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PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITY OF MEDICINAL PLANTS AGAINST OPPORTUNISTIC *CANDIDA ALBICANS* OF FEMALE HIV POSITIVE PATIENTS

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ABSTRACT

Medicinal plants constitute a very important natural resource used by indigenous medicinal systems for the last 300 years. 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. Three million people around the world die of AIDS each year. The incidence of fungal infections is increasing because of raising number of immunocompromised patient's widespread use of broad spectrum of antibiotics Antimicrobial activity of ethanol, ethyl acetate and DMSO extracts of *Adhatoda vasica, Acalypha indica, Acorus calamus ,Ocimum basicilicum* and *Vitex negundo* plants were investigated by agar well-diffusion method against HIV associated opportunistic *Candida*. The plant extracts showed better inhibitory activity against the tested organism. DMSO extracts produced much better activity when compared with ethanolic and ethylacetate extracts. Phytochemical screening of the plant revealed the presence of tannins, saponins, flavonoids, carbohydrates and alkaloids. This study creates social awareness among the female HIV patients who were infected by vaginitis caused by *Candida.*

Key Words: *Adhatoda vasica, Acalypha indica, Acorus calamus ,Ocimum basicilicum, Vitex negundo,* HIV associated opportunistic *Candida*, AIDS, and Social awareness.

Introduction

Three hundred million people around the world die of AIDS each year and so far, more than 25 million people have died of the disease. Today, atleast 33 million people are infected and there are more than 14,000 new infections everyday[1].AIDS is caused by Human Immuno Defeciency Virus(HIV) which is found in all cases of the disease. The incidence of fungal infections is increasing because of raising number of immunocompromised patient's widespread use of broad spectrum of antibiotics[2] *Penicillium chrysogenum, Trichophyton violaceum, Histoplasma capsulatum, Cryptococcus neoformans, Aspergillus spp., Blastomyces, Candida* spp., are the fungal pathogens mostly associated with HIV/AIDS patients with certain morbid symptoms. *Candida albicans* remains the most frequently isolated yeast pathogen, other *Candida* species such as *Candida glabarata, Candida krusei* and *Candida tropicalis* are emerging as opportunistic pathogens. The most common *Candida* infection treated by primary care physicians is vaginitis. Vulvovaginal Candidiasis(VVC) is the second most common cause of

vaginitis. There is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action[3]. Another big concern is the development of resistance to antibiotics in current clinical use and there has been an alarming increase in the incidence of new and reemerging infectious diseases. *Adhatoda vasica*, *Vitex negundo*, *Acalypha indica* ,*Acorus calamus* and *Ocimum basilicum* are greatly used for the treatment of vulvovaginal candidiasis.

Materials and Methods

Plant collection

Fresh leaves of five Plant materials belong to the following genera: *Adathoda vasica*, *Acorus calamus*, *Acalypha indica*, *Ocimum basilicum* and *Vitex negundo* were collected from the Herbal Garden of PRIST University, Thanjavur, Tamil Nadu, India, were identified by a botanist. The plant materials were cleaned and 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

The fresh leaves were dried in shade at room temperature and ground by using a blender. 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 Samples:

50 vaginal samples were collected from clinically suspected HIV patients of various Hospitals, Medical Colleges and Social Welfare Centres of Thanjavur district during the period from December 2009 to February 2010. All the patients were thoroughly evaluated by detailed history, clinical examination and biochemical parameters. The swabs from vaginal area of HIV positive female patients were collected under aseptic conditions. 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 ethylacetate and methanolic extract of the powdered specimens using standard procedures to identify the phytoconstituents as described by Trease and Evans (1989)[4] and Sofowara (1993)[5].

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 10^8cells/ml were spreaded on the medium. Antifungal activity test was then carried out by using hole-plate diffusion method. Holes were made on the medium using 6mm cork borer. The dried plant extracts were dissolved in dimethylsulfoxide(DMSO) 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. The inhibition zone is the area surrounding the hole and there is no growth of the inoculated microorganism. DMSO used as negative control.

Results

Isolated *Candida* from 50 female HIV patients with CD4 counts were represented in Table 1. The etiologies of opportunistic candidiasis in HIV patients were

represented in Table 2. Morphological characters seen in Microscopy were showed in Table 3.

Effect of Ethanol solvent on *Candida albicans*:

Antifungal activity of ethanol leaf extract of plant species against *Candida albicans* represented in Table 4. For *Vitex negundo*, the highest zone of inhibition(17mm) was noted in the concentration of 100µl followed by 16mm was noted in 50 µl. *C.albicans* showed resistant to 10µl concentration. For *Adhatoda vasica*, the highest zone of inhibition 21mm was noted in the concentration of 100µl followed by 18mm was noted in 10 µl. For *Acorus calamus*, the highest zone 20mm was in 100µl followed by 18mm in 10µl. For *Acalypha indica*, the highest zone 22mm was in the 100µl, followed by 21mm was in 10µl. For *Ocimum basilicum*, the highest zone 26mm was in the concentration of 100µl, followed by 18mm was noted in the concentration of 50µl.

Effect of Ethyl acetate solvent on *Candida albicans*:

Antifungal activity of ethyl acetate leaf extract of plant species against *Candida albicans* represented in Table 5. For *Vitex negundo*, the highest zone of inhibition about 17mm was noted in the concentration of 100µl. *C.albicans* showed resistant to the concentrations of 10µl(10mg/10ml) and 50µl concentration. For *Adhatoda vasica*, the highest zone of inhibition 18mm was noted in the concentration of 50µl(10mg/10ml) followed by 15mm was noted in the concentrations of 10 µl.*C.albicans* showed resistant to the concentrations of 100µl. For *Acorus calamus*, the highest zone of inhibition 19mm was noted in the concentration of 100µl 10mg/10ml, *C.albicans* showed resistant to the concentration of 50 µl and 10 µl of 10mg/10ml. For *Acalypha indica*, the highest zone of inhibition 26mm was noted in the concentration of 10µl(10mg/10ml), followed by 15mm was noted in the concentration of 50µl and *C.albicans* showed resistant to the concentrations of 100µl. For *Ocimum basilicum*, the highest zone of inhibition about 17mm was noted in the concentration of 50µl, followed by 15mm was noted in the concentration of 50µl and minimum zone of inhibition about 15mm was noted in the concentration of 10 µl and *C.albicans* showed resistant to the concentrations of 100µl (10mg/10ml).

Effect of DMSO solvent on *Candida albicans*:

Antifungal activity of DMSO leaf extract of plant species against *Candida albicans* represented in Table 6. For *Vitex negundo*, the highest zone of inhibition 22mm was noted in the concentration of 100µl followed by 18mm in the concentration of 10µl and minimum zone of inhibition 13mm was noted in the concentration of 50µl. For *Adhatoda vasica*, the highest zone of inhibition 20mm was noted in the concentration of 100µl followed by 16mm was noted in the concentrations of 10µl. For *Acorus calamus*, the highest zone of inhibition 25mm was noted in the concentration of 100µl,followed by 23mm was noted in the concentration of 50µl and minimum zone of inhibition 15mm was noted in the concentrations of 10µl. For *Acalypha indica*, the highest zone of inhibition 23mm was noted in the concentration of 10µl, followed by 21mm was noted in the concentration of 50µl and minimum zone of inhibition 18mm was noted in the concentrations of 100µl. For *Ocimum basilicum*, the highest zone of inhibition 20mm was noted in the concentration of 100µl followed by 18mm was noted in the concentration of 50µl and *C.albicans* showed resistant to the concentrations of 10µl.

Compared with ethyl acetate extracts, ethanolic extracts produced better antifungal activity. DMSO extracts produced much better activity when compared with ethanolic and ethylacetate extracts.

PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS

Phytochemical screening of the leaf extracts of all medicinal plants represented in Table 7 . The positive

results were observed for the leaf extract of *Vitex negundo* for tannins,saponins, flavanoids, phytosterols, alkaloids(Wagner's test), Carbohydrate (Molisch's test).The positive results were observed for the leaf extract of *Adhatoda vasica* for tannins, saponins, flavanoids, phytosterols, alkaloids(Wagner's test), Carbohydrate (Molisch's test). The positive results were observed for the leaf extract of *Acorus calamus* for tannins, saponins, flavonoids, phytosterols, alkaloids(Wagner's test), Carbohydrate (Molisch's test). The positive results were observed for the leaf extract of *Acalypha indica* for tannins, saponins, flavonoids, phytosterols, alkaloids(Wagner's test), Carbohydrate (Molisch's test). The positive results were observed for the leaf extract of *Ocimum basilicum* for tannins, saponins, flavonoids, phytosterols, alkaloids(Wagner's test), Carbohydrate (Molisch's test).

Discussion:

AIDS is a life threatening disease caused by a virus HIV. Antifungal activity of the plant materials showed different inhibition spectrum against the isolated opportunistic *Candida* spp. The increasing incidence of AIDS and the recent development of a new treatment strategy for patients with hematologic malignancies and organ transplants have led to steady increases in the number of immunocompromised patients with fungal infections[6]. Development of effective antifungal agents against systemic infections caused by this pathogen has been hindered by an unidentified insusceptibility of the organism to many metabolic inhibitors. The impermeability of *Candida albicans* cells has been considered the major cause for the refractivity of the organism to antifungal agents. In those cases in which mechanisms of resistance to specific agents have been elucidated, changes in membrane composition, the loss of transport, or modification of an enzyme target for the tested drug have been documented[7]. Medicinal plants are valuable natural resource and regarded as potentially safe drugs[8]. The activity of the plants tested in the present study was not only inhibitory to the fungal organisms but was also fungicidal. Such activities have been previously reported for other plants and the extent of the fungicidal activity has been evaluated by the time-kill experiments[9]. Studies by Okemo *et al* (2001)[10] indicated that the crude extracts of the neem plant *Azadirachta indica* killed a whole population of *C. albicans* at a concentration of 8 mg/ml in 24 h while Patel and Coogan, (2008)[11] found that *Dodonaea viscose* extracts killed all the *C. albicans* strains within 30 s. In the present study, *W. salutaris* was able to completely kill *C. albicans* cell at a concentration of 0.4 mg/ml. This indicates the possibility of compounds from this plant to kill fungal organisms with special reference to *Candida* sp. at lower concentrations than the crude extract. This activity could be due to the compounds such as muzigadial and warburganal previously isolated from this plant in other studies[12].

Table 1: Agewise distribution of HIV Positive Patients

AGE GROUP	No of patients	%	No of Positive Cases	%
0-20	13	26	6	12
20-40	33	66	19	38
40-60	3	6	3	6
60-80	1	2	-	-
Total	50		28	56

Table 2: Etiologies of Opportunistic Vaginal Candidiasis in HIV Positive Patients

S. No.	Etiology	Number of Suspected cases	Positive Cases	CD4 Count
1.	Vaginitis	31	21	250-400
2.	Thrush	11	4	350-600
3.	Diabetes mellitus	4	2	500-650
4.	Chronic fatigue syndrome	4	1	400-550

Table 3: Morphological Characters of Isolated *Candida Albicans*

ISOLATED FUNGAL spp.,	MORPHOLOGY
Candida albicans	Produced moist opaque creamy colony on saborauds dextrose agar. Clusters of round blastoconidia are present at some septae. Thick walled chlamydospores seen.

Table 4: Antifungal activity of Ethanolic of medicinal plants against *Candida albicans*

Name of Medicinal plant	Zone of inhibition (mm)		
	Extract amount 10-100mg (10mg/10ml)		
	10µl	50µl	100µl
Vitex negundo	-	16	17
Adathoda vasica	18	15	21
Acorus calamus	18	15	20
Acalypha indica	21	16	22
Ocimum basilicum	15	18	26

Table 5: Antifungal activity of Ethyl acetate Leaf extract of medicinal plants against *Candida albicans*

Name of Medicinal plant	Zone of inhibition (mm)		
	Extract amount 10-100mg (10mg/10ml)		
	10µl	50µl	100µl
Vitex negundo	-	-	17
Adathoda vasica	15	18	-
Acorus calamus	-	-	19
Acalypha indica	26	15	-
Ocimum basilicum	15	17	-

Table 6: Antifungal activity of DMSO Leaf extract of medicinal plants against *Candida albicans*

Name of Medicinal plant	Zone of inhibition (mm)		
	Extract amount 10-100mg (10mg/10ml)		
	10µl	50µl	100µl
Vitex negundo	18	13	22
Adathoda vasica	16	15	20
Acorus calamus	15	23	25
Acalypha indica	23	21	18
Ocimum basilicum	-	18	20

Table 7: PHYTOCHEMICAL SCREENING OF LEAF EXTRACT OF *Vitex negundo*

S. No.	Phytochemicals	Observation	***V.negundo***	***A. vasica***	***A.calamus***	***A.indica***	***O.basilicum***
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.	Phenoloic compounds						
	i) Ferric Chloride	No colour changes observed.	+	-	-	-	+
	ii) Gelatin	No precipitate formed	-	-	-	-	-
8.	Alkaloids						
	i) Mayer's test	No precipitate formed.	-	-	-	-	-
	ii) Wagner's test	Reddish brown precipitate observed.	+	+	+	+	+
	iii) Hagner's test	No precipitate formed	-	-	-	-	-
9.	Carbohydrates						
	i) Molisch's test	Violet ring formed	+	+	+	+	+
	ii) Fehling test	No precipitate formed	-	-	-	-	-
	iii) Benedict's test	No precipitate formed.	-	-	-	-	-

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