Antimycotic Activity of Four Plant Extracts and its comparison with Fluconazole in the Treatment of Otomycosis

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Abstract: Otomycosis is a superficial mycotic infection of the outer ear canal caused by many saprophytic fungi that could be considered as causative agents. Detection of the fungal agents in the external auditory canal could be valuable to determine the potential risk of otomycosis. The aim of this study was to identify the mycoflora of the human auditory canal in 60 healthy girl students in College of Applied Medical Sciences at AL- Dawadmi City. During two weeks, a total of 60 ear samples of healthy students were randomly collected by sterile swabs and cultivated on Sabouraud Dextrose Agar (SDA medium). Fungal isolates were identified using conventional methods and chromogenic media. Otomycosis was confirmed in 17 students (28.3 %),10 students from 17 students of otomycosis had mixed fungal - bacterial infections (16.6%) and no organism was isolated in 43 students (71.6%). *Candida albicans* (35.2%) followed by *Aspergillus niger* (29.4%) were the dominant fungal involved in otomycosis. The activity of water and ethanolic extracts of four plants (Neem, olive, garlic and ginger) was determined against isolated fungi in comparison to Fluconazole. Extracts of garlic were the most effective followed by olive. The lowest activity was recorded by neem and ginger extracts. *Candida albicans* is the most sensitive organism under the action of ethanolic garlic and olive extracts. The antifungal activity of fluconazole is highest in comparison to the antifungal activity of plant extracts.

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1. Introduction

Otomycosis is a superficial fungal infection of the external auditory canal. Otomycosis is sometimes associated with bacterial infection in its status as an opportunistic infection. The epidemiology of otomycosis is global; however, the hot, humid, windy and dusty environment of the tropics and subtropics makes otomycosis more prevalent in these regions (Joy *et al.,* 1980). Otomycosis infection may be acute, subacute or chronic and usually accompanied by inflammation, itching of the ear, otalgia, aural fullness, hearing impairment and tinnitus. The inflammation of ear is associated with superficial epithelial exfoliation and formation of masses of debris containing hyphae, which further worsen the discomfort and sometimes culminate into frank suppuration in the affected ear (Kaur *et al*., 2006) Most cases of fungal ear infection, with tympanic membrane perforation, middle ear and sometimes whole temporal bone involvement are associated with immunosuppressant (Strauss,1991). Using a lot of broad-spectrum antibiotics, persistent otorrhoea, trauma, using steroids, alterations in immunity, loss of cerumen, hearing aids, dermatological diseases and swimming have been documented as risk factors (Joy *et al*., 1980, Kurnatowski and Filipiak, 2001 and Martin *et al*., 2005). A wide variety of fungal species play an important role in the causation of otomycosis; the most common fungi include *Aspergillus* and *Candida* species. Other less frequently involved fungi include *Penicillium*, *Mucor* and *Rhizopus* species (Kaur *et al*., 2000and Pahwa *et al*., 1983). Pure or mixed fungal isolates could be responsible for either a unilateral or bilatral otomycosis (Pradhan *et al*., 2003).

Otomycosis accounts for about 10% of otitis external cases (Boijrab *et al*., 1996 and Dibb, 1991). According to the host and the fungal species involved, the typical aspect of otomycosis may be modified. Few numbers of licensed antifungal agents are available and used in human being treatment. Using systemic drugs is limited to treat man due to their high toxicity and problems of residues in products intended for human consumption (Araujo *et al.,* 2009). Different pharmacological antifungal agents have been recommended to control otomycosis. (Aly, 1997; Agwa *et al.,* 2000), but recently using of some natural plant extracts has been emerged to inhibit the harmful causative microorganisms. The antimicrobial and antitoxin properties of some medicinal plants, herbs, and their components have been studied and documented since the late 19th century (Safari, 2006). These natural plants include garlic, lemon grass, datura, acacia, a triplex, ginger, black seed, neem, basil, eucalyptus and alfalfa (Omar and Abd-El-Halim, 1992; Aly *et al.,* 2000; Aly and Bafiel, 2008). The natural plants are safe to human and ecosystem than

the chemical antifungal compounds, and can be easily used by the public who used them for thousands of years to enhance flavour and aroma of foods as well as its economic value (Shelef *et al*., 1980; Shelef, 1983).

Ancient people also recognized the value and importance of these plant materials in medicine and pharmacology. Plant extracts have been used traditionally to treat a lot of infectious diseases which caused by bacteria, fungi, protozoa and viruses (Soylu *et al*., 2005; Yoshida *et al*., 2005; Nejad and Deokule, 2009). A lot of reports are available in vitro and in vivo efficacy of plant extract against plant, animal and human pathogens causing fungal infections (Natarajan *et al*., 2003). The activity of some plant extracts against otomycosis of human can be very well visualized from the reports of Venugopal (1995). About 60% of Rwandese medicinal plant extracts (267 plant extracts) used by traditional healers to treat different microbial infections were active against dermatophytes (Vlietinck *et al*. 1995). Hitherto more than 300 different biologically active substances have been isolated from plant extract, among them organosulphur compounds such as allicin, azoenes and diallyltrisulfide. Eugenol **is** a phenolic compound which considered as the most important biologically active compound found in many plant extract (Kähkönen *et al*., 1999; Aly and Bafiel, 2008).

Several studies have cited the antifungal properties of certain medicinal plants. Biswas *et al*. (2002) observed that the extracts of neem leaves (Azadiracta indica) and neem seed oil kernels are effective aganist certain fungi including *Trichophyton*, *Epidermophyton*, *Microspor,* *Trichsporon*, *Geotrichum* and *Candida*.

Olive leaf extract contains significant quantities of phenolic compounds, such as oleuropein, oleuropeoside and hydroxytyrosol that inhibited a variety of microorganisms (Markin *et al.,* 2003). This coincides with the natural ability of olive tree to protect itself from microbial attack using a variety of antimicrobial substances. Garlic (Allium sativum) is one of the most important plants which was used. Garlic is utilized as folk medicine in many countries for its antimicrobial properties. In garlic extracts different biologically active substances such as organosulphurous compounds like alliin and aliicin, sterols, flavones and polyphenolcarboxylic acids were found (Parvu *et al.*, 2009).

Ginger (Zingiber officinale Roscoe, Zingiberacae) is a medicinal plant which widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hybertention, dementia, fever and infectious diseases. The antimicrobial activity of ginger has been described and studied by Onyeagba *et al.* (2004).

Polyenes, triazoles, nucleoside analogues, and echinocandins are four main classes of drugs that used for the treatment of fungal infections.

The polyenes family includes amphotericin B and nystatin. The triazoles family, better known as azoles includes fluconazole, miconazole and clotrimazole. The mechanism of action of the polyenes and azole families involves an essential chemical component called ergosterol which found in the fungal cell membrane. The drug binds to ergosterol and creates a polar pore in the fungal membranes. This causes ions (predominantly K+ and H+) and other molecules to leak out of the cell, leading to its death. The nucleoside analogues such as flucytosine work by interfering with nucleotide synthesis; a key step in cell energy production, metabolism, and signalling. Finally, the echinocandins are a novel class of antifungal agents. Their mechanism of action involves interference with cell wall biosynthesis. Their use in otomycosis has not been reported. The objectives of this study were to identify the mycoflora of healthy human ear canal and to evaluate the antifungal activities of four medicinal plants in comparison to the fluconazole against pathogenic of otomycosis.

2. Materials and Methods

1- Isolation and identification of isolates

During two weeks (winter January 2014), a total of sixty samples of external auditory canal were randomly collected from 60 healthy female students by using sterile swabs. A specific questionnaire was prepared for each case, which containing personal information, history of otalgia, residual water in the ear canal after bathing or swimming and a mycological report. This study was carried out at the Collage of Applied Medical Sciences at Al-Dawdmi Governorate in Shaqra University.

The specimens from each ear canal were cultivated on Sabouraud Dextrose Agar (SDA medium) supplemented with chloramphenicol (250 mg/l) and streptomycin (300 mg/l) and on SDA free from antibiotics. Then the cultures were incubated at 25°C for 7 days and 37°C for 2 days. After incubation period, mycological examination of the fungal isolates was conducted based on the colony morphology and microscopic characterization. CHROMagar *Candida* media (Rambach, France) were used to identify *Candida spp*. The investigation was carried out in safety conditions to minimize contamination with airborne microorganisms. All fungi were isolated and stored on Sabouraud Dextrose Agar (Oxoid) slants in the refrigerator at 4 °C prior to use.

2- Medicinal plant materials

Samples of four medicinal plants, i.e. Neem leaves (*Azadiracha indica*), olive leaves (*Olea europaea*), ginger plant rhizomes (*Zingiber officinale*) and blubs of garlic (*Allium sativum*) were collected during December 2014 from the market of AL-Dawadmi Governorate, Saudi Arabia. The plants were brought to the laboratory using vasculum in order to avoid loss of water or dryness in plants.

3-Preparation of aqueous and organic medicinal plant extracts

Aqueous and organic extractions were prepared by macerated 10 grams of each dried medicinal plant using blender. The produced powder was mixed with 100 ml of either distilled water and organic solvent (ethyl alcohol), (1:10 w/v). The resultant extract was filtered through a glass wool filter and then rinsed with a small quantity (about 50 ml) of 96% ethyl alcohol. The extracted solutions were evaporated under reduced pressure at 45 °C. Subsequently, the extracts were diluted by distilled water and stored in the deep freezer at -10 °C.

4-Antimicrobial activity

Antimicrobial activity of extracts were estimated by using the agar well diffusion assay method as described by Holder and Boyce (1994). Dimethyl sulfoxide DMSO was used as a negative control and fluconazole was used as a positive control. The plates were done in triplicates and were incubated at 28 °C. The antimicrobial activity was calculated on the basis of diameter of zone of inhibition, which was measured after seven days of incubation and the mean of the three readings were presented. The plant extracts and the standard antifungal agents were dissolved in DMSO, 100% biologically inert substances.

5-Determination of Antifungal Activity:

Fungal conidial suspension was prepared by the method of Guleria *et al*., (2006). Conidia were isolated from the 10 days old culture of the selected fungal cultures by flooding culture plates with 5ml of sterile distilled water and conidia were dislodged by using L-shaped glass rod. Spore suspension was then prepared in liquid Sabouraud dextrose medium to obtain a concentration of 3x 10^5 conidia/ml. Agar-well diffusion method was used for determination of antifungal activity in which the spore suspension was inoculated with molten Sabouraud Dextrose Agar. Wells (6mm in diameter) were cut from the agar with a sterile borer and 25, 50 and 100ul of (2%) plant extract solutions and fluconazole (positive control) were delivered into them. The plates were incubated at 25°C for seven days after which diameter of zones of inhibition (DIZ) were measured.

3. Results

The ages of the students ranged between 19-22 years. Otomycosis was confirmed in 17 students (28.3 %),10 students from 17 students of otomycosis had mixed fungal- bacterial infections (16.6%) and no organism was detected in 43 students (71.6%). All of the positive cases had unilateral otomycosis (28.3%). The isolated fungal species from the external ear canal comprised seven genera including *Candida* (9 cases,52.9%), *Aspergillus* (7cases,41.1%), *Penicillium* (3cases, 17.6%), *Cryptococcus* (1 cases, 5.8%), *Cunninghamella* (1 case,5.8%) *Cladosporium* (1 case, 5.8%) and *Geotrichum* (1 case, 5.8%) Fig (1). *Candida albicans* (35.2%) followed by *Aspergillus niger* (29.4%) were the dominant fungal involved in otomycosis (Table 1) Fig (2).

Staphylococcus species were reported as the dominant microbial pathogen in seven positive cases (11.6%), *Micrococcus* species in three cases (5%). add.

None of positive cases in this study suffered from any disease and immunocompromised conditions (receving chemotherapeutic agents or systemic steroids). Pruritus (70.5%) was the most common symptom followed by otalgia (58.8%) (Table 2). Positive cases (58.8%) gave history of frequent scratching of the external ear canal with either the tip of their fingers and objects like matchstick, pen cover, broom stick, or cotton bud. One positive case had hole in her eardrum.

Table (1): Number and percentage of different genera and species of mould and yeasts from 17 cases examined by culture

Isolated Fungi	Number of cultures	Percent
Candida spp.	9	52.9%
Candida albicans	6	35.2%
Candida tropicalis	3	17.6 %
Aspergillus spp.	7	41.1%
A.niger	5	29.1%
A.flavus	2	11.77%
Penicillium spp.	3	17.6 %
P. canescens	3	17.6 %
Cryptococcus sp.	1	5.8%
Cladosporium herbarum	1	5.8%
Geotrichum candidum	1	5.8%
Cunninghamella sp.	1	5.8%

Table (2): Symptomatology of otomycosis

Symptoms	Incidence (%)
Pruritus	70.5%
otalgia	58.8%

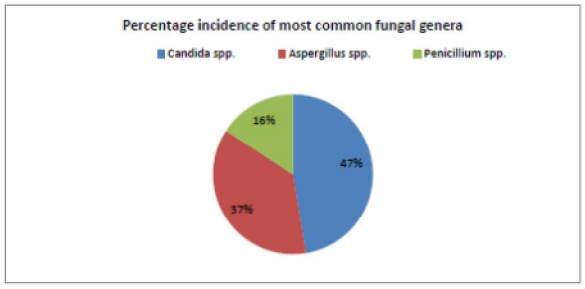

Fig (1): Percentage incidence of most common fungal genera

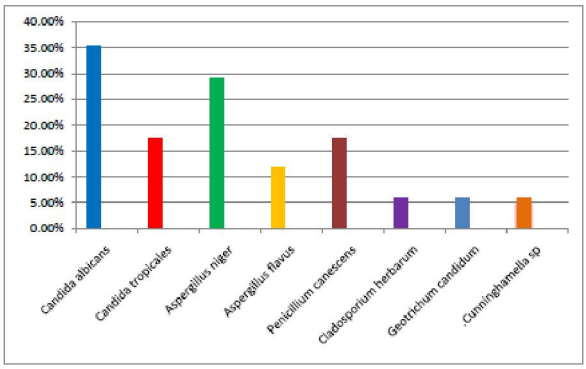

Fig (2): Percentage incidence of different fungal species.

In the present study the antifungal activities of different plant extracts versus fluconazole against nine fungal species that were isolated from ear of students (*Candida albicans*, *Candida tropicalis, A.niger, A.flavus P.canescens, Cryptococcus sp, Cladosporium herbarum, Geotrichum candidum* and *Cunninghamella sp.*) were studied and the following results were obtained; for *C. albicans,* the maximum inhibitory effect was recorded by using 100µl of ethanolic garlic and olive extracts with a DIZ values (53.6 and 53.3mm, respectively). The zone of inhibition using 100 µl of positive control, fluconazole was 78.3 mm. The minimum inhibition zone was recorded by using 50µl of water neem extract (25mm). For *C. tropicalis* the highest antifungal activity was achieved by using 100µl of ethanolic garlic extract, with a DIZ value 45mm followed by 50µl and 100µl of ethanolic olive extract, whereas the DIZ was 41mm. Lowest DIZ value 26.6 mm was recorded by using 50 µl of ginger water extract and the fluconazole showed DIZ 50mm.

The maximum inhibition by using 100 µl of ethanolic garlic for *Aspergillus niger* was 40 mm, followed by 100µl of ethanolic olive extract which achieved 36.6 mm of inhibition zone, the diameter of inhibition zone with fluconazole was 50mm but the lowest value of inhibition 8.3 mm was recorded at 25 µl of ethanolic ginger extract.

For *A.flavus,* the maximum inhibition zone was recorded by using 100µl of ethanolic garlic extract with the value of 36.6 mm, the minimum inhibition zone11.6mm was achieved at 50 µl of water extract of ginger. But the DIZ was 75mm under the action of 100µl fluconazole.

Table (3): The antifungal activity of Neem and Olive extracts in comparison to Fluconazol against isolated fungi

Fungal isolates	The antifungal activity of Neem leaves extracts (Diameter of the inhibition zone, mm) aganist different pathogenic fungi									The antifungal activity of Olive leaves extracts (Diameter of the inhibition zone, mm) aganist different pathogenic fungi								
	Neem extracts						Fluconazole (Positive control)			Olive extracts						Fluconazole (Positive control)		
	Water			Ethanol						Water			Ethanol					
	25µl	50µl	100µl	25ul	50µl	100µl	25µl	50µl	100µl	25µl	50µl	100µl	25µl	50µl	100µl	25µl	50µl	100µl
Aspergillus niger	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±41.6	±50	±NI	±16.6	±25	±NI	±25	±36.6	±IN	±41.6	±50
A.flavus	±NI	±16. 3	±25	±NI	±16.6	±33.3	±NI	±33.3	±75	±NI	±8.3	±16	±NI	±16.6	±25	±IN	±33.3	±75
Candida albicans	±NI	±25	±33.6	±NI	±28.3	±38.3	±16.6	±33.3	±78.3	±NI	±33.3	±41.6	±NI	±50	±53.3	±16.6	±33.3	±78.3
C.tropicalis	±NI	±33.3	±25	±NI	±33.3	±33.3	±16.6	±41.6	±50	±NI	±33.3	±33.3	±NI	±41.6	±41.6	±16.6	±41.6	±50
Penicillium canescens	±NI	±16.6	±20	±NI	±33.3	±33.3	±16.6	±33.3	±50	±NI	±25	±33.3	±NI	±35	±40	±16.6	±33.3	±50
Cryptococcus sp.	±NI	±NI	±8.3	±NI	±NI	±8.3	±41.6	±50	±58.5	±NI	±25	±33.3	±NI	±30	±41.6	±41.6	±50	±58.5
Cladosporium herbarum	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI
Geotrichum candidum	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	NI	±NI	±NI	±NI	±NI
Cunninghamella sp.	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	NI	±NI	±NI	±NI	±NI
NI= No Inhibition																		

Table (4): The antifungal activity of Ginger and Garlic extracts in comparison to Fluconazol against isolated fungi

Fungal isolates	The antifungal activity of Ginger rhizome extracts (Diameter of the inhibition zone, mm) against different fungi									The antifungal activity of Garlic bulb extracts (Diameter of the inhibition zone, mm) against different fungi								
	Ginger extracts						Fluconazole (Positive control)			Garlic extracts						Fluconazole (Positive control)		
	Water			Ethanol						Water			Ethanol					
	25µl	50µl	100µl	25µl	50µl	100µl	25µl	50µl	100µl	25µl	50µl	100µl	25µl	50µl	100µl	25µl	50µl	100µl
Aspergillus niger	±NI	±8.3	±26.6	±8.3	±25	±33.3	±NI	±41.6	±50	±NI	±26.6	±38.3	±NI	±30	±40	±IN	±41.6	±50
A.flavus	±NI	±11.6	±28.3	±NI	±16.6	±33.3	NI	±33.3	±75	±NI	±23.3	±30	±NI	±25	±36.6	±IN	±33.3	±75
Candida albicans	±NI	±28.3	±33.3	±NI	±31.6	±38.3	±16.6	±33.3	±78.3	±NI	±33.3	±41.6	±NI	±50	±53.3	±16.6	±33.3	±78.3
C.tropicalis	±NI	±26.6	±31.6	±NI	±33.3	±38.3	±16.6	±41.6	±50	±NI	±30	±40	±NI	±33.3	±45	±16.6	±41.6	±50
Penicillium canescens	±NI	±16.6	±20	±NI	±33.3	±33.3	±16.6	±33.3	±50	±NI	±26.6	±35	±NI	±30	±35	±16.6	±33.3	±50
Cryptococcus sp.	±NI	±NI	±8.3	±NI	±NI	±10	±41.6	±50	±58.5	±NI	±25	±33.3	±NI	±26.6	±36.6	±41.6	±50	±58.5
Cladosporium herbarum	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI
Geotrichum candidum	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI
Cunninghamella sp.	NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI	±NI
NI= No Inhibition																		

The maximum DIZ using 100µl of ethanolic olive extract for *Penicillium canescens* was 40mm and the minimum DIZ was 16.6 at 50 µl of water ginger extract. The positive control showed 50 mm of inhibition zone at 100µl.

100 µl of ethanolic extracts of olive and garlic achieved highest activity against *Cryptococcus sp*. (41.6 and 36.6 mm, respectively) but the minimum inhibition (8.3mm) was recorded under the action of 100 µl of water extracts of neem and ginger and ethanolic extract of neem. The fluconazole achieved 58.5 mm of inhibition zone at 100µl. All plant extracts failed in the inhibition of three fungal species, *Cladosporium herbarum, Geotrichum candidum* and *Cunninghamella sp.*

4. Discussion

Otomycosis is a recognised clinical problem in the tropical and desert regions of the world.

This could be as a result of the high degree of humidity, windy and heat in the regions. Besides, large proportion of the population is made up of outdoor labourers who constantly being exposed to the dusty environment. The habit of cleaning the ear with feathers, matchstick and contaminated finger tips is known to encourage the inoculation and growth of the spores of fungus on the moist external auditory canal especially in patient with poor personal hygiene (Lakshmipati and Murti, 1960).

Some cultural practices for females such as the head scarf and "hijab" commonly worn by all Muslim females may be play an important role in infection with otomycosis. This practice is associated with the prolonged covering of the external auditory canal which increase the humidity within the ear canal and hence predisposes to otomycosis, using of dryer after washing and setting of hairs by women also increases the humidity in the external auditory canal and this encourages otomycosis infection. (Brobby,1992)

Diagnosis of otomycosis is usually carried out by clinical findings with pruritus (70.5%) being the most common symptom followed by otalgia (58.8%) these results agree with the results which recorded by Barati *et al*. (2011) since the pruritus and otalgia were (65% and 55%, respectively). In this study, presumed diagnosis of otomycosis was confirmed by laboratory findings in 28.3 %. This result is nearly close to the result reported by Pontes *et al*., (2009) whereas the ototmycosis detected in 19.4% patients. Other studies reported that 65%, 74.7% and 78% of the patients as positive for otomycosis. (Ozcan *et al*., 2003, Kaur *et al*., 2007 and Aneja *et al*., 2010).

The aetiological agents of otomycosis found in our study were *Candida*, *Aspergillus*, *Penicillium, Cryptococcus*, *Cunninghamella*, *Cladosporium* and *Geotrichum species.*

Candida and *Aspergillus* species were the most common aetiological agents. The majority of the fungal pathogens isolated from the ear swabs in this study belonged to the taxon *Candida* represented by *Candida albicans* (35.2%).

In addition to this *A.niger* represented 29.1% and this is consistent with the results recorded by Jia *et al*., (2011) who found that the majority of the fungal pathogens isolated from the ear swabs belong to the taxon *Aspergillius*, represented by *A.niger*, *A.terreus*, *A.flavus*, *A.fumigatus*. The taxon Candida, represented by *C.albicans* and *C.lusitaniae*, all belonging to the anamorphic fungi.

In the south Eastern part of China, are similar to India, Turkey and other countries where the majority of the pathogenic fungi involved in otomycoses

belonged to Aspergilli (Aneja *et al*., 2010 and Ozcan *et al*.,2003). *A. niger* has been found to be the most common aetiological agent and recorded as the major cause of otomycosis (Ozcan *et al*., and Hurst, 2001). Kaur *et al*. (2000) reported *A.fumigatus* as the most common cause of otomycosis. *A. fumigatus* has been considered more pathogenic than *A.niger* due to production of haemolytic exotoxin which has the ability to alter skin resistance (Mugliston *et al.,*1985*). Aspergillus* is a saprophytic fungi which grow rapidly and production of large number of small, dry, easily aerosolised conidia make it a significant contaminant with regard to air quality and potential human exposure- related illness. Aspergilli are common in air borne dust, and their heavy growth is aided by cerumen. Further more, the normal ear canal has a pH in the acidic side, the common pathogenic Aspergillus experience optimal growth at a pH of 5-7 (Mugliston, 1985 and Yehia *et al.,*1990).

The percentage frequency of *Penicillium* species is moderate (17.8%). *Penicillium* species have world wide distribution in the nature and their spores are floating in the air, occasionally causing otomycosis (Richardson *et al*., 2003) although not being a common cause of it (Mahmoudabadi, 2006 and Ozcan *et al*.,2003). In the study of Ozcan *et al*., (2003) *Penicillium sp.* was the most dominant isolate (32.5%).

The percentage frequency of *Cryptococcus sp., Cladosporium herbarum, Geotrichum candidum* and *Cunninghamella sp.* was very low (5.9%). These results nearly resemble to the results that were recorded by Bassiouny *et al*.,1986 and Jia *et al.,*2011 whereas the frequencies of *Cldosporium herbarium* was 1.98 %, *Cryptococcus sp*. Was 1.74% and *Cunninghamella sp.* was 0.9%.

Staphylococcus species were reported as the dominant microbial pathogen in seven positive cases (11.6%), *Micrococcus* species in three cases (5%). This is consistent with the results recorded in the previous study by Baratia *et al*., (2011) who found that the percent of *Staphylococcus spp*. was 14% and the percent of *Pseudomonas sp*. was (5.3%).

Many investigations were carried out to discover plant products that inhibit the pathogenic fungi which infect humans because the plant extracts can inhibit the fungal growth without harming the host. The results of antifungal effect of four plant extracts in comparison to fluconazole against otomycosis revealed that, the ethanol extract of garlic bulb was the best to suppress the growth of *Candida albicans*, *C.tropicalis*, *Aspergillus niger* and *A.flavus* (53.6 – 36.6mm), followed by ethanol extract of olive leaves (53.3- 33.3mm). But ethanol olive extract showed higher activity against *Cryptococcus sp* and *Penicillium canescens* (41.6 and 40 mm, respectively) than ethanol garlic extract. These results confirmed the previous studies that the garlic is considered as a wonderful medicinal plants owing to its multiple biological properties, including antifungal activity and the extract of garlic was the most effective against *Candida* and *Aspergillus spp.*

The antifungal activity of garlic is attributed to the action of sulphur-containing compound-allicin (Chudzik *et al*.,2010).

The activity of ethanol olive extracts may be due to oleuropein, eleonic acid and other qualities that are benefit to treat humans otomycosis (Bokhari,2009).

The neem extracts failed to achieve good antifungal activity against tested fungi this result was agreed with the results that were recorded by (Villanueva *et al*.,2008) who found that the activity of neem extract is low against *Aspergillus flavus*, *A.niger* and *Candida albicans.*

The lowest activity of antifungal against tested fungi was recorded with water and ethanolic ginger extracts. This result is comparable with other studies which have shown that ginger has pronounced inhibitory activities against *Candida spp*. (Atai *et al*.,2009).

Fluconazole is an azole antifungal agent that have a broad spectrum of activity. This family of chemical components is effective in treating the most common aetiological agents of otomycosis. In this study the antifungal fluconazole exhibit highest activity against the fungi of otomycosis than ethanol extracts of garlic and olive (78.3 – 50 mm). *Candida albicans* and *Aspergillus flavus* were very sensitive for fluconazole. This result is nearly resembling to result that were reported by Nog *et al*.,1999 and Cohen *et al*.,1990 whereas fluconazole has shown an efficacy of 95—100% in vitro against *Aspergillus* species and *Candida albicans.* Fluconazole has also a systemic effect, and their high concentration in the skin, as well as its slow elimination justifies the use of this preparation in fungal infections of the ear. There were no cases of local sensitivity to fluconazole, and the infections seemed to resolve faster and display a lower recurrence rate. Topical medication is generally enough to that patients with otomycosis (Jia *et al*., 2011). *Cladosporium herbarum, Geotrichum candidum* and *Cunninghamella sp.* were found to be resistant to fluconazole.

Conclusions:

Many species of fungi have been identified as the cause of otomycosis with *Candida* and *Aspergillus spp*. being the most common culprits. This study supports the traditional medicine use of different plant extracts in treating different infections caused by pathogenic fungi in Saudi Arabia.

Recommendations:

Periodic examination of the ear in specialized clinics of the most important factors that reduce the incidence of otomycosis.

This study also suggests that a great attention should be paid to medicinal plants which are found to have plenty of pharmacological properties.

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