Effect of Garlic extract on the growth of Candida albicans Isolated from patients attending dermatology clinics in Misurata-Libya

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Abstract:

The study was conducted on patients attending the dermatology clinics at Misurata hospital center during the period between April to August of 2018. One hundred and twenty (120) samples were collected to isolate and identify Candida albicans. Microscopic examination (light and scanning microscopy) was carried out to identify the morphology of the isolate, also different concentrations of alcoholic and aqueous garlic extracts (20%, 30% and 40%) were screened for their efficacy in inhibiting of vegetative growth of C. albicans. The. Positive fungal infection was found in 75 sample. The most common fungal pathogens, collected by swabs from the skin, scalp, mouth, vagina and nail clippings, were **Candida** species and other filaments fungal , with C.albicans being the predominant isolate (35%).The antifungal susceptibility testing showed that The most common fungal pathogens, were **Candida** species and other filaments fungal. C.albicans being the predominant isolate (35%).The antifungal susceptibility testing showed that alcoholic garlic extracts had high efficacy inhibition growth of C. albicans, where the inhibition growth percentage was 97.5% at a concentration of 40mg/ml and without significant difference compared to the Nystatine, while- the high percentage of inhibition by aqueous extracts of garlic was 10.46% at a concentration of 20 mg/ml with a very high significant difference compared to the Nystatine. The antifungal effect of alcohol extracts of garlic is better than the aqueous extract for C.albicans(**p** < 0.001). garlic extract has a significant antifungal effect towards C.albicans **.**

Key words: Candida albicans, Garlic extract, Dermatology clinics

Introduction

In recent years, the incidence of infections caused by dermatophytes and other fungi has increased considerably especially among pediatric and geriatric populations [1]. C. albicans among other dermatophytes are a major causative agent for superficial dermatomycoses like candidiasis. Candida species are the most common human commensal that able to cause a broad spectrum of disease in hosts, emphasized that Candida albicans has become one of the opportunistic fungal pathogen that colonizes mucosal surface of the oral and vaginal cavities in host. Antifungal agents such as griseofulvin, azole derivatives and Nystatine may become of little use in the treatment of dermatophytoses as a result of the development of fungal resistance. prolonged duration of treatment and side effects, there exists a clear demand for additional antifungal with therapeutic potential. [2].Thus, many research programs have been conducted to find out new natural and synthetic compounds with antifungal properties and minimum side effects . In this context, attention has focused on the antifungal activity of medicinal plants and their constituents due to their potential biological properties. Herbal medicines have been important sources of products in

developing countries for treatment of common infection including fungal disease. Discovery of antimicrobial activities of garlic (Allium sativum) has a long history and it is reported on different microorganisms [3] .

Aim of paper

This work was conducted to isolation C.albicans from patients attending dermatology clinics in Misurata Libya, and to evaluate the effect of ethanolic and aqueous Garlic extracts on the growth of C.albicans.

Materials and Methods

Fungal strains collection:
The fungal pathogen (C. albicans) was obtained from patients, collected from oral, vaginal, hairs, nails and skin. Samples, during April to August 2018 from dermatology clinics in Misurata- Libya, after the sterilization of infected area by ethanol 70% from patients attending to dermatophytes clinical following the methods mentioned by [4]. Samples were collected in clean polyethylene bags, transferred to the laboratory immediately for further processing.

Identification of the fungal pathogen
The Identification of the fungal pathogen based on morphological colony isolates were characterized according to standard methods, mentioned by [5 ; 6]. Two techniques of pathogen isolate were used, in this study, as follow:

-Direct examination: The microscopic examination of the specimens was performed following the treatment with an aqueous solution of KOH (10%) to examine the presence of fungal filaments mentioned by [7]. **Serum Germ Tube Technique:** Triplicate sets of test tubes containing 1.0 mL of pooled human serum were inoculated with 2-3 colonies of Candida sp. The tubes were inoculated at 37°C for 3 hours after which a drop of the suspension was placed on labeled microscope slides for examination of germ tubes[8].

-Culture of samples: All samples of Candida were cultured on Sabouraud dextrose agar (S.D.A) with chloramphenicol, the plates were incubated at37°C ± 2, for up to two weeks then examined microscopically twice a week, for evidence of fungal growth. The fungal isolates were sub-cultured used the same media, then the isolates were examined by using lactophenol cotton blue [3]
Collection and preparation of experimental plant:
Fresh garlic (Allium sativum) was collected from the local markets in Misurata city, cleaned, shade-dried, and cut into small pieces, ground by **mortar** and **pestle** at that point set in clean holder as powder for consequent investigations.

Ethanolic Garlic extracts:

An amount of 40g from each powdered sample was mixed alone with 160ml of ethanol (70%) [9]. The mixtures macerated by shaker, left 24h for soaking, and then filtered through two layers of muslin cloth and by whatman No1. Afterword, the extract concentrated by rotary evaporator under 40C° and collected. An amount of one

gram of dried extract dissolved in 10ml distill water to obtain stock solution of 100mg\ ml the solution was further diluted to give concentrations of 20mg\ml, 30mg\ml, and 40mg\ml.

Aqueous Garlic extracts:

Aqueous extract was completely prepared according to the method described by [10]. 40g of the dry sample was places in the beaker, then 160 ml of distilled water was added. The mixture was macerated by shaker and allowed to stand for 24h for soaking. Afterword, the mixture was filtered through two layers of muslin cloth and by whatman No1. The extract was concentrated by a rotary evaporator at 60C˚, then collected in sterile screw capped bottle. An amount of 10ml of distill water was adding to one gram of the extract powder to produce (100 mg/ml) standard stock solution. Serial dilutions prepared from the stock solution to get concentrations of (20, 30 and 40 mg/ml).

Preparation of media:

The culture media (S.D.A) was used as a conventional medium for the growth of Candida [11]. The media was prepared by weighting appropriate quantity of the agar powder (20g), peptone (10g) and dextrose (40g) in 1000 ml distilled. Bring to the boil to dissolve completely, the media was Autoclaved at 121ºC for 15 minutes, cooled up to 45°C,included a drops of chloramphenicol, 250mg/L as broad spectrum antibacterial was added to the media then poured into petri dishes, about 20 ml every plate, leaved to solidified for subsequent studies.

Antifungal assay of the Garlic extract against C. albicans:

The tested microbe C. albicans was removed aseptically with an inoculating loop and transferred to a test tube containing 5 ml sterile distilled water. Sufficient inoculums were added until the turbidity was equal to 0.5 McFarland (10^8 colony-forming units: CFU mL^{-1}) standard. 1 ml of the cells suspension from the test tube was added to 15-20 ml of S.D.A before setting aside the seeded agar plate [12]. Antifungal assay of the C. albicans was carried out by using the method mention by [13]. An amount of 1ml of each concentration (20, 30 and 40 mg\ml) add to 20ml of (S.D.A) were separately and uniformly swabbed across a culture plate which were incubated at 37°C. Patented medicine namely Nystatine was used for comparison as positive control. The respective solvents (100%) without plants extracts were also used as negative controls. Each experimental result was determined by the average of tree replicates. The percentage of growth rate of the tested fungus was measured by program **(image j for area measurements)**. the vegetative growth inhibition rate of the tested fungus was calculated using the following equation: Vegetative growth inhibition rate (%) = ((control – treatment)/ control) X 100

Statistical analyses

All data will be presented as a mean. Statistical analysis were performed using One-way analysis of variance (ANOVA) followed by Dunnett comparison test. SPSS statistical package version 17 were used for statistical analyses. A differences in the means with $P < 0.05$ was considered statistically significant. T test analysis were also done between each concentration.

Results and Discussion

Isolation of C. albicans and other filamentous fungi from selected samples.

The results showed in Table (1) clarified that pathogenic fungi represented by C. albicans recorded 35 isolates with percentage of 46.7% which selected from 75 isolates. Most oral samples isolates were C. albicans recorded (21) isolates followed by vaginal then skin scraping. 40 isolates of other filamentous fungi were isolated from different sources with occurrence percentage of 53.3%.

Table (1): Total numbers of isolates from 120 specimens collected from patients attending to dermatology clinics in Misurata- Libya

Positive Microorganisms isolates	Numbers of Isolates from different Specimen types					The Percentage from (75) total positive isolates
	oral samples	Vaginal samples	Skin scrapings	scalp samples	Nails samples	
C. albicans	21	10	4	-	-	46.7%
other filamentous fungi	5	7	9	14	5	53.3%

The observation of C. albicans had the highest incidence rate (46.7%) among the other isolates. The same result was reported by [14].The result of this study also illustrated that the oral samples of C.albicans represent highest incidence followed by vaginal samples, this is due to several factors such smoking, immunity of patients, age, infection with diseases, prolonged antibacterial therapy, corticosteroid use, surgical procedures, poor nutritional status, aggressive cancer chemotherapy and the extensive use of antimycotic drugs for therapeutic courses has led to a change in the relative prevalence of various species of Candida.

Morphological characteristics of the pathogenic fungus (C.albicans)

The pathogen isolated C.albicans from patients was identified based on key demonstrated by[15]. The colonies were appeared on S.D.A medium as raised, glossy, smooth, glabrous yeast-like, white to cream colored and circular colonies. The microscopic morphology of C. albicans showing budding spherical to ovoid blastoconidia. C. albicans grow in several different morphological forms, ranging from unicellular budding yeast to true hyphae (pseudomycelium) with parallel-side wall and germ tube formation. (Fig.1a and b)

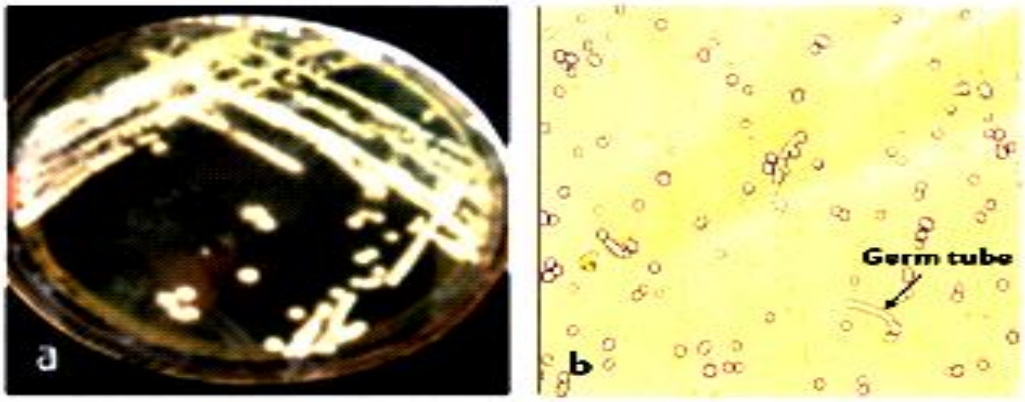

Fig. 1. (a)Colonies of C. albicans growing on (S.D.A). (b):Germ tube of C. albicans under light microscope.

In this study, the isolated Candida was sub cultured on S.D.A [16] mentioned that S.D.A is commonly used for the isolation of Candida spp. Based on the appearance of colonies grown on a tested media, the colonies were cream-white with a soft consistency and also the surfaces and margins of the colonies were round with convex and smooth (Fig.1.a), the same result have been reported [17]. More comprehensive diagnosis methods are necessary to fully confirm the diagnosis of C. albicans. Thus, a germ tube test was carried out, in this study, to differentiate C. albicans from Candida spp. This germ tube test is widely known as presumptive test for identification of C. albicans. Germ tubes were formed under certain environmental conditions; the yeast cell produces a germ tube which is the initial morphological form of hyphae (Fig.1.b). Therefore, this result agrees with [18].

Antifungal activity of ethanolic and aqueous garlic extracts against C. albicans

The effect of ethanolic garlic extracts against C. albicans as show in table (2) and .Fig (2) was dependent on the dose of the extract. Hence, with increasing concentrations of ethanolic garlic extracts, the fungal growth reduced. In the concentration of 40 mg/ml, few numbers of colony were appeared. The concentration of 40 mg/ml of ethanolic garlic extracts showed a higher antifungal activity recorded 97.5%, inhibition growth percentage of C. albicans and was more effective than both employed garlic extracts 20 mg/ml and 30 mg/ml, which recorded (80% and 84.4%) respectively. The results showed that C.albicans was strongly inhibited by the antifungal Nystatine, which was used as a positive control, the inhibition growth percentage recorded 100%. The statistical analysis, ANOVA ONE WAY, shown in table (2) illustrated that ethanolic garlic extracts inhibit C. albicans and the growth of C.albicans was highly significant reduced (P=0.001). It was observed that the inhibitory effect increased as ethanolic garlic extract concentrations increased. Analysis of variance (ANOVA ONE WAY, LSD and Dunnett test) showed that there were highly significant differences among ethanolic garlic extract concentrations (P=0.001). from the result it can be seen that C.albicans was showed slightly inhibited by the aqueous garlic extracts with different concentrations 20mg/ml, 30mg/ml and 40mg/ml, recorded inhibition percentage of 10.46%, 3.49% and 5.73% respectively, when compared with nystatine. The result of this study clarified that ethanolic garlic extracts

showed stronger inhibition activity than the aqueous garlic extracts against C.albicans.

Table (2): Antifungal activities of different concentrations of garlic extracts in comparable with Nystatine against C. albicans using agar plate method

The pathogen	% of inhibition growth by Ethanol garlic extracts				% of inhibition growth by Water garlic extracts				Nystatine (Positive control)
	20 mg/ml	30 mg/ml	40 mg/ml	Ethanol (Negative control)	20 mg/ml	30 mg/ml	40 mg/ml	Water (Negative control)	
C. albicans	80 **	84.4 **	97.5	0%	10.46 ***	3.49 ***	5.73 ***	0%	100%

***statistically very highly significant. **statistically highly significant

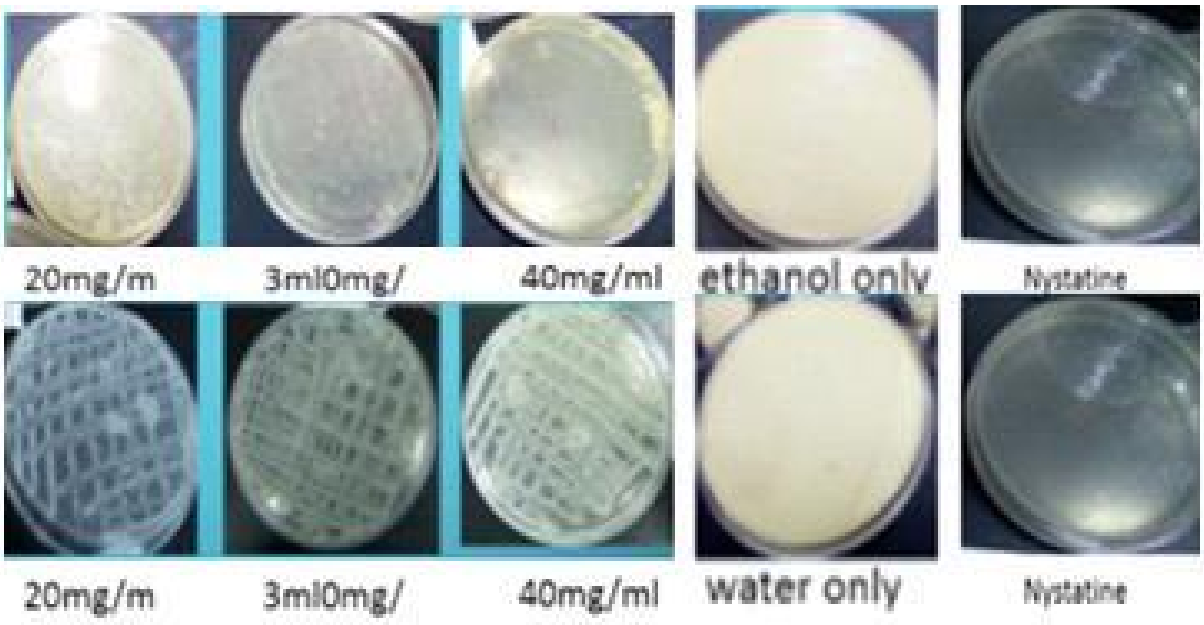

Fig (2): Effect of different concentrations of garlic extracts (ethanol and water) on the growth of C.albicans on (S.D.A) comparable with Nystatine.

In the present study, Garlic (Allium sativum) has been shown to inhibit the growth of C. albicans, the inhibition may be related to allicin or ajoene which controls the performance of some enzymes that are important to C.albicans. Our results clearly indi-

cate that the ethanol garlic extract showed higher inhibitory activity and 40% ethanolic garlic extract had a similar effect compared with Nystatine. This result in agreement with a study done by[19]. In contrast, the aqueous garlic extracts show less antifungal activity than the ethanolic extract against C. albicans, this may be due to increasing solubility of active compounds in ethanol which is in agreement with [20].As noted from the results that the less concentration of ethanolicgarlic extract gave less inhibitory activity against C.albicans and this is may be due to the lack of concentration of the active substance in low concentrations and therefore less effect in the inhibition of fungus[21].

From the results, there is a clear variation in the effect of garlic extracts, an aqueous garlic extract indicate that it has a lower antifungal activity against C. albicans where as ethanolic garlic extract showed stronger antifungal activity against C. albicans this result in consistent with [22] which explained that the activity of ethanol is due to its ability to extract polar compounds in general such as phenols, flavonoids and other compounds.

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