Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(3); 319-322

DOI number: 10.25258/phyto.v9i2.8081

ISSN: 0975-4873

Research Article

Phytochemical Analysis and *In Vitro* Antifungal Studies of Medicinal Plants *Elephantopus scaber*, *Cyclea peltata* and *Artemisia japonica*

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Received: 11th Jan, 17; Revised: 24th Feb, 17; Accepted: 10th March, 17 Available Online: 25th March, 2017

ABSTRACT

Introduction: The traditional folk knowledge of treatment have great role in the modern medicine. Based on the folk knowledge, the present work was under taken to study the antifungal activity of three plants *Elephantopus scaber*, *Cyclea peltata* and *Artemisia japonica* in our locality. Objective: To gave scientific validation to folk knowledge and propose the extract with promising antifungal activity for further identification of active molecules for the development of new therapeutics in future. Methodology: Disc diffusion method using various solvent extracts of each plant was utilized. The zone of inhibition and minimum inhibitory concentration were calculated. Results: The plants *Cyclea peltata* and *Artemisia japonica* posses better antifungal activity. Conclusion: The plant extract with antifungal activity used for further purification, identification and toxicity studies of active plant constituents may be used as lead compounds for the development of new therapeutic agents with good efficacy and least side effect in modern medicines against fungal infections.

Keywords: anti fungal, *Elephantopus scaber*, *Cyclea peltata* and *Artemisia japonica*, *Candida albicans*.

INTRODUCTION

The traditional knowledge of ayurveda have inevitable role in the treatment of many infectious diseases since ancient period. Kerala is rich source of wide range of medicinal plants[1]. The tribes and the traditional physicians have their own natural herbal remedies to cure various disease conditions. Medicinal plants have wide variety of chemical constituents with vast unexplored activity which help in the treatment of many complex diseases[2]. Antifungal studies of medicinal plants have significant role in the modern research. The ethno pharmacological and pharmacokinetic analysis of plant constituents provide scientific validation and help in the development of new therapeutic agents.

Candida is the most common opportunistic pathogenic fungus causing infections worldwide[3,4]. The common species are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, etc[5]. *Candida albicans* is the most common pathogenic strain within the group[6,7]. The biofilm formation ability of candida species increases the intensity of the pathogenic conditions[8]. The frequency of occurrences candidiasis is increased in immunosuppressive patients such as HIV patient, organ transplant patients and neonatal[7,9]. Candida species resistant to commonly used drugs amphotericin B and fluconazole[10,11]. The efficacy and side effect of conventional drugs are questionable. The plant molecule have inevitable role in the modern medicine, a wide range of potential phytochemicals present in plants with good efficacy and least side effect used for treatment against many diseases[12]. The present study evaluating the antifungal activity of three plants selected based on the folk knowledge.

MATERIALS AND METHODS

Selection and identification of the plants

Based on the folk knowledge from books, direct conversation with tribal disease healers and also through personal contact with various traditional physicians three plants available in our locality with unexplored antifungal effects were selected for the present study.

Authentication of plants

Selected plants were authenticated by botanist Dr. Udayan from Sreekrishna college Guruvayoor, Thrissur, Kerala, India and the voucher specimens were deposited as KVASU/CM/001 (*Elephantopus scaber*), KVASU/CM/002 (*Cyclea peltata*) and KVASU/CM/003 (*Artemisia japonica*) at the Department of Clinical Veterinary Medicine, Mannuthy, Kerala Veterinary and Animal Sciences University, Kerala, India.

Preparation of plant materials

Whole plant of all the three selected plants was collected in bulk quantity from places in and around Mannuthy, Thrissur, Kerala. Plants after collection were washed twice in tap water to remove the dirt and other foreign materials and then in sterile distilled water. Shade dried the plants for about one to two weeks. Whole plant was chopped into small pieces using scissors and again allowed to dry in shade. The dried materials were then ground to coarse powder separately using electric grinder without heating the plant materials and stored in air tight bottles.

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Table 1: Percentage of yield obtained.

Plant No	Solvent used	Yield (%)
	Aqueous	25
	Petroleum benzene	4.2
	Chloroform	3.92
KVASU/CM/001	Acetone	1
	Ethyl acetate	6.3
	Methanol	1.56
	Aqueous	36.4
	Petroleum benzene	5.96
	Chloroform	3.6
KVASU/CM/002	Acetone	7
	Ethyl acetate	1.96
	Methanol	3.84
	Aqueous	27.2
	Petroleum benzene	5.28
	Chloroform	5.85
KVASU/CM/003	Acetone	10.42
	Ethyl acetate	3
	Methanol	2.57

Preparation of plant Extracts

Hot aqueous extract

Weighed about 100gm of each plant, covered in a muslin cloth and kept immersed in one liter of water taken in a dish overnight. It was boiled in simmer for two days, removed the extract, filtered using 0.45µm sterile filters and concentrated by evaporating it in water bath at 50^0C. The extract was weighed and the yield was calculated.

Solvent extraction using Soxhlet apparatus

Weighed 25gm of each plant and filled it in thimble separately and placed it in soxhlet apparatus. Solvent extracts were taken subsequently by pouring various solvents based on polarity from highly polar to least polar and heating it to the boiling point of the respective solvent. The extracts so obtained was concentrated using rotary vaccum flash evaporator and yield of respective extracts were calculated and stored it in vials at refrigeration temperature. Various solvents used were petroleum benzene, chloroform, acetone, ethyl acetate and methanol subsequently. The phytochemical constituents were analyzed using standard protocols[13].

Fungal culture

Freeze dried samples of fungal cultures, *Candida albicans* (MTCC 1637) was procured from MTCC Chandigarh for invitro analysis.

Revival of freeze dried cultures

Sterile water 0.4 ml was added to the freeze dried culture in the vial and kept for 20 minutes for revival. It was then transferred to the culture media, Sabourauds dextrose agar and Sabourauds dextrose broth prepared following sterile precautions and kept at 25^0C for 24 hours. The growth was then preserved at refrigeration temperature for further use.

Antifungal studies on Candida albicans

The culture was maintained in Sabourauds dextrose broth. About 50µl of the culture was transferred to sabourauds dextrose agar in the petri dish and swabbed it on to agar surface. Five sterile discs were placed on the agar and a ketoconazole disc (10µgm/disc) was placed as control. Required quantity of the plant extract was diluted with the same solvent which used for the extraction and various concentrations of the extract (1µgm, 2 µgm, 3 µgm, 4 µgm and 5 µgm) were added on the sterile discs. The plates were then labelled and incubated at 25ºC for 24 hours. Growth of candida organisms and zone of inhibition was calculated after the incubation period.

Minimum inhibitory concentration

Plant extracts which were found to have effect on *Candida albicans* were selected to find out the minimum inhibitory concentration. Minimum Inhibitory Concentration was performed by a serial tube dilution technique. Initially 10 fold dilution of the plant extract was made. Sabourauds dextrose broth 100µl added to each tube. Culture of *Candida albicans* maintained in Sabourauds dextrose broth adjusted to 0.5 McFarland unit was added to all the tubes, mixed well and the absorbance was read at 630nm. The tubes were then incubated at 25ºC for 24 hrs and the absorbance was read after 24 hours and the minimum inhibitory concentration was calculated[14].

RESULTS AND DISCUSSION

Based on the folk knowledge the plants were selected for the study, identified and authenticated by botanist Dr. Udayan. The total percentage yield of various extracts of each plant was calculated and presented in Table No.1. The results of Phytochemical analyses of different extracts are presented in Table No 2. Disc diffusion method was used for the *In vitro* analysis of antifungal activity of the different plant extracts and zone of inhibition were measured and presented in Table No. 3. The extracts of plant *Elephantopus scaber* posses no antifungal effect in the present study. The extracts of methanol, chloroform, Ethyl acetate and acetone of plants *Cyclea peltata* and *Artemisia japonica* have better zone of inhibition. Odaya et al., 2016[15] reported the antifungal activity of *Cyclea peltata* leaves. Setzer et al., 2004[16]; Nedorostova et al., 2009[17] reported the anti microbial effect of *Artemisia douglasiana, Artemisia nilagirica, Artemisia vulgaris L. Artemisia absinthium* L. and *Artemisia dracunculus* L earlier according to our knowledge this is the first report antifungal activity of the plant *Artemisia japonica*. The minimum inhibitory concentration of active extracts was calculated and presented in Table No. 4. The methanolic extract of the *Cyclea peltata* have the least minimum inhibitory concentration of 9 µ gm compared to other extracts. The result obtained from *Artemisia japonica* revealed that the acetonic extract posses least minimum inhibitory concentration of 6 µ gm compared to other extracts. Based on the present study it was revealed that KVASU/CM/002 (*Cyclea peltata*) and KVASU/CM/003 (*Artemisia japonica*) in our locality has the anti fungal activity. The phytochemicals present in the active extracts (chloroform, ethyl acetate methanol and acetone) may be responsible for the antifungal activity. Based on this report it concluded that after the purification

Table 2: Results of phytochemical Analysis.

Plant no	Extract	Steroids	Alkaloids	Saponin	Glycosides	Diterpene	Tannin	Flavanoids
	Aqueous	+	-	-	+	+	-	-
	Chloroform	-	-	-	-	-	-	-
	Acetone	-	-	+	-	+	-	
KVASU/CM/0	Ethyl acetate	+	-	-	-	-	-/+	+
01	Methanol	-	-	-	+	+	+	
	Aqueous	+	-	-	-	-	-	-
KVASU/CM/0	Acetone	-	-	-	-	-	-	-
02	Ethyl acetate	-	-	+	-	-	-	
	Methanol	+	+	-	+	+	+	
	Aqueous	+	-	+	+	+	-	+
	Chloroform	-	-	+	+	-	-	
KVASU/CM/0	Acetone	+	-	-	+	+	+	
03	Ethyl acetate	+	-		-	-	-	
	Methanol	+	-	-	+	-	+	

Table 3: Zone of inhibition of various extracts.

Plant no	Extract	1	2	3	4	5	Ketoconazole
	Aqueous	-	-	-	-	-	29mm
	Petroleum benzene	-	-	-	-	-	29mm
KVASU/CM/001	Chloroform	-	-	-	-	-	29mm
	Acetone	-	-	-	-	-	29mm
	Ethyl acetate	-	-	-	-	-	29mm
	Methanol	-	-	-	-	-	29mm
	Aqueous	-	-	-	-	-	29mm
	Petroleum benzene	-	-	-	-	-	29mm
KVASU/CM/002	Chloroform	-	-	-	13mm	13mm	29mm
	Acetone	-	-	10mm	12mm	15mm	29mm
	Ethyl acetate	-	-	-	13mm	14mm	29mm
	Methanol	-	-	11mm	13mm	16mm	29mm
	Aqueous	-	-	-	-	-	29mm
	Petroleum benzene	-	-	-	-	-	29mm
KVASU/CM/003	Chloroform	-	-	-	11mm	11mm	29mm
	Acetone	-	-	12mm	14mm	17mm	29mm
	Ethyl acetate	-	-	-	12mm	13mm	29mm
	Methanol	-	-	-	10mm	11mm	29mm

Table 4: Minimum inhibitory concentration values of different extracts on *Candida albicans.*

Sl no	Plant extract		MIC (µ gm)
	Plant no	Extract	
1	002	Methanol	9
2	002	Ethyl acetate	12
3	002	Chloroform	11
4	002	Acetone	10
5	003	Methanol	10
6	003	Chloroform	11
7	003	Ethyl acetate	12
8	003	Acetone	6

identification and toxicity studies of active plant constituents may be used as lead compounds for the development of new therapeutic agents with good efficacy and least side effect in modern medicines against fungal infections.

ACKNOWLEDGMENT

The authors thankfully acknowledge the financial support received from ICAR New Delhi. Dr. Udayan from Sreekrishna college Guruvayoor, Thrissur, Kerala, India for his support and Department of Clinical Veterinary Medicine, Kerala Veterinary and Animal Science University, Mannuthy. Kerala, India.

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