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The Effect of Ginger Extracts on Bacterial Isolates from Patients with Suppurative Otitis Media and Externa: In Vitro Study

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Abstract

Background Otitis media is a group of complex inflammatory disorders affecting the middle ear, which can be acute or chronic. Otitis externa is an inflammation on the skin of the external auditory canal usually associated with secondary bacterial and/or fungal infection of macerated skin and subcutaneous cellular tissue.

Objective To determine the effect of ginger extracts on bacterial isolates from patients suffering from otitis.

Methods Two hundred patients suffering from suppurative otitis media and externa, who were attending to ENT Department, Al-Imamein Al-Kadhimein Medical City. The powder of ginger rhizomes was soaked with the solvent left in a shaking water bath at 40 °C for 24 hours and then filtered using Whatman No.1 filter paper. Each extract was concentrated using a rotary evaporator with vacuum to get the final crude extract after the procedure of ginger extract was done. The activity of this extract was tested against bacterial isolates from patients with otitis.

Results The results of this study revealed that otitis externa was less common infection than the other types of otitis 29 (14.5%), while acute otitis media comprised 96 (48.0%), and chronic suppurative otitis media consisted of 75 (37.5%). The most common bacterial isolates that caused otitis were *Pseudomonas spp*. followed by *Staphylococcus spp*. The results showed that, there are different effects among (chloroform, methanol, and aqueous) extracts of ginger against pathogenic bacteria.

Conclusion Generally, ginger extracts had a good effect on isolated bacteria. Chloroform extract was the most effective one, followed by methanol extract, while aqueous extract showed the least activity in this regard.

Keywords Otitis externa, otitis media, ginger extraction

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List of abbreviations: AOM = Acute otitis media, CSOM = Chronic suppurative otitis media, DMSO = Dimethyl sulfoxide, OE = Otitis externa, OM = Otitis media

Introduction

Ear infection is one of the most common diseases occurring throughout the world. Different etiological agents are responsible for ear infections [(1)]. Ear infections are three types; the first type is otitis externa (OE) that intricate the outer ear and ear canal. In OE, the ear seems to be painful when touch and tugging, it is also called swimmers ear. Otitis media (OM) is the second type in ear infection; in which the ear is contaminated with fluid behind the ear drum, in the habitually air filled in space of middle ear. The infection of middle ear is very common in childhood. In some cases, this condition

requires a surgical procedure called myringotomy and tube insertion. The third type is otitis interna, which involves the inner ear including sensory organs [2].

Ginger the rhizome of Zingiber officinale (family Zingiberaceae) is globally one of the most common spices and has been used as a culinary agent for over 1,000 years in Asia. The Zingiberaceous plants are characterized by their tuberous or non-tuberous rhizomes, which have strong aromatic and medicinal properties [3]. Recently, in Iraq, ginger was experimentally cultivated during one year with the attempt to grow and distribute the plant [4]. The main active components in ginger from its phenolic substance are gingerol and shogaol, while zingiberene is obtained from ginger oil [5]. Ginger has a wide range of action on the human body and has been found to be effective in the treatment of cataract, heart disease, migraines, struck amenorrhea, athletes foot, bursitis, chronic fatigue, cold, flu, coughs, depression, dizziness, fever, kidney stones [6]. Powdered derived ginger root is made into capsules and sold in pharmacies for medical use [7].

This study was carried out to determine the effect of ginger extracts on bacterial isolates from patients suffering from otitis.

Methods

Samples collection

Two hundred patients suffering from suppurative otitis media and externa who were attending ENT department, Al-Imamein Al-Kadhimein Medical City were enrolled in this study from November 2016 to the end of April 2017. Ear swabs specimens were collected from each patient in sterile swabs. All specimens were transported to the laboratory for processing and investigations at the same day. Samples processing occurred according to standard operating procedures which included: Cultivation on culture plates (blood, chocolate, MacConkey) agar for the isolation of bacterial pathogens. Gram staining for bacteria, biochemical tests as diagnostic tools for bacterial pathogens.

Preparation of ginger extract

Five hundred grams of dried rhizomes of ginger (Zingiber officinale) was purchased from local market in Baghdad, Iraq and authentication was done in Department of Pharmacognosy/ College of Pharmacy, AL-Mustansyriah University. The dried rhizomes were grind into very fine powder using a heavy-duty grinder. The powder of ginger rhizomes was divided into 12 portions then each portion extracted sequentially with three solvents beginning with the non-polar solvent and ascending to the most polar solvent (chloroform, methanol and distilled water, respectively) with a ratio of 1:7 W/V (30 gm of powder/ 210 ml of solvent); the extraction repeated twice for each solvent and the process of extraction used was the cold method, i.e., maceration. The powder of ginger rhizomes was soaked with the solvent and left in a shaking water bath at 40 °C for 24 hours and then filtered using Whatman No.1 filter paper to take the clear extract. Each extract was concentrated using a rotary evaporator with vacuum to get the final crude extract. The extract powder was kept in sterile bottles, labeled accordingly and stored in the refrigerator [8]. One gm of the crude extracts of (chloroform, methanol and aqueous) ginger extracts were dissolved in 10 ml DMSO (Dimethyl sulfoxide) to became the concentration 100mg/ml as stock solutions.

Antibacterial test of extracts using agar well diffusion method

The antibacterial activity of different extracts against bacteria was evaluated by using agar well diffusion method [9]. Isolated colonies were selected from nutrient agar plates culture and transferred to 3 ml of 0.85% normal saline to a density equivalent to the turbidity of the (0.5) McFarland standards. A sterile cotton swab was dipped into the bacterial suspension; excess fluid inoculum from the swab was removed by pressing the swab firmly on the side wall of the tube above the fluid level. Streaking of the inoculum was done over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60º each time to

ensure an even distribution of inoculum as a final step, the rim of the agar was swabbed. The plates were left at room temperature for 15 minutes to allow for any excess surface moisture to be absorbed. Wells of 5 mm was punctured with the help of a sterilized cork-porer into the pre-solidified Mueller Hinton agar plates containing the test organism. Using the micropipette, 20 µl of each extract (chloroform, methanol, and aqueous) was poured into the different wells of the inoculated plates. DMSO well was used as a negative control and bacterial plates were incubated at 37 °C for 24 hrs. The diameters of inhibition zones were measured, later on [10].

Statistical analysis

Data of this study were entered using EPI INFO7 Windows Version and analyzed by using statistical package for social sciences (SPSS) version 20. Descriptive statistics were presented as frequencies, percentage (%), means and standard deviation (SD). Chi square test was used to estimate the association between two categorical variables. Level of significance of ≤ 0.05 was considered as significant. Analysis of variance (ANOVA) used for comparison among more than two groups. Paired samples T test used for comparison between two groups.

Results

Gender and age distribution of patients with ear infection

A total of 200 patients suffering from otitis were enrolled in this study. The mean age of patients was (30.04) with standard deviation (20.41), ranged from 7 days - 80 years old. It was found that a half of patients were males as 109 (54.5%) and 91 (45.5%) were females. the first group which is the largest group included 50 patients (25%) when the age factor was (≤10) years old, while the seventh which is a last group of (>60) years included 16 patients (8%) (Table 1).

Table 1. Classification of patients with otitis regarding age groups

Age group	NO.	Percentage %
<10 years	50	25.0
11-12 years	23	11.5
21-30 years	35	17.5
31-40 years	25	12.5
41-50 years	30	15.0
51-60 ears	21	10.5
>60 years	16	8.0

Bacterial isolates from patients with otitis

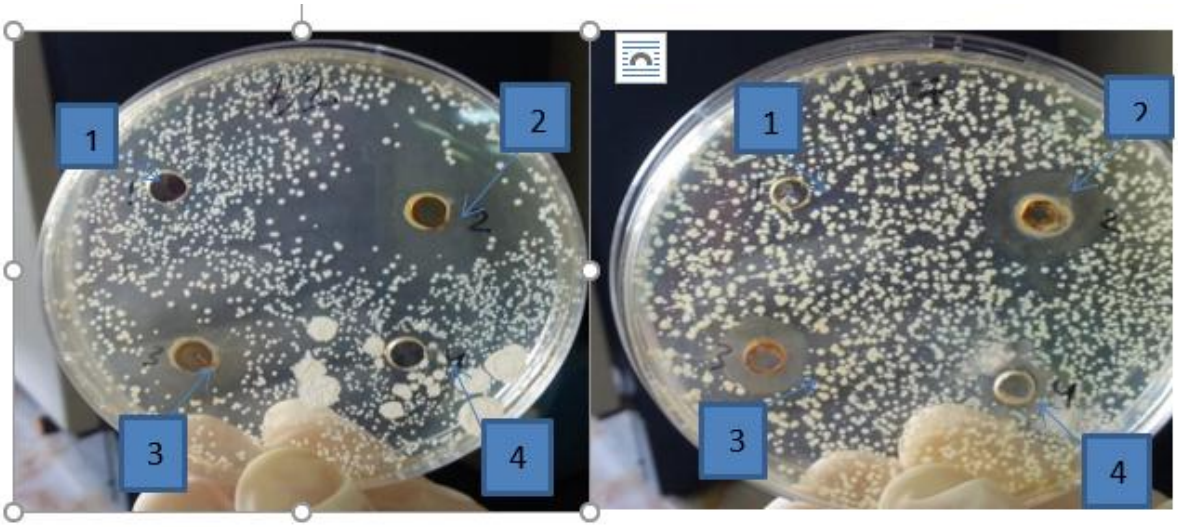
Table (2) shows the most bacteria isolates from patients suffering from ear discharge were *Pseudomonas spp.*, followed by *Staphylococcus aureus*. In this study also isolated *Ewingella americana* and *Pasturella pneumoniae* this first study isolate these bacteria.

Antibacterial activity of (chloroform, methanol, and aqueous) ginger extracts:

The antibacterial activity of ginger extract was prepared with different solvents (aqueous, methanol, and chloroform) tested against pathogenic bacteria species. Table (3) demonstrates the most effect of extract was chloroform ginger extract on the most bacterial isolate and less or no effect was aqueous ginger extract.

Table 2. Percentages of bacterial isolated from patients with otitis

Bacterial culture	Otitis media		Otitis Externa	Total
	AOM	CSOM		
Citrobacter spp.	1 (1.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)
E. coli	4 (4.2%)	5 (6.7%)	3 (10.3%)	12 (6.0%)
Enterobacter spp.	1 (1.0%)	1 (1.3%)	0 (0.0%)	2 (1.0%)
Ewingella americana	1 (1.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)
Klebsiella spp.	4 (4.2%)	3 (4.0%)	2 (6.9%)	9 (4.5%)
Morganella morganii	1 (1.0%)	0 (0.0%)	0 (0.0%)	1 (.5%)
Pasturella pneumoniae	4 (4.2%)	2 (2.7%)	2 (6.9%)	8 (4.0%)
Proteus spp.	5 (5.2%)	1 (1.3%)	0 (0.0%)	6 (3.0%)
Pseudomonas spp.	20 (20.8%)	29 (38.7%)	3 (10.3%)	52 (26.0%)
Serratia spp.	2 (2.1%)	1 (1.3%)	0 (0.0%)	3 (1.5%)
Staphylococcus aureus	17 (17.7%)	9 (12.0%)	3 (10.3%)	29 (14.5%)
Staphylococcus epidermidis	5 (5.2%)	2 (2.7%)	4 (13.8%)	11 (5.5%)
Staphylococcus capitis	5 (5.2%)	3 (4.0%)	2 (6.9%)	10 (5.0%)
Sterptococcus viridans	3 (3.1%)	1 (1.3%)	0 (0.0%)	4 (2.0%)
Streptococcus pneumoniae	2 (2.1%)	0 (0.0%)	1 (3.4%)	3 (1.5%)
Streptococcus pyogenes	1 (1.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)
No growth	20 (20.8%)	18 (24.0%)	9 (31.0%)	47 (23.5%)
Total	96 (100%)	75 (100%)	29 (100%)	200 (100%)



(A): Staphylococcus aureus. (B): Pseudomonas spp.

Figure 1. Antibacterial effect of aqueous extract of ginger, chloroform, and methanol extract. 1. Negative control (DMSO), 2. Chloroform extract, 3. Methanol extracts, 4. Aqueous extract

Table 3. The antibacterial activity of ginger extracts against pathogenic bacteria isolated from ear discharge

Bacterial isolate	Means of diameters of inhibition Zones (mm)			
	Chloroform	Methanol	Aqueous	Negative control
Citrobacter spp.	16	16	8	0
E. coli	15.92±4.5	11.25±4.79	6.25±3.05	0
Enterobacter spp.	9±1.41	8±1.41	0	0
Klebsiella spp.	12.56±4.16	12.56±4.56	5.22±3.96	0
Morganella morganii	12	13	0	0
Pasturella pneumoniae	11.88±5.96	8±6.99	7.38±4.81	0
Proteus spp.	13.33±2.42	7.17±3.6	8.33±1.37	0
Pseudomonas spp.	14.92±3.8	11.1±3.71	8.44±3.1	0
Serratia spp.	11.33±3.06	9.67±2.52	5.67±4.93	0
Staphylococcus aureus	14.45±5.88	11.66±3.88	3.07±4.07	0
Staphylococcus epidermidis	12.27±5.06	11±6.78	4.18±4.87	0
Staphylococcus capitis	15.8±2.94	13.8±3.71	5.4±4.99	0
Streptococcus viridans	13.75±2.63	11±3.37	4.5±5.26	0
Streptococcus pneumoniae	15.33±3.21	13.67±2.08	2.67±4.62	0

Discussion

In the present study, results indicated that males expose for OM more than females may be because males are more exposed to different conditions in work such as dust, humidity, and may be more actively involved in outdoor activities, hence to be more exposed to contaminated environment. On the other hand, females wearing of scarfs may be considered an important factor to decrease infection. This result agrees with other obtained by Almamory et al. (2014) [11] who mentioned that the rate of ear infection in males was higher than females, while disagree with that obtained by Khammas et al. (2010) [12] who mentioned that the rate of ear infection in females was higher than males. In the current study, all age groups could develop otitis with significant differences, the highest infection rate was so cases occurred in the age group (≤10) years were (50) cases, the plausible explanation of these result that children and infants may have low resistance to infection, and because of relative short and straight Eustachian tube [13], the lower immune system of children compared to adults, and the fact that bacteria adhere better to epithelial cells of children than adults [14]. This result agrees with other study done by Jayakar et al. (2014) [15] who proved that there were significant differences in the distribution of age in ear infection.

Bacterial isolates from patients with otitis

The current study revealed that the percentage of AOM was (48.0%), while CSOM was (37.5%), with lower rate of infection with OE, which was (14.5%). This study agrees with other done by Ayub et al. (2015) [2] who found that OM was the most frequent type of ear infection. The number of bacterial isolates was (153), it was found that the highest common bacterial isolates among patients with otitis was *Pseudomonas spp*. as 52 (26%), followed by *Staphylococcus aureus* as 29 (14.5%). A study done by Ibrahim (2013) [17] and AL-Ataar (2015) [18] who proved that *Pseudomonas spp.* and *Staphylococcus aureus* were the most commonly cause of CSOM and in another study done by Jaafar et al. (2014) [19] *Proteus spp.* in CSOM was more frequent than *Staphylococcus aureus. Streptococcus pneumoniae* and *Streptococcus pyogenes* isolated only from AOM cases in this study, which agrees with other study done by Almamory et al. (2014) [11]. Concerning *Morganella morganii* these

bacteria was isolated from AOM only, while in another study done by Chirwa et al. (2015) [20] these bacteria were isolated from CSOM.

Antibacterial activity of (chloroform, methanol, and aqueous) ginger extracts

The average diameter of inhibition zones by chloroform and methanol extracts against *Citrobacter spp*. (16 mm), while aqueous extract was (8 mm). These results disagree with Ogbonna et al. (2014) [21] who found that aqueous extract of ginger was found to have potent antimicrobial activity against *Citrobacter spp.* with mean diameter as (38 mm). This discrepancy of results might be due to the presence of different phyto-compounds, which may include terpenoides, alkaloids and phenolic compounds that may interfere with the results [22]. In *E. coli*, it was shown that chloroform extract was gave the largest mean of diameter of inhibition zones as (15.92±4.5 mm), this difference in responses might be due to the chemical compound found in chloroform that dissolved some compound that found in ginger. This study disagrees with Yassen and Ibrahim (2016) [23] who mentioned the mean of diameter of inhibition zones of methanol extracts were (15 mm). Methanol extract of ginger showed a stronger effect on *Morganella morganii* as (13 mm) than chloroform extract as (12 mm) with no effect of aqueous extract, there were no previous compatible studies to compare with these results. Chloroform extract gave the largest mean of diameter of inhibition zones in Pseudomonas spp. as (14.92±3.8 mm), ginger known to contain resins and volatile oils, which may be responsible for its potent antimicrobial activity against for all bacteria [26], this result disagrees with another by Abdulzahra and Mohammed (2014) [24] who found that chloroform extract has no effect at all against *Pseudomonas spp*. In case of *Staphylococcus aureus*, the diameters mean of chloroform extract gave the largest inhibition zone, followed by methanol extract, while aqueous extract of ginger gave the lesser inhibition zones chloroform and methanol ginger extract will have an effect on external membrane of Gram-negative, because bacteria renders then to be highly hydrophilic surfaces, whereas the negative charge of the surface of the Gram-positive wall may reduce their resistance to antibacterial compounds [25]. This study agrees with Ahmed et al. (2012) [25] who mentioned that chloroform and methanol extracts, were exhibited highly antimicrobial activity against Staphylococcus aureus. Ginger rhizome has several components which have antibacterial and antifungal effects, gingerol and shagol identified as more active agents of ginger [26].

From this study it is concluded that the most frequent of bacteria was isolated from patients with otitis was *Pseudomonas spp.* followed by *Staphylococcus aureus*. The percentage of AOM was the highest among the other types of infection and the lower one was OE. The age group ≤10 years old were the highest among other groups in developing otitis. Chloroform extract of ginger was the most effective as antibacterial followed by methanol extract, while aqueous extract was the weaker.

From this study authors recommended further study needed to investigate the bioactive materials by column chromatography and to identify the concentration of active constituent by HLPC. Also, further study needed to explain the mechanism of action.

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Authors contribution

Mohammed: Msc student, Dr. Al-Attraqchi: supervision, Dr. Al-Hasseni: sample collection, Dr. Sahib: consultation of the pharmaceutical part of research.

Conflict of interest

The authors declare that they have no competing interests.

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