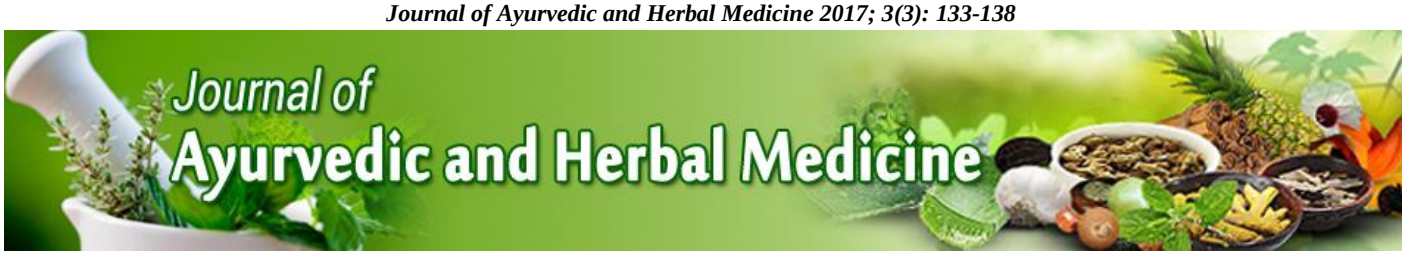

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Sensitivity of *Candida albicans*to aqueous and alcohol extracts of four medicinal plants

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ABSTRACT

*Candida albicans*adimorphic fungus that exists as a commensal of warm-blooded animals including humans. *Candida albicans* infection raises a number of challenges including resistance of *Candida albicans* to the commonly used antifungaland the higher cost of the antifungal agents.The aim of this study is to test the fungus *Candida albicans* sensitivity to some medicinal plants namely, *Solenostemma argel* (Hargel*), Matricaria chamomilla* (Chamomile), *Origanum majorana* (Marzanjosh) and *Guiera senegalensis* (Gebbish). The pure culture of the fungus was obtained from the Medical laboratory - University of Gezira, Sudan. The plants were obtained from Wad Medani herbal local market. Both aqueous and alcoholic extracts were prepared from the four herbs (20%, 35% and 75%) concentrations and (2%,3%,4%,5%,6% and 10%). The sensitivity was tested, using the disc diffusion test that measures the inhibition zones produced by the different herbs at the different concentrations and extraction methods used against the *C.albicans*. The anticandidal activity ofthe aqueous extract of the different herbs did not detect, regardless of the concentration used, while the alcoholic extracts showed remarkable anticandidal activity and the herbal plant *Guiera senegalensis* gave thehighest inhibition (14.3mm) at the highest concentration (10%), followed by *Matricaria chamomilla* with an average inhibition zone of 14 mm and*Solenostemmaargel*with average inhibition zone of 13 mm and *Origanum majorana* with average inhibition zone of 12.6 mm. Significant differences were found between the four herbs at the different concentrations (P value = 0.002).Moreover, increasing the concentration was found to improve the antifungal activity of the herbs and gave better fungal inhibition.Further studies are recommended to investigate the role of the different extraction solvents on the efficiency of the herbs.

Keywords: *Candida albicans*, Herbs, Antifungal, Gezira area, Sudan.

INTRODUCTION

*Candida albicans*a dimorphic fungus that exists as a commensal of warm-blooded animals including humans[1].It is an oval yeast with a single bud. It is a part of a normal flora of mucous membrane of the upper respiratory, gastrointestinal and female genital tracts[2].

Candida albicans infections (candidiasis) are very infrequent in healthy individuals. Candidiasis may be divided into superficial (such as oral and vaginal thrush hand chronic mucocutaneous candidiasis) anddeep-seated (such as *Candida*-due myocarditis and acute disseminated *Candida septicemia*) and represents a major clinicalproblem[1].

Candida albicans infections faces a number of problems including limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of Candida to commonly used antifungal, relapse of Candida infections and non-cost effective antifungal agents[3].In order to alleviate the problem of reduced availability of drugs needed to treat candidiasis, traditional medicine derived from plants are still being used. This prompted the search for novel and active anti-C. albicans agents from plant sources[4].

The plant hargel (*Solenostemma argel*) is a member of the family Asclepiadaceae that comprises numerous medicinal plants[5]. Phyto-chemicals of medicinal properties from Hargel shoots had been reported by many workers[6-9] reported that the aqueous extracts of hargel have antifungal and antibacterial properties.

Chamomile is a daisy (Asteracea)-like flower that grows indigenously in Europe, North West Asia, North Africa, besides being cultivated in North America and in many parts of the world[10]. *Matricaria chamomilla L.* belongs to a major group of cultivated medicinal plants. It contains a large group of therapeutically interesting and active compound classes[11].

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The species *Origanum majorana* L.is an aromatic, perennial, herbaceous plant belonging to the family Lamiaceae. The plant has been used as a flavoring and herbal spice from time immemorial. The plant is reported to possess antibacterial activity[12].

Gebbish (*Guiera senegalensis*) is one of the plants that have not been scientifically exploited fully. It is a shrub found abundantly in the savanna region of West Africa, The leaves of the plant are used against dysentery, cough, and malaria fever. A tea made from its leaves is prescribed through oral route to treat eczema (1 liter per day) [13].

The objective

The objective is to study the sensitivity of *Candida albicans*to Hargel (*Solenostemma argel)* – chamomile *(Matricaria chamomilla)* - Marzengosh (*Origanum majorana*) and Gebbish (*Guiera senegalensis*) aqueous and alcoholic extracts and which give better effect and the effect of increasing and decreasing the concentration.

MATERIALS AND METHODS

The media used in this study is Sabouraud dextrose agar (SDA). It was prepared by adding 65 grams of dehydrated sabouraud dextrose to 1000 ml distilled water and it is added then it autoclaved for 15 minutes[14].

The dried plants samples were collected from herbal local marketat Wad Medani city. Each dried plant was grinding and saved in bottle as a powder.

Preparation of *candida albicans*

The sample of *candida albicans*was given as stock from the Medical laboratory, University of Gezira in petri dish as culture. And then was made subculture in SDA media and incubated then transferred and preserve in the Refrigerator.

Water extraction

The water extract was prepared by soaking dried powder plants in distilled water (50g/500ml), incubated overnight at room temperature, except in *Origanum majorana which* extracted directly without sucking overnight. A stock solution was prepared, from which the different concentrations (20%, 35% and 75%) were made.

All the suspensions were filtered by using a filter paper and the pumper machine.

Ethanol extraction

The ethanol extract was prepared by soaking 10 mg of each plant for 48 hours in 200 ml absolute ethanol 98% at room temperature. The samples were carriedto a rotary evaporator to remove ethanol under pressure. The crude extracts werekept in refrigerator in glass bottles until the further experiments. In the experiments the crude dissolved in distilled water in test tube.

All extracts was diluted successfully with (2% - 3 % - 4% - 5% - 6% - 10%) concentrations.

Sensitivity test

The solid medium diffusion technique using the filter paper discs was used for screening the anti candidal activity of *Solenostemma argel – chamomilla – Origanum majorana* and *Guiera senegalensis.* 1mL of the *Candida albicans* suspension was uniformly spread on the sterile sabouraud dextrose agar petri dishes. Filter paper discs (Whatman n. 1, diameter 6mm) were soaked with the extract and placed on the inoculated agar[15, 16]. The system was incubated at 35-37°C/24 hours. At the end of the incubation period, the bacterial growth inhibition zones diameters were measured using the calipers and expressed in millimeters. When observed growth inhibition zones with diameter equal to or more than 10 mm diameter, then it was considered as positive antibacterial activity. The control was negative control with normal culture[17].

Statistical analysis

All values were expressed as mean ± SEM and difference between the means of were considered significant at $p<0.05$. (ANOVA) SPSS with two ways WAS used. Package version 18.0 for Windows was used for the analysis.

RESULTS

Aqueous extractions of *Solenostemma argel* (Hargel)*, Matricaria chamomilla* (Babonj), *Origanum majorana* (Marzangosh) and *Guiera senegalensis* (Gebbish) plants in different concentrations (20 % - 35% - 75 %) were not effecting against *candida albicans*and the growth around the disk is not affected.

Ethanolic's extractions of *Solenostemma argel* (Hargel)*, Matricaria chamomilla* (Babonj), *Origanum majorana* (Marzangosh) and *Guiera senegalensis* (Gebbish) plants show an anti candidal activity as depicted in Table (1).

Table 1: Anti microbial activity of ethanolic extract of (*Matricaria chamomilla (A) –Origanum majorana* (B) *Solenostem maargel*(C) and *Guiera senegalensis*(D) against *Candida albicans. Inhibition z*one' s diameter was measured in (mm).

Plant	Inhibition zone with diameter (mm)																		
	Extracts concentration																		
	2%			3%			4%			5%			6%			10%			
A	10	8	7	7	7	7	10	10	9	8	8	14	10	12	13	14	14	14	
B	5	10	9	7	9	10	10	10	7	10	11	10	10	10	12	12	11	15	
C	7	12	11	8	12	11	10	11	12	11	12	11	12	12	12	12	13	14	
D	10	11	11	11	12	12	11	12	13	11	13	14	12	13	15	12	13	18	

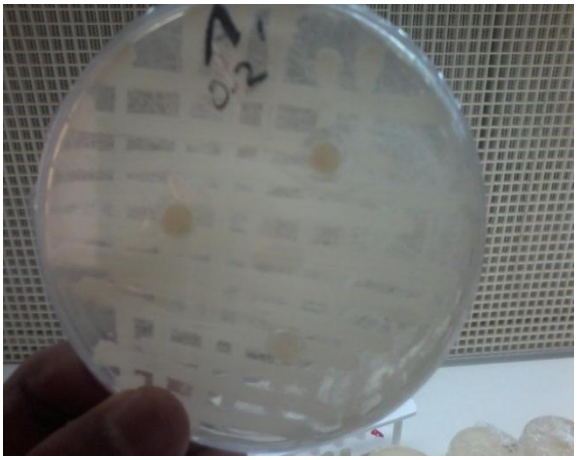

Figure 1: *Chamomilla* with 2% inhibit the growth of *C. albicans* in the region around the disk.

Figure 2: Chamomilla with 3% inhibit the growth of *C. albicans* in the region around the disk.

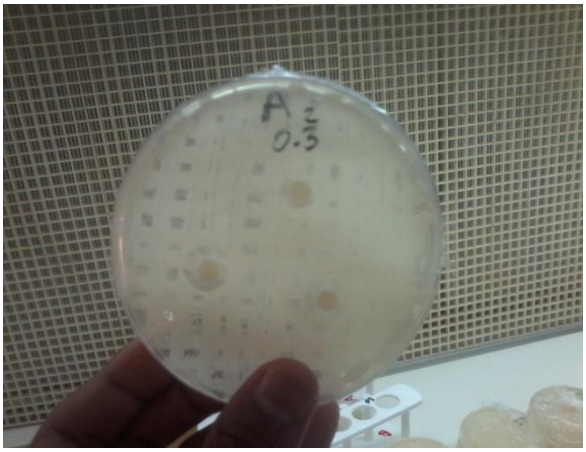

Figure 3: *Chamomilla* with 6 % inhibit the growth of *C. albicans* in the region around the disk.

Figure 4: *Origanum majorana* with 2% inhibit the growth of *C. albicans* in the region around the disk.

Figure 5: Origanummajorana with 3% inhibit the growth of *C. albicans* in the region around the disk.

Figure 6: *Origanum majorana* with 10 % inhibit the growth of *C. albicans* in the region around the disk.

Figure 7: *Solenostem maargel* with 2% inhibit the growth of *C. albicans* in the region around the disk.

Figure 8: *Solenostemma argel* with 5 % inhibit the growth of *C. albicans* in the region around the disk.

Figure 9: *Solenostemma argel* with 10 % inhibit the growth of *C. albicans* in the region around the disk.

Figure 10: *Guiera senegalensis* with 3% inhibit the growth of *C. albicans* in the region around the disk.

Figure 11: *Guiera senegalensis* with 4% inhibit the growth of *C. albicans* in the region around the disk.

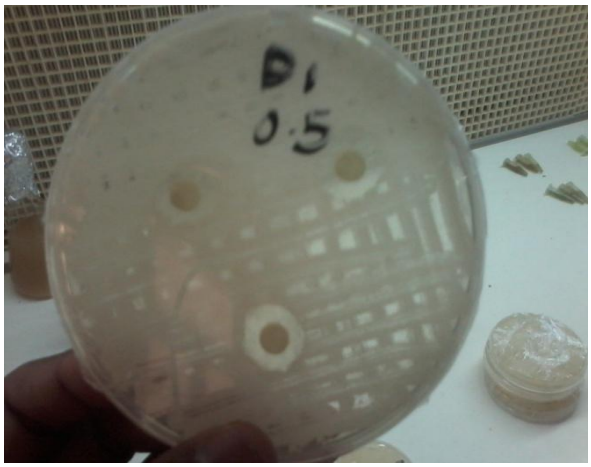

Figure 12: *Guiera senegalensis* with 5% inhibit the growth of *C. albicans* in the region around the disk.

Static analysis

Table 2: The statement of the existence of a real impact of the different herbs and to clarify effective of the plants. The analysis shows that there is Significant difference between the results (P value = 0.002).

	ANOVA				
Degree	**Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	75.042	3	25.014	5.494	.002
Within Groups	309.611	68	4.553		
Total	384.653	71			

P value = 0.002 significant

Table 3: The impact of the different concentration of the different herbs.

	ANOVA				
Degree	**Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	155.736	5	31.147	8.980	.000
Within Groups	228.917	66	3.468		
Total	384.653	71			

P value = 0.000 significant

Table 4: Tests between Subjects Effects Dependent on Variable. R squared more than 0 explain the regression dependent on Variable.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	253.319[a]	23	11.014	4.025	.000
Intercept	8602.347	1	8602.347	3144.005	.000
Plant	75.042	3	25.014	9.142	.000
Concentrate	155.736	5	31.147	11.384	.000
plant * concentrate	22.542	15	1.503	.549	.898
Error	131.333	48	2.736		
Total	8987.000	72			
Corrected Total	384.653	71			

a. R Squared = .659 (Adjusted R Squared = .495)

DISCUSSION

Medicinal Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones[18, 19, 20].

A solvent with polar molecules like water tends to dissolve other substances having polar molecules, as well as substances that form ions when dissolved. This is the case because the charges or partial charges of the solvent molecules and solute molecules attract one another. Ethanol is a very polar molecule due to its hydroxyl (OH) group, with the high electro negativity of oxygen allowing hydrogen bonding to take place with other molecules. Ethanol can dissolve both polar and non-polar substances[21].

(*Solenostemma argel*) is a desert plant of traditional medical uses in Sudan. The antimicrobial properties of *Solenostemma argel* were reported by[22, 9].

Solenostemma argel aqueous extracts with different concentrations (20% – 35% – 75%) did not show any anti candidal activity. The differences in the extract yields from the plant materials ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants. Chemical investigations, and phytochemical of *S. argel* revealed the presence of numerous biochemical ingredients, flavonoids, kaempferolquercetin, rutin, flavanones and alkaloids[23]. Thus, the extracted compounds depend on the nature of the plant and the solvents used.

Solenostemma argel ethanolic extracts showed anti candidal effect with different concentrations (2% – 3% – 4% – 5% - 6% – 10%), so these extracts had compounds that were effective against *C. albicans*. Higher anti candidal activity of *Solenostemma argel* ethanolic extracts was recorded in the highest concentration (10%), while less antifungal activity was observed at the 2% concentration. It was also found, that increasing the concentration of the extract, gave better inhibition than decreasing the concentration. These findings were comparable with the previous findings of[22] who found that alcoholic extracts of *Solenostemma argel* have a marked antifungal activity. Although, [24]concluded from their studies for determining the antimicrobial activity of *solenostemma argel* extraction with methanol/water in different proportions, that the different fractions of *Solensotemma argel* extracted by chloroform/methanol possess an antimicrobial activity to some Gram positive and Gram negative bacteria in a variable manner and a weak fungicidal activity.

Matricaria chamomilla pharmaceutically has anti-inflammatory and antipyretic agents, beside antitumor activities[25] reported that, the extracts of *Matricaria chamomilla* have antiseptic, antibacterial and antifungal properties. In this study, *Matricaria chamomilla* aqueous extracts with deferent concentration (20% – 35% – 75%),have not shown any anti candidal activity. Although, approximately 120 secondary metabolites have been identified in chamomile, including 28 terpenoids, 36 flavonoids and other compound[26]. These extracted compounds depends on their nature, thus, their dissociation in the solvent vary[27]. Reported that the quality of *Matricaria chamomilla* depends on the concentration of the alcohol since water increase the degree of enzymatic degradation.

In this study, the ethanolic extraction of *Matricaria chamomilla* showed an anti candidal activity, it was found that ahigher inhibition rate of candida was found at a concentration of 10% and less inhibition recorded at a concentration of 2%, and this shows that increasing the concentration leads to greater anti candidal effect while decreasing the concentration leads to reduced anti candidal effect, these findings agreed with[28]*who* reported that an ethanolic extract and essential oils of German chamomile inhibited the growth of both Staphylococcus and *C. albicans*.

When the sensitivity of *Candida albicans* was tested for the aqueous extracts from *Origanum majorana* with concentrations (20% – 35% - 75%) it also showed no effect on the growth of *Candida albicans*. *Origanum majorana* was known to contain polyphenols such as carvacrol, including numerous flavones and the essential oil of *Origanum majorana* is composed primarily of monoterpenoids and monoterpenes[29]. Carvacrol has anti microbial activities, but carvacrol is insoluble in water this can justify why no result was obtained from the aqueous extract of *Origanum majorana* on *Candida albicans* in this study.

The ethanolic extraction of *Origanum majorana* showed anti candidal activity, with ahigher inhibition at a concentration of 10% and less inhibition at a concentration of 2%.

Previous study conducted by[30] suggested that the essential oil of *O. majorana* possess antibacterial activity, moreover, the work conducted by[12] revealed that the leaves of marjoram have antimicrobial activity against *Bacillus anthracis, Proteus vulgaris, Salmonella stanley, S. newport, Streptococcus agalactiae, S. guneus and Aspergillus fumigatus.* They also concluded that methanol extract of *O. majorana* has a strong microbicidal property and superiority over commercial microbicides, may prove it to be an effective herbal protectant against a wide spectrum of pathogenic bacteria and fungi. Moreover, [31] reported that the ethanol extracts of *Origanum majorana* is effective against both *C. albicans* and *Aspergillus niger*.

Guiera senegalensis aqueous extracts with different concentration (20% – 35% – 75%) did not show any anti candidal activity. *Guiera senegalensis* (family *Combrataceae*, local name in Khartoum – Ghubeish) is known to have flavonoids (catechin, myricitrin, rutin and quarterin), saponins alkaloids, tannins, mucilage, kaempferol, apigenin, rutin, gallic acid. kaempferol have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, antidiabetic, antiosteoporotic, estrogenic/antiestrogenic, anxiolytic, analgesic, and antiallergic activities[32]. Kaempferol is insoluble in water and soluble in ethanol and methanol. Ethanolic extraction of *Guiera senegalensis* showed anti candidal activity, it was found that ahigher inhibition of *Guiera senegalensis* rate was found at a concentration of 10% and less inhibition record rate at the concentration of 2%, and this shows that increased concentration leads to greater anti candidal effect and reducedconcentration leads to reducedanti candidal effect.

Leaves, young shoots and galls of *G senegalensis* are used in Burkinabe folk medicine for their antibacterial andantifungal properties[33]. It has also been reportedthat crude methanolic extracts of *G senegalensis* exhibit antimicrobial properties on bacteria and fungi[34].

reported that the methanolic extract of *Guiera senegalensis*is active against *C. albicans* and it has an anti candidal activity[35].

When analyzing the results obtained statistically it was found that there were significant differences between the various plants species (P value = 0.002)

*Guierasenegalensis*ethanolic extract is more active and gave better results in the 10 % concentration with anaverage inhibition zone of 14.3 *Matricaria chamomilla ethanolic extract gave better result in 10% with average inhibition zone 14mm Solenostem maargel* ethanolic extract *gave better result in 10% with average inhibition zone 13 mm* and the inhibition *Origanum majorana* ethanolic extract *gave better result in 10% with average inhibition zone 12.6 mm.*

CONCLUSION

The aqueous extraction of the four medicinal herbs (*Matricaria chamomilla – Origanum majorana – Solenostem maargel and Guiera senegalensis*) did not give detectable results against *C. albicans*, while alcoholic extracts gave anti-fungal activity against *C. albicans* vary depending on the herb andthe effectwas higher with increased concentrations.

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Conflict of interest – Authors have no conflict of Interest.

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