

Assessment of antimicrobial activity of cerumen (earwax) and antibiotics against pathogenic bacteria isolated from ear pus samples

Iffat Naz

Department of Biology, Scientific Unit,
Deanship of Educational Services,
Qassim University, Buraidah, Qassim,
Kingdom of Saudi Arabia

Abstract

The present study is focused on the assessment of the antimicrobial activity of cerumen and antibiotics against bacteria isolated from ear pus samples. Thus, a total of 50 ear pus samples were collected from infected patients using sterile swabs and were screened using pure culture techniques. Total of 04 different bacterial isolates were identified while, the prevalence data revealed that *Pseudomonas spp.*, were dominant (58%, n = 29) among isolated bacteria followed by *Staphylococcus spp.*, (22%, n = 11), *Escherichia coli* (14%, n = 7) and *Proteus spp.*, (6%, n = 3). Further, bioassay revealed that *Pseudomonas spp.*, and *Staphylococcus spp.*, were most sensitive to Clindamycin (94.73%) while displayed resistant to Ciprofloxacin and Ampicillin. Similarly, *E. coli* and *Proteus spp.*, were most sensitive to Ciprofloxacin (92.8-95.21%) as compared to the other antibiotics. Moreover, antibacterial activity of cerumen was also assessed against test organisms and its maximum activity was observed against *Pseudomonas spp.*, (90% equivalent to Clindamycin potency) and *Staphylococcus spp.*, (60% equivalent to Amoxicillin potency) while least effective against *E. coli* (36%) and *Proteus spp.*, (22%). Thus, it was concluded that the antibacterial activity of cerumen might be due to the presence of potential chemicals i.e. flavonoids and terpenoids.

Introduction

Earwax (cerumen) is a grey brown or yellowish waxy substance secreted in the outer one third of ear canal and, is a protective barrier between the external environment and deep external auditory canal.¹ Chemical composition of earwax includes 60% desquamated, 12-20% saturated and unsaturated fatty acid and 6-10% cholesterol.²⁻⁵ It is a secretion of specialized sets of glands, like sebaceous glands that secrete sebum (combi-

nation of fatty acid). Another gland i.e. apocrine sweat glands release secretion that combines with the sebum to form cerumen. It picks up discarded cells, ear follicles and may contain dust or other debris, but the resulting compound forms earwax or cerumen.⁶

There are two different types of genetically determined earwax such as wet type and dry type. The dry type is most common in Asians and Native Americans and has a grey brownish colour while the wet type can be found in Caucasians and Africans and has a brown or dark colour.⁷ About 30-50% of South Asians, Central Asians and Pacific islanders have the dry type of cerumen. Cerumen type has been used by anthropologist to track human migratory patterns, such as those of Eskimos.⁸ Further, the study of earwax has shown controversy, as some authors have suggested that earwax has high level of nutrients which help in microbial growth and other hold a view that earwax has some compounds that inhibit the growth of microorganisms and prevent ear infections.^{9,10} Ear infection in any form has a varied microbial etiology, which influences the selection of an efficacious of anti-microbial agents. According to WHO survey, 42 million people worldwide have hearing loss where major cause is otitis media. Infection of the ear can be classified depending upon the site: otitis externa (infection of external ear) and otitis media (infection on middle ear).¹¹ Otitis is a bacterial infection of the ear canal caused by rupture in the normal skin or cerumen, which is a protective barrier in the presence of elevated humidity and temperature.¹² It is commonly known as *swimmer's ear*, though anything that disrupts this protective lipid layer can lead to the introduction and proliferation of bacteria.¹³ Trauma from cleaning the ears with fingernails or cotton buds has been identified as the most common predisposing factor locally.¹⁴ Moreover, the two most common bacterial species isolated from the external auditory canal of normal individuals are the *Staphylococcus* species (*Staphylococcus auricularis*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Staphylococcus capitis*) and the *Corynebacterium* species (*Turicella otitidis* and *Corynebacterium auris*). The third most frequently recovered bacteria are the *Streptococci* and *Enterococci*. Together, they account for more than 90% of the normal flora in the external auditory canal.^{14,15} *Pseudomonas aeruginosa*, *S. epidermidis* and *S. aureus* are the most common pathogenic bacteria isolated in acute diffuse otitis externa locally.¹⁶ Doree and Burton,¹⁷ studied 2039 subjects with acute otitis externa and isolated similar bacterial species. Further, the same research group also identi-

Correspondence: Iffat Naz, Department of Biology, Scientific Unit, Deanship of Educational Services, Qassim University, Buraidah, 51452, Qassim, Kingdom of Saudi Arabia (KSA).

Tel.: +966 533897891

E-mail: iffatkhattak@yahoo.com ;

I.Majid@qu.edu.sa

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fied the *Microbacterium* species (*Microbacterium otitidis* and *Microbacterium alconae*) as key pathogens in acute otitis externa.¹⁷ The present research study was designed to isolate and identify pathogenic bacteria from the infected ear pus clinical samples and then, to estimate the antimicrobial activity of cerumen and antibiotics against the isolated pathogenic bacteria.

Materials and Methods

Sample collection

A total of 50 ear pus samples were collected from infected patients admitted in tertiary care hospital at district Peshawar, KPK, Pakistan, using sterile swabs under proper hygienic conditions. The samples were labeled bearing the person code, age, sex and date. Collected samples were suitably diluted and were streaked on nutrient media. After incubation, these were subjected for isolation of pathogenic bacteria using standard technique.

Cerumen collection

Earwax or cerumen samples were collected from both male and female healthy persons of approximately same age groups, using sterile ear buds and the amount of cerumen was measured in milligram (mg). Then the samples were pooled and dissolved in sodium bicarbonate-glycerol buffer (pH 8.2) at 1-5% concentration.

Isolation and identification of pathogenic bacteria

Isolation and identification of pathogenic bacteria from the collected samples were carried out using standard bacteriological procedures *i.e.* cultural characteristics, microscopy and different biochemical tests (catalase, oxidase, urease, indole, coagulase and triple sugar iron tests) according to the Berge's Manual of Determinative Bacteriology 9th Edition.

Antibiotic susceptibility assay

Antibiotic susceptibility assay was performed according to Kirby-Bauer disc diffusion assay in order to determine the efficacy of narrow and broad-spectrum antibiotics. Antibiotic selection was based on standard guidelines and 5 different commercially available antibiotics disc *i.e.* Amoxicillin, Ampicillin, Ciprofloxacin, Clindamycin and Gentamycin were used in the present study against test organisms. In antibiotic sensitivity assay, Mueller-Hinton agar plates were prepared by spreading 0.1 ml of diluted inoculum of each test bacterial isolate over media surface. After spreading, was allowed to dry for 5 min and then with the help of sterilized forceps, antibiotic discs were placed gently on the surface of bacterial lawn at equal distance. The plates were then incubated at 37°C for 24 h, after incubation the antibiotics sensitive bacteria have made clear rings or zone of inhibition around the discs, which was then measured in millimeter (mm) to evaluate the *in vitro* potency of antibiotics against test organisms.

Qualitative analysis of chemical constituents of cerumen (earwax)

Cerumen was analyzed for different chemical constituents such as flavonoids, terpenoids and phenolic compounds using standard procedure as described below.

Flavonoid determination

About 2-3 ml of aqueous extract of cerumen was taken in labelled test tubes and then 2% NaOH was added to it drop by drop. After this, concentrated HCl was added to the solution. If color of the solution was disappearing after addition of HCl, then it indicated the presence of flavonoids.¹⁸

Phenolic compounds determination

Aqueous extract of cerumen was used for determination of phenolic compounds through Ferric chloride test. Almost 1 ml of aqueous extract was mixed with 2 ml distilled water and only a few drops of 10% Ferric chloride was added to it. If blue and green color appeared after addition of Ferric chloride, then it is indication of the presence of phenolic compounds and if there was no coloration then the test was considered as negative for phenolic compounds.¹⁸

Terpenoids determination

Aqueous extract of cerumen was used for the existence of terpenoids through Salkowski test. About 2 ml of chloroform was mixed thoroughly with the sample extract solution and then 3 ml of concentrated sulfuric acid was in safety mixed to form a layer. The presence of terpenoids was shown by the appearance of reddish brown coloration of the border.¹⁸

Determination of antibacterial activity of cerumen (earwax)

The antibacterial activity of cerumen extracts against pathogenic bacterial isolates were evaluated by utilizing agar well diffusion method.

Nutrient agar plates were prepared and approximately, 50 µl inoculum of every selected pathogenic bacterial specimen were homogeneously spreaded on specified plates with the help of glass spreader. After the period of five minutes, three wells of about 8 mm diameter were bored in the media plates, one well was used for positive control (antibiotic), one well was used for negative control (distilled water) while the remaining one was used for earwax extract. The equal volume (50 µL) of controls as well as extract were poured into these wells. The plates were incubated for 24 h at 37°C. After incubation, zones of inhibition were measured in millimeter (mm) to evaluate the potency of earwax extract against test pathogenic bacterial specimens.

Results

Bacteriological analysis of ear pus samples and prevalence of isolated bacteria

In the present study, 50 ear pus samples were screened for the presence of various bacterial pathogens and 4 different pathogenic bacterial isolates *i.e.* *Pseudomonas spp.*, *Staphylococcus spp.*, *E. coli* and *Proteus spp.*, were identified and characterized by pure culturing technique as shown in Table 1. While, the data regarding the prevalence of various bacterial species is shown in Figure 1. Among the isolates, n = 29 (58%) were *Pseudomonas spp.*, which was the most prevalent bacterial species while, n = 11 (22%) samples were *Staphylococcus spp.*,

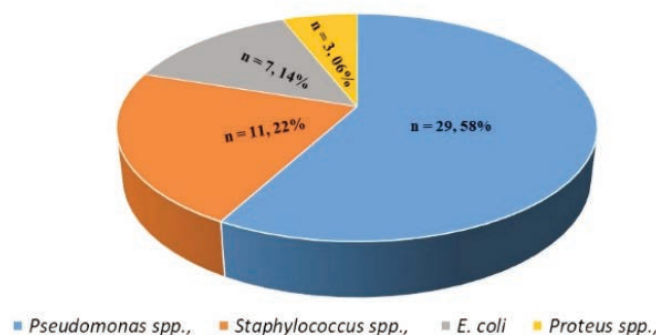


Figure 1. Percentage distribution of isolated bacteria in ear pus samples (n = 50).

Table 1. Bacteriological analysis of ear pus samples.

Isolates	Gram's Reaction	Catalase	Oxidase	Coagulase	Urease	Indole	TSI test	Identified Organisms
1	-	+	+	-	-	-	NC	<i>Pseudomonas spp.</i>
2	+	+	-	+	+	-	K/A, AG	<i>Staphylococcus spp.</i>
3	-	+	-	-	-	+	K/A, AG	<i>E. coli</i>
4	-	+	-	-	+	-	NC	<i>Proteus spp.</i>

AG = Acid and gas; + = Positive; - = Negative; A = Acid production; K = alkaline reaction; NC = No change; reduction; K/A = Red/yellow; K/NC = Red/no color change.

and $n = 7$ (14%) for *E. coli*. The least prevalent bacterial species detected was *Proteus* which was found to be 6% ($n = 3$).

Antibiotics susceptibility profile of isolated bacteria

The antibiotics susceptibility profile of isolated bacterial species was drawn by using agar disc diffusion method while percent value was calculated using CLSI zone of inhibition as standard values.¹⁹ The results regarding the antibiotic susceptibility profile of *Pseudomonas spp.*, against the antibiotics used is shown in Figure 2. The data suggested that *Pseudomonas spp.* was most sensitive to Clindamycin (94.73%) followed by Amoxicillin (82.2%), Gentamycin (76.47%) while has shown resistance to Ciprofloxacin and Ampicillin, where the potency was 33.28 and 40% respectively. The results regarding the antibiotic susceptibility profile of *Staphylococcus spp.*, against the antibiotics used is shown in Figure 3. According to results, *Staphylococcus spp.*, was most sensitive to Clindamycin (94.73%) followed by Gentamycin (94.44%), Amoxicillin (55.55%) while resistant to Ciprofloxacin and Ampicillin, and have shown potency of 31.4 and 43.3% respectively.

The results regarding the antibiotic susceptibility profile of the tested antibiotics against *E. coli* is shown in Figure 4. It was observed that *E. coli* was most sensitive to Ciprofloxacin (95.21%) followed by Clindamycin (94.11%) and Amoxicillin (92.85%) while displayed resistant to Gentamycin and Ampicillin, where the potency was 33.3 and 46.6% respectively.

The results regarding the antibiotic susceptibility profile of the tested antibiotics against *Proteus spp.*, is shown in Figure 5. According to results, *Proteus spp.*, was most sensitive to Ciprofloxacin (92.85%) followed by Clindamycin (92.11%) and Gentamycin (83.28%) while displayed resistant to Amoxicillin and Ampicillin, and demonstrated efficacy of 54.7 and 66.6% respectively.

Qualitative analysis of chemical constituents of cerumen (earwax)

Aqueous extract of cerumen was tested for qualitative analysis of chemical constituents including flavonoids, phenolic compounds and terpenoids using standard procedure and results are shown in Table 2. It was observed that aqueous extract of cerumen have contained different chemicals i.e. flavonoids and terpenoids while, phenolic compounds were absent in the extract.

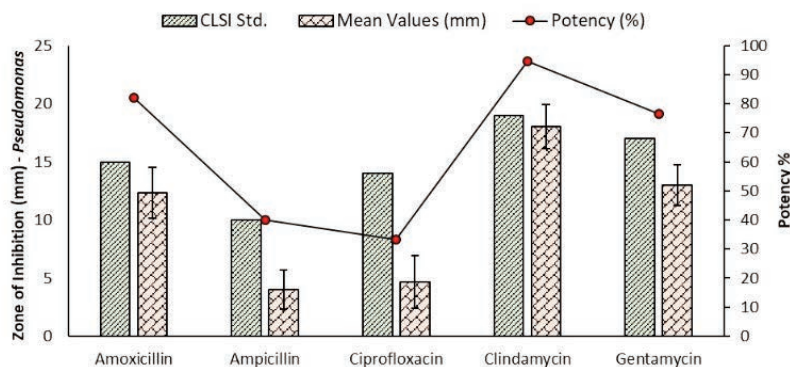


Figure 2. Antibiotics susceptibility profile against *Pseudomonas spp.*

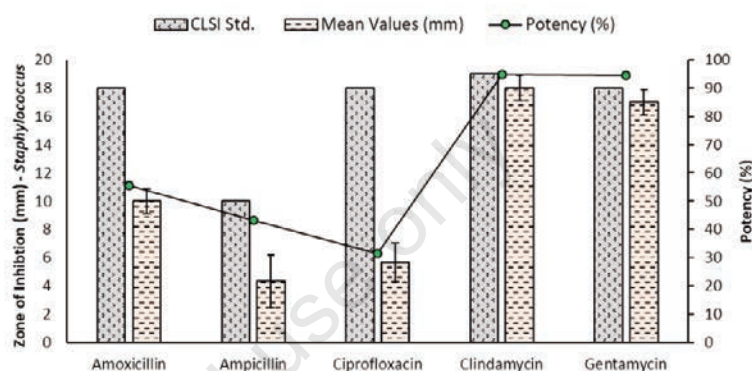


Figure 3. Antibiotics susceptibility profile against *Staphylococcus spp.*

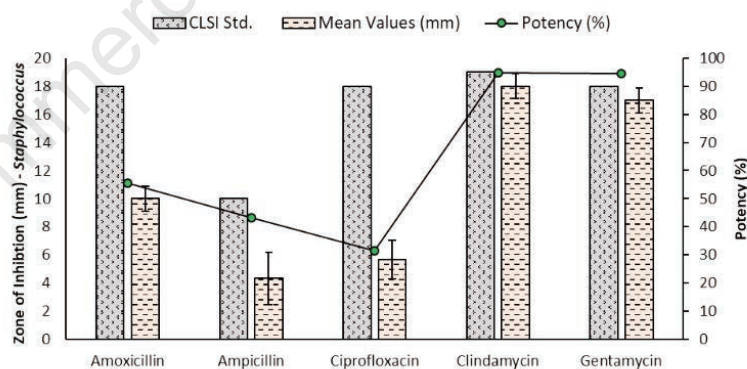


Figure 4. Antibiotics susceptibility profile against *E. coli*.

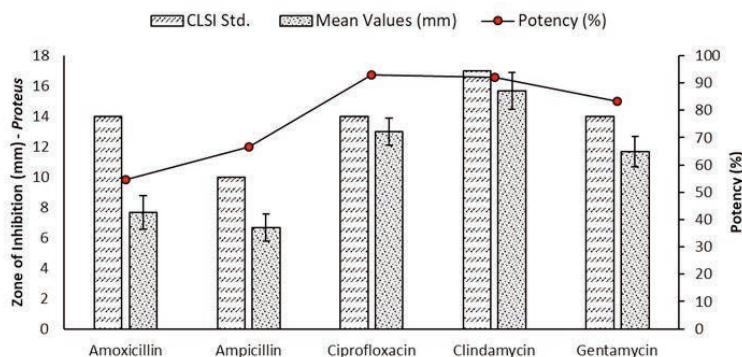


Figure 5. Antibiotics susceptibility profile against *Proteus spp.*

Determination of antibacterial activity of cerumen (earwax)

Table 3 depicted that cerumen displayed maximum zone of inhibition against *Pseudomonas* and *Staphylococcus spp.*, while it was less effective against *E. coli* and *Proteus spp.* In addition to this, Table 3 also illustrates the bactericidal effect of cerumen against test organisms. Where as 90% bactericidal activity was observed against *Pseudomonas spp.*, followed by 60% against *Staphylococcus spp.*, 36% against *E. coli* and 22% against *Proteus spp.*

Discussion

The pathogens present in ear pus samples pose a serious threat of infections which may leads to some acute and chronic infectious diseases if proper care is not taken.³ The use of antibiotics to stop and cure the infections in ear is the major mean in modern medicinal practices.¹⁰ The present research study was focused on isolation and identification of pathogenic bacteria from infected ear pus samples and then to determine the activity of commercially available antibiotics and earwax against isolated bacteria. Herein, 50 ear pus samples were collected from infected patients at district Peshawar, Pakistan using sterile swabs under proper hygienic conditions. Samples were then processed and different bacterial isolates were identified through pure culturing technique. Isolated bacteria were identified according to Bergey's Manual of Determinative Bacteriology (9th Edition). On the basis of microscopic and biochemical analyses, 04 different bacterial isolates were identified, out of which one was Gram positive (*Staphylococcus spp.*) while three were Gram negative (*Pseudomonas spp.*, *E. coli* and *Proteus spp.*). Further, the data regarding the prevalence of various bacterial species depicted that *Pseudomonas spp.*, were 58% (n = 29) among isolated bacteria followed by *Staphylococcus spp.*, (22%, n = 11), *E. coli* (14%, n = 7) and *Proteus spp.*, (6%, n = 3). Microbial infection in ear might be due to maintaining sub-standard hygienic conditions. Different researchers

also conducted similar studies and identified different pathogenic bacteria from the ear pus using conventional culturing technique and concluded that *P. aeruginosa* was the most widely recognized bacteria.^{11,20}

The antibiotics susceptibility profile for isolated bacterial species were drawn by using agar disc diffusion method while percent value was calculated using CLSI standard values.¹⁹ In the present study, 5 different commercially available antibiotics were evaluated for antimicrobial activity against the prevailing bacterial isolates with the aim to be used for the prevention of different clinical complications. The results of the experiments revealed that *Pseudomonas* and *Staphylococcus spp.*, were most sensitive to Clindamycin, Amoxicillin and Gentamycin while these displayed resistant to Ciprofloxacin and Ampicillin. Devi *et al.*²¹ also conducted similar study and found that Clindamycin, Amoxicillin and Gentamycin were more effective antibiotic against both Gram-positive and Gram-negative bacteria isolated from the infected ear pus samples. However, in the present study, *E. coli* and *Proteus spp.*, were most sensitive to Clindamycin, Amoxicillin and Ciprofloxacin while these bacteria displayed resistant to Amoxicillin and Gentamycin. Similar results were also reported by Vaghela *et al.*²² A possible reason for antibiotic resistance might be irrational use of antibiotics therefore, it was suggested that antibiotics must be used after performing susceptibility test and by doing this, the phenomenon of antibiotic resistance might be reduced to greater extent. Furthermore, Magnet *et al.*²³ also described that genetic variation among bacterial isolates was the most significant reason for antibiotic resistance and also suggested that

all these complications could be minimized up to some extent by maintaining hygienic condition in daily life.

Efficacy of antimicrobial properties of human earwax has been a subject of debate for different researchers since many years.²⁴ Researchers believed that absence of earwax mediates an alkaline environment which promotes growth of different microbes within ear and hence resulted into different infections.^{21,25} In fact, earwax has been shown to have significant antibacterial and anti-fungal properties.²⁶ Earwax is a grey brown or yellowish waxy substance secreted in the outer one third of ear canal. It is a protective barrier between the external environment and deep external auditory canal.¹ Table 3 depicted that cerumen displayed maximum zone of inhibition against *Pseudomonas* and *Staphylococcus spp.*, while it was less effective against *E. coli* and *Proteus spp.*

The current research investigation demonstrated that cerumen has antibacterial properties, which played significant role in the protection of the external auditory canal. Interestingly, Schwaab *et al.*²⁷ proved that cerumen of some mammals possess anti-*Staphylococcal* and anti-*Micrococcal* activities. In the current study, it was observed that for *Pseudomonas spp.*, cerumen showed 90% of activity, which was equivalent to Clindamycin (94% effect), for *Staphylococcus spp.*, cerumen showed 60% of activity, which was approximately equivalent to Amoxicillin (56% effect), for *E. coli* cerumen showed 36% of activity, which was equivalent to Gentamycin (33%) while *Proteus spp.*, displayed resistant pattern against cerumen (22% of activity). Our findings on the bactericidal effect of cerumen on *Pseudomonas* were in alignment

Table 2. Qualitative analysis of chemical constituents of cerumen (earwax)

Components	Aqueous Extract Cerumen (Earwax)
Flavonoids	+++
Phenolic compounds	-
Terpenoids	++

- = absent; + = little bit color; ++ = Intermediate; +++ = Strong

Table 3. Bactericidal activity of cerumen (earwax) against test organisms.

Bacterial Isolate	Conc. of cerumen (%)	Diameter of disc (D) (mm)	Diameter of zone (d) (mm)	Zone of inhibition (ZI = D-d)	Potency %
<i>Pseudomonas spp.</i>	3	8	32	24	90
<i>Staphylococcus spp.</i>	3	8	24	16	60
<i>E. coli</i>	3	8	14	6	36
<i>Proteus spp.</i>	3	8	12	4	22

with the findings of Swain *et al.*²⁸ However, some researchers found insignificant bactericidal effect and stated that *Pseudomonas* was not a normal commensal of the ear canal, and thus might not be recognized by the immune system of the ear canal.^{29,30} Yang *et al.*³¹ reported inconsistent bactericidal activity of cerumen against *S. aureus*. Different researchers reported significant bactericidal activity of human cerumen to almost coincide with the results of current study.^{25,26} However, other studies have reported a lack of bactericidal effect of cerumen on *P. aeruginosa*.³²

In addition to this, bactericidal activity of cerumen might be due to the presence of potential chemicals *i.e.* flavonoids and terpenoids in the aqueous extract of cerumen (earwax). According to Harith *et al.*¹⁸, important chemicals that were vital in combating bacterial isolates were terpenoids and flavonoids because of high affinity of these constituents to aqueous and organic solvents. In addition, these secondary metabolites also possess multiple biological and therapeutic activities such as treatment of neurodegenerative diseases, vasodilatory action, antimicrobial, antibacterial, antiviral and anti-inflammatory.³³

Conclusions

In the current study, different bacterial isolates were identified in ear pus samples using conventional culturing techniques. It was concluded that *Pseudomonas spp.*, (58%) was the most prominent and prevalent bacteria identified and isolated from all ear infected clinical samples and followed by *Staphylococcus spp.*, (22%), *E. coli* (14%) and *Proteus spp.*, (6%). From bioassay, it was concluded that *Pseudomonas* and *Staphylococcus* were most sensitive to Clindamycin, Amoxicillin and Gentamycin while have shown resistance to Ciprofloxacin and Ampicillin. On the other hand, *E. coli* and *Proteus spp.*, were most sensitive to Clindamycin, Amoxicillin and Ciprofloxacin while these bacteria were resistant to Amoxicillin and Gentamycin antibiotics. The other part of the present research tries to put forward that apart from being a physical barrier, cerumen also acts as protective coating over the external auditory canal due to its antibacterial properties. In the current study, it was concluded that cerumen displayed strong antibacterial activity against *Pseudomonas spp.*, (90% potency) and *Staphylococcus spp.*, (60% potency) which might be due to the presence of potential chemicals *i.e.* flavonoids and terpenoids. Hence, it has been recommended that routine wax removal or ear cleaning is not mandatory unless impacted

wax is leading to earache or conductive hearing loss. Furthermore, proper care and preventive measures should be taken for patients with ear infections in order to minimize the chances of infections.

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