Antimicrobial activity of banana peel (*Musa paradisiaca* L.) in different solvent extracts

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Abstract
Banana peel is an outermost part of banana fruit and it is considered as waste material. It has been extensively studied that banana peel consists novel ingredients. The authentication of biological properties of these ingredients is beneficial for commercial applications of banana peel. This study was performed to investigate the qualitative analysis of phytochemical and antimicrobial activity of banana peel. Qualitative analysis of phytochemical in different solvent extracts was carried out by different tests and antimicrobial activity was performed by agar well diffusion method. Alkaloids, glycosides, tannins, phenols, saponins, flavonoids and terpenoids were observed in extracts prepared in polar and non-polar solvents. Most of the phytochemicals were present in ethanol extract of banana peel. Aqueous, methanol, ethanol and ethyl acetate extracts exhibited antimicrobial activity against *B. subtilis E. coli S. aureus* and *S. typhi.* Among all solvent extracts, ethanol extract exhibited prominent antimicrobial activity against all studied strains while ethyl acetate extract showed lower antimicrobial activity. Ethanol extract exhibited the highest activity (Inhibition zone 22 ± 0.1) against *S. aureus* while ethyl acetate extract showed the lowest activity (Inhibition zone 6 ± 0.2) against *B. subtilis*. Chloroform and petroleum ether extract not exhibited antimicrobial activity. The phytochemicals of banana peel could be utilized as an alternative source of antimicrobial agent.

Key words: *M. paradisiaca* (Banana peel), *B. subtilis E. coli S. aureus, S. typhi,* Flavonoids.

1. Introduction

Banana *(Musa paradisiaca* L.) is a tropical fruit plant belongs to *Musaceae* family and it is most popular fruit cultivated in many countries of the world [1]. Different parts of banana such as pulp, flower, stem, and leaves have medicinal properties [2]. Banana peel is considered as waste product of banana and it is used various industrial applications including pulp and paper, bio-fuel production, cosmetics, bio-sorbents, organic fertilizer, energy related activities, environmental clean-up and biotechnology related processes [3, 4]. It consisting bioactive compound such as tannins, flavonoids, alkaloids, phlobatannins, glycosides, and terpenoids. These bioactive compounds exhibit various biological activities such as antidiabetic, antioxidant, anti-inflammatory and antibiotic [5]. Unripe banana fruits are used for treatment of dysentery and diarrhea disease [6]. Microorganisms have been identified as serious mediators to cause food spoilage or food borne diseases [7]. Chemically synthesized preservatives have been utilizing to control food poisoning pathogens and food spoilage but they exert negative influence like hazardous to human health, deposition of chemical residues in food, gaining of microbial resistance against used chemicals and unpleasant side effects on human health [8, 9]. In view of this, the searching of preservatives from natural sources is a better option to develop effective and health safer food preservatives. In this context plant extracts could be utilized as antimicrobial agents for prevention of food preservation [10, 11]. Some studies have been investigated that banana peel exhibit antimicrobial and antifungal activities [12, 13]. In comparison of these studies, we have evaluated the antimicrobial activity in banana peel against pathogenic bacteria. The assessment of antimicrobial activity in different solvent extracts of banana peel against microorganisms was performed by using agar well diffusion method.

2. Materials & Methods

2.1 Chemicals and reagents

Mayer's reagent, Fehiling's solution, ammonia, ferric chloride, sodium hydroxide, sulphuric acid, chloroform, petroleum ether, ethyl acetate, ethanol, methanol, agar, yeast and peptone were purchased from HiMedia.

2.2 Preparation of Sample

Riped bananas (*Musa paradisiaca* L.) were purchased from local market of Ahmednagar Maharashtra, India. Banana peels was removed from fruits and disease free peels were selected. Collected peels were completely dried in incubator at 50 ^{0}C and after drying peels were crushed into fine powder by grinder mixer. The fine powder was preserved at room temperature.

2.2 Preparation ofextracts

5 gm of banana peel powder was soaked (1:20 w/v) in water, methanol, ethanol, ethyl acetate, chloroform and petroleum ether at room for 3 hrs. The suspensions were filtered through whatman filter paper and obtained filtrates were concentrated by using rotary evaporator. The residue of each solvent was dissolved in DMSO (10 mg/ml) and preserved at room temperature.

2.3 Qualitative analysis of chemical constituents

The analysis of chemical content in each extract was performed using following tests.

I. Test for alkaloids: In a test tube1 ml extract and 1 ml conc. sulphuric acid was mixed with few drops of Mayer's reagent. The formation of green colour was indicated the presence of alkaloids.

II. Test for glycosides: In a reaction mixer containing 1 ml extract, 2 ml chloroform and 10 % ammonia solution were mixed properly. The formation of pink colour indicates the presence of glycosides.

III. Test for tannins and phenols: One millilitre of extract was mixed with 2 ml 5% ferric chloride. The development of greenish black colour indicates the presence of tannins and phenols.

IV. Test for flavonoids: In a test tube 1 ml extract was mixed with 10 % sodium hydroxide. Yellow colour was observed after dropwise addition of dil. hydrochloric acid, indicates the presence of flavonoids.

V. Test for saponins: In a test tube 2 ml extract was mixed with 4 ml water and shaken vigorously, formation of foams indicates the presence of saponins.

VI Test for terpenoid: In a reaction containing 1 ml extract, 3 ml chloroform, few drops of conc. sulphuric acid. The reaction mixture was allowed to stand for few minutes. Generation of reddish brown colour indicates the presence of terpenoid.

VII. Test for reducing sugar: One millilitre of extract was mixed with 3 ml Fehling's solution and incubated in water bath for 5 minutes. The formation of brick red precipitation indicates the presence of reducing sugars.

VIII. Test for carbohydrates: Two millilitre extract was mixed with, 2 ml Molisch's reagent and mixed well followed by addition of conc. sulphuric acid along the side of test tube was added. Change in colour of solution indicates the presence of carbohydrates.

2.4 Bacterial strains

Two gram positive (*Bacillus subtilis, Staphylococcus aureus*) and two gram negative (*Escherichia coli, Salmonella typhi*) pathogenic and food poisoning bacterial cultures were obtained from Department of Microbiology, Ahmednagar College, Ahmednagar (M. S.) India.The selected bacterial strains were sub cultured at 35°C in Mueller Hinton agar slants. The viable cell count was measured at 580 nm using spectrophotometer. The cell culture was taken into sterile saline water and cell count was attained of 10^7CFU/ml.

2.5 Antibacterial activity in extracts

The agar well diffusion method was used to evaluate antimicrobial activity of different solvent extracts. The 50 ml of sterilized nutrient agar medium was poured into each sterile petri plate and allowed to solidify. Each bacterial culture was consistently spread over media by using a sterile cotton swab. Thereafter, wells were made in agar medium by using sterile cork borer. Sterile extract (250 µl) of each solvent was transferred into separate wells using micro pipette. The plates were kept in the fridge at 5°C for 2 hrs to permit plant extracts diffusion then incubated at 37 ^{0}C for 48 hrs. The antimicrobial activity was visually observed and zone of inhibition was recorded.

3. Result and Discussion

Phytochemicals are group of secondary metabolites that are derived from natural resource especially with plants origin, involved in their growth and development and exhibit various biological properties like antioxidant and anticancer [14, 15]. The analysis of chemical constituents in different extracts of aqueous, methanol, ethanol, ethyl acetate, chloroform and petroleum ether revealed that banana peel contains alkaloids, carbohydrates, cardiac glycosides, flavonoids, tannins, terpenoids, saponins and phenols/ polyphenols. Banana peel is well known to have various phytochemicals such as alkaloids, flavanoids, carbohydrates, proteins, Tannins, Terpenoid, Saponins, Glycosides and Anthroquiones [16].

Table 1: Qualitative detection of chemical constituents in different solvent extracts of banana peel (*Musa paradisiaca* L.); (+) indicates presence of phytochemical and (-) indicates absence of phytochemical

Sr. No.	Tests	Aqueous	Methanol	Ethanol	Ethyl Acetate	Chloroform	Petroleum Ether
1	Alkaloids	+	+	+	+	+	-
2	Glycosides	+	+	+	+	+	-
3	Reducing Sugar	+	+	+	-	-	-
4	Carbohydrates	+	+	+	-	-	-
5	Tannins & Phenols	+	+	+	+	_	-
6	Saponins	+	+	+	-	+	-
7	Flavanoids	+	+	+	+	-	-
8	Terpenoids	-	-	-	+	+	-

Table 1 shows that the presence/absence of phytochemicals extracted in different solvent extracts from banana peel. All phytochemicals were detected in ethanol extract except terpenoids. Similarly, all phytochemicals were detected in aqueous and methanol extracts except terpenoids. None of phytochemicals were detected in petroleum extract. Tannins, Phenols and flavanoids were found in almost all solvents except chloroform and petroleum ether. Alkaloids and glycosides were detected in all solvents except petroleum extract. Carbohydrates and reducing sugar only detected in polar solvents. Similarly, terpenoids were detected only in ethyl acetate and chloroform extracts.

Table 2: Extract yields and antibacterial activity of different solvent extracts of banana peel (*Musa paradisiaca* L.)

Extracts	% yield	Inhibition zone (mm) against pathogenic bacteria			
		B. subtilis	*E. coli*	*S.aureus*	*S.typhi*
Aqueous	2.60	12 ±0.2	6±0.1	14±0.2	8 ±0.3
Methanol	2.70	11±0.1	9 ±0.2	6 ±0.3	15±0.1
Ethanol	5.18	18±0.3	16±0.1	22±0.1	14±0.1
Ethyl Acetate	2.14	6±0.2	10±0.2	7±0.2	9±0.2
Chloroform	1.12	Nil	Nil	Nil	Nil
Petroleum Ether	1.08	Nil	Nil	Nil	Nil

Table 1 revealed that an extracted residue in different solvents and ethanol was found to have more extractive efficacy from banana peel as compared to other solvents. Phytochemical result of previous study of Ehiowemwenguan et al. (2014) has showed that ethanol is a better solvent for extraction of bioactive chemicals in banana peels including alkaloids, glycosides, tannins, saponins, flavonoids and volatile oil [12]. Extractability of banana peel phytochemicals in petroleum ether was very low as compared to other solvent. The extracted percent yields in different solvents were in order of ethanol > methanol > aqueous > Ethyl Acetate > Chloroform > Petroleum ether as shown in table 1.

Antimicrobial activity of different solvent extracts of *banana peel* was tested against *B. subtilis, S. aureus, E. coli,* and *S. typhi* (Table 2). The different extracts such as aqueous, methanol, ethanol and ethyl acetate exhibited antibacterial activity against these pathogenic bacteria while chloroform and petroleum extracts not exhibited antimicrobial activity. Ethanol extract showed the highest antimicrobial activity while ethyl acetate extract showed lowest activity against all strains. This observed data indicates that the compounds soluble in ethanol have more bioactive potency than other solvents. It was possibility that among

detected chemical constituents the phenolic compounds, tannins and flavonoids have antibacterial activity. It has been investigated that phenolic compounds in plants like catechol, caffeic acid and resveratrol possess antimicrobial activity [17]. Tannins exhibit antimicrobial activity by inhibition of extracellular microbial enzymes, dispossession of the substances required for microbial growth or direct interfere in microbial metabolism through inhibition of oxidative phosphorylation [18]. Ethanol extract showed antimicrobial activity in order of *S. aureus*>*B. subtilis*>*E. coli* >*S. typhi* while methanol extract showed antimicrobial activity in order of *S. aureus*>*B. subtilis*> *S. typhi*> *E. coli.* The aqueous extract showed maximum antibacterial activity against *S. aureus* while minimum antibacterial activity showed against *E. coli.* Ethyl acetate extract exhibited maximum antibacterial activity against *E. coli* while minimum activity exhibited against *B. subtilis.* Flavonoids are a group of phenolic compounds have been extensively studied to have antibacterial activity and synthetic derivative of flavonoids exhibit antimicrobial activity against multidrug-resistant gram-positive and gram-negative bacterial including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* [19]. Ehiowemwenguan et al. (2014) reported the antibacterial activity in banana peel against both gram-positive and gram-negative bacteria [12]. Similarly, Kapadia et al. (2015) have investigated banana peel extract exhibit antimicrobial activity against periodontal pathogens (*P. gingivalis*, *A. actinomycetemcomitans*) [13].

4. Conclusion

The present study revealed that the banana peel is a source of phytochemicals such as alkaloids, glycosides, tannins, phenols, saponins, flavonoids and terpenoids. The different solvents extracts such as aqueous, methanol, ethanol and ethyl acetate exhibit antimicrobial activity against *B. subtilis, E. coli, S. aureus* and *S. typhi.* Banana peel could be utilized an alternative source of antimicrobial agent as preservative for prevention of food spoilage and food borne diseases.

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