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Research Article

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Antifungal Activity & Phytochemical Analysis of *Acalypha Indica* L On Opportunistic Fungal Pathogens Associated With Hiv

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Abstract: Various medicinal plants extensively used as an Ayurvedic Medicine for over many years for various illnesses. They are extensively used as Indian traditional medicine to cure various human ailments. The World pandemic of AIDS has been with us for more than twenty five years and shows no signs of abatement. The incidence of fungal infections is increasing because of raising number of immunocompromised patients widespread use of broad spectrum of antibiotics. Medicinal plants constitute a very important natural resource used by indigenous medicinal systems for the last 300 years. Antimicrobial activity of ethyl acetate, diethyl ether and methanol extracts of *Acalypha indica* were investigated against HIV associated opportunistic fungal pathogens. The plant extracts showed better inhibitory activity against the tested organism. Ethylacetate extracts produced much better activity when compared with methanolic and diethylether extracts. Phytochemical screening of the plant revealed the presence of various compounds. This study creates social awareness among the HIV patients who were infected by various fungal infections.

Key words: AIDS, immunocompromised patients, *Acalypha indica*, HIV patients.

INTRODUCTION:

The breakdown of body immune system is the hallmark of HIV infection. Infections which are rarely seen in those with normal immune systems are deadly to those with HV. This makes AIDS patients susceptible to a variety of opportunistic infections. People with HIV can get many infections called opportunistic infections. OIs are caused by various pathogenic microorganisms such as bacteria, fungi, virus and parasites [1]. Infections with opportunistic pathogens have been one of the hallmarks of the AIDS since the beginning of the epidemic. Diseases caused by bacteria and fungi are responsible for higher death rates significant proportion found in HIV population [2].

Fungal pathogens can lead to many of the complications seen in advanced HIV disease and are commonly identified in HIV infected population with decreased immune function. While infections with these organisms can be fatal, appropriate identification and management of the condition can result in reduced mortality and the opportunity for effective management of HIV disease with highly

active antiretroviral therapy (HAART). The common genera of fungal organisms affecting individuals with AIDS include *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Penicillium* and *Tinea* etc.

Plants have a great potential for producing new drugs for human benefit. There is a continuous development of resistant strains which pose the need for search and development of new drug to cure diseases [3]. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds [4]. According to a report of WHO, more than 80% of world's populations depend on traditional medicine for their primary health care needs [5]. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [6]. Medicinal plants are finding use as pharmaceuticals, nutraceuticals, cosmetics and food supplements. Even as traditional source of medicines and they continue to play pivotal rule [7].

***Acalypha indica* Linn:** *A. indica* is an important medicinal plant in the Indian Ocean islands as well as in India for its expectorant properties. It also has significant antibacterial and antifungal activities, both against human and plant pathogens. The leaves, root, stalks and flower are used in medicine. It has cathartic, anthelmintic, expectorant, anodyne and hypnotic properties. The leaves possess laxative properties and are used in chronic bronchitis, asthma, consumption, syphilitic ulcers and Candidal vaginal infections. The leaves, root, stalks and flower are used in medicine. The plant contains the alkaloid acalyphine which is an active principle, Indian acalypha is a well known remedy in rheumatism [8].

Systematic position of *Acalypha indica* L.

Kingdom	:	Plantae
Order	:	Malpighiales
Family	:	Euphorbiaceae
Genus	:	*Acalypha*
Species	:	*indica*
Common Name	:	Kuppai meni.

MATERIALS AND METHODS:

Fresh leaves of *Acalypha indica* collected from the Herbal Garden of PRIST University, Thanjavur, Tamil Nadu, India, were identified by a botanist. The plant materials were cleaned, shade dried and powdered. Fresh plant material were washed with tap water, air dried, homogenized to a fine powder and stored in air-tight bottles.

Extraction:

Two hundred and fifty gram of each plant powder was soaked in 1.25-1.5 L of 95% ethanol for 5 days at room temperature. The mixture was mixed daily for regular infusion. After a five day period,

the extract was filtered by using Whatman filter paper No.1. The filtrate was dried by using rotary evaporator at 60^0C. The dried extract was stored in sterile glass bottles at -20^0C until using.

Collection of Biosamples: 250 biosamples were collected from clinically suspected HIV patients of various Hospitals, Medical Colleges and Social Welfare Centres of Thanjavur and Trichy districts. All the patients were thoroughly evaluated by detailed history, clinical examination and biochemical parameters including CD4 count. All the strains were confirmed by cultural characteristics and maintained in slants for further use.

Phytochemical screening: Qualitative Chemical tests were carried out on the methanolic extract of the powdered specimens using standard procedures to identify the phytoconstituents as described by Trease and Evans (1989)[9] and Sofowara (1993)[10].

Antifungal Sensitivity Tests: Saborauds agar medium was prepared and autoclaved at 121^0C under 15 psi pressures for 30 minutes. After cooling to about 65^0C, 25ml of the medium poured in Petri dishes. The plates kept at room temperature for solidification and stored at 4^0C until using. Inoculum containing 108 cells/ml were spreaded on the medium. Antifungal activity test was then carried out by using hole-plate diffusion method [12]. The dried plant extracts were dissolved in respective solvent to final extract amounts of 10 to100mg/ml. Each hole of about diameter 6mm in each plate was filled with 10,50 and 100µl of plant extract separately. The inoculated agar plates were incubated at 37^0C for 24 hours. After the incubation period, the diameter of inhibition zone to each hole was measured in millimeter.

Thin layer chromatography (TLC) : TLC and bioautography [13] were done to separate the antimicrobial principles, the crude leaf extracts showed high antifungal activity was subjected to TLC. Here the solvent used were 5 and 10% methanol in chloroform. 1 mg/ml concentration of the effective plant extract was spotted on the TLC plates and dried. It was then run with both ultraviolet and iodine chamber. Thin layer chromatography was performed on Merck TLC F254 plates, with chloroform: Methanol (95:5) as mobile phase. Developed chromatography plates of crude extract was dried overnight sprayed with a suspension of actively growing cells of bacteria and fungi and incubated at 37 and 24°C respectively in a chamber at 100°C relative humidity for 18 hrs. Plants were sprayed with MTT-3 (4,5-Dimethyl thiazol-2.5- Diphenyl tetrazolium Bromide) (5 mg/ml). Clear zones on the chromatogram indicate inhibition of growth after incubating for hours at 37°C.The separated components were visualized under visible and ultraviolet light (254 and 360 nm). The Retention Factor (RF) value was calculated by using the following formula.

$$RF = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

RESULTS AND DISCUSSION:

Eight types of fungal pathogens were identified. They were *Aspergillus*, *Blastomyces*, *Candida*, *Cryptococcus*, *Fusarium*, *Histoplasma* and *Penicillium*. The predominant isolates include *C.*

albicans, *A. niger*, *A. flavus*, *H. capsulatum*, *P. marneffei*, *C. neoformans*, *F. moniliforme*, *A. terreus*, *B. dermatidis* and *F. solani* (Table 1). Table 2 showed the qualitative screening of *A. indica* Linn. The results of the phytochemical analysis (Table 2) showed that the methanolic extract of medicinal plant extracts showed that *A. indica* showed positive results for saponins, flavonoids, glycosides, phenolic compounds, fixed oils and fats, alkaloids and protein and aminoacids. The effective bioactive compound of *A. indica* was noted to be Acalyphin, a cyanogenic glucoside. There is no previous study conducted evaluating the anti-fungal property of *A. indica*. Oksana *et al*. (2007) reported that flavonoids (quercetin, kaempferol, isorhamnetin, isoquercitrin), phenolic derivatives (gallicin, gallic, syringic, and caffeic acids), and coumarin (scopoletin) have potent anti-fungal activity against *Microsporum* spp. and *Trichophyton* spp. (Oksana *et al.*, 2007). The ethylacetate, methanol and diethyl ether extracts of *A. indica* showed variable inhibition zones. The results were statistically substantiated with one-way ANOVA(Table 3). The 'F' values of all extracts were significant at 5% level. It showed that the ethylacetate extracts all plants were used for controlling the fungal pathogens. Fungal infections are among some of the most common opportunistic infections that occur in HIV patients. Most people have been exposed to the disease causing fungi because they are everywhere. However, infections only occur in individuals who have weakened immune systems that cannot prevent the fungus from growing. Invasive fungal infections are most common opportunistic infections associated with significant morbitity and mortality for patients with HIV infection, and the risk of invasive fungal infections varies with host immunity as well as environmental exposure [14].

The diethyl ether extracts of herbal plants showed different zones of inhibition on the fungal pathogens. The 'F' values of *A. indica* and *A. calamus* only significant, others were non-significant at 5% level. It reveals the plant extracts showed non-significant result were only moderate to control the pathogens when compared with the extracts shown significance result (Table 3). The methanol extracts of herbal plants showed different zones of inhibition on the pathogens. The results substantiated with ANOVA showed the 'F' value of *A. indica* only non-significant at 5% level. Other plants were significant to control the fungal pathogens very well (Table 3). Gangadevi *et al.* reported that ethyl acetate extract of leaves and roots of *Acalypha indica* recorded higher activity than the methanol and hexane earlier reported that the endophytic fungi isolated from the leaves of *A. indica* elicited promising antibacterial activity against the three human pathogenic bacteria [15].

TLC and Bioautography of *A. indica:* Six lanes namely A, B, C, D, E, F and G indicated the separation of various components in the extract. Growth inhibition zones, indicated in lane G, showed the presence of active fungicidal components, well separated from each other, in at least one major and six minor positions of bioautogram. Rf values for *A. indica* detected by TLC and bioautography were found to be 0.16, 0.26, 0.39, 0.5, 0.11, 0.78 and 0.97. Therefore, suggesting thereby the presence of antimicrobial active substances in the specific bands.

CONCLUSION:

To conclude that, this study would definitely create awareness among the HIV positive patients for taking control or preventive measures based on herbal plants against the common opportunistic bacterial and fungal infections causing pathogens. However, due to changing pattern of infections

depending upon the degree of immunosuppression, constant monitoring of infections in HIV positive population is important for better management and to improve the quality of life of such patients. Alternative to antibiotics, the patients should be recommended to take the herbal based medicines particularly *A. indica* surely minimise the fungal related OI and increase the life span of HIV patients.

Table 1 Isolated fungal pathogens of HIV^+ Patients

S. No.	Fungi	Identified predominant spp.	Number of fungi isolated	Percentage
1.	*Aspergillus* spp.	*A. flavus*	8	7.84
		A. niger	18	17.65
		A. terreus	2	01.96
2.	*Blastomyces* spp.	*B. dermatidis*	2	01.96
3.	*Candida spp.*,	*C. albicans*	48	47.06
4.	*Cryptococcus* spp.	*C. neoformans*	4	03.92
5.	*Fusarium* spp.	*F. moniliforme*	3	02.04
		F. solani	2	01.96
6.	*Histoplasma* spp.	*H. capsulatum*	9	08.82
7.	*Penicillium* spp.	*P. marneffei*	6	05.88

Table 2 Qualitative phytochemical screening of medicinal *Acalypha indica* Linn

S.No	Phytochemicals	Result	Observation
1.	Tannins	+	Appearance of brownish green colour
2.	Saponin	+	Appearance of foam
3.	Flavanoids	+	Appearance of yellow colour
4.	Terpenoids	-	No colour change was observed
5.	Glycosides	+	No ring formation
6.	Phytosterols Libermann Burchard's test	+	An array of colour changes was observed.
7.	Phenolic compounds i) Ferric Chloride ii)G elatin	 - -	 No colour changes observed. No precipitate formed
8.	Alkaloids i) Mayer's test ii) Wagner's test iii) Hagner's test	 - + -	 No precipitate formed. Reddish brown precipitate observed. No precipitate formed
9.	Carbohydrates i) Molisch's test ii)Fehling test iii)Benedict's test	 + - -	 Violet ring formed No precipitate formed No precipitate formed.

+ = Present; - Absent.

Table 3 One-way ANOVA for various extracts of *Acalypha indica* with respect to fungal pathogens

Name of the extract used	Groups	Sum of Squares	Df	Mean Square	'F' value	5% limit
Ethylacetate	Between Groups	93.000	3	31.000	3.244	S
	Within Groups	398.800	16	24.925		
	Total	491.800	19			
Diethyl ether	Between Groups	56.150	3	18.717	4.030	S
	Within Groups	290.800	16	18.175		
	Total	346.950	19			
Methanol	Between Groups	74.550	3	24.850	5.648	S
	Within Groups	70.400	16	4.400		
	Total	144.950	19			

Df – Degree of freedom

S - Significant

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