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IN-VITRO ANTI-FUNGAL ACTIVITY OF *GARLIC* OIL ON THE COMMON ISOLATES OF *CANDIDA SPECIES*

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Abstract

Background: The growing interest of using Garlicis seriously investigated over several years as a natural antimicrobial agent. Candidias is an opportunistic systemic fungal infection caused by gram positive yeast form fungus *Candida.* More than 90% of invasive infections due to *Candida* are attributed to five species *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*. In the previous decades drug resistant strains of *Candida* makes the problem significant to be considered for alternative treatment strategy. **Objective:** To evaluate *In-vitro* anti-fungal activity of (Garlic) oil on the common isolates of Candida species. **Method:** Experimental study was conducted in University of Gondar, North West Ethiopia. Fresh bulbs of garlic were purchased from local markets in Gondar. The cloves were separated and peeled to obtain the edible portion. The edible portion was chopped and macerated using clean pestle and mortar. The homogenate was centrifuged and supernatant was then filtered by Whatman filter paper to get a crude oil of garlic. 10µl, 15µl, 20µl, 25µl 30µl and 35µl of the oil added to commercially prepared blank disk (Hi-Media, Mumbai, 6mm in diameter). The commonly isolated *Candida spp such as, C. albicans*, *C. tropicalis* and *C. krusei*., which were procured from Gondar Teaching Hospital, Microbiology Laboratory, used to determine the anti-fungal activity of garlic oil using disc diffusion method. **Results:** The result showed that an extremely sensitive activity against on the common isolates of *Candida* against different concentrations of garlic oil. The minimum activity showed was 28mm inhibition zone for 10µl and maximum of 37mm inhibition zone for 35µl of garlic oil against *Candida krusei*. Whereas, 33mm inhibition zone for 10µl and maximum of 42mm inhibition zone for 35µl and 31mm inhibition zone for 10µl and maximum of 45.5mm inhibition zone for 35µl of garlic oil against *Candida albicans* and *Candida tropicalis*, respectively. Generally, all the isolates were extremely sensitive for garlic oil compared to standard anti-fungal agents' ketoconazole and amphotericin B. The minimum inhibition zone diameters of standard antifungal agents were 23.75mm and 22mm for ketoconazole and amphotericin B against Candida isolates, respectively.MIC and MFC results have shown that 4% for *C.albicans* and *C.tropicalis* and 2%for *C.krusei*, respectively. **Conclusion:** The study revealed an extremely increased antifungal activity of garlic oil against Candida isolates compared to standard antifungal agents, which suggests that garlic can be a potential alternative treatment for Candida infection.

Keywords: Garlic oil, Anti-fungal activity, *Candida spp.*

Introduction

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Man has used natural products of animals, plants and microbial sources for thousands of years in either pure form or crude extracts to threat many diseases by using its wonderful healing power.[1,2] Garlichas been used as a spice, food and folk medicine since ancient times. Investigations have been undertaken to provide a scientific basis for its medicinal use and several have reported its medicinal, insecticidal, anti-microbial, anti-protozoan and anti-tumor properties.[3] It is cultivated practically throughout the world, appears to have originated in Central Asia and know spread to all over the world.[4]

Garlic contains active component allicine and adjoine and around two hundred unusual chemicals including other sulfur compounds, several enzymes and minerals responsible for its medicinal effect but adjoine was superior to allicin in antifungal activity.[1] Mechanism of action of garlic is unknown, but different Scholars give their suggestions such as, Barone&Tansey proposed that garlic acts by inactivating essential thiols and adetumbi proposed that through blockage of lipid synthesis is likely to be an important feature of the anti-Candidal activity of garlic.[5] It has been suggested that development of resistance to allicin arises 1000 fold less easily than it does to certain antibiotics.[4] Variations in composition of garlic and genetic disparity among bacteria and fungi of the same or different species have been found responsible for the few inconsistencies inthe antibacterial and antifungal properties of garlic extract.[6]

Since the early 1980s, fungi have emerged as major causes of human disease, especially among the immune compromised.[7,8] Candidiasis is an opportunistic fungal infection caused by gram-positive yeast form fungus known as Candida. This yeast normally found in small amount in human body. Infection will occur if there is an over growth of Candida and in different cases of immunity suppression. Candida species are the fourth most common cause of nosocomial bloodstream infections in the developed world.[7] Of greater concern are invasive fungal infections which have much lower incidence rates than superficial infections but have alarmingly high mortality rates, despite treatment with anti-fungal drugs. Patients with compromised immune systems (associated with cancer and HIV) and those undergoing invasive clinical procedures (stem cell, bone and organ transplants) are a predisposing factor susceptible to these invasive, life-threatening infections, and mortality rates mostly range from 46 to 75% depending on the invading pathogen and the underlying condition.[7,9] About 75% of women will get a vaginal yeast infection called vulvovaginitis during their lifetime, and 90% of all people with HIV/AIDS develop oral Candida infections most commonly infants, elderly people and those with a weakened immunity.[9-11]

More than 90% of invasive infections are caused due to five *Candida spp*namely: *C.albicans (the most prevalent 1/3 of world population), C. glabrata*, *C. parapsilosis (*of limited pathogenicity*), C. tropicalis (*prevalent in the immune suppressed patients*)* and *C. krusei*.It is estimated that neither*C. glabrata nor C. krusei* showed a consistent increase or decrease in isolation rate. However, increased rates of isolation of *C. tropicalis* (an increase from 4.6% to 7.5%) and *C. parapsilosis* (an increase from 4.2% to 7.3%) were observed between 1997 and 2003.[12] In Ethiopia 82% of HIV patients are exposed to candidiasis and isolates such as, *Candida albicans* 12.2%, *Candida tropicalis* 7.7% and *Candida krusiee* 4.7% were resistant to fluconazole, ketoconazole and itraconazole as estimated by study in North -West Ethiopia in 2013.[10]

Yeast like fungi's are resistant to antibiotics and difficult to control in those with depressed immunity.[13] The limitations of most commonly used licensed antifungal drugs such as, development of resistances, drug-related toxicity, hazardous drug interactions, or insufficient bioavailability has been reason for the growing interest of using garlic as a natural antifungal agent.[3] Garlic serves as an ideal replacement of drugs because; it is affordable plant to fight fungal infections.[13] Also, since garlic is most effective on the preventative level, it can be recommended for daily use.[12] On determining intrinsic variation in activity of Garlic on different geographic variation and comparing to its prevalence and the development of drug resistant strain of Candida there is almost no study conducted about the in vitro effect of garlic oil on *Candida spp*in North-West Ethiopia. Therefore, the objective of this study was to evaluate *In-vitro* anti-fungal activity of *(*Garlic) oil on the common isolates of *Candida*

species. So, the study generates information that will help the modern medicine by reducing morbidity and mortality.

Methods and materials

Study area

The study was conducted in College of Medicine and Health Science, School of Biomedical and Laboratory Sciences, University of Gondar, North-West Ethiopia from March 2014 to May 2014.

Study design and period

Experimental study was conducted to evaluate the antifungal effect of garlic oil on the common isolates of *Candida spp*from March 2014 to May 2014.

Source of population

Known cultures of *Candida species* isolated from infected patients were used in this study.

Study population

Three isolates of *Candida* specious such as, C. *albicans, C.tropicalis* and *C. krusie*, were included.

Sample size and sampling techniques

By using a non-probability convenience sampling technique a total of three *Candida species (Candida albicans, Candida tropicalis and Candida krusie),* was obtained from the University of Gondar Hospital Microbiology Teaching Laboratory.

Dependent variable

The anti-fungal activity of garlic oil on *Candida species*

Independent variables

Candida species, amount of garlic

Inclusion criteria

Candida species those can be grown on CHROM agar and easily differentiated based on colony characteristics and pigment production will be included.

Exclusion criteria

Candida species which can grow on CHROM agar but needs further biochemical test for species identification was excluded.

Garlic oil sample collection and preparation

Fresh bulbs of garlic were purchased from local markets in Gondar. The cloves was separated and peeled to obtain the edible portion. The edible portion was chopped and macerated using clean pestle and mortar. The homogenate was then filtered by passage through Whatmann filter paper to get a crude oil of garlic, which was considered as 100% concentration. This was collected in a sterile vial then centrifuged for 8 hrs and supernatant is stored at 4°C until used.

Test organism preparation

The three Candida species were obtained from Microbiology Diagnostic Laboratory, Gondar University Hospital. Candida colonies appear with pink, green and bluish green color that are *C.krusie,C.albicans, C.tropicalis* respectively in the CHROMagar. The area of sample collection and Indian ink procedure to check it is not Cryptococcus was performed as a confirmatory test. Then by sterile inoculating wire loop suspend the picked colonies in 3-4 ml Trypton Soya Broth in a sterile tube. The turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard and incubate at37^{o}Cuntil use. The suspensions of Candida spp. was prepared from mature cultures grown at 37 °C on CHROMagar.[14]

Anti-fungal susceptibility testing

Antifungal susceptibility testing was performed for all Candida isolates using modified disc diffusion method; by adding 2% glucose to Mueller-Hinton agar to accelerate growth of some Candida species. Suspension was prepared by Trypton Soya broth from colony of incubated CHROMagar. Then the suspension was compared to 0.5 McFarland standards which is appropriate standard for susceptibility determination. The Candida suspension was streaked on modified Mueller-Hinton surface using moist cotton swab from the suspension then allowed to dry for 15 minutes. The blank discs prepared by garlic oil with ,10µl,15µl,20µl,25µl,30µl and 35µl along with standard antifungal discs (ketoconazole and Amphotericin B) was placed on Mueller-Hinton agar using flamed forceps, incubate the plate for 72 hour at 37^{0}C,finally the zone of inhibition was measured using zone scale reader and interpretation was done as mentioned below. Each assay was performed in triplicates and mean value was considered.[15, 16]

Determination of MIC & MFC

The minimum inhibitory concentration (MIC) is the lowest concentration of extract that produce no visible fungal growth (turbidity) .MIC value of the garlic oil was determined by preparing 6ml dilution of trypto soya broth with garlic oil in different concentration of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16% and 18% in sterile tubes and by adding 100µl of Candida suspension to each tube.6ml tryptosoya broth with 100µl Candida suspension for positive control and 6ml tryptosoya broth with no Candida suspension for negative control was simultaneously maintained for each spp and Incubated aerobically at37^0c for 72 hr. The lowest concentration of the extract yielding no growth on the plate was recorded as the minimum fungicidal concentration (MFC).sub culturing from each tube on to fresh modified MH agar plate and incubating at 37^0c for 72 hr is the process to determine MFC of the garlic oil.[17]

Quality Control

Controlling the quality of a given experiment is mandatory to get good result, so, this study used different methods to reduce error. Sterile blank disks, autoclaving materials and agars, clove garlic rinsing with 70% alcohol was done to reduce contaminants. Standard drugs are also be used as a control for garlic activity test. Suspensions are also checked by 0.5% McFarland standard.[17]

Data analysis and interpretation

The mean of each concentration was used and the results presented in tables, figure and description to explain the resistance and susceptibility pattern of the *Candida spp*.

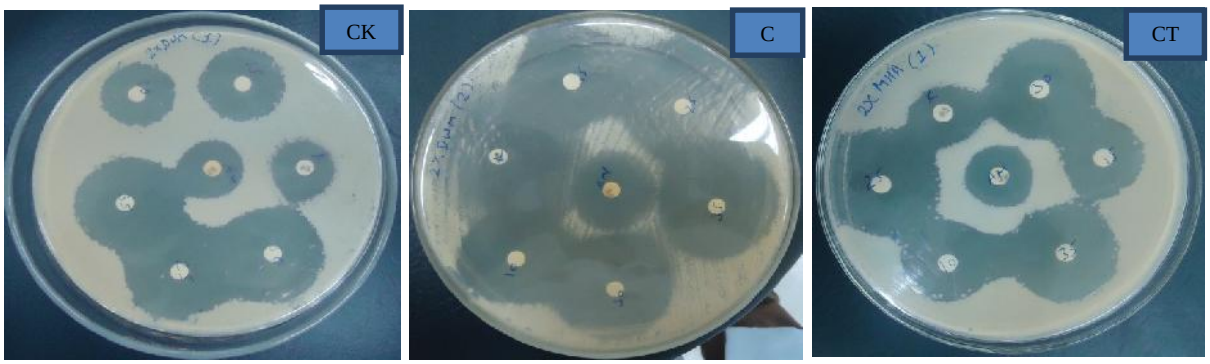
Result

The result of the *in vitro* antifungal activity of garlic extract has shown as follows (Table 1)**.** The present study has revealed that high zone of inhibition of garlic oil against common *Candida* isolates in Ethiopia. The result showed that an inhibition zone on the common isolates of Candida in different mm against disk with10µl, 15µl, 25µl, 30µl, 35µl. The minimum activity showed was 28mm inhibition zone for 10µl and maximum of 37mm inhibition zone for 35µl of garlic extract against *Candida krusei.* Whereas, 33mm inhibition zone for 10µl and maximum of 42mm inhibition zone for 35µl and 31mm inhibition zone for 10µl and maximum of 45.5mm inhibition zone for 35µl of garlic extract against *Candida albicans* and *Candida tropicalis* respectively. From the isolates *Candidakrusei* show the last small inhibition zone than *Candida albicans* and *Candida tropicalis* with the smallest amount of Garlic oil which is 10µl. The largest inhibition zone 45.50 was reported from *Candida tropicalis* in 35µl of garlic oil.

Table No. 01: Anti-fungal activity of *oil* on the common isolates of *Candida sps.*.

Amount of Garlic oil in single blank disc	Inhibition zone diameter in mm		
	C. krusei	***C. albicans***	***C. trophicalis***
10µl	28.00	33.00	31.00
15µl	29.67	34.00	37.00
25µl	37.00	42.33	40.00
30µl	36.30	42.00	39.00
35µl	37.00	42.00	45.50
Kt	26.27	29.00	23.75
Amp	22.00	27.67	24.33

** The inhibition zone diameter in mm is mean values from experiment done in triplicates; Kt: Ketoconazole;*



Amp: Amphotericin B

Fig. No. 01: Antifungal susceptibility of garlic oil against Candida spp. (Disc diffusion method)
CK-*Candida krusie*; CA-*Candida Albicans*; CT- *Candida tropicalis*

MIC and MFC for Garlic oil

MIC and MFC result for Garlic oil has shown as follows in (Table 2 and 3).The present study has revealed that with a very small percent of Garlic oil there is good anti-fungal activity each tubes for *C.albicans* and *C.tropicalis* are not turbid except the tube with 2% means 4% is MIC for *C.albicans* and *C.tropicalis.* Each tube prepared for *C.krusie*does not show turbidity.

Table No. 02: Minimum inhibitory concentration of garlic oil against *Candida spp*

	Garlic oil (%)								
Candida spp	**40%**	**30%**	**20%**	**10%**	**8%**	**6%**	**4%**	**2%**	**Control**
Candida albicans	N	N	N	N	N	N	N	**T**	**T**
Candida tropicalis	N	N	N	N	N	N	N	**T**	**T**
Candida krusie	N	N	N	N	N	N	N	**N**	**T**

** T: Turbid N: Not turbid*

Minimum Fungicidal concentration result has showed in (Table 3) this study revealed the killing capacity of garlic oil at 4% concentration was achieved for*C.albicans* and *C.tropicalis. However,* C.krusie was not grown on each plates prepared with garlic oil. The amount of colonies in *C.tropicalis*is more than *C.albicans as shown* in the figure (2). MFC for *C.krusie is not determined.*

Table No. 03: Minimum fungicidal concentrations of Garlic oil against Candida sps

	Garlic oil (%/CFU)								
MFC	**40%**	**30%**	**20%**	**10%**	**8%**	**6%**	**4%**	**2%**	**Control**
C.alibican	0	0	0	0	0	0	0	**G**	G
C.tropicalis	0	0	0	0	0	0	0	**G**	G
C.krusie	0	0	0	0	0	0	0	0	G

0: No growth, G: Growth, Minimal inhibitory concentration, MFC: Minimal fungicidal concentration,

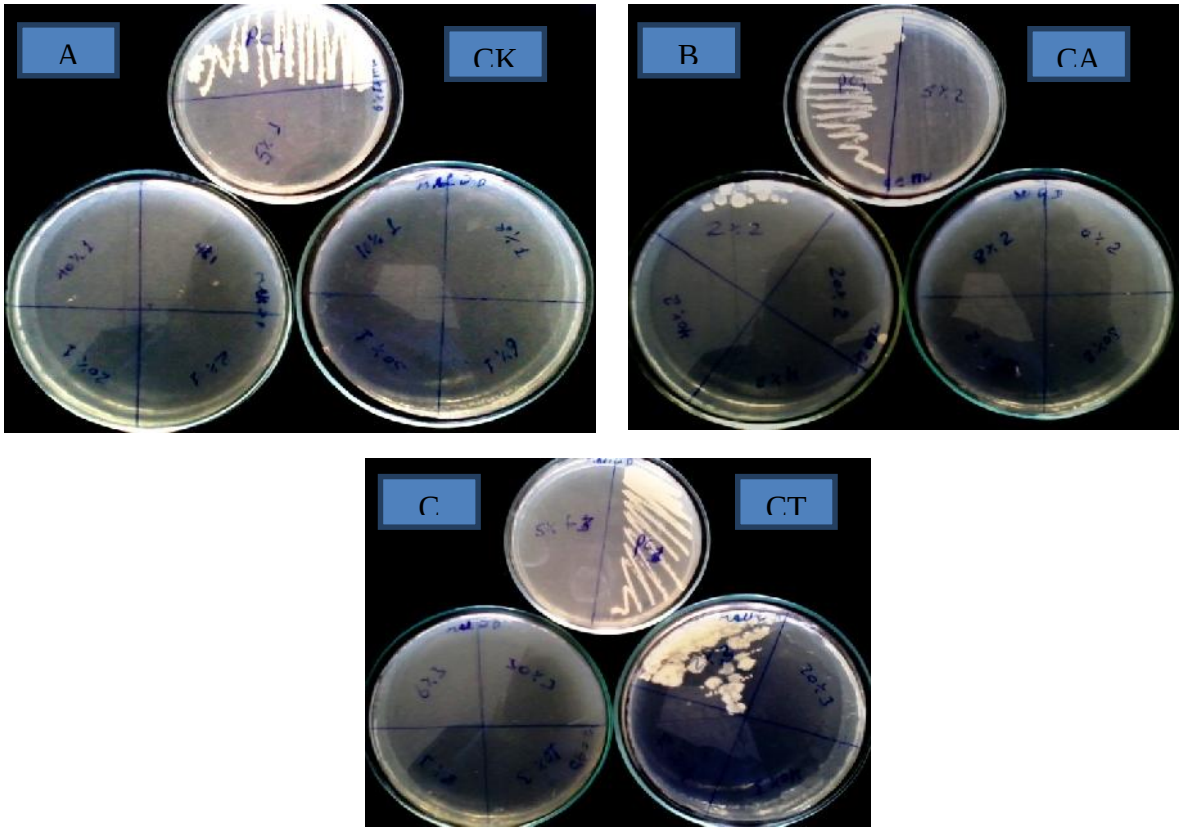


Fig. No. 02: Minimum fungicidal activity of garlic oil against Candida spp.
A:*Candidakrusie*; **B:***Candida Albicans*; **C:** *Candida tropicalis*

Discussion

This study was carried out to determine the in-vitro anti-fungal activities of Garlic oil on the common isolates of *Candida* spp and showed extremely good activity on growth inhibition of the common isolates of Candida but, making comparison to discuss with other literatures was difficult because the study used different laboratory method from most of presented studies. The present study have similar report with the study conducted in Japan, which is Adjoiene strongly inhibit growth rate of *Candida krusie,Candidaalibican and Candida tropicalis*<20µg/mL,[18] study in Malaysia have good fungicidal effect[19] and the study conducted by Tensay & Appelton[18] & Atikin and Moorie[20] have showed large area of inhibition.

The current study show *Candida krusie* is easily inhibited Candida *spp* by Garlic oil than *Candida albicans* and *Candida tropicalis* in the reported MIC result but, on the reverse to this, study from Iran reported that Garlic with standard drug Fluconazole reduce the MIC of the drug larger means 4 fold in *C. tropicalis*than *Candida albicans* and *Candida krusie*,[21] this may be occurred because of genetic disparity among Candida spp from country to country.[6]

On the other hand some studies have shown totally different activity of the garlic in comparison to the present study which means one study from California reported garlic juice with10-120µg/mL don't show any inhibitory effect on *Candida albicans*[20] and the other from South Africa on the drug resistant oral *Candida sps* there is weak anti-fungal effect to the drug resistant *Candida.*[22]

Conclusion

From the current study, it is concluded that there is potential evidence for the antifungal activity of garlic oil against common isolates of Candida spp, such as *Candida krusie*, *Candida albicans* and *Candida tropicalis*. On this regard, garlic oil offer alternative source of medication or can be used as a dietary supplement along the standard anti-fungal agents for patients with Candidiasis to obtain synergistic effect.

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