

# IN VITRO ASSESSMENT OF ANTIFUNGAL POTENTIAL OF APPLE CIDER VINEGAR AND ACETIC ACID VERSUS FLUCONAZOLE IN CLINICAL ISOLATES OF OTOMYCOSIS

Hayder Badr Jabir M.B.Ch.B,FICMS\*

Fatin Naeem Abbas B.Sc,M.Sc\*\*

Rana Muhsin Khalaf M.B.Ch.B, M.Sc\*\*\*

## ABSTRACT

**Background:** There has been an increase in the prevalence of otomycosis in recent years. This has been linked to the extensive use of antibiotics, use of immunosuppressant drugs and steroids for a variety of malignant and immunological diseases. Treatment of otomycosis is challenging, and requires a close follow-up; in addition treatment with topical or systemic antifungal drugs might be associated with problems of resistance, mucosal irritation, and systemic toxicities. *Candida albicans* and *Aspergillus niger* are the most commonly identified organisms.

**Aim:** the aim of this study is to investigate the antifungal potential of apple cider vinegar and acetic acid in a hope to find a safer alternative to traditional antifungal drugs in treatment of otomycosis.

**Patients and methods:** ear swabs were collected from 18 patients attending otolaryngology outpatient clinic-Al-Habboobi Hospital who presented with aural symptoms suggestive of otomycosis.fungal identification was done depending on microscopical and colonial morphology. Antifungal effect of the tested agents was assessed by agar well-diffusion assay.

**Results:** 18 samples were examined, 13 (72%) of them were positive for fungal growth, 6 were *Aspergillus niger* , 1 was *Aspergillus flavus*, 2 were *Candida albicans*, and the other 4 were Non- *Candida albicans*.

Apple cider vinegar(5%) inhibits growth of *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and Non- *Candida albicans* with average diameter of inhibition zones of 15mm, 13mm, 17.5mm, 17 mm respectively, while the average diameters noted with acetic acid 2% were 10.5mm, 11mm, 13mm, 13.5mm respectively. Fluconazole (5mg/ml) inhibits growth of the *Aspergillus niger*, *Aspergillus flavus* with average diameters of 14mm,20mm respectively,while cause no inhibition of *Candida albicans* and Non- *Candida albicans*.

**Conclusion:** Apple cider vinegar and acetic acid have significant antifungal activity at the tested concentrations against *Aspergillus niger*, *Aspergillus flavus*, and fluconazole resistant *Candida albicans* and Non- *Candida albicans*.

**Key words:** otomycosis, apple cider vinegar, acetic acid, *Candida*, *Aspergillus*

## INTRODUCTION:

Otomycosis, also known as fungal otitis externa, has been used to describe a fungal infection of the external auditory canal and its associated complications sometimes

involving the middle ear<sup>1</sup>.

**Epidemiology:** The prevalence rate has been quoted as 9% of patients presenting with signs and symptoms of otitis externa.<sup>1</sup> Prevalence is also related to the

\* Otolaryngology. Department of basic sciences/ College of Nursing- Thi-Qar University.

\*\* Department of microbiology/ College of medicine- Thi-Qar University.

\*\*\* Pharmacology. Department of pharmacology/ College of medicine- Thi-Qar University.

## In Vitro Assessment Of Antifungal Potential Of Apple Cider Vinegar And Acetic Acid Versus Fluconazole In Clinical Isolates Of Otomycosis

geographical area, as otomycosis is most commonly present in tropical and subtropical humid climates.

**Predisposing factors:** Various factors have been proposed as predisposing factors for otomycosis, including extensive and sometimes unnecessary use of antibiotic eardrops for the treatment of otitis media and otitis externa, use of broad-spectrum antibiotics, humid climate, absence of cerumen, local trauma. Instrumentation of the ear, immunocompromized host.<sup>2</sup> Other predisposing conditions include eczema, allergic rhinitis, and asthma.<sup>3,4</sup>

**Clinical features:** The cardinal features are ear discomfort/pain, intense itching and a feeling of a 'wet' ear with a blocked sensation. Otoscopy reveals congestion and oedema of meatal skin. At the early stages of infection, dry fungal growth can be clearly seen (white/black spores of *Aspergillus* infection) or there may be a creamy deposit (*Candida* infection). At a later stage, one will see dirty white debris akin to a wet blotting paper filling up the entire canal over an inflamed meatal skin<sup>5</sup>.

**Microbiology:** Many species of fungi have been identified as the cause of otomycosis. *Aspergillus niger* and *Candida albicans* are the most common causative agents of otomycosis. *Aspergillus* is considered the predominant causal organism in tropical and subtropical regions<sup>6</sup>.

### **Treatment:**

Otomycosis is a chronic recurring mycosis. The ear canal should be cleared of debris and discharge.<sup>2</sup> Suction can be used if available. Cleaning may be required several times a week. Analgesia is required. If there is an irritant or allergen it must be removed. Burrow's solution or 5% aluminum acetate solution should be used to reduce the swelling and remove the debris<sup>1</sup>. An aqueous solution of 1% thymol in metacresyl acetate, or iodochlorohydroxyquin should be considered if drying the ear does not work satisfactorily<sup>7</sup>.

Antifungal ear drops are of value<sup>8</sup>. There is no consensus on treatment but evidence

supports the use of topical ketoconazole<sup>1</sup>. Clotrimazole and econazole drops are very effective but may be needed for 1 to 3 weeks<sup>9</sup>. Clioquinol is both antibacterial and antifungal and may be used as eardrops with hydrocortisone in the formulation of Locorten Vioform<sup>TM10</sup>.

### **Tested agents:**

#### **Apple cider vinegar (ACV):**

**Description:** Apple cider vinegar (ACV) is a solution of acidic acid produced by fermentation of apples.

**Contents:** Scientists have measured ninety different substances in apple cider vinegar such as thirteen types of carboxylic acids, four aldehydes, twenty ketones, eighteen types of alcohols, eight ethyl acetates etc. It also contains important minerals, trace elements and vitamins (as listed underneath) as well acetic acid, propionic acid, lactic acid and malic acid, enzymes, amino acids as well as roughage in the form of potash and apple pectin. Minerals and trace elements: Potassium, Calcium, Magnesium, Phosphorous, Chlorine, Sodium, Sulfur, Copper, Iron, Silicon, Fluorine. Vitamins: Vitamin C, Vitamin E, Vitamin A, Vitamin B1, Vitamin B2, Vitamin B6, Provitamin beta-carotene<sup>11</sup>.

Benefits of Organic apple cider vinegar<sup>12, 13</sup>:

- Cardiovascular health: Pectin in the apple cider vinegar is a fiber which helps to eliminate bad cholesterol from the body, and in regulating blood pressure
- Weight reduction, also due to its pectin content
- Antifungal and antibacterial activity: Due to the malic acid content in Apple cider vinegar, it helps in fighting fungal and bacterial infections.
- Apple cider vinegar is high in potassium content, and organic apple cider vinegar diet can help greatly to reduce any potassium deficiencies of the body.

- Beta-carotene, an antioxidant present in organic apple cider vinegar helps to maintain a wrinkle free skin.
- Maintain the correct PH balance in the body.

#### **Acetic acid:**

Acetic acid (plain, white household vinegar) is an antibiotic that treats infections caused by bacteria or fungus. It is commonly prescribed home remedy for treatment of otitis externa.

Acetic acid is used to treat infections in the ear canal. This medication will not treat a middle ear infection (otitis media).

Treatment involves profuse and frequent irrigation of the ear with 2% acetic acid<sup>14</sup>.

## **Patients and methods:**

Eighteen patients attending the outpatient otolaryngology clinic/Al-Habboobi Hospital -Thi-Qar province with symptoms suggestive of otomycosis were involved in this study. All patients had one or more of the aural symptoms (itching, otalgia, hearing loss).

#### **Methods:**

##### **Clinical sampling:**

Otomycosis was diagnosed by careful history taking and otoscopic examination. Ear swabs of the affected ears were collected and sent for microbiological assessment.

##### **Microbiological procedures:**

##### **Fungal identification:**

Fungal isolates were identified on the basis of colonial morphology, lactophenol cotton blue wet-mount microscopy, and slide culture in accordance with standard procedures. Germ-tube testing and morphology on cornmeal agar were also performed to enable the accurate identification of *Candida* species<sup>15</sup>.

The presence of fungal elements in stained smears was re-confirmed by fungal culture. Any kind of clinical materials, especially liquid samples (swabs, pus) should be examined as quickly as possible. Swabs did not require processing and were directly used for culture. Swabs were

rolled and inoculated over the surface of Sabouraud's Dextrose Agar with chloramphenicol (SC). Cultures were incubated at laboratory ambient (25-27°C)<sup>15,16</sup>.

#### **Agents and Test controls:**

Apple cider vinegar 5% and acetic acid 2% were purchased from local market. Negative controls included use of distilled water, although no antifungal activity was noted with it. Positive control included use of a common antifungal drug, fluconazole (Flucomin capsules 150 mg-mission limited/India) which was dissolved in distilled water (DW), it was placed in a separate well of each plate to compare the fungal susceptibility to the tested agents.

#### **Agar well diffusion assay:**

Plates of Sabouraud's Dextrose Agar media were seeded with a suspension of actively growing yeast cells. Suspensions of approximately  $6 \times 10^6$  yeast cells per ml in sterile normal saline compared with Macfreland tube were prepared<sup>17,18</sup>. And about 1.5 ml of it was uniformly seeded on nutrient media in 12x1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded properly.

Wells of 6mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers. A fixed volume of fluconazole, apple cider vinegar and acetic acid and DW were loaded in the wells using sterilized dropping pipettes and diffusion was allowed at room temperature for 2 hours. After preincubation, the plates were incubated aerobically at 30 C for 24-48 hours. Antifungal activities were determined by measuring diameter of inhibition zone (DIZ) in mm. each experiment was repeated thrice and the average values of antimicrobial activity were calculated<sup>19</sup>.

## **Statistical analysis:**

Data are expressed as average, statistical significance between groups was determined by unpaired student's t-test using  $p < 0.05$  as a criterion for significance.

## **RESULTS:**

18 samples were examined, 13 (72%) of them were positive for fungal growth, 6(46%) of fungal isolates were *Aspergillus niger*, 1 (8%) was *Aspergillus flavus*, 2(15%) were *Candida albicans*, and the other 4(31%) were Non- *Candida albicans*, as shown in table (1) and figure(1).

Apple cider vinegar inhibits growth of *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and Non- *Candida albicans* with average diameter of inhibition zones of 15mm, 13mm, 17.5mm, 17 mm respectively, while the average diameters noted with acetic acid 2% were 10.5mm, 11mm, 13mm, 13.5mm respectively. Fluconazole (5mg/ml) inhibits growth of the *Aspergillus niger*, *Aspergillus flavus* with average diameters of 14mm, 20mm respectively, while causes no inhibition of *Candida albicans* and Non-*Candida albicans* as shown in table (2,3) and figure (2,3).

## **DISCUSSION:**

Although rarely life-threatening, otitis media presents a challenging and frustrating entity for both patients and otolaryngologists for it frequently requires long term treatment and follow-up, yet the recurrence rate remains high<sup>20</sup>.

To date there is no FDA approved antifungal otic preparation for the treatment of otitis media. Many agents with various antimycotic properties have been used, and clinicians have struggled to identify the most effective agent to treat this condition<sup>21</sup>.

Several problems might be encountered with antifungal drugs, first; resistance; as fungi may become resistant to antifungal drugs due to target gene mutations, enzyme modification or to development of pump system that expels the drug out of the fungal cell<sup>22</sup>. second; as less data exist regarding the safety of the use of ototopical medications in the presence of a tympanic membrane perforation<sup>15</sup>, topical antifungal drugs are potentially irritant to middle ear mucosa. Third; toxicity, as antifungal

drugs can cause systemic toxicities as hepatotoxicity and nephrotoxicity. These problems necessitate searching for safer effective remedies with known antifungal activity.

Apple cider vinegar is a commonly prescribed antifungal agent in folk medicine for treatment of fungal skin, ear and vaginal infections. In the present study apple cider vinegar (5%) showed significant antifungal effect ( $p < 0.05$ ) against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and Non- *Candida albicans* when compared with the negative control. Such results agreed with<sup>23</sup> who found that topical application of apple cider vinegar is effective treatment of *Pityrosporum ovale* folliculitis. The antifungal activity of apple cider vinegar might be attributed to its malic acid, acetic acid contents or to other non identified ingredients.

Acetic acid (2%) was commonly prescribed remedy for fungal otitis externa, in vitro study revealed significant antifungal effect ( $p < 0.05$ ) against examined fungal species, this result support its clinical use in otitis media<sup>14</sup>.

Fluconazole, is a triazole antifungal drug which is widely prescribed for treatment of different mycoses, for its better fungal selectivity and relatively improved adverse effect profile, this study revealed that fluconazole showed antifungal activity close to that of apple cider vinegar and acetic acid against *Aspergillus niger*, but it showed better activity against *Aspergillus flavus*. For *Candida albicans* and Non-*Candida albicans* fluconazole showed no antifungal activity at 5, 20 and 100 mg/ml. This result reflects the important growing problem of antifungal resistance, on the other hand apple cider vinegar and acetic acid exerted significant antifungal action ( $p < 0.05$ ) against these resistant strains.

## **CONCLUSION:**

Apple cider vinegar and acetic acid have significant antifungal activity at the tested concentrations against *Aspergillus niger*,

*Aspergillus flavus*, and fluconazole resistant *Candida albicans* and Non-*Candida albicans*. Such activity may suggest promising effective alternative or synergistic remedy to the popular antifungal drugs in treatment of otomycosis.

### RECOMMENDATIONS:

- In vivo evaluation of the antifungal effect of apple cider vinegar is required
- Investigation of the apple cider vinegar irritant potential to middle ear mucosa as well as the possible ototoxic
- 

potential of both apple cider vinegar and acetic acid, because data regarding such potentials are lacking.

- Investigation of the possible additive or synergistic antifungal effect of apple cider vinegar and acetic acid with other antifungal drugs.
- Understanding the molecular basis of antifungal activity of apple cider vinegar and acetic acid to identify their specific antifungal targets.

Table (1): types and percentages of fungal isolates.

organism	Number of isolates	Percentile %
<i>Aspergillus niger</i>	6	46%
<i>Aspergillus flavus</i>	1	8%
<i>Candida albicans</i>	2	15%
Non- <i>Candida albicans</i>	4	31%

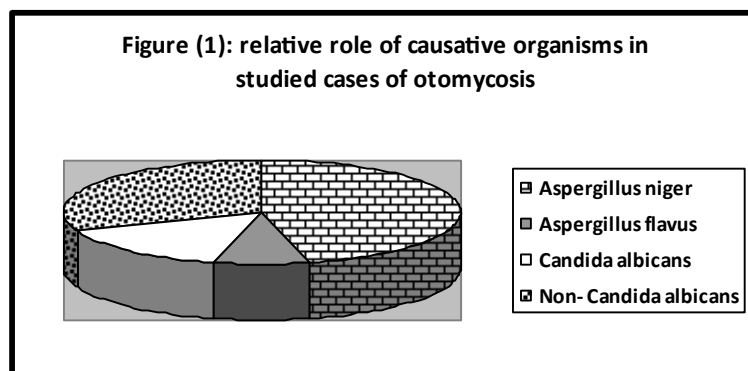


Table (2): averages diameters of inhibition zones (DIZ) for apple cider vinegar 5% in comparison with negative and positive controls.

Organism	DIZ for the tested agents (mm)		
	Apple cider vinegar (5%)	Fluconazole(5 mg/ml)positive control	D.W(Negative control)
<i>Aspergillus niger</i>	15	14	0
<i>Aspergillus flavus</i>	13	20	0
<i>Candida albicans</i>	17.5	0	0
Non –candida albicans	17	0	0

**In Vitro Assessment Of Antifungal Potential Of Apple Cider Vinegar And Acetic Acid Versus Fluconazole In Clinical Isolates Of Otomycosis**

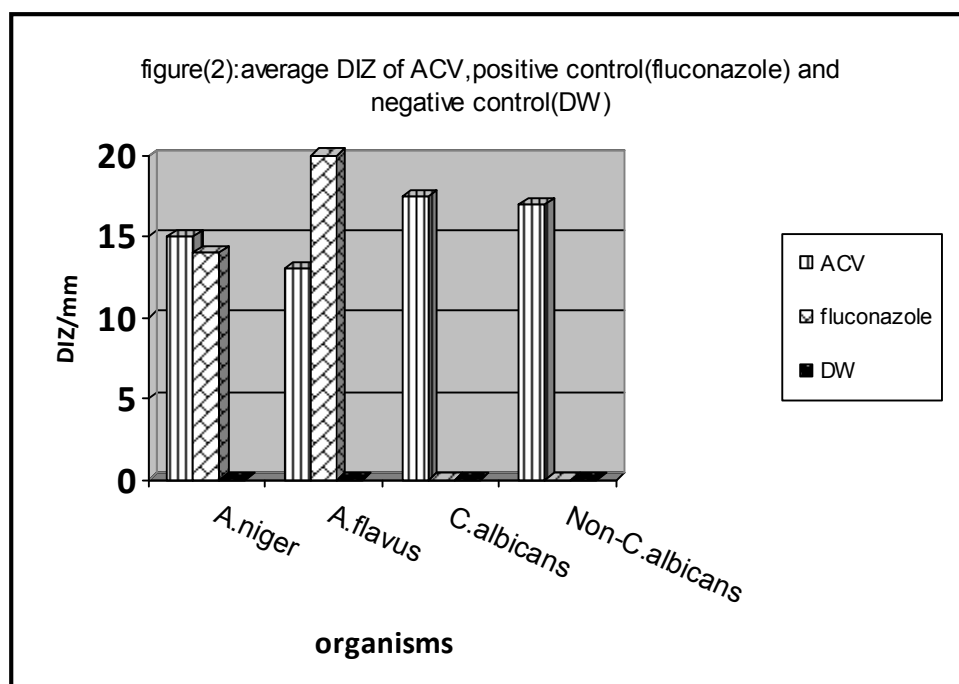
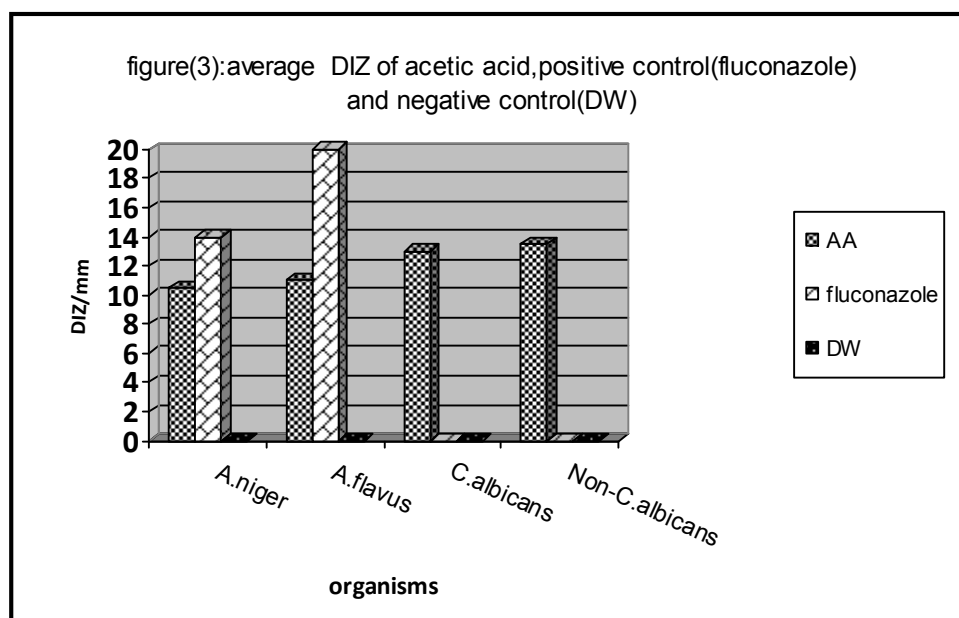


Table (3): averages diameters of inhibition zones (DIZ) for acetic acid 2% in comparison with negative and positive controls.

Organism	DIZ for the tested agents (mm)		
	Acetic acid (2%)	Fluconazole(5 mg/ml)positive control	D.W(Negative control)
Aspergillus niger	10.5	14	0
Aspergillus flavus	11	20	0
Candida albicans	13	0	0
Non –candida albicans	13.5	0	0



## REFERENCES:

1. Ho T, Vrabec JT, Yoo D, et al; Otomycosis: clinical features and treatment implications. *Otolaryngol Head Neck Surg.* 2006 Nov;135(5):787-91. [abstract]
2. Stern JC, Lucente FE. Otomycosis. *Ear Nose Throat J* 1988;67:804 –10.
3. Garry JP; Otitis externa. *eMedicine*, November 2007.
4. A. Jackman, R. Ward, M. April, J. Bent, Topical antibiotic induced otomycosis, *Int J Pediatr Otorhinolaryngol* 69 (2005) :857—860.
5. Management of Common Otological Diseases at a Peripheral Medical Set Up  
Gp Capt RC Kashyap MJAFI 2003; 59 : 332-336
6. V.J. Jadhav, M. Pal, G.S. Mishra, Etiological significance of *Candida albicans* in otitis externa, *Mycopathologia* 156 (2003) 313—315
7. Georgiev V; *Opportunistic Infections: Treatment and Prophylaxis* 2003
8. Bassiouny A, Kamel T, Moawad MK, et al; Broad spectrum antifungal agents in otomycosis. *J Laryngol Otol.* 1986 Aug;100(8):867-73. [abstract]
9. P. Kurnatowski, A. Filipiak, Otomycosis: prevalence, clinical symptoms, therapeutic procedure, *Mycoses* 44 (2001) 472-479.
10. Maher A, Bassiouny A, Moawad MK, et al; Otomycosis: an experimental evaluation of six antimycotic agents. *J Laryngol Otol.* 1982 Mar;96(3):205-13. [abstract]
11. [www.healthandfitnessforums.com/apple.html](http://www.healthandfitnessforums.com/apple.html)
12. Corwin Brown: Health Benefits of Apple Cider Vinegar – Maintain Health. *articlesbase*, 2008.
13. pradhnya Tajni: Organic Apple Cider Vinegar. *Buzzle.com*. published 1/14/2010.
14. Jack L. Pulec, Christian Deguine : Otomycosis - Otoscopy Clinic - Brief Article, *Ear, Nose & Throat Journal*, June, 2002
15. Ravinder Kaur, Nalini Mittal, Manish Kakkar: otomycosis a clinicomycological study. *Ear, Nose & Throat Journal* , August, 2000
16. Ali Zarei Mahmoudabadi: *Mycological Studies in 15 Cases of Otomycosis: Pak J Med Sci* October - December 2006 Vol. 22 No. 4 486-488)
17. Abu-Mejdad M.J. Najwa: Evaluation of activity of some plant extracts against some fungi causes superficial cutaneous mycoses. 2005.
18. Crockett, C.O., F. Guede-Guina, D. Pugh: *Cassia alata* and the preclinical search for therapeutic agents for the treatment of opportunistic infections in AIDS patients. *Cellular and molecular biology*, 1992, 38: 799-802.
19. G.H. Shahidi Bonjar: anti yeast activity of some plants used in traditional herbal-medicine of Iran, *Journal of biological sciences* 4(2): 212-215, 2004.
20. Mugliston T, O'Donoghue G.: Otomycosis: a continuing problem. *J Laryngol Otol* 1985;99:327–33.
21. T.J. Martin, J.E. Kerschner, V.A. Flanary: Fungal causes of otitis externa and tympanostomy tube otorrhea, *Int J Pediatr Otorhinolaryngol* 69 (2005) 1503—1508
22. Finkel, Richard; Clark, Michelle A.; Cubeddu, Luigi X: *Lippincott's Illustrated Reviews: Pharmacology*, 4th Edition 2009.
23. *Pityrosporum Folliculitis – FAQ*. *Acne.org*

## التقييم المختبري لقابلية خل التفاح و حامض الخليك المضادة للفطريات مقارنة بالفلوكونازول في عزلات سريرية لفطريات الاذن.

حيدر بدر جابر\*، فاتن نعيم عباس\*\*  
رنا محسن خلف\*\*\*

### الخلاصة:

**تمهيد:** لقد ازدادت معدلات الاصابة بفطريات الاذن في السنوات الاخيرة و ذلك نتيجة للاستخدام الواسع للمضادات الحيوية و الادوية المثبطة للمناعة و الستيرويدات . يشكل علاج فطريات الاذن تحديا لكل من الطبيب و المريض لما يستدعيه من متابعة مستمرة بالاضافة الى المشاكل المرتبطة باستخدام الادوية المضادة للفطريات منها مشكلة مقاومة الفطريات للادوية، واحتمال تسبب هذه الادوية بتهييج الغشاء المخاطي للاذن الوسطى، كذلك احتمالية حدوث بعض التأثيرات السامة لهذه الادوية. تعتبر الكانديدا و الاسبرجلس اكثر انواع الفطريات تسببا في التهاب الاذن الفطري.

**الهدف:** هدف هذه الدراسة هو تحري قابلية كل من خل التفاح و حامض الخليك المضادة للفطريات مقارنة بالفلوكونازول في عزلات سريرية لفطريات الاذن كخطوة لأيجاد علاج بديل اكثر امان و فعالية من الادوية التقليدية المستخدمة.

**المرضى و طريقة العمل:** تم اخذ مسحات الاذن من ١٨ مريض مصاب باعراض فطريات الاذن يزورون العيادة الخارجية لامراض الانف و الاذن و الحنجرة في مستشفى الحبوبى العام. تم تشخيص الفطريات المسببة اعتمادا على الشكل المجهرى وخصائص المستعمرة. تم تحري التأثير المضاد للفطريات للمواد المستخدمة بطريقة الحفر بالاغار.

**النتائج:** تم فحص ١٨ عينة، ١٣ (٧٢%) كانت موجبة للنمو الفطري، ٦ منها اسبرجلس نايجر، ١ اسبرجلس فليفس، ٢ كانديدا البيكانس و ٤ نون كانديدا البيكانس.

وجد ان خل التفاح (٥%) و حامض الخليك (٢%) لهما تأثير مثبت معتد به على كل من اسبرجلس نايجر، اسبرجلس فليفس، كانديدا البيكانس و نون كانديدا البيكانس حيث كانت معدلات اقطار التثبيط ١٥، ١٣، ١٧، ٥، ١٧، ٥ ملليمتر على التوالي، بينما معدلات اقطار التثبيط لحامض الخليك ١٠، ٥، ١١، ١٣، ٥، ١٣، ٥ ملليمتر على التوالي. بالنسبة للفلوكونازول (٥ ملغ/مل) سبب تثبيط معتد لكل من اسبرجلس نايجرو اسبرجلس فليفس حيث كانت معدلات اقطار التثبيط ١٤ و ٢٠ على التوالي، بينما لم يسبب اي تثبيط ملحوظ ضد كانديدا البيكانس و نون كانديدا البيكانس.

**الاستنتاج:** ان لخل التفاح و حامض الخليك تأثير مضاد للفطريات معتد به في التراكيز المختبرة ضد كل من اسبرجلس نايجر، اسبرجلس فليفس و كانديدا البيكانس و نون كانديدا البيكانس المقاومة للفلوكونازول.

**مفتاح الكلمات:** فطريات الاذن، خل التفاح، حامض الخليك، كانديدا، اسبرجلس.

\* فرع العلوم الاساسية- كلية التمريض-جامعة ذي قار  
\*\* فرع الاحياء المجهرية- كلية الطب-جامعة ذي قار  
\*\*\* فرع الادوية-كلية الطب-جامعة ذي قار