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Antifungal Potentially of Commercial Vinegar, Apple Cider Vinegar, *Eucalyptus* Oil and Locasten Ear Drops in Controlling Otomycosis Caused by *Aspergillus niger*

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ABSTRACT

This study aims to evaluate the antifungal activity of commercial vinegar, apple cider vinegar, *Eucalyptus* oil and pharmaceutical Locasten ear drops, containing clotrimazole in controlling otomycosis caused by *Aspergillus niger*. A total of 60 patients exhibiting otomycosis diagnosis were selected for fungal isolation. Fifty seven isolates were identified as *Aspergillus niger*. Different concentrations of an aqueous extractions of *Eucalyptus* leaves in addition to commercial vinegar, apple cider vinegar and Locasten ear drops were applied to determine antifungal activity against *Aspergillus niger*. The antifungal activity was estimated as the decrease in the fungal dry weight (%). Activates of commercial vinegar, apple cider vinegar and *Eucalyptus* oil were also compared against Locasten ear drops. Results showed that by adding 1ml, 3ml and 5ml of tested substances at pH 6.0 and incubated at 35^{o}c for 10 days exhibit antifungal activities against *A. niger* with more or less similar degrees. Also results showed that the addition of 5ml of commercial vinegar, apple cider, Locasten ear drops and *Eucalyptus* oil resulted in inhibition percentage of 99.5%, 98.5%, 98.4% and 95.0% respectively. Furthermore the concentration of 1ml of Locasten, commercial vinegar, apple cider and *Eucalyptus* oil resulted in inhibition percentage of 73.2%, 66.8%, 65.4% and 37.1% respectively. The highest inhibition activity 99.5% was caused by commercial vinegar solution at concentration of 5ml.

The authors recommended to apply commercial vinegar as a promising antifungal treatment for controlling otomycosis.

Keywords: Otomycosis , *Aspergillus niger*, Antifungal activity, vinegar, *Eucalyptus* oil.

1. INTRODUCTION

The ear is the organ of balance and hearing. Four main parts are known to constitute the ear structure; outer ear consisting of auricle or pinna which is the most outer part of the ear, and outer auditory canal represents the tube that connects the external ear to the middle ear, tympanic membrane (eardrum), which separates between the external ear and the middle one, middle ear consisting of Ossicles including three small bones that are connected and transmit the sound waves to the inner ear and Eustachian tube which is a mucus lined canal that links the middle ear with the back of the nose and helps to equalize the pressure in the middle ear. Equalized pressure is needed for the proper transfer of sound waves and inner ear which consists of cochlea, this contains the nerves for hearing and vestibule for balance [1]. Otomycosis is an ear infection caused by some specific fungi . It's also known as fungal otitis externa. About 1 in 8 of otitis external infections is fungal in origin [2]. Otomycosis usually affects the outer ear canal. In some cases, it may also affects the middle ear. It's more commonly seen in tropical and subtropical parts of the world and it is more common in hot climates and in those who partake in aquatic sports. The most common environmental causes of otomycosis are contaminated water and prolonged use of topical antibacterial agents. Otomycosis can become a chronic condition if not adequately treated, or if it does not respond to treatment [3].

Many different fungal species have been reported as the etiology of otomycosis [4, 5]. Most of them are belonging to *Aspergillus spp*. and *Candida spp*., with their prevalence influenced by environmental factors and climatic conditions [6, 7]. Among them the genus *Aspergillus* is recognized as the leading cause common of otomycosis more so than *Candida* species. Globally , *A. niger* and *C. albicans* are frequently isolated in otomycosis cases, particularly in Africa where *A. niger* is dominant, followed by *A. fumigatus, A. flavus, A. terreus* and *A. nidulans* [6 – 12]. These *Aspergillus* species thrive in tropical and subtropical regions due to their virulence factors, which contributes to their ability to cause infections [13, 14]. *A. niger* can produce the mycotoxin''ochratoxin A'' [14] enhancing its pathogenic potential. Studies have showed that *Aspergillus* accounts for nearly 70% of fungal otitis externa cases [11]. Other fungi like *Mucor spp*. [6, 15], *Rhizopu spp*. [10, 6] and *Penicillium spp.* [6, 16] are less common. Among all, *A. niger* remains the most frequent pathogen, followed by *A. flavus* and *A. fumigatus* [17, 18, 19].

In Egypt, a wide variety of medicinal and aromatic plants are widely accessible throughout the country. Among these, *Eucalyptus* species are particularly prevalent. The essential oils extracted from *Eucalyptus* leaves are known for their diverse biochemical properties, in addition to their aroma and functional characteristics. Members of the *Eucalyptus* genus (family *Myrtaceae*) are often employed to treat infections caused by microbes. The foliage of *Eucalyptus* citriodora contains approximately 1.36% essential oil, primarily composed of citronellal (57%), citronellol (15.89%), citronellyl acetate (15.33%), and other minor constituents [20]. This essential oil exhibits a broad range of biological activities, including antimicrobial [21], antifungal [22], anticandidal [23], antibacterial [24], expectorant, and cough-relieving properties [25].

Natural substances are considered beneficial due to their antimicrobial and antioxidant activities [26, 27]. For example, apple cider vinegar is recognized for its various pharmacological properties such as antidiabetic effects [28, 29], potential to prevent Alzheimer's disease [30], antioxidant capabilities [26], its role in weight management, and its ability to regulate blood glucose levels [31]. Vinegar has traditionally been utilized to manage a variety microbial infections, such as otomycosis, and fungal infections of the toenails and fingernails, especially those resistant to conventional medications. The antifungal effect can be enhanced by applying pure apple cider vinegar directly to the infected area, either alone or combined with substances like tea tree oil, olive oil, or hydrogen peroxide. The acidic nature of vinegar inhibits fungal growth within the ear. Additionally, a mixture of vinegar and alcohol in equal parts may alleviate otomycosis symptoms during early infection stages [32]. Standard treatment for

fungal otitis externa generally involves thorough cleaning followed by topical antifungal therapies. Ear drops containing agents like fluconazole and clotrimazole are commonly used to treat various fungal infections of the ear [33, 34]. This study investigates the antifungal effectiveness of commercial vinegar, apple cider vinegar, *Eucalyptus* oil, and Locasten ear drops in managing fungal otomycosis.

2. STUDY AREA

The present study was conducted at the Otolaryngology Outpatient Clinic of Mansoura University Hospitals. Patients who were clinically diagnosed with otomycosis were selected to participate in the investigation.

3. MATERIALS AND METHODS

3.1 Selection of patients: The otolaryngology outpatient clinic at Mansoura University Hospitals were selected to carry out the present study for 3 months in 2021. Patients clinically diagnosed with otomycosis were selected which presented with different complaints(aural pain, itching, otorrhea, with or without hearing loss, and their examination revealed erythema, fungal debris and creamy or blackish aural discharge). The patients that had recent history of antifungal topical medication were excluded from our study. Sixty patients were included that clinically diagnosed as otitis externa. The ages of the patients ranged between 10-60 years. Once the clinical diagnosis was confirmed, samples were collected from the external auditory canal by using sterile cotton swabs under aseptic conditions. The samples were kept in ice box and transferred to the laboratory for further studies.

3.2 Strain isolation and identification: Fungal isolation and identification were conducted in the Microbiology Laboratory, Faculty of Science, Port Said University. A total of 60 clinical samples were collected from patients, and each sample was divided into two portions for mycological analysis. For direct microscopic examination, one portion was treated with 10% potassium hydroxide (KOH) on a glass slide. The presence of otomycosis was confirmed by the visualization of septate hyphae, aseptate conidiophores, and fruiting structures characteristic of Aspergillus species. The second portion was cultured on Sabouraud Dextrose Agar (SDA) plates supplemented with 0.05 mg/ml of chloramphenicol (AppliChem GmbH, Darmstadt, Germany) and incubated at 35°c. The plates were monitored daily for seven days and up to 10–15 days to allow the appearance of fungal colonies. Subculturing was performed to develop pure isolates, which were identified based on macroscopic colony characteristics and microscopic morphology. The isolate identification was based on the monographs of Raper and Fennell 1965 [35], Amaia et al. 2005 [36], and Nagamani et al. 2005 [37]. Although molecular confirmation was not performed in this study owing to some technical limitation in our laboratory, classical morphological methods were applied based on Raper and Fennell's, Amaia et al. and Nagamani et al. monographs. These monographs remain a widely accepted approach for the routine identification of *Aspergillus* species. The isolated fungal strains were preserved on SDA slants and stored in sterile Eppendorf tubes containing 10% sterile glycerol in distilled water for future reference and research.

3.3 Preparation and maintenance of spore suspensions: The purified fungal cultures were maintained on Czapek-Dox agar at 4◦c. The preparation of spore suspensions were carried out according to the method of Hopwood et al. 1985 [38] as follows:

The surface of a well-sporulated culture was scraped and suspended in sterile water supplemented with 0.05% TritonX-100 to minimize the hydrophobicity of the spores surface. The crude spores suspension then filtered through cotton wool to remove fugal mycelia and pieces of agar medium. The filtrates were centrifuged at 3000 rpm for 10 minutes, then washed once in sterile water to remove the

dissolved substances of growth medium. The washed spores were separated by centrifugation and re suspended in 5ml 20% glycerol and kept at -20◦c in a screw capped tube for subsequent procedures.

3.4 Preparation of antifungal materials:

3.4.1 Vinegar solution: Both commercial vinegar and apple cider vinegar used in this study were purchased from the local market in Port Said city, Egypt.

3.4.2 Pharmaceutical locasten ear drops: The commercially available antifungal medication Locasten was utilized in this study. It belongs to the antifungal class of drugs used for treating fungal infections affecting the skin and body, including otomycosis. Its mechanism of action involves disrupting fungal cell membrane synthesis, thereby inhibiting fungal growth. According to the manufacturer's information, the active ingredient in Locasten is clotrimazole at a concentration of 10 mg/ml.

3.4.3 Preparation of *Eucalyptus* oil extract: The *Eucalyptus* leaves were collected from wild *Eucalyptus* trees at Dakahliya governorate, Egypt. The leaves were washed using distilled water and left to dry for 7 days, crushed manually, transformed to the powder form using electric grinding and kept for distillation and oil extraction. The oil was extracted using Soxhlet apparatus operating via a steam distillation method. Fifty grams of the powdered leaves were mixed with 500 ml of distilled water, heated to 100 °c and subjected to distillation for one hour. Boiling began within 5 minutes leading to vapour formation. The vapour was cooled down with the help of condensed. The condensed material of oil and water mixture was collected. The water was separated by using of the rotary evaporator and the *Eucalyptus* oil was purified and kept at a temperature less than 25 °c in containers away from sunlight for subsequent procedures.

3.5 Testing antifungal activities of pharmaceutical Locasten ear drops, apple cider vinegar, commercial vinegar and *Eucalyptus* oil extract: The antifungal activities of Locasten ear drops, apple cider vinegar, commercial vinegar, and *Eucalyptus* oil extract were evaluated using the dry weight method in Czapek-Dox broth medium. Fifteen 100 ml Erlenmeyer flasks, each containing 50 ml of sterilized Czapek-Dox liquid medium, were inoculated with 1 ml of a fungal spore suspension containing 4×10^6 CFU/ml. The flasks were divided into five groups, each consisting of three replicates. Four groups were assigned to the different antifungal agents, while one group served as a control. Three concentrations (1 ml, 3 ml, and 5 ml) of each antifungal agent were tested. All groups were incubated at 35 °C for 10 days. After incubation, fungal mycelia were harvested, dried to a constant weight, and the results were recorded as fungal dry weight (mg/ml). Each experiment was conducted in triplicate.

3.6 Statistical Methodology: The data were statistically analyzed to compare the significance of differences between paired means using the ANOVA test. Pairwise comparisons between each two groups were further conducted using the Post Hoc Tukey test.

4. RESULTS AND DISCUSSION

4.1 Patient selection: Results of patients selection (Table 1) showed that 60 patients with ages ranging from 10 to 60 years old has recorded as positive otomycosis cases. Thirty five male (58.33%) were reported, 25 of them were from rural (71.42%) and 10 from urban (28.57%). The positive female cases were 25 (41.66%), 20 of them were from rural (80%) and 5 were from urban(20%). The patients with otitis split into three groups according to their age; the first group have age from 10 to 18 years, the second group have age from 19 to 55 years and the third group have age more than 55 years. Ten male patients were of the age range from 10 to 18 years old (28.57%) , 20 patients of the age range from 19 to

55 years old (57.14%) and 5 patients were more than 55 years old (14.28%). Dealing with the female patients; 8 of them were of age range from 10 to 18 years old (32%), 10 of them were of age range from 19 to 55 years old (40%) and 7 patients were more than 55 years old (28%). Results also showed that patients suffering from hearing impairment were 3 male patients and 2 female patients of age from 19 to more than 55 years. These results have come in agreement with numerous earlier studies which reported the global prevalence of otomycosis among otitis externa cases to range between 9% to 30%. Predisposing factors for otomycosis include the presence of earwax (cerumen), frequent use of topical antibiotics and corticosteroids, humid environmental conditions, the use of instruments or self-cleaning practices involving foreign objects, immunosuppression, previous open-cavity mastoidectomy procedures, the use of hearing aids with occlusive molds, and the buildup of epithelial debris in the ear canal. Notably, the condition appears more frequently in individuals aged 20 to 30 years compared to children under 10 years. Typical symptoms include unilateral ear pain (otalgia), continuous ear discharge (otorrhea), itching (pruritus), and tinnitus. Patients may also experience gradual hearing loss due to fungal matter blocking the ear canal. Otoscopic inspection may detect black, fluffy fungal growth, particularly when *Aspergillus niger* is involved [39]. Dealing with the gender and age, the study showed that otomycosis occurs more commonly in males of the age 19-55 (20 patients) than females within the same age (10 patients). This can explained by some religious traditions which encourage female to wear head cover which may protect ears from environmental fungal infection and from high temperature and humidity. Moreover most females in Egypt spent more times at home rather than males. Also, some hormonal changes during pregnancy or menstruation may precipitate in infection occurrence. This result comes in contrast with the study of Kumar et al. 2005 who stated that Otomycosis occurs more commonly in females than males and our finding confirmed the results of other researchers which had been reported [40].

Table 1. distribution of patients according to gender, habitat area, age and hearing impairment.

Gender	**Male**			**Female**		
No. of patients	35			25		
Habitat& no. of patients	Rural 25 (71.42%) Urban 10 (28.57%)			Rural 20 (80%) Urban 5 (20%)		
Age & no. of patients	10 – 18 years	19-55 years	>55 years	10 - 18 years	19-55 years	>55years
	10	20	5	8	10	7
%	28.57%	57.14%	14.28%	32%	40%	28%
No. of patients with hearing impairment	0	1	2	0	1	1

4.2 fungal strains isolated from patients : Results of isolation and identification procedures are shown in Table (2) . Results showed that 57 patients were recorded to be suffering from *A. niger* infection including 33 males, 10 patients (10 – 18 years old), 18 patients (19 – 55 years old) and 5 patients (>55 years old). Regarding to females results showed that 24 patients were proved *A. niger* otitis infection (7 with the age of 10 – 18, 10 with the age of 19 – 55 and 7 of them with the age more than 55 years old). This result come in agreement with those of Miertusova and Simaljakova 2003 [41] and Nong et al. 1999 who stated that *A. niger* is the most common etiologic agent of otomycosis [42].

Table 2. frequency of *A. niger* isolated from different patients.

Gender	**Male**			**Female**		
Age	10-18	19-55	>55	10-18	19-55	>55
No. of isolates	10	18	5	7	10	7
%	30.3%	54.5%	15.2%	29.2%	41.7%	29.2%

4.3 Isolates identification: Results showed that the fungal colonies grown on Sabouraud Dextrose Agar (SDA) appeared as black, powdery masses with a white to pale yellow reverse, consistent with the characteristic features of *Aspergillus* species. Microscopic examination using lactophenol cotton blue stain revealed biseriate phialides radiating from the entire vesicle and the presence of globose black conidial heads, confirming the morphological identity of *Aspergillus niger* as described by Raper and Fennell, Amaia et al. and Nagamani et al. monographs. These morphological characteristics were sufficient for presumptive identification, although molecular confirmation is recommended for future work.

4.4 Antifungal activity of pharmaceutical Locasten ear drops, apple cider vinegar, commercial vinegar and *Eucalyptus* oil extract: Results showed that by adding 1ml ,3ml and 5ml of Locasten, apple cider, commercial vinegar and *Eucalyptus* oil at pH 6.0 and incubated at 35^{o}c for 10 days exhibit antifungal activities against *A.* niger with more or less similar degrees. The highest inhibition activity (99.5%) was caused by commercial vinegar solution at concentration of 5ml while the lowest inhibition (37.1%) was caused by *Eucalyptus* oil extract at concentration of 1ml. Numerous studies have explored the antifungal and antibacterial effects of acetic acid [43, 44, 45]. However, our results can explained by the fact that applying Vinegar, especially in the form of acetic acid, as effective and low-cost treatment for otomycosis creates an acidic environment that inhibits fungal growth. Most fungi thrive around neutral condition, so increase lowering of pH in ear canal can help to control the infection. The antifungal properties of acetic acid are well-documented, and it has been used in various medical applications for its ability to disrupt the cell walls of fungi, ultimately killing them [46]. Several studies and clinical trials have examined the efficacy of acetic acid in treating otomycosis. Erkan et al. stated that topical application of acetic acid 2 % and hydrocortisone combinations twice per day for three weeks were very effective [47]. Studies have shown that it is effective in reducing fungal load and alleviating symptoms when used consistently over a few weeks. Research comparing the effectiveness of acetic acid to other antifungal treatments, such as clotrimazole or miconazole, has shown that acetic acid can be equally effective, especially in uncomplicated cases. Jabir et al. 2011 [48] demonstrated that fluconazole exhibited antifungal activity comparable to that of apple cider vinegar against *A. niger*. Additionally, apple cider vinegar and acetic acid were effective against *A. niger*, *A. flavus*, and fluconazole-resistant *C. albicans* and non-*albicans* strains, suggesting they may be suitable alternatives to conventional antifungal medications. The more or less similar result produce apple cider vinegar contains acetic acid, similar to regular vinegar, but also includes other compounds like malic acid, vitamins and minerals, which might contribute to its antifungal properties. These findings align with those reported by Du et al. 2019 [49] and Kim et al. 2012 [50]. The polyphenolic content of apple vinegar, which varies depending on apple variety, ripeness, and geographic origin, also contributes to its antimicrobial potential [51]. Furthermore, the presence of secondary metabolites in plants provides natural defense mechanisms against fungi, bacteria, and viruses [52]. The broad-spectrum antimicrobial activity of natural products is largely attributed to their diverse bioactive compounds [53, 54]. The activity of *Eucalyptus* oil extract may due to the fact that it contains compounds such as eucalyptol (1,8-cineole), which exhibit antifungal properties

[54]. Research has shown that *Eucalyptus* oil can inhibit the growth of various fungal species. It has been used effectively in treating respiratory and skin infections against fungi like *Aspergillus* and *Candida*, common in otomycosis has been demonstrated in several studies [55 ,56, 57]. *Eucalyptus* oil is known for a boarder range of antimicrobial activity compared to vinegar and apple cider vinegar.

4.5 Effect of concentrations of antifungal agents on the fungal growth: Results showed that concentrations of Locasten, apple cider, commercial vinegar and *Eucalyptus* oil were exhibited antifungal activities against *A. niger* with different degrees. Also, it was reported that the addition of 5ml of tested substances caused the maximum antifungal inhibition , while the minimum one was recorded in case of addition of 1ml of solutions.

Concentration of 5ml of commercial vinegar, apple cider, Locasten and *Eucalyptus* oil resulted in inhibition percentage of 99.5%, 98.5%, 98.4% and 95.0% respectively, Concentration of 3ml of commercial vinegar, Locasten, apple cider and *Eucalyptus* oil resulted in inhibition percentage of 96.6%, 95.6%, 95.3% and 69.2% respectively and the concentration of 1ml of Locasten, commercial vinegar, apple cider and *Eucalyptus* oil resulted in inhibition percentage of 73.2%, 66.8%, 65.4% and 37.1% respectively. Results showed in Tables (3, 4, 5). It is cleared that the antifungal activities in controlling otomycosis where decreased as the concentrations of commercial vinegar, apple cider, Locasten and *Eucalyptus* oil were decreased. Several studies stated that different concentrations of vinegar can be effective in controlling otomycosis, with lower concentrations (2-4%) being safer for regular use while still effective against mild to moderate infections that have been found effective in clinical settings, reducing symptoms and eradicating the infection with less discomfort to patients. Higher concentrations may be reserved for more severe cases but should be used cautiously to avoid irritation to the ear canal. The study conducted by Gopal et al. demonstrated that the application of 10% acetic acid resulted in a complete reduction for *Aspergillus niger* [58]. Also, different concentrations of *Eucalyptus* oil can vary in their effectiveness against fungal infections. Studies have shown that different concentrations of *Eucalyptus* oil can vary in their effectiveness against fungal infections; low concentration (0.5-1%) is Suitable for mild antifungal effect and mild cases or as a preventive measure, moderate concentration (2-5%) can effectively inhibit the growth of fungi and appropriate for moderate infections, high concentration (5-10%) has Strong antifungal properties and effective in treating established infections and very high concentration (>10%) is potentially very effective in controlling fungal growth and generally not recommended for direct application in the ear without medical supervision [59, 60].

Table 3. Percentage of antifungal inhibition (%) of *A. niger* by adding 5 ml of tested substances.

Tested substances (5ml)	**Dry wt. replications**			**Average mg / ml**	**Percentage of inhibition**
	R1	**R2**	**R3**		
Control	690	710	670	690	
Locasten	8	12	14	11.33	98.4%
Apple cider	8	10	13	10.33	98.5%
Comm. Vinegar	2	5	4	3.66	99.5%
***Eucalyptus* oil**	32	38	33	34.33	95.0%

Table 4. Percentage of antifungal inhibition (%) of *A. niger* by adding 3 ml of tested substances.

Tested substances (3ml)	Dry wt. replications			Average mg /ml	Percentage of inhibition
	R1	R2	R3		
Control	690	694	686	690	
Locasten	25	37	30	27.33	95.6%
Apple cider	32	29	37	32.66	95.3%
Comm. Vinegar	20	23	27	23.33	96.6%
***Eucalyptus* oil**	200	222	216	121.33	69.2%

Table 5. Percentage of antifungal inhibition (%) of *A. niger* by adding 1 ml of tested substances.

Tested substances (1ml)	Dry wt. replications			Average mg /ml	Percentage of inhibition
	R1	R2	R3		
Control	680	677	683	680	
Locasten	187	181	178	182	73.20%
Apple cider	221	253	232	235.33	65.4%
Comm. Vinegar	212	243	223	226	66.8%
***Eucalyptus* oil**	420	433	430	427.66	37.10%

4.6 Statistical data analysis : Dealing with concentration ANOVA pairwise test showed that there is a significant correlation between inhibition activities caused by adding 5ml (Table 6) , 3ml (Table 7) and 1 ml (Table 8) of Locasten ear drops, apple cider vinegar, commercial vinegar and *Eucalyptus* oil extract ($p < 0.001$). The same result was reported in correlation of inhibition activity and the type of substance applied ($p < 0.001$).

Table 6. Statistical data analysis of antifungal inhibition of *A. niger* by adding 5 ml of tested substances.

Statistical analysis	Control	Antifungal drug	Apple cider	Comm. Vinegar	*Eucalyptus* oil
Min.	670.0	8.0	8.0	2.0	32.0
Max.	710.0	14.0	13.0	5.0	38.0
Mean	690.0	11.3	10.3	3.7	34.3
± SD	20.0	3.1	2.5	1.5	3.2
± SE	11.5	1.8	1.5	0.9	1.9
F (p)	**3196.641* (<0.001*)**				
P1		<0.001*	<0.001*	<0.001*	<0.001*
P2			1.000	0.843	0.073
P3				0.897	0.060
P4					**0.015***
% change from control		**-98.4%**	**-98.5%**	**-99.5%**	**-95.0%**

F: F for ANOVA test, Pairwise comparison bet. each two groups was done using Post Hoc Test (Tukey)
p_1: p value for **Control** and each other group
p_2: p value for **Antifungal drug** and each other group
p_3: p value for **Apple cider** and each other group
p_4: p value for **Comm. Vinegar** and ***Eucalyptus* oil**
*: Statistically significant at $p \leq 0.05$.

Table 7. Statistical data analysis of antifungal inhibition of *A. niger* by adding 3ml of tested substances.

Statistical analysis	Control	Antifungal drug	Apple cider	Comm. Vinegar	*Eucalyptus* oil
Min.	686.0	25.0	29.0	20.0	200.0
Max.	694.0	37.0	37.0	27.0	222.0
Mean	690.0	30.7	32.7	23.3	212.7
± SD	4.0	6.0	4.0	3.5	11.4
± SE	2.3	3.5	2.3	2.0	6.6
F (p)	**5850.054* (<0.001*)**				
P1		<0.001*	<0.001*	<0.001*	<0.001*
P2			0.995	0.650	<0.001*
P3				0.443	<0.001*
P4					<0.001*
% change from control		**-95.6%**	**-95.3%**	**-96.6%**	**-69.2%**

F: F for ANOVA test, Pairwise comparison bet. each two groups was done using Post Hoc Test (Tukey)
p_1: p value for **Control** and each other group
p_2: p value for **Antifungal drug** and each other group
p_3: p value for **Apple cider** and each other group
p_4: p value for **Comm. Vinegar** and ***Eucalyptus* oil**
*: Statistically significant at $p \leq 0.05$.

Table 8. Statistical data analysis of antifungal inhibition of *A. niger* by adding 1ml of tested substances.

Statistical analysis	Control	Antifungal drug	Apple cider	Comm. Vinegar	*Eucalyptus* oil
Min.	677.0	178.0	221.0	212.0	420.0
Max.	683.0	187.0	253.0	243.0	433.0
Mean	680.0	182.0	235.3	226.0	427.7
± SD	3.0	4.6	16.3	15.7	6.8
± SE	1.7	2.6	9.4	9.1	3.9
F (p)	**1095.521* (<0.001*)**				
P1		<0.001*	<0.001*	<0.001*	<0.001*
P2			0.001*	0.004*	<0.001*
P3				0.825	<0.001*
P4					<0.001*
% change from control		**-73.2%**	**-65.4%**	**-66.8%**	**-37.1%**

F: F for ANOVA test, Pairwise comparison bet. each two groups was done using Post Hoc Test (Tukey)
p_1: p value for **Control** and each other group
p_2: p value for **Antifungal drug** and each other group
p_3: p value for **Apple cider** and each other group
p_4: p value for **Comm. Vinegar** and ***Eucalyptus* oil**
*: Statistically significant at $p \leq 0.05$.

5. CONCLUSION

The present study concludes the following:

- Pharmaceutical Locasten ear drops, apple cider vinegar, commercial vinegar, and *Eucalyptus* oil extract demonstrated varying degrees of controlling of otomucosis caused by *Aspergillus niger*.
- The highest inhibitory effect was observed using commercial vinegar at a concentration of 0.1 ml/ml.
- Commercial vinegar at a concentration of 0.1 ml/ml was reported as the most effective one in controlling *Aspergillus niger* induced otomycosis.
- The lowest otomycosis controlling degree was recorded using *Eucalyptus* oil extract at a concentration of 0.01 ml/ml.
- The most surprising result is that commercial vinegar (0.1 ml/ml) exhibited stronger antifungal activity than the pharmaceutical Locasten ear drops.

The authors are recommended to apply commercial vinegar as a promising antifungal treatment for controlling otomycosis induced by *Aspergillus niger*.

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