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Antifungal Activity of Derum (Juglans Regia L. Bark) Extracts Against Candida Albicans Isolates (In Vitro Study)

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Abstract

Background: Derum is a one of "chewing sticks" existing in nature, collected from stem bark of walnut tree (Juglan regia L.). In some countries used mainly by women as tools for cleaning teeth and as coloring material for cosmetic purpose.

The objective of performed study was aimed at evaluating the antifungal potential of two extracts from Juglans regia (L.) bark (water and water-methanol) against pathogenic Candida albicans strains.

Materials and methods: Different concentrations of derum extracts were prepared by (water and water-methanol) methods, swap of saliva were collected from volunteers of patient at dental hospital, from which candida albicans were isolated,purified and diagnosis according to morphological characteristic and biochemical test. In vitro; experiments were prepared to assess the effect of different type and concentrations of derum extracts on growth of candida albicans by agar diffusion methods in comparison with nystatin and amphotericin B.

Results: The result showed, candida albicans isolates sensitive to both types of derum extracts, which improved, by inhibition growth of candida albicans and inhibition were increased with the increasing concentrations from 0.5% to 50%. Nystatin inhibition growth of candida albicans bettter than both types of derum extracts (water and alcohol) and amphotericin B; but Candida albicans more sensitive to both types of derum extracts than amphotericin B.

Conclusion: Candida albicans were sensitive to different concentrations of (water and water-methanol) extracts of derum.

Key words, Candida albicans, Juglans regia bark extracts, Antifungal activity.

Introduction

"For many centuries different populations and cultures around world have been using various tools, ranging from porcupine bones to chewing sticks to clean their teeth and gum" [1,2]. Relatively easy to access and the popularity of "chewing sticks", that have made a different communities were used it as tools for plaque control; although "chewing sticks" obtained from different sources, many components of chewing sticks and other related plants were reported to have beneficial biological properties, including significant anti-bacterial and anti-fungal activity [3,4]. Derum is a one of "chewing sticks" existing in nature, collected from stem bark of walnut tree (Juglan regia L.). In some countries used mainly by women as tools for

cleaning teeth and as coloring material for cosmetic purpose[5]. Its extracts showed broad-spectrum antimicrobial activity. It inhibition microorganisms including gram-positive bacteria like (Staphylococcus aureus and Streptococcus mutans), gram-negative bacteria like (Escherichia coli and Pseudomonas aeruginosa) and different types of pathogenic yeast for example (Candida albicans); derum extract also increase the pH of the saliva [6,7]. Different extract of juglan regia L. bark show potent antioxidant activity[8].

"Candida is a genus of yeast, commonly, it is part of the normal flora of the mouth, skin, intestinal tract and vegina"[9,10]. But sometimes in individuals with predisposing factors, which present among immunodeficiency, diabetic, age extreme radiotherapy, AIDS, treated with antibiotic and pregnant women, denture wearing and the use of orthodontic appliance, candidiasis is the earliest infection manifest for million people[11,12]. Candida abicans strains consider the most commonly species that causing invasive candidiasis of skin surface, mucosal membrane, urinary tract infection and even life threating by systemic invasions with candidaemia[13]. The most common treatment is antifungal agents, such as (fluconazole, itraconazol, miconazol and ketoconazol) and polyenes (amphotericin B or nystatin)[14]. Unfortunately, antifungal therapy is very limited in addition to prolong uses of antifungal treatment will produce problems of side effects for example hematological, hepatic, and/or renal toxicity and may be lead to emergence of strain resistant[15]. The challenge has been to develop effective strategies for treatment of candidiasis and other fungal disease. Lower side effects of very types of herbal extracts have suggested them as sources of new pharmaceuticals[16]. Recently, many plant extracts have therapeutic value with respect to oral diseases [17].

The present study performed to test the effect of different concentrations of derum extracts(water and alcohol) on growth of candida albicans in comparison with other two anti-fungal therapy which are nystatin and amphotericine B.

Materials and methods

Preparation of derum extracts:

The stem bark of juglan regia L. obtained from local market. Derum sticks were cut into small pieces and crunching to powder using a roller-mill. water and water-methanol extracts was prepared by adding 100g of small partials from plant material to 500ml of solvent (deionized water and methanol 99.8%) and allowing the mixture to stand 48 hours at room temperature[18]. Then it was filtered by using filter paper (No. 1). The solvents were evaporated at 40^{o}C by means of rotary evaporator to get crud extracts, then the both extracts dissolved in water to prepare different concentrations.

Fungal isolates:

Isolates of candida albicans strain had been obtained from oral cavity of patients with orthodontic appliance in dental hospital by swabbing method; no patient was under antifungal drugs. A sterile cotton swab (NipponMenbo,Tokyo,Japan) was immediately cultured into sabouraud dextrose agar (Hi Medic, Mumbai, India)to elicit isolated; which incubated at 37^{o}C for 24-48 hour. Colonies of Candida albicans strain purified and diagnosis according to morphological characteristic and biochemical test.

The anticandidal potential of investigated extracts was screened by using disk diffusion method of Noumi et al with minor modifications[19]; according to Noumi et al inoculum was prepared by adding colonies of candida albicans strain to normal saline 0.85% and adjusted to McFarland 0.5 turbidity standard which contain 10^6cell\ml. Adequate amount of Mueller Hinton agar (Hi-Media) (with 2% glucose were distributed into sterile plates and allow solidifying under aseptic condition. The organism suspension was inoculated with sterile spreader on surface of solid medium in plates. Four wells of equal size and depth had been prepared in each agar plate; each well was filled with 0.1 ml of the test agents. The plates were incubated for at 37^OC for 24 h. Zone of inhibition which is clear zone of no growth were measure across the diameter of each well. No zone indicated complete resistance of fungi to the agents. As control positive nystatin 100,000 IU/ml (Lifepharma, Italfarmaco, Group - Italy) and amphotericin B 10 µg/ml (Fungizone, BioBasic, Inc., Toronto, Canda) were used.

Statistical Analysis:

Data manipulating and anaylasis were done by using SPSS program version 21.

Result

Sensitivities of candid albicans to different concentrations of derum extracts (water and alcohol), nystatin and amphotericin B were tested according to Agar Well Technique.The results showed that both type of derum extracts had same color dark brown as showed at **(figure1)**. Candida albicans were sensitive to water and water-methanol extracts of derum by inhibition growth of candida albicans strain and the sensitivity increased with increasing concentrations start from (0.5%) to (50%) which more effective, candida albicans were more sensitive to nystatin compare to both types of derum extracts and amphotricien B as showed at **(figure2)**.

Table (1), showed mean and Standard Deviation(SD) for sensitivity of candida albicans to different agents.

Table (2), showed comparison between both type of derum extracts (water and methanol) at same concentration , statistically no significant difference for inhibition growth of candida albicans between 0.5% and 1% concentration and high significant difference between 5%,10%,15%,and 20% , but a significant difference was found between 30%,40% and 50%.

Table (3), preformed to compere each concentration of either water or water methanol extracts with nystatien and amphotricine B , for water extract, candid albicans sensitive to nystatin higher than water extracts, statically with a highly significant difference. The result showed that candida albicans sensitive to water extracts higher than amphtricin B at 40%and 50% with highly significant difference. On comparison water-methanol extracts with nystatien, candida albicans sensitive to nystatien more than water- methanol extract with highly significant difference from 0.5% to 30% concentrations and a significant difference at 40% and 50% but when comparsion with amphotericin B, the result showed that water methanol extract better than amphotricic B for inhibition growth of candida albicans with highly significant difference from

5%to 50% with no significant difference at concentration 0.5% and 1%. The result showed that nystatin better than amphotericin B for inhibition growth of candida albicans with highly significant difference.

Discussion

A variety of chemotherapeutic agents have been investigated for a possible ability to control oral pathogens. Herbal materials are one of groups which have been studied in such investigations. The present study was designed to obtain information on the microbial effect of juglan regia bark on candida albicans and findings have validated the presence of such effect. At present study evidence support an antifungal effect of derum on candida albicans. Polyphenol and derivatives are not only antimicrobial compounds isolated from juglan regia bark [5,7], but juglon and glycosides are amongst strongest antimicrobials constituents of juglan regia bark [20,21]. The anti-microbial effects of derum was observed on gram-positive, gram-negative and fungi [6,7]. Inspite of, there is a drib information exist about antifungal attributes of derum spatially against candida albicans some study report that (water and alcoholic) extracts of juglan regia were inhibited in vitro growth, candia albicans strains [7,16,19,22], and that coincidence with this study. The antifungal effect of derum against candida strain may be through the following mechanisms; The number of hydroxyl group $(OH)^-$ on the some constituents of derum specially for the polyphenol were believed to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation result in increased toxicity [23]. Now there are many of researches about using phenolic substance in order to inhibit the growth of fungi [24, 25],as we know the main candida virulence factors are exoenzymes production, biofilm-formation, adherence and dimorphism[26,27,28]; many studies improved the effect of phenolic acids against these factors[29,30,31]. Another mechanism is by the inhibition of biofilm formation of candida albicans; once this biofilm is inhibited, the fungi cell will lose their connection to each other and the surface which apparently can cause cell death [32,33]. Juglon has believed to inhibit abroad spectrum of microorganism including bacteria, and fungi including candida albicans. Many recent studies have focused on the toxicity effect of juglon toward microorganisms [1]. The mechanisms for the toxic effects of juglon is not clearly understood, several pathway have been proposed which include that it may be cause death of cell, disruption cell cycle , modification of DNA, inhibition synthesis of mRNA, alkylation of thiol or amine groups of essential protein , and decreasing level of p53 (tumour suppressor) [34,35]. Juglon can also be reducing enzyme in mitochondria or cytoplasm to form a semi-quinone radical [36].

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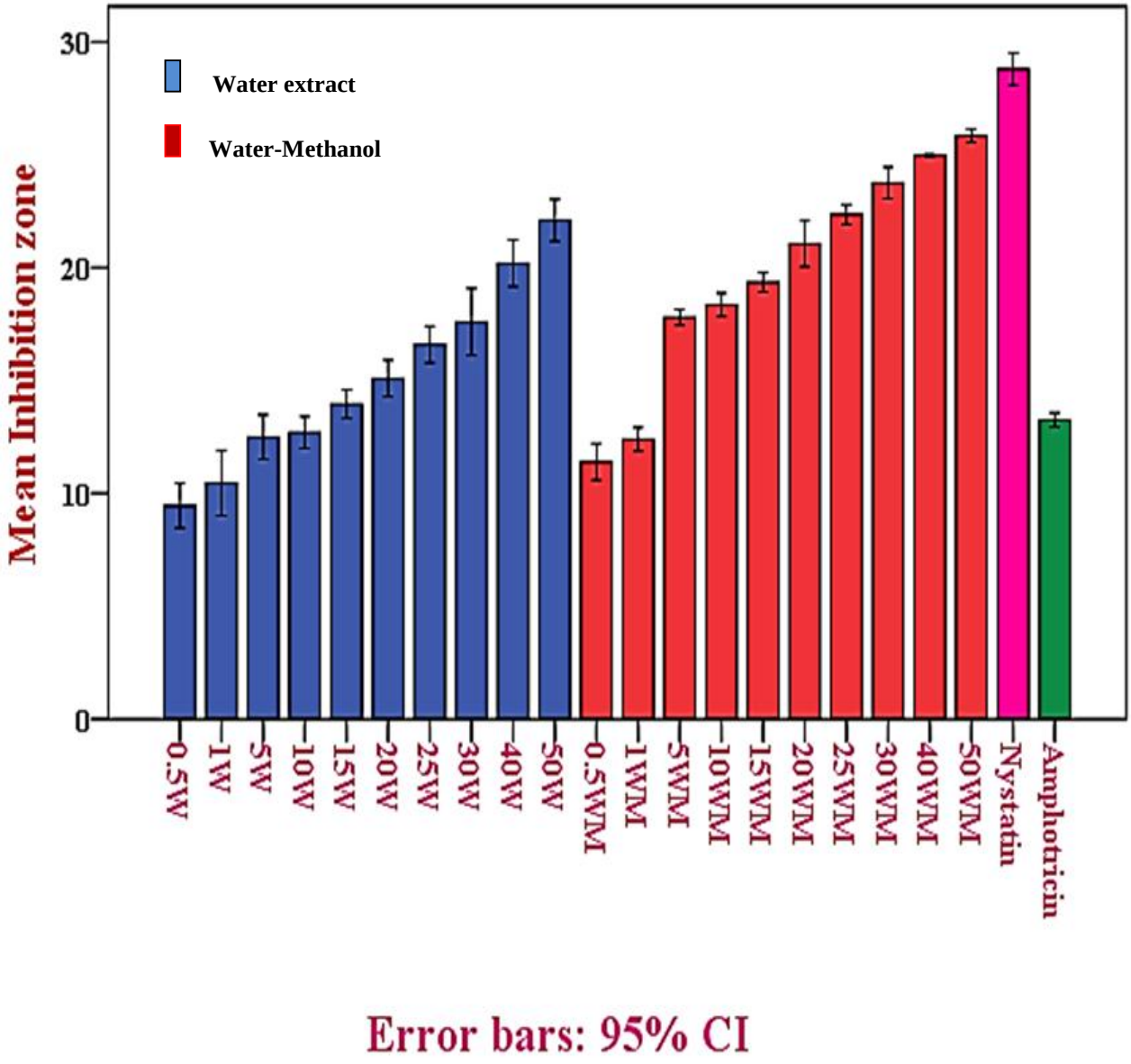
Figure (1):Type of extract (a) Water extract (b) Water-Methanol extract



Figure(2): Mean of inhibition zones of candida albicans (mm) to different agents (Agar well diffusion technique)

Table (1) Mean and Standard Deviation(SD) for inhibition zones of Candida Albicans to different agents.

Concentrations	Mean(mm)			±SD		
	Extract			Extract		
	Water	Water-Methanol	Total	Water	Water-Methanol	Total
0.5%	9.46	11.40	10.43	.50	.50	.50
1%	10.46	12.40	11.43	1.00	1.00	1.00
5%	12.50	17.80	15.15	5.00	5.00	5.00
10%	12.70	18.36	15.53	10.00	10.00	10.00
15%	13.96	19.36	16.66	15.00	15.00	15.00
20%	15.10	21.06	18.08	20.00	20.00	20.00
25%	16.60	22.36	19.48	25.00	25.00	25.00
30%	17.60	23.76	20.68	30.00	30.00	30.00
40%	20.20	24.98	22.59	40.00	40.00	40.00
50%	22.10	25.84	23.97	50.00	50.00	50.00
Total	15.07	19.73	17.40	3.99	4.74	4.95
Nystatin	28.8			0.570		
Amphotricin	13.26			0.251		

No. of Isolates: 5

Table (2) Comparison between two types of Derum extracts according to Candida Albicans sensitivity.

Concentration	Group1	Group2	Sig.
0.5%	**Water**	**Water-Methanol**	**0.157 (NS)**
1%	**Water**	**Water-Methanol**	**0.386 (NS)**
5%	**Water**	**Water-Methanol**	**0.001 (HS)**
10%	**Water**	**Water-Methanol**	**0.000 (HS)**
15%	**Water**	**Water-Methanol**	**0.000 (HS)**
20%	**Water**	**Water-Methanol**	**0.000 (HS)**
25%	**Water**	**Water-Methanol**	**0.000 (HS)**
30%	**Water**	**Water-Methanol**	**0.003 (S)**
40%	**Water**	**Water-Methanol**	**0.006 (S)**
50%	**Water**	**Water-Methanol**	**0.006 (S)**

P<0.05 Significant(S), P<0.001 High significant(HS), P>0.05 N0 significant(NS)

Table (3) Comparisons between different concentrations of Water and Water-Methanol extracts with Nystatin and Amphotricin B .

Group	Conc.	MD		Sig.	
		Nystatin	Amphotricin	Nystatin	Amphotricin B
Water	0.5%	-19.34	-3.80	.000(HS)	.008(S)
	1%	-18.34	-2.80	.000(HS)	.132(NS)
	5%	-16.30	-.76	.000(HS)	.921(NS)
	10%	-16.10	-.56	.000(HS)	.938(NS)
	15%	-14.84	.70	.000(HS)	.672(NS)
	20%	-13.70	1.84	.000(HS)	.068(NS)
	25%	-12.20	3.34	.000(HS)	.005(S)
	30%	-11.20	4.34	.000(HS)	.029(NS)
	40%	-8.60	6.94	.000(HS)	.001(HS)
	50%	-6.70	8.84	.000(HS)	.000(HS)
Water-Methanol	0.5%	-17.40	-1.86	.000(HS)	.065(NS)
	1%	-16.40	-.86	.000(HS)	.252(NS)
	5%	-11.00	4.54	.000(HS)	.000(HS)
	10%	-10.44	5.10	.000(HS)	.000(HS)
	15%	-9.44	6.10	.000(HS)	.000(HS)
	20%	-7.74	7.80	.000(HS)	.000(HS)
	25%	-6.44	9.10	.000(HS)	.000(HS)
	30%	-5.04	10.50	.000(HS)	.000(HS)
	40%	-3.82	11.72	.003(S)	.000(HS)
	50%	-2.96	12.58	.004(S)	.000(HS)
	Nystatin		15.54		.000(HS)

P<0.05 Significant(S), P<0.001 High significant(HS), P>0.05 N0 significant(NS)