Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Comparative Analysis of Antibacterial Activity of Banana Peel at Different Stages of Ripening

Shadma Anjum[1*], Shanthy Sundaram[2]

[1]*Centre of Food Technology, University of Allahabad, Prayagraj-211016, Uttar Pradesh, India*
[2]*Centre for Biotechnology, University of Allahabad, Prayagraj-211016, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received: 16 April, 2022
Revised: 04 December, 2022
Accepted: 22 December, 2022
Published: 30 January, 2023

Keywords:
Musa paradisica, Antibacterial, Flavonoid, Tannin, Ripening.

DOI:
10.25004/IJPSDR.2023.150101

ABSTRACT

The present study investigated the phenolic compounds like tannin and flavonoids that provide banana peel extracts their antibacterial properties against bacterial pathogens, i.e., *Enterobacter. aerogenes*, *Pseudomonas. aeruginosa, Salmonella. typhimurium, Staphylococcus aureus and Bacillus subtilis* by using HPLC (High-performance liquid chromatography). The pathogenic bacteria affect skin (*S. aureus*), soft tissues, gastrointestinal tract (*P. aeruginosa)* and urinary tract (*E. aerogenes)* etc. Banana (*Musa paradisica*) peels at different stages of ripening (unripe, ripe, leaky ripe) were treated sequentially with 70% acetone, which was partitioned in order of polarity with water, ethyl acetate, chloroform, and hexane. Textural properties of fruit peel were determined by texture analyzer. The antibacterial activity of the samples was evaluated by the agar cup diffusion method, and inhibitory concentrations were measured by the MIC (minimum inhibitory concentration) method. The approach of formulating new drugs through natural sources has proved to be the most successful strategy. Many active metabolic products with biological significance are obtained from them. The findings of the present investigation suggested that the unripe banana peel sample had higher antibacterial activity than the ripe and leaky ripe (over-ripe). Higher MIC were obtained with water fractionate and 70% acetone extracts of banana peel against gram-negative bacteria, *E. aerogenes* and *P. aeruginosa*. Water fractionate has the highest inhibitory activity against gram-negative bacteria (*E. aerogenes* and *P. aeruginosa*) compared to gram-positive bacteria (*S. aureus* and *B. subtilis).* The ethyl acetate, chloroform and hexane fractionate of unripe, ripe and leaky ripe banana peels do not exhibit antibacterial activity. The highest concentration of tannin in unripe banana peels is one of the responsible phenolic components for its antibacterial activity. While certain flavonoids also exhibit antibacterial activity in banana peel, as the ripening proceeds. The present study discusses the essential information on banana peel, including its varieties, effect of ripening, solvent condition, pharmacological actions, and relevance in pharmaceutical industries. Banana peels, like tannin and flavonoid, exhibit broad-spectrum antimicrobial properties against all tested bacterial strains. Most of the compounds are polar in nature and water gives optimum conditions for extraction of these compounds from banana peel. It has the potential to be a nutraceutical preparation alternative and is beneficial to the pharmaceutical sector by providing the alternative of chemically synthesized drugs

Introduction

Ancient Indian texts on Ayurveda describe the medical benefits of bananas, which have been found to be useful in treating a wide range of illness like irregular kidney function, depression and neurodegenerative disease. Bananas with high potassium and low sodium may reduce the risk of high blood pressure and stroke.[1] Amongst fruits, banana is the second largest produce after citrus, accounting for around 15%.[2]

India's participation in the world's banana production is 27%, contributing to about 37% of the total fruit production.[3] The main by-product of banana processing

***Corresponding Author:** Dr. Shadma Anjum

Address: Centre of Food Technology, University of Allahabad, Prayagraj-211016, Uttar Pradesh, India

Email ✉**:** Shadmaanjum@yahoo.co.in

Tel.: +918530745459

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

industry is peel, which represents almost 30% of fruit.[4] However, no attention is being paid to banana peel, which is unfortunate because they have medicinal properties due to the presence of various polyphenols and bioactive molecules.[2, 4] Phenolic compounds like tannins and flavonoids have been reported for antimicrobial activity.[5] Studies have shown that fruit peel contains various phenolic components which are effective against pathogenic microorganisms.[6,7] It is commercially vital if by-products produced by fruit processing industry could be reused. However, very few literature have been published for antioxidant and antibacterial property of banana peel.[8] Polyphenols, carotenoids, and other antioxidants found in banana peel help your body fight cancer-causing free radicals. Banana peel helps treat crohn's disease and irritable bowel syndrome and boosts digestive health. High level of tryptophan combined with the B6 in banana peel helps relieve depression.[9] Banana peel is a natural oxalate oxidase source that helps degrade kidney stone.[10] In this study, the effect of phenolic compounds present in banana peel during maturation, ripening and senescence were observed against diverse range of human pathogenic bacteria comprising of both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis)* and gram-negative (*Enterobacter aerogenes*, *Pseudomonas aeruginosa, Salmonella typhimurium)*.

The antibacterial activity in this raw but natural product have medicinal properties which can be of immense benefit in the pharmaceutical industry because product obtained from banana peel will be cheap and without any side effect. Apart from antioxidant, antibacterial and anti-cancerous activity, the pharmaceutical industry have developed great interest in utilizing banana peel as an alternative to chemically synthesized drugs. Current economic pressure and the escalating cost of substrates for microbial growth and production necessitate the exploration of organic substrates for microbial production by pharmaceutical industries. Banana peel can be utilized as a substrate for citric acid production by *Aspergillus niger*. Banana peel, a natural super-disintegrant that can be used as a pharmaceutical excipient for oral drug delivery due to the exhibition of faster drug dissolution, satisfies all the standards of fast dissolving tablet. Bananas peel also an economical and feasible alternative carbon source for the cultivation and growth of probiotic lactobacilli. [11]

Considering all the aforementioned aspects, including its varieties, effect of ripening, solvent condition, pharmacological actions, and its relevance in pharmaceutical industries, the present investigation entitiled "Comparative analysis of the antibacterial activity of banana peel at different stages of ripening" was conducted.

Materials And Methods

Sample Collection and Extraction

Banana (*Musa paradisica*: local name Bhusawal keli) fruits were purchased from local market of Kaushambi, Uttar Pradesh, India. The whole fruit of banana was washed thoroughly under running tap water, dried on paper towel. The peel tissues of fresh unripe green, ripe yellow, and leaky ripe brown with traces of yellow banana fruit (200 g) were heated in 750 mL of distilled water for 5 minutes. Banana peel was homogenized with 70% acetone twice at room temperature using pre-chilled pestle and mortar for 48 hours under shaking conditions using an electrical shaker. The combined extracts of 70% acetone extracts were then filtered and centrifuged at 4°C in Beckman refrigerated centrifuge machine for 15 minutes at 15,000×g. The supernatant was concentrated to 150 mL.[12] This aqueous extract was partitioned into chloroform ($CHCl_3$) and water, using a separator and then extracted with aqueous saturated ethyl acetate. Fractionate and 70% acetone extract were concentrated using a rotary evaporator with the water bath set at 40°C. The percentage yield of extracts ranged from 7 to 19% w/w. The antibacterial activities of hexane, chloroform, ethyl acetate aqueous fractions and 70% acetone extract were measured at different stages of ripening of peel.

Monitoring of Different Ripening Stages of Banana Peel

Determination of Color

In this study, visual colors of banana peel at different ripening stages were measured using X-rite colorimeter (USA) and expressed in terms of the L' a' b" system. Colorimeter was calibrated using a white reference standard tile, as described by Rangana.[13]

Assessment of Rheological Properties of Banana

Penetrometry experiments were carried out using textural properties analyzer TAXT2i (Stable Microsystems, USA), connected to a data acquisition system, fitted with a 49.03 kg trigger force with a needle probe moving at a rate of 2 mm/s to a depth of 15 mm. The maximum force applied to break up the peel stand for peel hardness (expressed in N). The force/time curve slope stands for fruit firmness (expressed in N S^{-1}) as described by Breene.[14]

Preparation of Test and Standard Solution

The extract, standard antibiotics and standard ascorbic acid were dissolved in freshly distilled DMSO (Dimethyl sulfoxide) and used for *in-vitro* antibacterial activity determination. Antibiotic sensitivity was tested in nutrient agar plates by using broad spectrum antibiotic, kanamycin.

Determination of Total Phenolic Content

The amount of total soluble phenolics was determined according to the Folin–Ciocalteu's method[15] with slight modification. The decrease in absorbance of the resulting solution was measured spectro-photometrically at 517 nm. The reaction mixture consisted of 0.5 mL of the extract (10 mg/mL), 5 mL of distilled water, and 0.5 mL of the Folin-Ciocalteu's reagent. After a period of 3 minutes, 1-mL of saturated 5% sodium carbonate solution was added. The 10 mL volumetric flasks were shaken and allowed to stand for 1-hour. The absorbance was measured at 725 nm (each measurement repeated three times) in a UV- visible spectrophotometer (the same equipment was used in the DPPH test). The following formula calculated the total content of phenolic compounds of extracts in gallic acid equivalents (GAE).[7]

$$C = c.\ V/m$$

Where: C—total content of phenolic compounds, mg/g plant extract, in GAE.
c —the concentration of gallic acid established from the calibration curve, mg/mL.
V—the volume of extract, mL
m—the weight of pure peel extract (gm).

Determination of Tannin Content

The amount of total tannin content was determined according to the Folin–Denis method[15] with slight modification. Banana peel powder (2 g) was transferred into a 250 mL conical flask and boiled in a flask gently for 30 minutes after adding water, centrifuged at 2000 rpm for 20 minutes and supernatant was collected in 100 mL volumetric flask to make up the volume 1-mL of sample extract was transferred to 100 mL volumetric flask containing 75 mL water. A total of 5 mL of Folin Denis reagent and 10 mL of sodium carbonate solution were added to the mixture and diluted to 100 mL with water. Further the mixture was shaken and absorbance was measured at 700 nm after 30 minutes. Standard graph was prepared by using 0–100 µg tannic acid.

Determination of Flavonoid Content

The amount of flavonoid was determined spectrophotometrically using a method based on the formation of a flavonoid-aluminium complex.[16] The extract (1-mL) at a concentration of 1-mg/mL was added to a 10 mL volumetric flask and made upto a volume of 5 mL with distilled aqueous. At zero time, 0.3 mL of 5% (w/v) sodium nitrite was added to the flask. After 5 minutes, 0.3 mL of 10% (w/v) $AlCl_3$ in ethanol was added followed by 2 mL of 1 M NaOH at 6 minutes and the volume was made upto 10 mL with distilled water. The absorbance at 510 nm was read immediately. Gallo-catechin, catechin, and quercitin was chosen as a standard and the levels of total flavonoids were determined in triplicate and expressed as catechin equivalents in mg/100 g of fresh weight (fw) with slight modification.

$$TFC = \frac{R^* D.F^* V^* 100}{W}$$

Where,
R- Result obtained from the standard curve
D.F- Dilution factor
V- Volume of stock solution
100- For 100 gm dried peel
W- Weight of peel used in the experiment

HPLC Analysis

The coloum size, mobile phase, and flow rate were slightly modified from Durgawale *et al.* (2016) for quantitative estimation of tannin by HPLC analysis.[17] The PTFE syringe filter, 0.45 mm, Whatman, UK, was used to filter the extracts, whereas, the loop size were 10 µL. Mobile phase (Acetonitrile: water 15: 85) was optimized and found to be optimum for better separation and identification of flavonoid 1-mL of sample (10 µL extract + 990 µL mobile phases) was injected through syringe for HPLC analysis. Gallo-catechin, gallic acid, catechin and tannin were identified in the samples by comparing their retention times (R_t) with the standards of gallo-catechin, gallic acid, catechin, quercitin and tannic acid.

Preparation of Standard Solution

Gallo-catechin, catechin, quercitin, tannic acid and gallic acid were used as a standard to screening of compound responsible for antioxidant property. Standard were prepared by dissolving 1-mg of each standard in 1-mL of HPLC grade water. The concentration of stock solution was 1000 µg/mL. The concentration of working solution was 20, 60 and 100 µg/mL prepared from stock solution. Each of these drug solutions (10 µL) was injected three times into the column, the peak area and retention times were recorded.

Chromatographic Equipment and Condition

The chromatographic analyses were performed with genesis 7 micron C-18 (ODS), Phenomenex reverse phase column on a (300 x 4.6 mm i.d., 7 µm), Metrohm Technologies solvent degasser. Gradient elution of mobile phase comprising of, using 0.05M acetonitrile: water (HPLC Grade) and methanol: water (HPLC Grade) as mobile phase in the ratio of 15:85(acetonitrile: water), at a flow rate of 0.40 to 0.90 mL/min at temperature 38 to 45°C and pressure 4.2 to 7.5 MPa run for 10 minutes. Prior to injection of analyte, the column was equilibrated for 30–40 minutes with mobile phase.

The system consist of interface part 830 IC, 2 sets of (818 IC) pump, separation centre (820 IC) and a detector part (lambda 1010) UV-absorbance detector operated at 282 nm at 1000 psi and the system was equipped with data acquisition software. The column oven temperature was 38°C.

Chromatogram of sample (70% acetone extract and water extract) was calculated by the following formula. By using

following formula quantitative estimation of tannin by HPLC is calculated. All the value considered are mean values of triplicate injection of sample (2 mg/mL) and standard (2 mg/mL).

$$\text{Conc. of sample} = \frac{\text{concentration of standard x AUC of sample}}{\text{AUC of standard}}$$

Conc. of sample= X mg/mL of screening compound in extract.

Antibacterial Activities

Bacterial Strains

"Pure bacterial cultures were obtained from National Collection of Industrial Microorganisms (NCIM) National Chemical Laboratory, Pune, India and maintained on nutrient agar overnight in rotatory shaker at 37°C temperature.[18] Two strains of gram-positive (*S. aureus and B. subtilis*) and three strains of gram-negative (*E. aerogenes and S. typhimurium and P. aeruginosa*) bacteria is used to evaluate antibacterial potency of banana peel extracts.

Preparation of Standard Bacterial Suspension

Fresh stock suspensions of the bacterial strains *S. aureus, B. subtilis, P. aeruginosa, E. aerogenes* and *S. typhimurium* were used at a concentration of 10^8–10^9 per mL of the stock suspension by means of the surface viable counting technique.

Antibacterial Assay of Natural Extracts

The agar well diffusion method was used to determine the antibacterial activity of the prepared extracts [19] in agar plates containing 1.5% nutrient agar in the basal layer and 0.8% nutrient soft agar layer on top, the well is made on upper layer. 0.8% nutrient agar was incubated with 10 µL of bacterial culture of each type. The surface of the solid media was inoculated with specific bacteria from its broth culture. The wells were made using a cork borer in the solid medium and four different concentrations of the extract (2, 4, 6, and 10 mg/mL) were added into the wells. The plates were then incubated at a specific temperature (30 ± 1°C for *B. subtilis*, and 37°C for other bacterial strains) for 24 hours. The control used was kanamycin, which is a broad-spectrum antibiotic. The screening of antibacterial activity of the extracts was assessed by measuring the diameter of the clear zones surrounding the wells (including the disc diameter) in mm.

Minimum Inhibitory Concentration Determination

The minimum inhibitory concentration (MIC) is defined as the lowest concentration (ppm) of the banana peel extracts against standards in agar plates showing no visible bacterial growth. The soft nutrient agar was then added on a petridish containing 15 mL hard agar (as mentioned above). The samples were dissolved in DMSO (dimethyl sulfoxide) and the extracts at different concentrations ranging from (2 to 10 mg/mL) of 70% acetone, water, ethyl acetate, chloroform and hexane fractions of banana peel were tested separately for each bacterium.

Statistical Analysis

The antibacterial activities of all three stages of banana peel (*Musa paradisica* var. Bhusawal keli) were indicated by clear zones of growth inhibition. All experiments were performed in triplicates and results were presented as Mean ± SD (Standard deviation). Analysis of variance was performed using two-way ANOVA and the significant differences ($p < 0.05$) between the means were performed to determine the effect of solvent polarity and ripening stages on the bioactive compounds and antibacterial property of banana peel.

Results And Discussion

The antibacterial activity of 70% acetone extracts and their fractions in water, ethyl acetate, chloroform and hexane were assayed *in-vitro* by the agar cup diffusion method against five bacterial strains.

Standardization of Banana Peels Samples

Texture Analyses of Banana Peel at three Different Stages of Ripening

Textural characteristics that include hardness/firmness, cohesiveness, springiness and chewiness of banana peel showed changes as the ripening proceeded. Unripe peels are significantly ($p < 0.05$) more firm and harder than ripe and leaky. Fig. 1 (a) showed that firmness (956 ± 2.12 to 32 ± 0.042) and chewiness (484.84 ± 1.63 to 17.23 ± 1.28) initially increased and then decreased as the ripening proceeded, while, springiness (1.002 ± 1.53 to 1.05 ± 0.017) and cohesiveness (0.582 ± 0.003 to 0.509 ± 0.01) decreased in the later stages of ripening. These parameters were taken for the optimization of banana peel samples for further studies.

Determination of Color Value at three Different Stages of Ripening

The variation in a* and S versus the time of storage were reported in Fig. 1 (b), where the parameter a* increased from -0.1866 ± 1.63 (green) to 2.875 ± 1.28 (yellow) during the first eight days of storage. During the following days, it decreased steadily at the constant rate of over 2.87 ± 1.28 (black). Furthermore the L* value increased gradually from 10.57 ± 2.45 without ethylene treatment to 19.57 ± 0.169 after 7 days of storage with ethylene. This value slightly decreased after 15 days of storage. During ripening, the color of the banana peel changed gradually from yellowish green to yellow color and the brightness of the color due to the carotenoids started developing. Ripe stage of banana peel showed more brightness compared to unripe and overripe samples of banana peel.

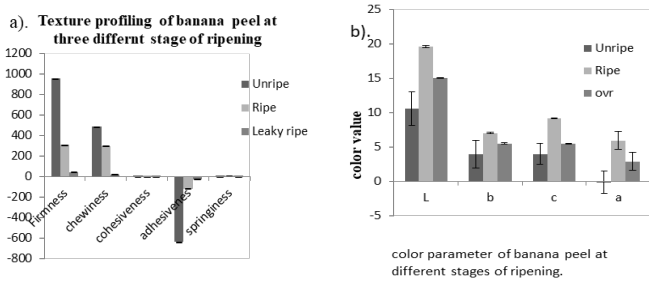

Fig. 1: Standardization of banana peel sample at different stages of ripening (a) Texture profiling of unripe, ripe and leaky ripe banana peel at three different stages of ripening (b) Color value of banana peel at three different stages of ripening. *Mean values of three replications ± standard error. L^a value denotes lightness, a^b value denotes redness, bc value denotes yellowness, Chroma $(C^*_{ab})^d$ values denote color functions of unripe, ripe and leaky ripe sample of banana peel.

Effect of Polar Extracts of Banana Peel for Antibacterial Activity

According to Fig. 2 (a), the MIC of a 70% acetone extract of unripe banana peel was less than 2 mg/mL (Zone of Inhibition: 7.5 ± 0.082 mm) against gram-positive *B. subtilis*, while the zone of Inhibition for *S. aureus* was 0.002 ± 0.00 mm and the MIC for *P. aeruginosa* was between 2 and 4 mg/mL (zone of inhibition: 0.024 ± 0.00 mm) as shown in Fig. 2 (b).

In Fig. 2 (b) water fractionate of unripe banana peel exhibited less than 2 mg/mL MIC, against gram-negative *P. aeruginosa* (zone of inhibition 12.5 ± 0.00 mm) and *E. aerogenes*(15.2 ± 0.057 mm) whereas, with gram-positive *B. subtilis* (zone of inhibition 0.012 ± 0.00 at 4 mg concentration) and *S. aureus* (zone of inhibition 0.002 mm ± 0.00 at 2 mg concentration and 0.75 ± 0.086 mm at 4 mg concentration), the MIC was in the range of 45 mg/mL shows in Fig. 2 (a). Fagbemi *et al.*[20] reported the MIC of *E. aerogenes* ranged from 4–512 mg/mL for unripe banana peel. Similarly Mokbel and Hashinaga[9] reported the high value of banana peel because of its antibacterial activity. In present work, water extract (2 mg/mL) of unripe banana peel shows the higher efficiency for antibacterial activity against *E. aerogenes*. The results of antibacterial screening [Fig. 2 (a) and (b)] indicated that the highest inhibition was observed in water fractionate against *E. aerogenes* (MIC < 2 mg/mL) followed *P. aeruginosa.* Ababutain *et al.* (2015) investigation support the present study that water followed by other polar solvent (ethanol, followed by acetone) was the best solvent for the active substance which have antibacterial potential. He also had reported that carbohydrate, glycosides, tannin, phenolic compound and gum were present in aqueous extract in good quantity[21]

In Fig. 3(a) (b), the result shows that 2 mg/mL is the minimum inhibitory concentration of 70% acetone extract of ripe banana peel to inhibit P aeruginosa (.934 + 0.02 at 2 mg concentration), *E. aerogenes* (zone of inhibition 2.2 + 0.001 at 2 mg concentration) and *B. subtilis* (zone of inhibition 4.4 +0.002), whereas higher concentration was required to inhibit S.aureus (zone of inhibition 0.06+0.004at 4mg concentration)The result also displays that 2mg/ml of water extract of ripe banana peel is effective concentration to exhibit minimum inhibition against S.aureus (zone of inhibition 0.03+0.0023) and P.aeruginosa (zone of inhibition 0.004+0.01) as shown in Fig. 3 (a) and (b)

Flavonoids and tannin were used as positive controls while, DMSO was used as blank control in experiments. Kanamycin was used as a standard to compare the antibacterial activity of test sample. Among gram-negative bacteria, highest inhibitory effect was observed with *E. aerogenes* (zone of inhibition 15.2 ± 0.057 mm at 2 mg concentration) followed by *P. aeruginosa* (Zone of inhibition 12.5 ± 0.00 mm at 2 mg concentration) by water extract of unripe banana peel. *E. aerogenes* can cause gastrointestinal infections, urinary tract infection (UTI), skin and soft tissue infection, respiratory infection, and adult meningitis, while *P. aeruginosa* causes infection in the urinary tract, respiratory system, dermis, soft tissue, gastro-intestine. From the above investigation, it is clear that, banana peel is potent and beneficial against skin and gastro-intestinal disease.

In contrast, 1.2–2 mg of water fractionates was sufficient to inhibit some gram-negative *E. aerogenes* (Zone of inhibition 0.401 ± 0.012 mm at 2 mg concentration) and *P. aeruginosa* (0.934 ± 0.02 mm at 2 mg concentration) as shown in Fig. 3 (b), while 1.2 mg of water extract of leaky ripe banana peel is effective for gram-positive *S. aureus* (Zone of inhibition 0.02 ± 0.00) and *B. subtilis* (Zone of inhibition 0.122 ± 0.001) shown in Fig. 3 (a) The type of solvent used for extraction influence the compound, responsible for antibacterial activity as reported by Bacon *et al.* (2017).[22]

S. typhimurium were least affected even at higher concentration, more than 5 mg/mL (Zone of inhibition 1.96 ± 0.0577 mm) of water fractionate of leaky ripe banana peel. The lack of inhibitory activities in ethyl acetate, chloroform and hexane fractions of banana peel,

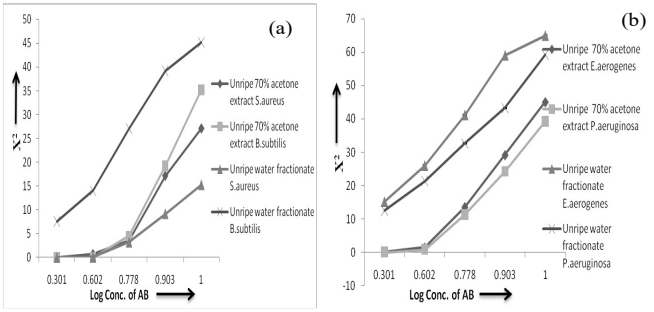

Fig. 2: Minimum inhibitory concentration in mg/mL of unripe banana peel extract (70% acetone and water fractionate) against (a) Gram-positive (*S. aureus, B. subtilis*) bacterial strain (b) Gram-negative (*E. aerogenes, P. aeruginosa*) bacteria strain. The data are displayed with mean + standard deviation (bars) of three replication.

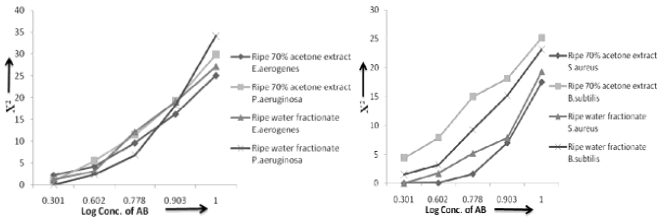

Fig. 3: Minimum inhibitory concentration in mg/ml of Ripe banana peels extract (70% acetone and water fractionate) against (a) Gram negative bacteria (b) Gram positive bacteria. The data are displayed with mean + standard deviation (bars) of three replication.

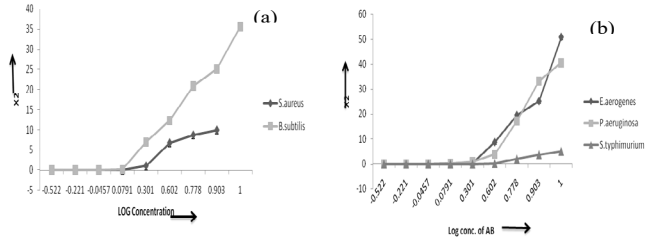

Fig. 4: Zone of inhibitions leaky ripe banana peel sample (ethyl acetate fraction, water fraction, chloroform fraction and hexane fraction) and negative control (DMSO+ water) against (a) Gram positive (*S. aureus, B. subtilis*) bacterial strain (b) Gram negative (*E. aerogenes, P. aeruginosa, S. typhimurium*) bacterial strain. Mean values of three replications ± standard error.

could be attributed to the extraction of active components of banana peel in polar solvent rather than non-polar solvent. The highest antibacterial activity of water extract of banana peel sample clearly indicated that the polar compounds inhibited the growth of food-borne bacteria *in-vivo*. High bioflavonoid and tannin content in extracts may be responsible for antibacterial activity.[23]

Effect of Tannin in Banana Peel for Antibacterial Activity

The results of extraction of phenol from unripe, ripe, and leaky ripe banana peel samples with 70% acetone (acetone/aqueous), water, chloroform, ethyl acetate and hexane fraction are shown in Fig. 5 (a). Low recovery of phenols in water fraction (741.5 ± 4 GAE mg/100 g) compared to 70% acetone (1729.85 ± 90.12 GAE mg/100 g) extract of unripe banana peel may be caused due to the oxidation of phenolic compounds by polyphenol oxidase.[24] Highest tannin content was found in unripe water fractionate in comparison to 70% acetone extract in the range of 6160 ± 0.12 to 4360 ± 0.057 mg/100 gm as shown in Fig. 5 (b) and (c) shows the zone of inhibition of different concentrations (10, 6, 4, 2 mg) of standard tannin against gram-negative *P. aeruginosa (*zone of inhibition 19.5 ± MIC < 2 and gram-positive *B. subtilis* by agar cup method. The result shows that tannic acid (positive control) exhibits broad spectrum antibacterial activity. This result was compared with test sample of unripe banana peel. Highest concentration of tannin in unripe banana peel is one of the phenolic components responsible for its antibacterial activity. Similarly, Çolak *et al.* (2010) reported that tannic acid exhibited broad spectrum antibiotic activity.[25] Tannins are high molecular weight, water soluble polyphenolic compounds.[26] Surojanamethakul *et al.* (1994) reported that variety and ripening, affect the amount of tannin content likely to support the above investigation.[27]

Effect of Banana Peel Flavonoids for Antibacterial Activity

The results of flavonoid content in terms of mg catechin, rutin and quercitin were determined in water fractionate of unripe banana peel were in the range of 78.94 ± 0.057, 66.43 ± 0.068 and 41.61 ± 0.057 mg equivalent /100 gm, whereas in acetone extract, the amount were 80.46 ± 0.023 mg catechin, 68.8 ± 0.096 mg rutin and 45.84 ± 0.073 mg quercitin equivalent/100 gm as shown in Fig. 6 (a). As the ripening proceeded, water fractionates of unripe banana peel showed highest correlation for flavonoids, phenol and tannin content than ripe and leaky ripe sample. Flavonoids, phenols and tannins were present in all the stages of banana peel while saponin and alkaloid were not detected as shown in Fig. 5 (a), (b) and 6 (a) (b) and (c) shows zones of inhibition against gram-negative (*P. aeruginosa*) and gram-positive (*B. subtilis*) using different concentrations of (10, 6, 4, 2 mg) standard flavonoid by agar cup method. Kapadia *et al.* (2015) reported that secondary metabolites like tannin, flavonoids, glycoside, alkaloid phlobatannin, and terpenoids found in banana peel are responsible for its antibacterial activity.[28]

Higher concentrations (10 mg/mL) of the water extracts of leaky ripe sample exhibited maximum zone of inhibition against *B. subtilis* (35.6 ± 0.115 mm) and *E. aerogenes* (50.88 ± 0.152 mm). But ripe banana peel (10 mg/mL) sample showed maximum zone of inhibition against *P. aeruginosa* (34.2 ± 0.165 mm in water fractionates and 29.9 ± 0.12 mm in acetone fractioned) compared to *E. aerogenes* (34.2 ± 0.165 mm in water fractionate and 29.9 ± 0.12 mm in acetone fractioned). The antibacterial activity in ripe and leaky ripe banana peel may be due to

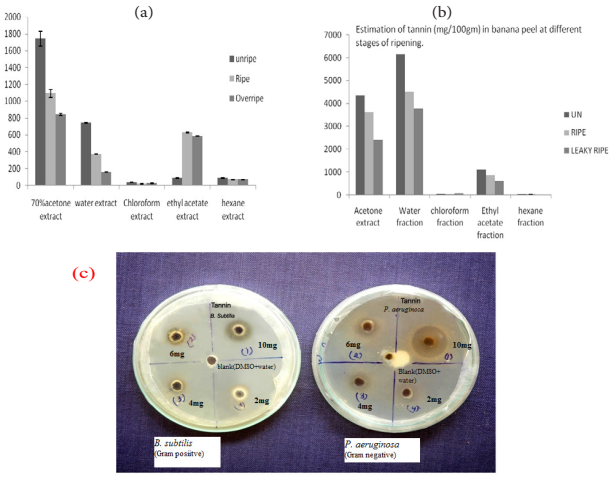

Fig. 5: (a) Total phenolics content in GAE/10mg **(b)** Tannin content in mg/100 gm (tannic acid) **(c)** Zone of inhibition of different concentration (10, 6, 4, 2mg) standard tannin against gram negative (*P. aeruginosa*) and gram positive (*B. subtilis*) by agar cup method.

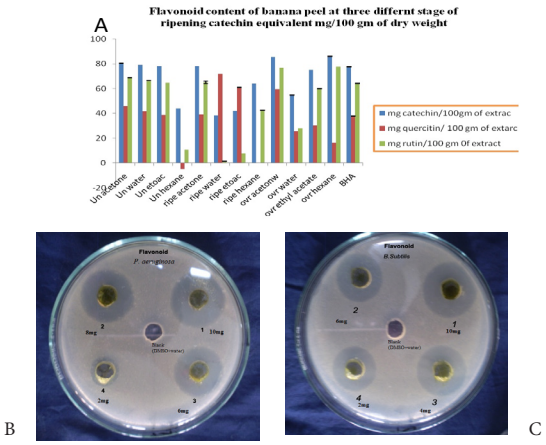

Fig. 6: (A) Total flavonoid content mg catechin, rutin and quercitin equivalent/100 gm dry weight in 70% acetone extract and different fractionate (water, ethyl acetate, chloroform and water) of banana peel at different stages of ripening (B) Zone of inhibition of different concentration (10, 6, 4, 2 mg) standard flavonoid against gram negative (*P. aeruginosa*) (C) Zone of inhibition of different concentration (10, 6, 4, 2mg) standard flavonoid against gram positive (*B. subtilis*) by agar cup method.

certain flavonoids like catechin, quercitin, rutin etc. This study further analyzed the above compounds by HPLC (high pressure liquid chromatography). Whereas ethyl acetate, chloroform and hexane fractionate of unripe, ripe and leaky ripe banana peels do not exhibit antibacterial activity.

Correlation Analysis

The antibacterial activity of unripe, ripe and overripe extract of banana peel was significantly correlated with standard antibiotics kanamycin determined by agar cup method as shown in Fig. 7 (a, b), 8 (a,b), 9 (a, b) and 10 at varying concentrations (10, 6, 4, 2 mg) of extracts and antibiotic (kanamycin). The highest correlation was observed between gram-positive *S. aureus* and gram-positive *B. subtilis* (R^2 = 89.63) and with gram-negative *P. aeruginosa* (R^2 = 89.11) at the different stages of ripening and at different concentrations of extracts.

Fig. 11 and 12 shows the correlation between diameter of the inhibition zone (mm) and total phenolic content (mg gallic acid equivalents) of different concentration of 70% acetone extract and water extract of unripe banana peel against four foodborne bacteria. In water extract of unripe banana peel, highest correlation between zone diameter and phenolic content were observed in gram-negative *P. aeruginosa* (R^2 = 0.992) followed by gram-positive *S. aureus* (R^2 = 0.967) where as in 70% acetone extract highest correlation were observed in gram-negative *P. aeruginosa* (R^2 = 0.954) and *E. aerogenes* (R^2 = 0.952) whereas least correlation was observed against gram-positive *S. aureus* (R^2 = 0.917). This directly indicates that phenolic content are highly concentrated and effective against *P. aeruginosa* in water extract of banana peel.

Kanamycin exhibited broad-spectrum antibacterial

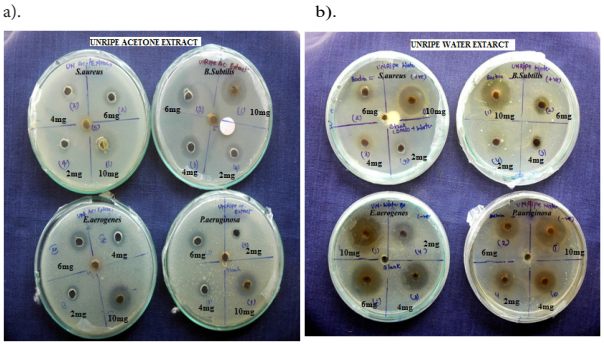

Fig. 7: Determination of Minimum inhibitory concentration using (A) 70% acetone extracts with concentration(10, 6, 4, 2 mg) (B) Water fractionate with concentration (10, 6, 4, 2) of unripe banana peel against gram positive (*S. aureus and B. subtilis*) and gram negative bacterial (*E. aerogenes and P. aeruginosa*) strain.

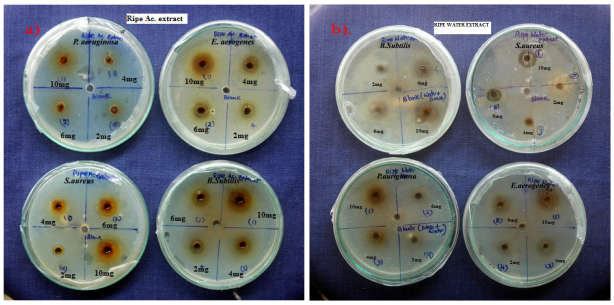

Fig. 8: Determination of Minimum inhibitory concentration using (A) 70% acetone extracts with concentration (10, 6, 4, 2 mg) (B) Water fractionate with concentration (10, 6, 4, 2 mg) of ripe banana peel and negative control (DMSO + water) against gram-positive and gram-negative bacterial strain.

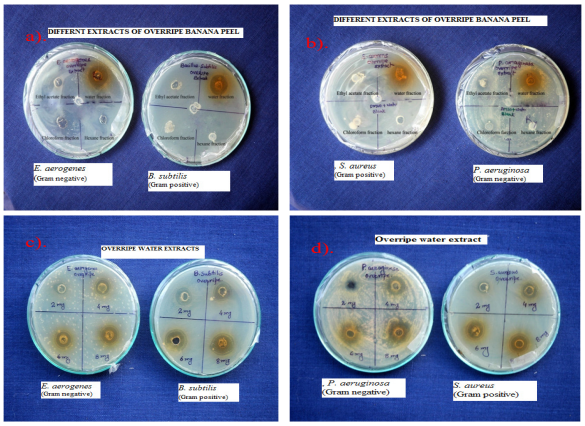

Fig. 9: (A) Zone of inhibitions of leaky ripe banana peel (ethyl acetate fraction, water fraction, chloroform fraction and hexane fraction) against *S. aureus* (gram-positive) and *P. aeruginosa* (gram-negative) (B) Zone of inhibitions of leaky ripe banana peel samples (ethyl acetate fraction, water fraction, chloroform fraction and hexane fraction) against *E. aerogenes* (gram positive) and *B. subtilis* (gram negative) bacterial strain (C) Minimum inhibitory concentration of water extract of leaky ripe banana peel against E. aerogenes (gram positive) and *B. subtilis* (gram negative) bacterial strain (D) Minimum inhibitory concentration of water extract of leaky ripe banana peel against *S. aureus* (gram-positive) and *P. aeruginosa* (gram-negative).

activity. Highest inhibitory effect was visualized for gram-positive *S. aureus* (Zone of inhibition 36 ± 0.08 at 2 mg) and gram-negative *E. aerogenes* (Zone of inhibition 15.2 ± 0.057 mm at 2 mg). The lowest concentration of extracts and standards used for the experiment was 2 mg/mL. *B. subtilis* (14.2 ± 0.035 mm at 4 mg) were least affected by kanamycin compared to others as shown in Fig. 10. When, compared it with banana peel, water extract of unripe banana peel exhibit maximum inhibition against gram-negative *E. aerogenes* (zone of inhibition 15.2 ± 0.057 mm) and

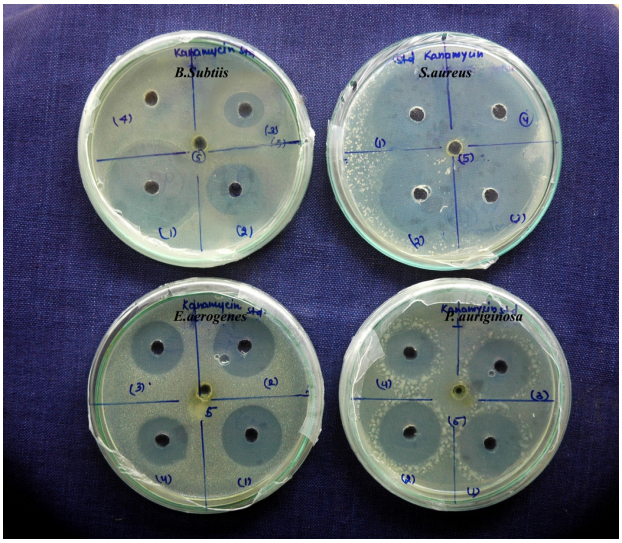

Fig.10: Determination, 6 mg of Minimum inhibitory concentration using (A) Different concentration (10, 4, 2 mg) of standard Kanamycin against gram positive and gram negative bacterial strain.

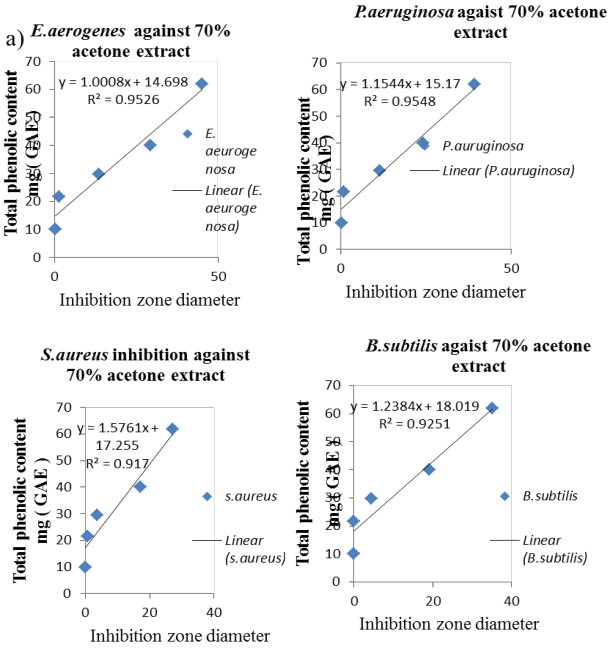

Fig. 11: Relationship between diameter of inhibition zone (mm) and total phenolic content (mg gallic acid equivalents) of different concentration of 70% acetone extract for four foodborne bacteria. (a). *E. aerogenes* (b). *P. aeruginosa* (c). *S. aureus* (d). *B. subtilis*

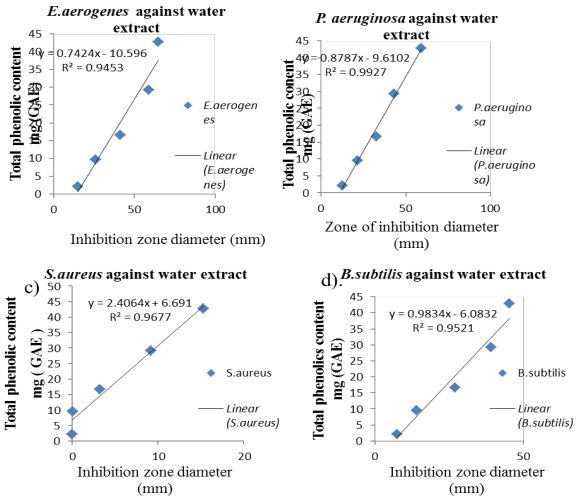

Fig. 12: Relationship between diameter of inhibition zone (mm) and total phenolic content (mg gallic acid equivalents) of different concentration of water extract for four foodborne bacteria. (a). *E. aerogenes* (b). *P. aeruginosa* (c). *S. aureus* (d). *B. subtilis*

P. aeruginosa (zone of inhibition 12.5 ± 0.00 mm) while 70% acetone extract shows maximum inhibition against gram-positive *B. subtilis* (zone of Inhibition: 7.5 ± 0.082 mm), whereas, *S. aureus* were least affected. To some extent, these results were similar to the previous studies reported by Rao *et al.* 2012; Mokbel and Hashinaga, 2005.[29,8] The difference in sensitivity could be attributed to different morphological constitutions of gram-positive and gram-negative bacteria.[30] Various metabolic compounds in banana peel show its broad-spectrum antibacterial activity similar like standard antibiotic.[25]

Analysis of Phenolic Compound by HPLC

Results of HPLC analysis [Fig. 13 (a), (b), (c)] determined at 280 nm, shows the presence of various constituents as evidence, by the chromatogram obtained at various retention times (3.24, 5.51, 6.11, 7.00, 8.68, and 9.08). These constituents were obtained in 70% acetone extract and water fractionate of unripe, ripe and leaky ripe banana peel.

A 1-mg of each standard (Gallo-catechin, catechin, gallic acid, tannic acid and quercitin) was dissolved in 1-mL of HPLC grade water. For quantitative estimation of tannin in 70% aqueous extract of banana peel tannic acid were used as a standard, 15% acetonitrile and 85% water with pH 4.5 as mobile phase. Calibration curve of mean area against concentration of varying concentration of tannic acid was plotted. The concentration of stock solution was 10 mg/mL. Working solution was 20, 60 and 100 µg/mL prepared from stock solution by taking its 10 µL and then injected three times into the column and diluted up to 1-mL with mobile phase (acetonitrile: water), the peak area and retention times were recorded.

In Fig. 13 (a, b, c), 10 mg/mL of water fractionate of unripe, ripe and leaky ripe banana peel (10 µL) diluted to 1-mL with mobile phase(acetonitrile: water)

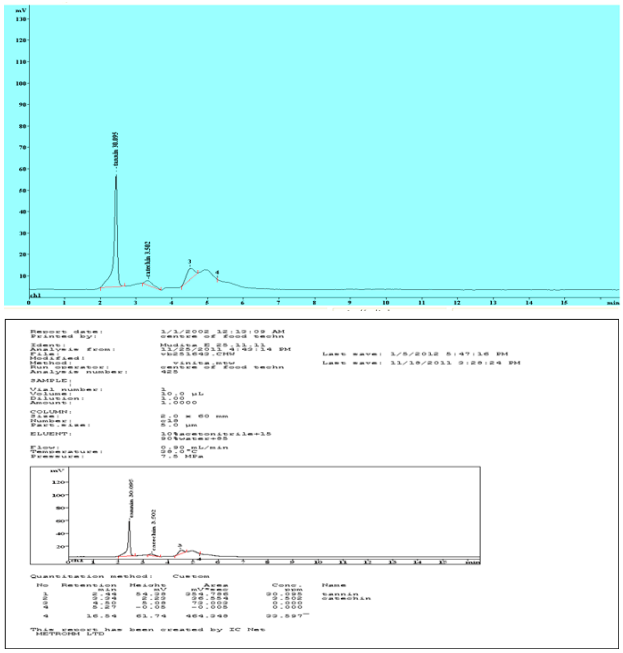

Fig. 13 (a): Chromatogram result of water extract of unripe banana peel extracts and its parameters and data records.

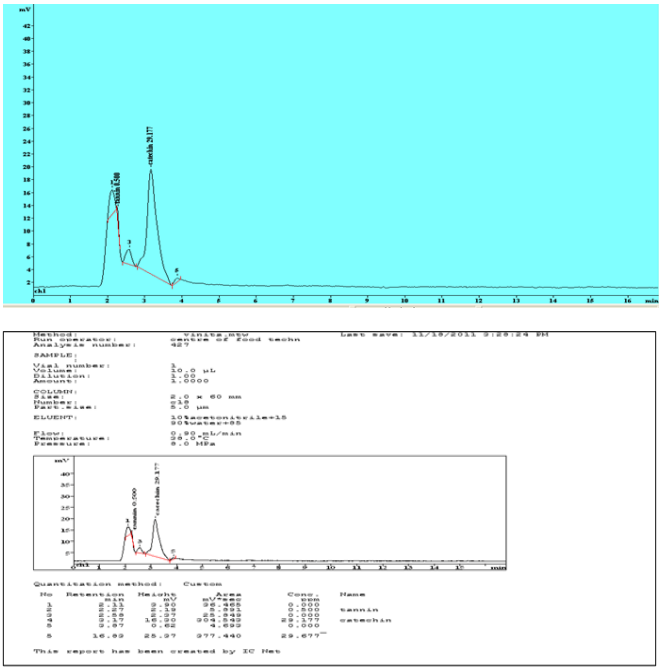

Figure 13 (c): Chromatogram result of water extract of leaky ripe banana peel extracts and its parameters and data records.

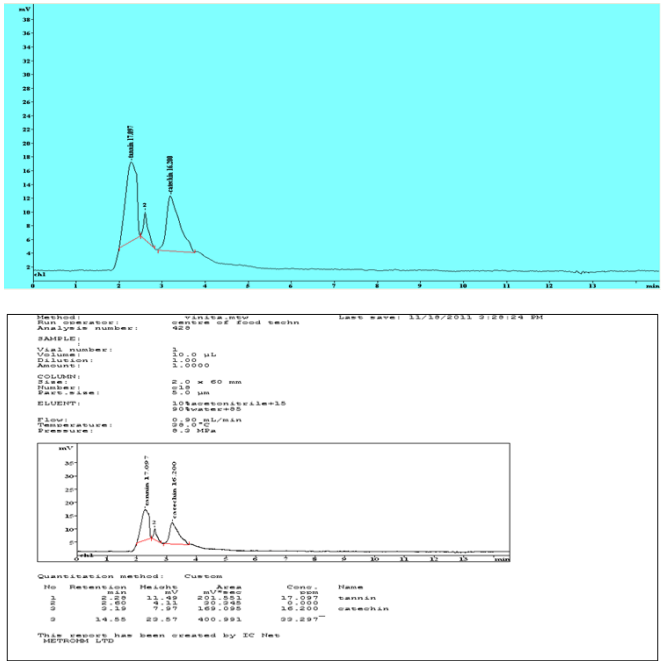

Figure 13 (b): Chromatogram result of water extract of ripe banana peel extracts and its parameters and data records.

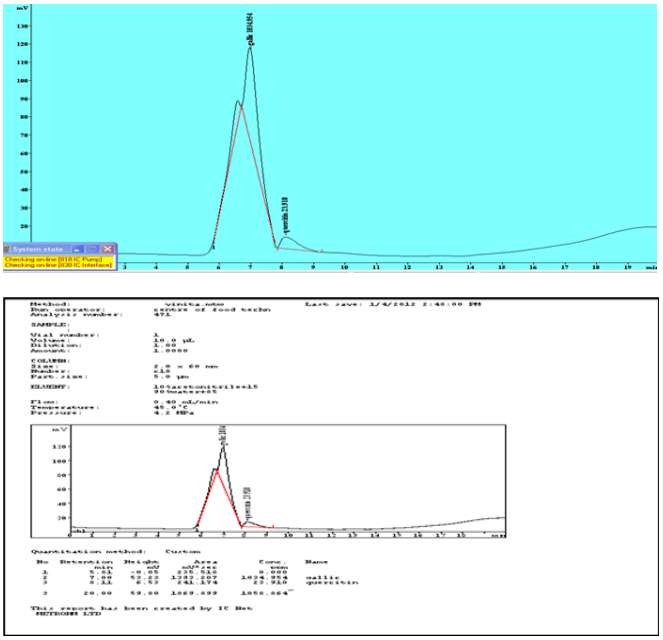

Figure 14 (a): Chromatogram result of 70% acetone extract of unripe banana peel and its parameters and data records.

(100 µg/mL) shows peak in the retention time similar to the retention time of tannin (2.44 minutes) and catechin (3.34 minutes mg). Significantly high tannin (30 µg/mL) content was seen in unripe banana peel (100 µg/mL) whereas, water fractionate of leaky ripe peel (100 µg/mL) shows high content of catechin (29.177 µg/mL). For quantitative estimation of tannin in 70% aqueous extract of banana peel using tannic acid as standard, 15% acetonitrile and 85% water with pH 4.5 as mobile phase. Calibration curve of mean area against the concentration of varying concentrations of tannic acid was plotted. The growth of bacteria was inhibited by group of tannins. It has been reported that tannin acid and propyl gallate, but not gallic acid, were inhibitory to foodborne bacteria, pathogenic bacteria and off flavor producing microorganisms. The antimicrobial properties associated with the hydrolysis of ester linkage between gallic acid and polypols hydrolyzed after ripening of many fruits. Tannins in these fruits thus

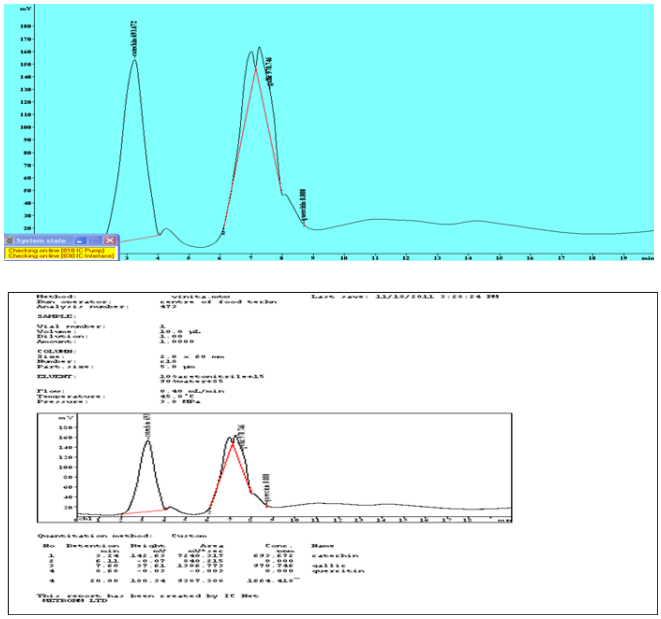

Figure 14 (b): Chromatogram result of 70% ripe banana peel extracts and its parameters and data records.

serve as a natural defense mechanism against infection.[17] In Fig. 14 (a) Peak obtained at retention time 7.00, was quite similar to retention time of gallic acid 7.05 in 70% acetone extract, while peak obtained at retention time 8.11 (Peak 3) is closer to the retention time of quercitin 7.93. The intensive peak of catechin (693.67 µg/mL) at retention time 3.14 and gallic acid (970.7 µg/mL) at retention time 7.60 were obtained in 70% acetone extract of ripe banana peel (1000 µg/mL) as shown in Fig. 14 (b). Whereas, mainly two peaks of gallic (303.24 µg/mL) and quercitin (1.778 µg/mL) were identified in 70% acetone extract of leaky ripe (1-mg/mL) banana peel while other were unidentified. The variation in antibacterial activity in aqueous extract of banana peel sample against gram-positive and negative bacteria at varying ripening stages may be due to the accumulation of tannin content and some other soluble sugar. Result from HPLC analysis (Fig. 13 a, b) strongly support that tannin in the responsible component for antimicrobial activity in water fractionate of banana peel. Tannins, are high molecular weight phenolic compounds which are present in many plants, including fruit pericarp.[31] The result was in line with the report given by Parseh *et al.* (2011) that tannin rich fruit could cure and prevent various illnesses. They inhibit bacterial growth and protease activity by damaging its cell wall and cytoplasm,[32,18] causing the rapid destruction of bacteria's vegetative structure and spores' more slowly.[33] Similarly, Çolak *et al.* (2010) also reported that tannic acid exhibited broad spectrum antibiotic activity. This study showed that banana peel in very mature stages is very potent antibacterial agent. Apart from that, certain flavonoids like catechin, gallo-catechin and quercitin are also responsible for the antibacterial property of banana peel as illustrated by HPLC analysis.

Conclusion

It is evident from the prior findings that banana peel appears to satisfy all the criteria for antibacterial agents, being both affordable and safe. In this study, banana peel was observed to have an antimicrobial effect on all bacterial isolates, similar to tannin and flavonoid and exhibit broad-spectrum antimicrobial properties. Unripe extract of banana peel exhibited broad-spectrum activity against gram-positive and gram-negative bacteria. From the above investigations, it can be concluded that the waste part of banana could be a potential candidate for nutraceutical preparation and also useful in the pharmaceutical industry to benefit society, as it can be utilized for its antibacterial and antioxidant properties. A banana a day can keep your skin and stomach safe all the way!

Acknowledgement

Miss Shadma Anjum would like to thank Maulana Azad National Fellowship for the financial. This work was funded by grants of UGC Scheme of Maulana Azad National Fellowship (MANF) 2010-2011 17.1/2010/MANF-MUS-UTT-5479. This work has also been supported by the Centre of Food Technology and Centre of Biotechnology, University of Allahabad under Research University Grant Scheme (MANF-UGC) (Project No: F1-17.1/2010/MANF-MUS-UTT-5479). I am pleased to thank the Centre for Biotechnology and Centre of Food Science and Technology, University of Allahabad for providing the facilities.

Conflict of interests

All other authors declare no conflict of interests.

Code Availability

HPLC Report has been created by IC NET metrohm ltd.

Consent of Participate

Verbal consent was solicited and obtained for pure bacterial culture from National Collection of Industrial Microorganisms (NCIM) National Chemical Laboratory, Pune, India and maintained on nutrient agar.

Reference

1. Kumar KPS, Bhowmik D, Duraivel S. Manivannan U. Traditional and medicinal uses of banana. J Pharmacogn Phytochem 2012; 1(3): 57-70.
2. Food and Agriculture Organization statistical databases (FAOSTAT). http://apps.fao.org. 2008.
3. Mohapatra D, Mishra S, and Sutar N. Banana and its utilization: an overview. J Sci Ind Res 2010; 69: 323–329.
4. Arora A, Choudhary D, Agarwal R, Singh V P. Compositional variation in β-carotene content, carbohydrate and antioxidant enzymes in selected banana cultivars. J Food Sci Technol 2008; 43: 1913–1912.
5. Jahan M, Warsi M K, Khatoon F. Concentration influence on antibacterial activity of banana blossom extract-incorporated