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Antimicrobial Screening of Fruit Extracts Against Microflora Isolated from Bluetooth Ear Buds

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Abstract: **Bluetooth accessories like earbuds, headphones, neckbands, and more have the potential to be a vehicle for the spread of dangerous pathogenic bacteria and fungi. The current study investigated the microbial diversity linked to Bluetooth devices along with antibiotic susceptibility against various fruit extracts viz. litchi, apple (*Malus domestica*), guava (*Psidium guajava*), pomegranate (*Punica granatum*), and the mango (*Magnifera indica*). The study aimed to investigate microbial growth associated with earbuds and headphones used by young people of Village Bakharwa Modinagar [GZB]. *Staphylococcus aureus, Aspergillus niger, Aspergillus fumigatus, Bacillus species, and Clostridium species*, were tentatively identified on basis of biochemical and morphological characteristics. Plant chemical biodiversity is a valuable potential resource of antimicrobials. With this aim five fruit extracts were investigated in the study and the findings showed strong antimicrobial activities can be found in mango (*Magnifera indica*) (12mm±0.002mm), guava (*Psidium guajava*) (15mm±0.002mm), pomegranate (*Punica granatum*) (10mm±0.002mm), litchi (*Litchi Chinensis)* (9mm±0.002mm) and apple (*Malus Domestica*) (13mm±0.002mm). The present investigation may provide a promising subject for further in vitro and in vivo studies to develop a potent safe disinfecting spray for ear buds.**

Keywords: **Bluetooth devices, bacteria, medicinal plants, antimicrobial activity.**

1. Introduction

Mobile phones are now considered essential and integrated tools at workplaces. The smart phones serve a wide range of applications including calculators, information, epidemiology, entertainment, recording data and news. The use of earbuds among young adults and students is on the rise, as they can be worn when you are working your hands are not free. These earphones if not cleaned regularly can be colonized by bacteria and fungi which are normal inhabitant from skin of ear, head and some environmental contaminants. Combined with the fact that cleaning and disinfection is not a common practice The debris get accumulated on ear phones and they serve as nourishment for microbes. When these microbes outnumber a permissible limit, they cause otitis externa. *Bacillus spp., vibrio, Staphylococci,* were the bacteria isolated from the earbuds, *while Aspergillus spp., Rhizopus spp*. were the fungi isolated. The use of earbuds or headphones has been suggested as a possible factor for external ear canal infection since they can increase the temperature and humidity of the canal, create the potential for skin abrasion, and provide a vehicle for the introduction of organisms into the canal skin. Studies have shown that continuous use of earphones can cause damage to the ear canal, resulting in ear pain and hearing loss.

Plants have an incredible ability to produce an ample variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins [3]. These biomolecules are the foundation of plant-derived antimicrobial substances (PDAms) [2]. Some natural products are highly proficient in the treatment of bacterial infections [1]. There are numerous plants and natural products which have antibacterial, antifungal, and antiprotozoal effect that could be used either systemically or locally. Medicinal properties of plants have also been preferred throughout the world, due to their potent pharmacological activities, low toxicity, and economic viability, when compared with synthetic drugs. In India, there are about 45,000 plant species that have reported medicinal properties. This large number of plants offers a varied number of phytochemicals which can be screened as natural antimicrobial agents to solve the problem of rising antimicrobial resistance.

Guava (*Pisidium guajava* L.) is a fruit plant belonging to the family *Myttacease*, guava leaves, roots, and used for prevention and treatment of diarrhea guava also showed a significant antibacterial activity against food borne diarrhea causing bacteria which are *Staphylococcus* species, *Shigella* species. and *Pseudomonas* species. Guava is also used as anti-inflammatory and antiseptic as well as treatment of diabetes, hypertension, pain fever, respiratory disorders, gastroenteritis, diarrhea and dysentery.

Pomegranate (*Punica granatum)* is an Punica that is a member of the *Lythraceae* family. Punica granatum (pomegranate) is one of the Mediterranean medicinal plants that has been used for generations in treating ulcers, diarrhea, and male infertility. Pomegranate are rich in antioxidants and flavonoids, both of which are known to prevent free radicals from damaging your cells.

Mango (*Mangifera Indica*) this plant, which is also known as Aamra is a member of the mangifera family of (Anacardiaceae).

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This fruit has been used in the Ayurevedic system for medicine. Various parts of plant are used as a dentrifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tinic, laxative and diuretic and to treat diarrhea, dysentery, anaemia, asthma cough, hypertension and piles.

Apple (*Malus domestica*) belongs to rosaceae. There is now considerable scientific evidence that these bioactive substances present in apple and peel have the potential to improve human health, for example contributing to preventing cardiovascular disease, diabetes, inflammation, and cancer.

Lychee (*Litchi chinensis*) belongs to the Sapindaceae family, lychee leaves, roots, and used for prevention and treatment of reduce swelling and reduce pain. It might also stimulate the immune system and work as an antioxidant. People use lychee for cough, fever, pain and other conditions.

The present work designed to investigate the antimicrobial effects of of Methanol, Acetone, N-hexane and Ethanol extracts of five fruit plants Mangifera Indica [mango], Psidium guajava [guava], Punica granatum [pomegranate], Malus domestica [apple against several microbial strains which are associated with earbuds and headphone used among youngsters of Village Bakharwa Modinagar [GZB] so as to explore natural antimicrobial agents to disinfect earbuds so as to maintain their cleanliness and keep them microbes free.

2. Materials and Methods

1) Collection of Sample

Samples were collected aseptically with sterile swabs moistened with sterile normal saline and by rolling over the exposed surfaces of the earpiece. One maximum care was taken to ensure that all the areas must be swabbed.

2) Sample inoculation

After collection, the samples were immediately transported to the laboratory, serially diluted and third and fourth dilution is inoculated on Nutrient Agar and Mac-Conkey's agar, Mannitol Salt agar and Potato dextrose agar and plates were incubated aerobically at 37°C for 24 hours. After incubation, plates were examined for growth and colonial morphology of the isolates. Gram-positive and Gram-negative bacteria were identified as per standard microbiological procedures.

3) Plant material

In the investigation, the whole plant of Guava *(Psidium guajava*), Mangifera Indica[mango], Psidium guajava [guava], Punica granatum [pomegranate], Malus domestica [apple] fruits were collected from field market of Modinagar. The fruits are air dried at room temperature at 20 °C for 5 days. Two hundred and fifty grams of dried powdered sample was extracted by one liter of ethanol 70% at 30 °C for 48 h and filtering through Whatman No. 4 filter paper.

The plants were extracted by rotatory evaporation at 50 °C till complete dryness occurs. The total extract was dissolved in water in a concentration of 500 mg/ml and stored at −20 °C for further use.

4) Antimicrobial susceptibility test

The well-diffusion method was employed for assessment of antimicrobial susceptibility. To check for antibacterial activity, the plant extracts were tested on Mueller Hinton II plates. 5 mm diameter wells with a sterile borer were drilled into the media before the plates were streaked with bacteria. A sterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level to remove any excess inoculum before applying the test bacterium to all plates, which had already been adjusted to the 0.5 McFarland standard solution. To achieve a uniform distribution of the inoculum, the agar plate's surface was streaked across the entire sterile agar surface before being swabbed one more around the rim. The excess moisture on the plates is allowed to evaporate for 3 to 5 minutes.

Following the bacterial inoculation of the plates, 70uL aliquots of each test extract were poured into each well. Three plates were used for each extract to select a particular bacterium, with the identical extract being applied to each plate. Controls were kept for each bacterial strain in which the extract was replaced with pure solvents. The plates are tagged, parafilm-sealed, and placed in an incubator preheated to 37°C. Each plate was checked for inhibitory zones after a 24-hour incubation period. The millimetre measurements of the inhibitory zones were taken with a ruler. The outcomes were the average of at least three separate trials that were all conducted in parallel.

5) Minimal inhibitory concentration (MIC)

In dilution technique, two-fold serial dilutions of the extracts were prepared in concentrations ranging from 625 to 10,000 µg/mI. From each dilution of each extract, one milliliter of was mixed with 9 ml of Muller Hinton Agar. Ten microliter of each standardized broth cultures (1.5×10^8 CFU/ml) was cultivated on the surface of the plates containing various concentrations of the extracts. The plates were then incubated according to growth requirement of each organism and observed for any visible bacterial growth. MIC was the lowest concentration of extract that resulted in no visible growth on the surface of the agar.

6) Minimum bactericidal concentration (MBC)

Blocks of agar plates showing no growth after MIC tests were inoculate to fresh nutrient broth (acting as the recovery medium) for the determination of the MBC. The broths were incubated according to growth requirement of each organism. The absence of turbidity in the recovery medium was evidence of bactericidal activity.

7) Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by two-tailed *t*-test after Bonferroni error correction of the predictive value. *P* values less than 0.05 were considered statistically significant. Microsoft Excel 2010 statistical package was used for all analyses.

3. Results and Discussion

From 60 specimens collected, 55 pure bacterial culture have been obtained. The distribution of bacterial isolates from the ear buds was as follows: 10 (25%) Gram positive cocci (GPC) *(Staphylococcus* spp., *Streptococcus* spp.) and 25 (75%)

Gram-positive bacilli (GPB) (Fig. 1). The detected organisms were 15 (32.5%) Gram negative bacilli (GNB) (*Pseudomonas aeruginosa)* 5(9%) Gram negative cocci as shown in fig. 1.

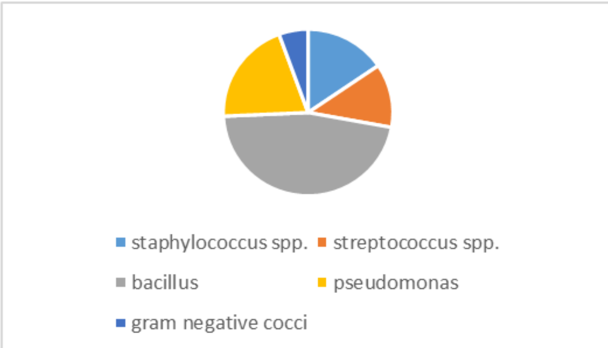

Fig. 1. The distribution of bacterial isolates from the ear buds

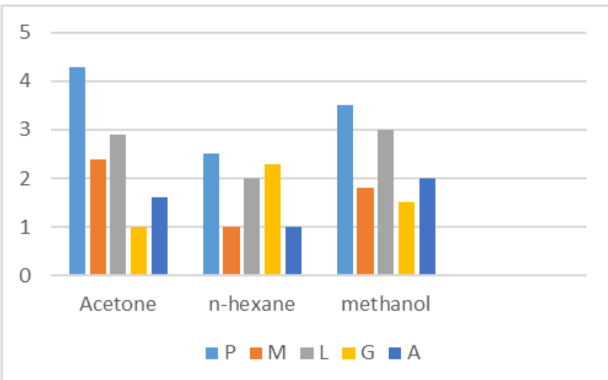

Fig. 2. Antimicrobial activity (in mm) of plant extracts against tested bacterial isolates

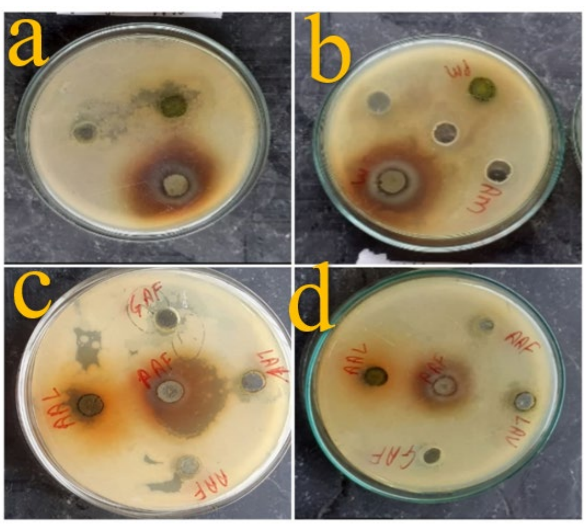

Fig. 3. Antimicrobial activity of plant extracts (a) Pomegranate, (b) Mango, (c) Litchi, (d) Guava against tested bacterial isolates

Methanol, n-hexane extracts represented good activity against most of the isolates while the acetone extract of plants showed a lesser activity against nearly all of the isolates. Table 1 shows the MIC and MBC of 5 isolates for Methanol, n-hexane extracts of *Mangifera indica, Psidium guajava, Punica granatum, Malus domestica*. High MIC value was recorded for *Staphylococcus* spp. with 10mg/ml to 20mg/ml and *Streptococcus* spp. with 16 to mg/ml. On the other hand, lower MIC values were observed for Gram-positive bacilli with 7.5mg/ml to 15mg/ml respectively. The extracts showed the same bacteriostatic activity against Pseudomonas in the range of 13mg/mL to 26 mg/ml. The MBC of the extracts presented 20mg/ml to 40 mg/ml against gram negative cocci respectively.

Fig. 4. Antimicrobial activity of plant extracts. Apple against tested bacterial isolates

Table 1
MIC and MBC of fruit extracts against Staphylococcus spp.

	Fruit	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus spp.	Pomegranate	10	20
	Mango	25	50
	Litchi	15.6	33.7
	Guava	21	41
	Apple	15	30

Table 2
MIC and MBC of fruit extracts against Streptococcus spp.

	Fruit	MIC (mg/ml)	MBC (mg/ml)
Streptococcus spp.	Pomegranate	16.5	33
	Mango	10	20
	Litchi	20	40
	Guava	25	50
	Apple	25	20

Table 3
MIC and MBC of fruit extracts against Bacillus

	Fruit	MIC (mg/ml)	MBC (mg/ml)
Bacillus	Pomegranate	7.5	15
	Mango	10	20
	Litchi	13.5	27
	Guava	6.5	13
	Apple	18	35

Table 4
MIC and MBC of fruit extracts against Pseudomonas

	Fruit	MIC (mg/ml)	MBC (mg/ml)
Pseudomonas	Pomegranate	13	26
	Mango	50	60
	Litchi	30	50
	Guava	20	40
	Apple	19.8	39

Table 5
MIC and MBC of fruit extracts against Gram negative cocci

	Fruit	MIC (mg/ml)	MBC (mg/ml)
Gram negative cocci	Pomegranate	20	40
	Mango	25.5	51
	Litchi	28	56
	Guava	32	64
	Apple	21.4	42.8

Utmost Gram-positive bacteria are girdled by a coarse peptidoglycan cell wall. This structure, although mechanically strong, appears to offer little resistance to the prolixity of small motes similar as antibiotics. Gram-negative bacteria, compass themselves with alternate membrane, the external membrane, which functions as an effective hedge. The minimum inhibitory attention (MIC) and minimal bactericidal attention (MBC) of the excerpts against the five bacteria ranged from 10,000 to 20,000µglmI.as shown in table 1, 2, 3, 4, 5 and demonstrated in fig. 3, 4.

Phytochemical analysis revealed several classes of secondary metabolites similar as alkaloids, polyphenols, flavonoids, anthraguinones, coumarins, saponins, tannins, triterpenes and steroids. Differences were observed in the antibacterial conditioning of the extracts. These could be due to the differences in their chemical composition as well as in the medium of action of their bioactive ingredients. All the extracts are rich in secondary metabolites. The antibacterial effect for tannins attributed to its capability to reply with proteins to form stable water- undoable factors since bacterial cell wall made from proteins. In addition, it may bind to proline – rich protein and intrude with the protein conflation, also the antimicrobial action of saponins rendered leakage of proteins and certain of alkaloids due to its capability to intercalate with DNA of both Gram positive and negative bacteria and intrude with cell division, while flavonoids exertion is due to their capability to bind with intracellular and answerable proteins and to bind with bacterial cell walls and the antibacterial parcels of steroids are due to complex with membrane lipids and exerts its action by causing leakage of cellular contents.[3]. In this study the pomegranate showed antimicrobial activity at different concentrations to inhibit the growth of different microbes, identified in the study. these findings are in accordance with the findings of [11], who studied guava excerpts against food born pathogen and corruption bacteria that due to the phenolic factors which make them effective against the tested microorganisms. This result verified by [3] and these compliances matched also with that of the findings of [11], [15] who showed the antibacterial exertion of guava splint excerpts grounded on how the phenolic factors act particularly flavonoids. These compliances also agreed with [6] who displayed the antibacterial exertion of guava against food borne pathogens. This exertion is attributed to live tannins and leucocyanidin, also due other active factors like saponins, flavonoids, steroids, glycosides. This result agreed with results recorded by [8], [10]. Who recorded the seed extracts was effective only against three bacterial strains.

The study of the plant extracts found phytochemicals, which are known to have physiological and medicinal effects. For instance, tannins are polyphenolic substances that bind to proline-rich proteins and prevent protein synthesis while also possessing antimicrobial properties. According to considerable research, plants create hydroxylated polyphenolic chemicals called flavonoids in response to microbial infections, and these compounds have been shown to have antimicrobial activity in vitro against a variety of bacteria. Their power has been linked to their capacity to assemble complexes with bacterial cell walls, soluble extracellular proteins, and both. Although terpenoids are mostly employed for their fragrant properties, researchers have discovered that they may also be effective antibacterial agents. Glycosides called saponins have been discovered to have inhibitory effects on S. aureus, a gram-positive bacterium. As a consequence, the phytochemical characteristics showed that the methanol, ethanol, and acetone extract include chemical components that have been discovered to have antibacterial activities, which may have contributed to the findings from the antibacterial study.

4. Conclusion

The present investigation reveals the antimicrobial activity by using pomegranate, apple, litchi, guava and mango fruit extract. The five above-mentioned fruit extracts have an important antimicrobial exertion that inhibits the growth of five microbial populations of different bacterial orders; so, these plant extracts can be used as antiseptic or disinfectant after future investigations to conserve human health.

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