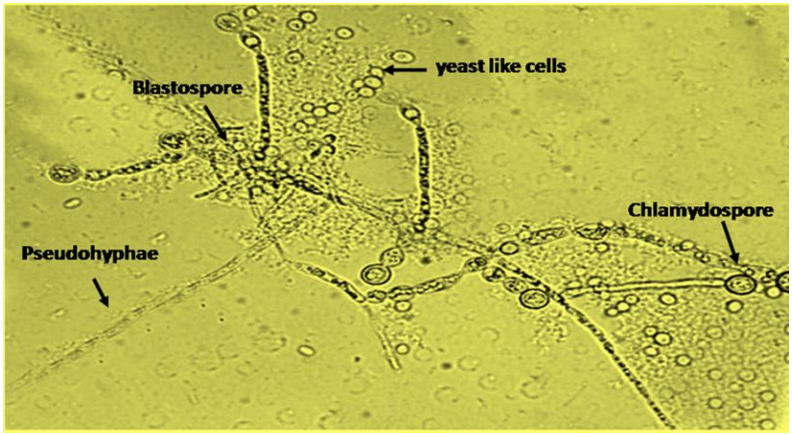


FIGURE-4.MICROSCOPIC VIEW OF C. *albicans*

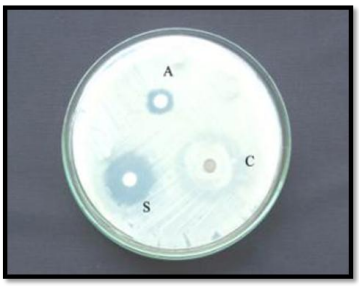


Oral swab smear showing pseudohyphae ,blastoconidia and chlamydoconidiaof *C. albicans*(40x)

Fig-5.Disc diffusion method for *A.aspera* leaves extract

Antifungal actvites of the leaves of the achyranthes aspera against C.albicans

Ethanolic extract of *A.aspera*Methanolic extract of *A.aspera*



E/M 2:18 combined fraction of the leaves of *A.aspera*

CONCLUSION

From the above results it is concluded that *A.aspera* methanolic leaves extracts have great potential to use a new phytomedicine against multiple resistant *Candida albicans*. Therefore selected compounds from these plants could be utilized by the pharmacological society for the development of novel drugs against oral *candidiasis*. Since our country is a rich source of the medicinal plants, used in this day, development of these phytomedicine is relatively inexpensive and less time consuming.

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