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ANTIBACTERIAL ACTIVITY OF HUMAN EARWAX AGAINST SELECTED PATHOGENIC BACTERIA

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Abstract

The human ear produces a waxy substance which is commonly known as earwax or "Cerumen". The earwax is formed in the outer ear canal. Present study was done antibacterial Activity of Human Earwax against Selected Pathogenic Bacteria. Earwax is a natural product of the ear, which acts as a natural protector of ear against various infection, water and small particles that may be harmful, dust, and even insects. It is made by the secretion of specialized sets of glands located in the ear canal, yellowish brown waxy substances. The earwax buffers readily the bacteria at different concentration. Freshly collected earwax suspended at a concentration of 3% glycerol and $NaHCo_3$ buffer (pH 8.2) showed antibacterial activity against some pathogenic bacteria. The antibacterial activity of earwax was evaluated against three bacterial pathogens by using agar well diffusion method. The organisms were tested for susceptibility against human earwax. On the basis of results it is concluded that human earwax was found to be more effective against *P. aeruginosa, S. aureus* and least was shown against *E. coli.* The present study revealed that the human earwax could be remedial antibiotics, particularly for controlling bacterial pathogens.

***Keywords*:** Antibacterial activity, Human earwax, *P. aeruginosa, S. aureus* and *E. coli*.

Introduction

The human ear produces a waxy substance which is commonly known as earwax or "Cerumen". The earwax is formed in the outer ear canal. The formation of earwax in the ear canal is situated between the fleshy part of the ear on the outside of our head and the middle ear. The colour of the earwax when it is formed in the ear canal is greyish-brown to greyish black, yellowish-brown to dark brown respectively. Cerumen is secreted from both ceruminous gland and sebaceous gland. Sebaceous glands secrete oily substances called "sebum". Sebum consist fat (lipids) and the dermis of dead fat producing cells. Earlier, it was believed that sebum protects and helps in waterproofing of hair and skin from being dry, brittle and cracked. The Sebaceous gland are found in hair-covered areas of the body where ducts open into hair follicles, deposit sebum to the hairs, and bring it to the skin surface along the hair shaft. This occurs in the outer portion of the ear canal where ear hairs are present. In other locations of the body without hair, sebum is brought to the skin surface pores in the skin. In the gland, sebum is produced within specialized cells and it released when these cells bust.

Ceruminous gland includes exocrine glands belonging to a category called sudoriferous or sweet glands, and these are two types (a) eccrine (b) apocrine. Exocrine sweet glands are smaller, not as deep and exit their secretions in pores of the skin, but these glands cover most of the body except ear canal. Apocrine sweet glands are modified sudoriferous glands. This are found larger, deeper and exit a thicker secretion along the surface of a hair shaft. This thicker secretion is called cerumen. According to the authors, it was reported that cerumen is determine through the thin layer chromatography. Cerumen contains squalene (6.4%), cholesterol esters (9.6%), wax esters (9.3%), triacylglycerols (3%), fatty acids (22.7%), cholesterol (20.9%), ceramides (18.6%), cholesterol sulphate (2%) and several unidentified polar components (7.5%) (Jeffrey *et al*., 1990). It can also contain amino acids, neurostearic acid, cerotic acid, triglyceride, hexone bases, immunoglobin, glycopeptide, copper and other compounds (Chai and chai, 1980).

Earwax have several important role such as: 1) Lubrication of the ear canal to prevent itchiness, drying and burning of skin it is because of the high lipid content of the sebum produced by the sebacous gland and reduces the incidence of tiny cracks that could trap the bacteria and allow an infection to flourish, 2) It can also help to maintain the acidic environment of the ear canal. This acidity kills fungi and bacteria and then shielding the ear from infection, 3) Finally cerumen help to keep the ear canal, serving as a self-cleaning agent, protecting by trapping and preventing objects from getting inside of ear and creating damage to the deep inner structures of the ear canal.While in other studies conducted up to 1960s, it was found that little evidance for supporting an antimicrobial role of cerumen, after more studies found that cerumen has a bactericidal effect on some strains of bacteria. Cerumen have been found to be effective in

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reducing the viability of a wide range of bacteria, including *Staphylococcus aureus, Pseudomonas aeruginosa,* and many variants of *E. coli.* These antimicrobial properties are due to the presence of saturated fatty acids, lysozymes and also to the relatively low pH (6.1) of cerumen (Okuda *et al.,* 1991). The aim of the study is show that earwax has rich levels of nutrients which aid in microbial growth rather than inhibit it. Earwax contains antimicrobial property which prevents external ear from infection and also it leads to kills certain foreign organisms which enter the ear canal. Thus, the present study was conducted to determine the antibacterial activity of human earwax (suspension) against selected human pathogens.

Materials and Methods

Human earwax was collected from different healthy people.Test pathogens were procured from the Microbial Culture Collection Bank, Department of Industrial Microbiology, JIBB, SHUATS, Prayagraj.

Preparation of Cerumen solution

The samples were collected in sterilized bottle using sterilized swabs. Then the amount of human earwax was measured to be 3g. Glycerol-sodium bicarbonate buffer solution was prepared 10ml of this buffer solution was taken in 5 test tube. Earwax samples were dissolved in this buffer solution to obtain its different concentration (0.2g/ml, 0.4g/ml 0.6g/ml, 0.8g/ml and 1g/ml) as per the standard. Stored the earwax Suspension in sterile test tube.

Preparation of Bacterial suspension

Nutrient broth was prepared, transferred into test tubes, autoclaved it. Inoculums were picked from slants *i.e. Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa* and inoculated in nutrient broth. This process held in laminar air flow. Then all broth culture incubated for 24 hr at 37°C and observed the growth.

Determine the antibacterial activity

To determine the different concentrations for the antibacterial activity of human earwax suspension against selected bacterial pathogens, which was conducted by well diffusion method. Nutrient agar poured into sterilised Petri-plates. After solidified the agar plates were labelled as corresponding to the cerumen concentrations. Using sterile cotton swabs, swabbing was done with fresh bacterial cultures on the surface of agar plates**.** Sterile the Cork borer with the help of alcohol and 5mm wells were cut in agar plates. With the help of a 100µl Micropipette transferred 50µl of earwax suspension in the first well of the agar plate and 50µl buffer (glycerol and $NaHCo_3$) as control in the second well. Earwax suspension transferred in each well according to different concentration. The procedure was repeated in all the 10 plates and two agar plates ware kept for control as organism control and media control. All the plates were incubated at 37°C for 24 hours. Zone of inhibition was measured in mm.

Results and Discussion

Up to 1960s, it was reported that little evidence for supporting an antibacterial role for cerumen, but more recent studies found that cerumen has a bactericidal effect on some strains of bacteria. Earwax is normally produced in the ear canal. It is made by the secretions of specialized sets of glands located in the ear canal. The wax produced forms a physiological barrier between the external environment and deep external auditory canal (Yassin *et al*., 1996). The findings of the present study revealed that human earwax shown antibacterial property against tested selected pathogenic bacteria. Currently it is reported that earwax is introduced for the detection of some psychotropic and antipileptic drugs (EngyShokey *et al.,* 2017). As a result the antibacterial activity of earwax was screened against the selected bacterial pathogens.

Antibacterial activity of human earwax

In the present study, antibacterial activity of earwax was observed against different pathogenic bacteria at different concentration. The study showed that antibacterial activity of human earwax was found to be effective against all the pathogenic bacteria. Human earwax was found to be more effective against *P. aeruginosa* at different concentrations level *P. aeruginosa* (0>18>18.5>21>25), *S. aureus* (0>10>11.5>16>18.5) and while least shown by *E. coli* (0>10>11>13>16) (Figure 1, 2, 3, 4 and 5).

In our study the cerumen inhibited the growth of bacteria at different concentration. The study demonstrated that cerumen has antibacterial properties, which plays important role in the protection of the external auditory canal. Many of authors proved that the cerumen of some mammals posses antistaphlococcal, antimicrococcal and antiherpes activities (Sokolov *et al.,* 1995). The bactericidal effect of cerumen on *S. aureus* and *E. coli* (Stone and fulghum, 1984), the antibacterial cerumen effect the growth of *S. aureus, P. aeruginosa* and *E. coli* (Lum *et al.,* 2009). On comparing the result of antibacterial activity of cerumen, we observed more effective against *P. aeruginosa, S. aureus* and least was shown in *E. coli.* The lowest concentrations of the human earwax that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism were recorded as the MIC value of the extracts. The MIC values of human earwax for different pathogenic bacteria ranged from 1000ug/ml respectively were prepared against different bacteria (*P. aeruginosa, S. aureus* and *E. coli*).

Bactericidal effect of cerumen was analysed for their antibacterial activity against *P. aeruginosa, S. aureus* and *E. coli* at different concentration by agar well diffusion method (Table 1). It was observed that microbial growth of different

microbes was independent bacteria for human earwax taking different concentration. The minimum inhibitory concentration (MIC) was determined as the lowest concentration inhibited the visible growth of *P. aeruginosa, S. aureus* and *E. coli* with the MIC (50µg/ml) bacteria for human earwax. *P. aeruginosa, S. aureus* lowest concentration of bacteria for human earwax inhibited the visible growth of *P. aeruginosa, S. aureus* and *E. coli* with the MIC (25µg/ml) for bacteria. These results indicated that the MIC of human earwax. The lowest concentration retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism recorded as the MIC values.

Conclusion

In this study, Human earwax was collected from different healthy people. The suspensions were prepared by dissolving human earwax in Glycerol and Sodium bicarbonate pH (8.2). Antibacterial activity of the earwax was evaluated against three human pathogens (*Staphylococcus aureus, E. coli, Pseudomonas aeruginosa*) by agar well diffusion method. On the basis of the given study following observations were made and conclusions drawn: (a) The human earwax showed more antibacterial activity against *Pseudomonas aeruginosa* as compared to *Staphylococcus aureus* and *E. Coli* (b) The solvent buffer showed no effect on the viability of the strain tested after incubation of 24-48 hr. In our study the earwax inhibited the growth of bacteria at different concentrations. The study demonstrated that human earwax has antibacterial activity, which plays a role in the protection of the external auditory canal and our result agree with Lum *et al*. (2009); Chai and Chai (1980) who demonstrated the bactericidal effect of earwax on two stains of *Pseudomonas aeruginosa.* Hence, it is concluded that this study on human earwax would lead to the establishment of some valuable compound that can be used to formulate new and more potent antimicrobial drugs of natural origin. Further studies are needed to identify the presence of antimicrobial peptides in human earwax which may lead to development of new concepts for the local treatment of external auditory canal diseases in the future.

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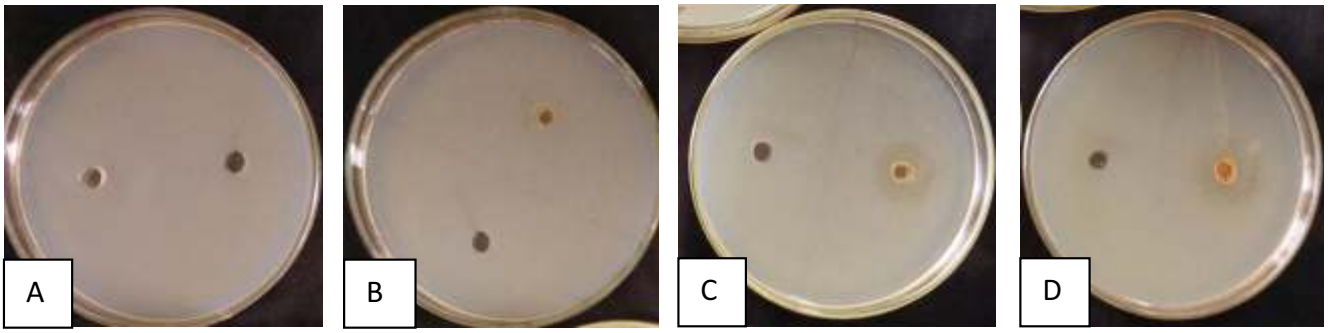

Figure 1: *S. aureus* (A- 0.4 mg/ml, B- 0.6 mg/ml, C- 0.8 mg/ml, D- 1 mg/ml)

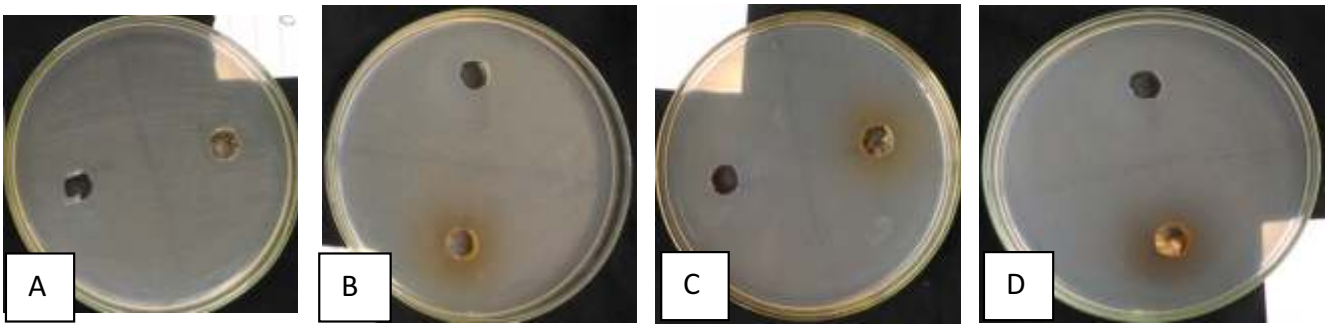

Figure 2: *P. aeruginosa* (A- 0.4 mg/ml, B- 0.6 mg/ml, C- 0.8 mg/ml, D- 1 mg/ml)

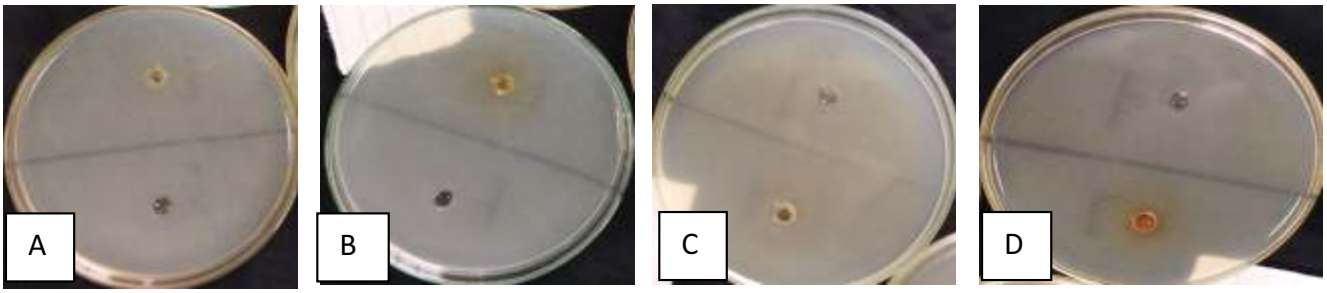

Figure 3: *E. coli* (A- 0.4 mg/ml, B- 0.6 mg/ml, C- 0.8 mg/ml, D- 1 mg/ml)

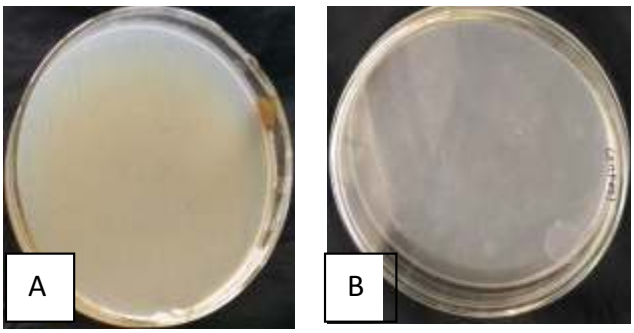

Figure 4: A-Organism control, B- Control

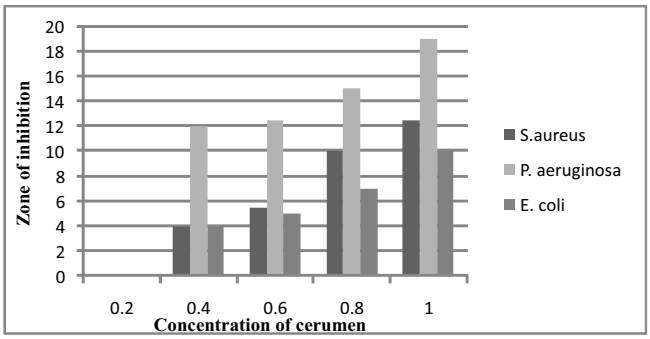

Figure 5: Antibacterial activity of human earwax against test organism

Table 1: MIC of human earwax against bacteria *P. aeruginosa, S. aureus* and *E. coli*

Concentration (µg/ml)	Human earwax against bacteria		
	P. aeruginosa	***S. aureus***	***E. coli***
1000	-	-	+
333	-	*	+
111	*	+	+
37	+	+	+
12	+	+	+
MIC	+	+	+