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ANTIBACTERIAL, ANTIOXIDANT AND PHYTOCHEMICAL ANALYSIS OF RIPE AND UNRIPE BANANA PEEL

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ABSTRACT
The banana peel processing was optimized by drying temperature. The extract was obtained by different solvent with respect to time for extraction. The drying process was done at room temperature for several weeks. The ground banana peel samples were extracted with different solvents *viz.* 80% ethanol, 70% acetone and distilled water. The extraction was performed by using magnetic stirrer for 30 minutes. Phenolic content was found highest in aqueous ripe sample with concentration of 84µg/ml and lowest in ethanol unripe with 13µg/ml. Disc diffusion method used to verify the existence of antimicrobial activities on different microbial isolates like *E. coli, S. typhi, Salmonella Shigella, E. faecalis, P. fluorescens, and B. subtillis, Staph* all. Results were obtained in acetone extract against *P. fluorescens* with zone of clearance having 27mm diameter whereas less zone of clearance was observed in ethanol extract against *E. faecalis* .with diameter of 11mm.Thin Layer Chromatography is used to determine the alkaloids and vitamin (E, C) in different extracts, Vit. C and tannic acid were spotted followed by high Performance Liquid Chromatography that is used to determine the bioactive compounds in banana peel extract. Salicyclic acid was detected in both the ripe and unripe aqueous samples using HPLC technique.

KEYWORDS: Antioxidant, Antibacterial, phytochemical, Banana peel, TLC, HPLC.

INTRODUCTION

Fruits and vegetables are an important part of good diet. Several known chronic diseases can be reduced by consumption of banana peel. Bioactive compounds are present in significant amount in fruits and vegetables which are negatively associated with their mortality and morbidity from cardiovascular and certain types of cancer. Fruits and vegetables waste and their byproducts are formed in great amount during industrial processing and hence represent a serious problem, as they exert harmful impact on environment (PM, Dewick *et al.*, 2002)

Banana is a genus of Musa and family of Musaceae. There are around 70 species of *Musa* with a broad variety of uses (Fereidoon S. Marrian, 2004). The common is banana scientifically known as *Musa sapientum.* It was originally from Malaysia and are now cultivated all over the tropical and subtropical continents. Banana plants are the world's biggest herbs, grown abundantly in many developing countries (Aurore *et al.*, 2009). Musa species trees are best cultivated in a highly organic soil with pH 5.5-7.0. This plant needs a lot of water to grow and yield bananas. A single banana fruit weigh approximately 125gram in over 122 countries worldwide banana is cultivated (Husain and William, 2010). The total production of banana fruit is 56.4 million metric tons in the cultivated area of 3.8 million hectares ranking it fourth behind rice, corn and milk (Arumugam and Manikandan, 2011).There are various industrial applications of banana including energy related activities, bio-sorbents, pulp and paper, cosmetics, bio-fuel production, organic fertilizer, environmental cleanup and biotechnology related processes (Bori *et al*., 2007).

Banana peel recorded stronger antioxidant activity and more quantity of phenolic compounds (Someya *et al*., 2002), higher in mineral contents and greater range of phenolics composition than banana pulp. As the potassium content of banana is higher than any other fruit, because of which they are naturally slightly radioactive (Amarnath and Balakrishnan, 2007).

Antifungal and antibiotic properties are found in the peel and pulp of fully ripe bananas (Brooks, 2008). The antibiotic acts against Mycobacteria (Omojasola and Jilani, 2009). A fungicide in the peel and pulp of green fruits is active against a fungus disease of tomato plants (Ponnuswamy *et al*., 2011). Norepinephrine, dopamine, and serotonin are also present in the ripe peel and pulp (Ratule *et al*., 2007). The norepinephrin and dopamine elevate blood pressure serotonin inhibits gastric secretion and stimulates the smooth muscle of the intestines (Anhwang *et al*., 2009). Tannic acid, gallic acid, vitamin C and B, volatile components are also present in banana peel

All parts of the banana plant have medicinal applications (Amit and Shailandra, 2006): the roots are administered in digestive disorders, dysentery and other ailments; banana seed mucilage is given in cases of diarrhea in India (Bhat *et al*., 2010a) ;the young leaves are placed as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and of the leaves are taken in dysentery and diarrhea and used for treating malignant ulcers (Girish and Satish, 2008) the astringent plant sap in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery and diarrhea, and it is applied on hemorrhoids, insect and other stings and bites; the flowers in bronchitis and

dysentery and on ulcers; cooked flowers are given to diabetics.

Banana peel contains fatty acids responsible for their antimicrobial activity. Vitamin A, Vitamin C , Vitamin E, Vitamin B6, âsitosterol, malic acid, succinic Acid, palmatic acid, gallocatechin, dopamine Magnesium, phosphorus, potassium, fiber, Iron are present in banana peel. (Sumathy *et al.,* 2011) Additionally, Flavonoids, tannins, phenols, steroids alkaloids, glycosides, saponines and terpenoids were found to be present in the peels of genus *Musa* which are associated with the antimicrobial activity of peel. These phytochemicals have been reported to exert multiple biological and pharmacological effects (antihypertensive, antidiabetic and anti-inflammatory activities). Bioactive compounds in plants are majorly responsible for their medicinal applications therefore the presence of these bioactive substances in banana peels suggests that the peels possess valuable medicinal potential yet to be explored (Ighodaroro *et al.,* 2009)

Against cancer and heart diseases banana should be considered as a functional food source banana is also considered to be a potential application natural antioxidant (Someya *et al*., 2009) Bananas peel are a good source of natural antioxidant which include vitamins and beta carotene. It contains number of antioxidants and minerals that can help the skin restore itself naturally. Natural antioxidants are primarily phenolic compound that may occur in all part of plant. They are multifunctional and can react as free radical terminators, metal chelators and single oxygen quenchers. The common phenolic compounds in banana peel are topopherols, flavonoids and other related compound such as phenolic acid. (Nur Atiah Binti Azmi 2010**).**

Phenolic compounds are secondary metabolites, which have been associated with flavor and colour characteristics of fruits and vegetables and are gaining considerable attention because of their potent antioxidant and health promoting properties (Kaur and Kappor, 2001). Banana peel could be good source of carbohydrates and fiber. The high fiber content also indicates that the peels could help treat constipation and improve general health and life style (Anhwange, B.A. *et al.,* 2009*).*

MATERIALS & METHODS

Collection of banana peels

Banana peels of both ripe and Unripe is collected from the local market, Aurangabad. It was dried at room temperature for several weeks and dried peels were blended using mechanical blender and made in fine powder. This powder of banana peel was stored in reagent bottle for further use.

Extraction of dried banana peel powder with different organic solvent

The dried banana peel powder was suspended in organic solvents. The organic solvents used are 80% ethanol, 70% acetone and distilled water.

Ethanolic Extract

10gram banana peel powder (ripen and unripen) is suspended in 80% 100 ml ethanol (80ml ethanol + 20ml distilled water) and kept on magnetic stirrer for half an hour and after vigorous shaking by stirrer it is kept for 48 hours incubation at room temperature.

Acetonic extract

10gram of banana peel powder (both ripe and Unripe) is suspended in 70% 100 ml acetone (70 ml acetone + 30 ml distilled water) and kept on magnetic stirrer for half an hour after vigorous shaking by stirrer it is kept for 48 hours incubation at room temperature.

Distilled water extract

10 gram of banana peel powder (both ripen and Unripen) is suspended in 100ml distilled water and kept on magnetic stirrer for half an hour after vigorous shaking by stirrer it is kept for 48 hours incubation at room temperature.

After the incubation of 48 hours the extracts were filtered by using muslin cloth both filtrate and residue were stored in reagent bottle for further analysis. (Ahmed M. Aboul-Enein *et al.,* 2016*)*

Purification of Extract

The banana peel extract was purified by using syringe filter. And the filtered purified extract was stored in autoclaved eppendroff tubes and stored in refrigerator for further analysis.

Phytochemical Analysis

Test for glycosides: to 0.5ml of extract 1ml of acetic acid was added and cool in water bath at 4^0C to this mixture 0.5ml of concentrated H_2SO_4 was added the formation of oil layer on the top indicated the presence of glycosides (Odebiyi and Sofowora, 1978).

Test for Alkaloides: To 1.5ml of the extract was added 0.5ml of 1% HCL. This resulting mixture was then treated with few drops of Meyer's reagent (0.13gm $HgCl_2$ and 0.5gm of potassium iodide in 10ml of distilled water) the presence of creamy white ppt confirmed the presence of alkaloides (Ogunkwe *et al*., 2004).

Test for Tannins: one drop of 5% (0.5gm $FeCl_3$ in 10 ml distilled water) $FeCl_3$ was added to 0.5ml of the plant extract. The presence of a dirty-green precipitate indicated the presence of tannins (Trease and Evans, 1996).

Test for Flavonoids: To 0.5ml of the extract was added 2 drops of ammonia solution (NH3+) followed by 0.5ml of concentrated HCl. The pale brown coloration of the entire mixture indicated the presence of flavonoids (Odebiyi and Sofowora, 1978).

Test for Steroids: To 0.5ml of the plant extract was added 0.5ml of concentrated tetraoxosulphate (vi) acid (H2SO4). Formation of red colour indicated the presence of steroids (Trease and Evans, 1996).

Antimicrobial assay

Preparation of Active cultures of microbial isolates

150ml of nutrient broth (Beef extract 0.15g, Yeast extract 1.3g, Peptone 0.5g, Nacl 0.5g, Distilled Water 150ml) was prepared and was autoclaved. In laminar air flow it was distributed in screw cap bottles approximately 20ml in each bottle and loopful culture of *E. coli, S. typhi, Salmonella Shigella, E. faecalis, P. fluorescens, B. subtillis, S. auras* and staph all inoculated in respective screw cap bottle and incubated at 37^0C for 48 hours. After incubation the bottles are stored at 4^0C for further use

Preparation of nutrient agar plates for disc diffusion assay

500ml of nutrient agar (Peptone 2.5g, yeast extract 1gm, NaCl 2.5g, Agar 7.5g,) was prepared and autoclaved it. Later, in laminar air flow this autoclaved nutrient agar was

poured in Petri plate approximately 20ml in each Petri plate and allows it to solidify. After solidification the paper disc diffusion assay was performed on these plates.

Paper disc diffusion assay:

Firstly autoclaved paper discs and forceps were taken. Then 10 microlitre of microbial suspension was spreaded with the help spreader. Spreader and forceps used were heat sterilized. Then paper discs were dipped into the peel extract and were placed centrally in the plates. Later, the plates were incubated at 37^0C for 24 hours and observed for zone of clearance (Mokbel and Hashinaga, 2005)

Antioxidant assay

Antioxidant assay was performed by using Phosphomolybdate reagent by using ascorbic acid as standard. The stock solution of ascorbic acid was prepared by using 0.5gm of ascorbic acid in 100ml from which dilutions are made ranging from 50mg/ml to 200mg/ml (Rashid *et al.*, 2013, Ahmed *et al*., 2013).

Total Phenolic Content

The total phenolic content in banana peel extract was found out calorimetrically by Folin Ciocalteu reagent using gallic acid as standard (VL *et al.*, 1965).

Detection of bioactive compounds present in the banana peel extract

Thin Layer Chromatography

TLC for tannic acid:

Tannic acid was used as a standard for TLC and spotted on TLC Plate along with peel extract by using capillary tube; the spots were allowed to dry after that TLC plate was kept in mobile phase. The mobile phase was used as follows: chloroform: methanol: water 5:5:0.5 and allowed to reach the $3/4^{th}$ of the TLC plate. Then the 10% H_2So_4 were used as a locating reagent and sprayed on TLC plate and the spots were observed under U.V. light (Matook Saif Mokbel and Fumio Hashinga, 2005).

TLC for vita. C

Vita. C is the water soluble vitamin and it was spotted on TLC by using ascorbic acid as a standard, the 0.1gm of ascorbic acid was dissolved in 0.5ml of water and spotted on TLC plate along with peel extract by using capillary tube. Then TLC plate was kept in mobile phase. The mobile phase was used as follows: chloroform: ethanol : Acetone: conc. Ammonium hydroxide 2:2:2:1 and allowed to reach $3/4^{th}$ of the TLC plate, then TLC plate were observed under U.V. light for the presence of vita C in the samples. Vita C gives dark blue colour spot under U.V. light.

TLC for vit. E

Vit. E is the fat soluble vitamin. The tablet of vit. E was used as standard and spotted on TLC plate along with the peel extract by using capillary tube. Then TLC plate was kept in mobile phase. The mobile phase was used as follows: Petroleum ether : ethyl acetate 19: 1 and allowed to run $3/4^{th}$ of the TLC plate, then the TLC plate were observed under ultra violet light for the presence of Vit. E in the sample (M. BAI *et al.*, 1993)

High Performance Liquid Chromatography

The HPLC was used to detect the presence of alkaloids in the banana peel extract by using salicylic acid as standard. (Prerna Khawas and Sanker Chandra Deka, 2014). The sample injected was 20 microliter which were made in 1.4ml 50% methanol glass vials were used to make sample and standard. The column used was of C18 type. The gradient solvent system was used A and B was used A- acidified water of ph 2.64 and B- acidified water: acetonitrile 20:80. Flow rate of mobile phase was maintained at 1.5ml/min, retention time was 40min at room temperature .UV detector set at wavelength 320nm. The standard was prepared by mixing 0.1g of salicyclic acid in 1.4ml of absolute methanol.

RESULTS

The banana peels were collected from local market from Aurangabad, dried at room temperature and grounded in fine powder this powder of banana peel is stored in reagent bottle for further use.

Extraction of banana peels

The peel powder was extracted by using different concentration of ethanol (80%), acetone (70%) and pure distilled water. Following figure indicating the results of given sample.

Phytochemical analysis

The presence of different phytochemicals in banana peel extract has been shown in Table 3. It is observed that different phytochemicals have different degree of solubility in different solvents according to the polarity of solvents.

TABLE 1: Phytochemical analysis

Phytochemicals	Ripe peel extract			Unripe peel extract		
	Acetonic	Aqueous	Ethanolic	Acetonic	Aqueous	Ethanolic
Alkaloids	+	+	+	+	+	+
Glycoside	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Steroids	+	+	+	+	+	+

Antimicrobial Assay

Enterococcus faecalis has been found frequently infected, root cannel treated teeth, it cause primary infection in root cannel treated teeth.

Our investigation for aqueous and acetone unripe peel extract shows more diameter in zone of clearance against *E. faecalis* as compare to aqueous and ethanolic ripe peel extract as shown in table 2.

Pseudomonas fluroescens is an unusual cause of disease in humans, and usually affects the patient with compromised immune system *P. fluroescenes* is also known cause of fin rot in fish. Aqueous extract shows the maximum antibacterial activity as shown in table 3. Whereas Acetone unripe extract shows antimicrobial activity against *Bacillus subtilis.*

TABLE 2: diameter of zone of clearance of *E.faecalis* against extract

Organic Solvent	Zone of clearance in mm	
	Ripe	Unripe
Acetone	-	24nm
Aqueous	14mm	20mm
Ethanol	11mm	13mm

TABLE 3: Zone of clearance in Diameter of *P. fluroscence* and *B. subtilis* with different extract

Extract	Bacterial Species	Banana peel	Zone of clearance in Diameter
Aqueous	*P. fluroescens*	Ripe	27mm
		Unripe	-
Acetone	*B. subtilis*	Ripe	-
		Unripe	8mm

FIGURE 1a. Zone of clearance of acetone. Extract against *E. faecalis*

FIGURE 1b. zone of clearance of ethanolic extract against *E. faecalis*

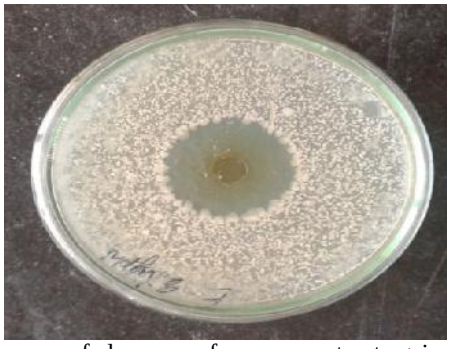

FIGURE1.c. zone of clearance of aqueous extract against *E. faecalis*

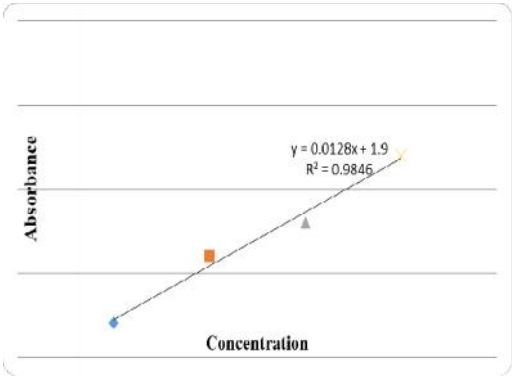

FIGURE 2. zone of clearance of acetone extract against *B. subtilis*

Antioxidant assay

The results of antioxidant assay have been shown in table 4 with the maximum antioxidant activity in acetone unripe and least in acetone ripe.

TABLE 4. concentration of samples obtained by antioxidant assay

Extraction Solvent	peel extract	Concentration (µg/ml)
Acetone	ripe	34µg/ml
	unripe	92µg/ml
Ethanol	ripe	66µg/ml
	unripe	50µg/ml
Aqueous	ripe	56µg/ml
	unripe	40µg/ml

Total phenolic content

The highest phenolic content was observed in aqueous ripe while least was observed in acetone unripe. Table 5 shows the concentration of phenolics in banana peel extract.

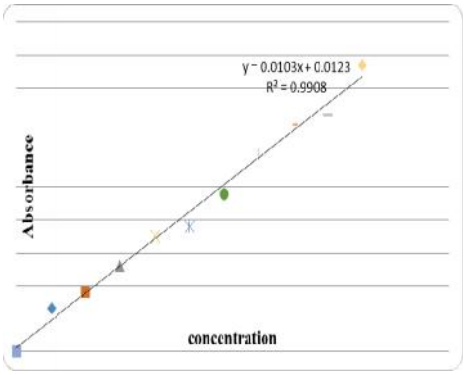

TABLE 5. concentration of samples obtained by Total Phenolic Content

Extraction Solvent	peel extract	Concentration (µg/ml)
Acetone	ripe	57 µg/ml
	unripe	73 µg/ml
Ethanol	ripe	35 µg/ml
	unripe	13µg/ml
Aqueous	ripe	84 µg/ml
	unripe	36 µg/ml

TLC for detection of bioactive compounds

TLC for tannic acid

Black spots were observed on TLC after spraying with 10% H_2SO_4. A results were obtained in all three extracts of aqueous, acetone, ethanol ripe samples, where as in unripe sample result was obtained only in acetone extract this confirms the presence of tannic acid in the banana peel extract.

FIGURE 4a. TLC of ripe sample Fig.4b. TLC of unripe sample

TLC of vitamin C

Blue spots were observed in ripe sample after observing under U.V. light hence this confirmed the presence of vitamin C in the sample.

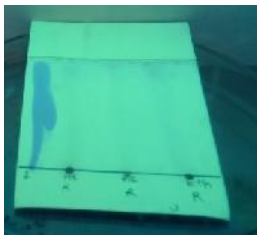

FIGURE 5. TLC of vitamin C

TLC of vitamin E

No spots were observed on the TLC plate under U.V. light it indicates that vitamin E is absent in banana peel extract

High Performance Liquid Chromatography

In HPLC analysis was done. It was carried out only for one solvent i.e. aqueous. Comparative study reveals that aqueous peel extract from ripe and unripe banana gives more proper result as compare to other extraction solvent. Ripe and unripe banana peels with aqueous extracts shows that unripe peel extract have high amount of phytochemicals present in it as compare to aqueous ripe banana peel extract. Salicylic acid was used as a standard which shows retention time at 17.6 min and unripe and ripe sample showed similar result with retention time 17.3 min and 17.2 min respectively .Hydroquinone was also detected with retention time at 6.24 min in unripe banana peel extract. p-Coumaric acid was detected with retention time at 27.02 min in ripe banana peel extract. Results were compared with the HPLC analysis performed by Prerna Khawas and Sanker Chandra Deka (2014).

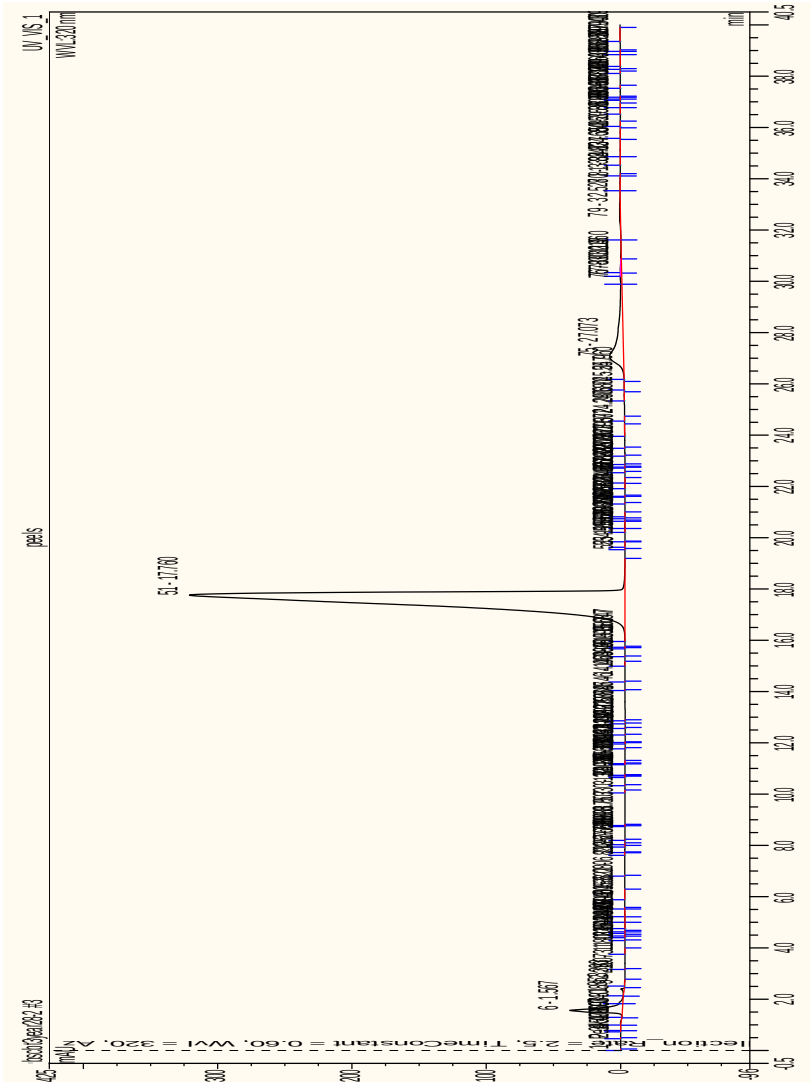

FIGURE 7a. HPLC analysis of standard salicylic acid

HPLC for aqueous extracted ripe sample

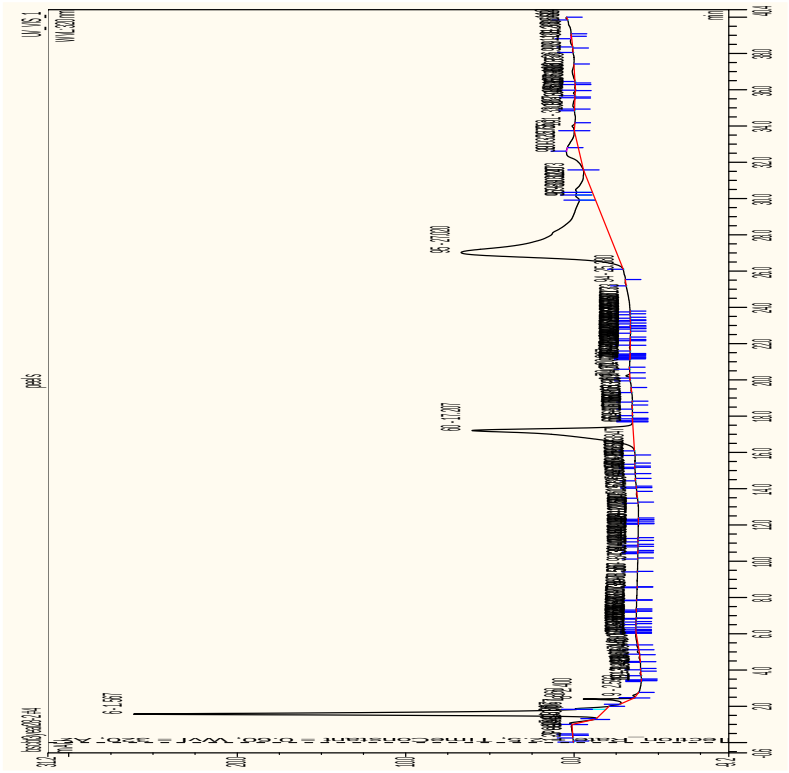

FIGURE 7b. HPLC analysis of aqueous ripe sample.

HPLC for extracted aqueous unripe sample

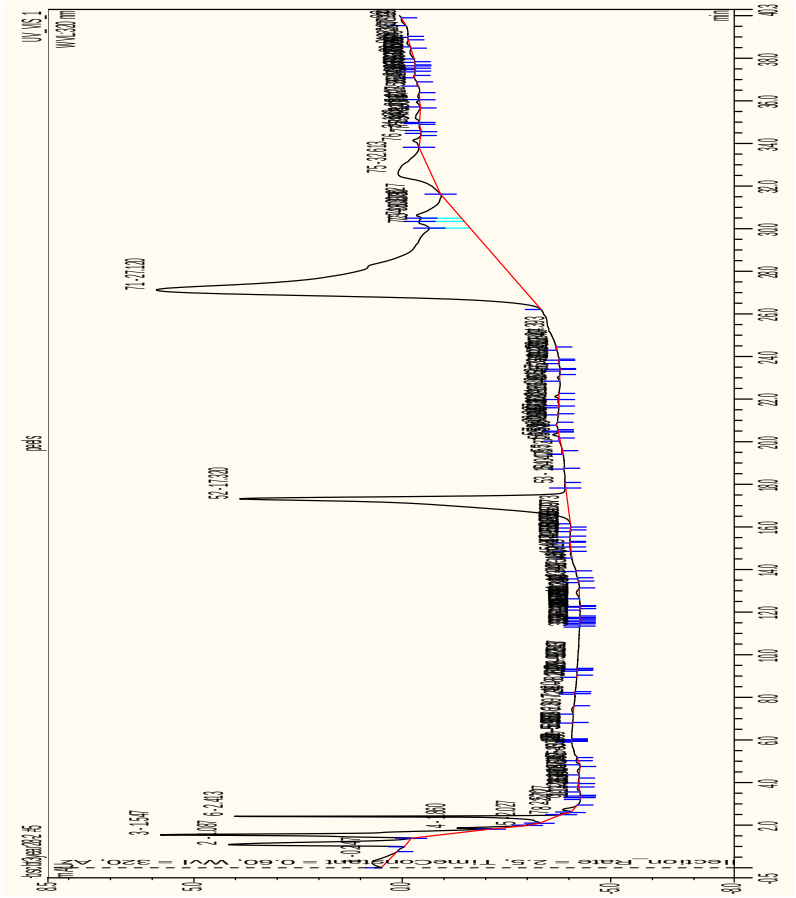

FIGURE 7C HPLC analysis of aqueous unripe sample.

DISCUSSION

80% ethanolic, 70% acetone and 100% distilled water ripe and unripe peel extract was carried out for further studies. Same result was obtained by Ahmed M. Aboul-Enein *et al.* (2016).

The phytochemical screening carried out on ripe and unripe banana peels showed peels contain some secondary metabolites such as glycosides, alkaloids, flavonoids, tannins and steroids. In general, secondary metabolites presences in plants have been reports by Rabe (2000) should be responsible for therapeutic activity.

All three extracts of unripe banana peels recorded significant antimicrobial activities, while ripe banana peel extracts recorded low activity. Singh and Bhat (2003) reported that flavonoids are responsible for the antimicrobial activity associated with some medicinal plants. The result of this project highlight the fact that organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles where either polar or non polar and they were extracted more through the organic solvent medium.

Total antioxidant activity of various extracts of ripe and unripe banana peels was studied. This assay gives an estimate of the overall antioxidant potential of banana peels. In the presence of extracts Mo (VI) is reduced to Mo (V) and forms green colored phosphomolybdenum V complex, it shows maximum absorbance at 765nm. According to the results, the acetone unripe and ethanol ripe extracts showed highest antioxidant activity, whereas acetone ripe and aqueous unripe showed least antioxidant activity. Similar studies by Gonzalez-Montelongo *et al.* showed the total antioxidant activity of banana peel extract under different solvent and incubation condition.

Phenols are secondary metabolites in plants and are known to possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities and also decrease cardiovascular complications SR *et al. (*2012) reported that Methanolic extract of banana peel has higher total phenolic content.

TLC was carried out by using aqueous, ethanolic and acetone extract of banana peels. The result reveals that appearance of spots indicates the presence of tannic acid and Vit. C in the extract. Ana maria Hossu and *et al.*, (2009) found the similar result.

In HPLC analysis, different varied Peaks shows, many phenolic compounds are found in the both ripe and unripe banana peels. The HPLC analysis of the phenolic compounds revealed that salicyclic acid and other phenolic compounds were present in both ripe and unripe samples. Khawas P. and *et al. (*2014) reported the same results with various developmental stages of banana.

CONCLUSION

In our studies on comparative analysis depicted that, unripe banana peels extract have more phytochemical content, as well as good antioxidant and antibacterial properties as compare to ripe banana peels extract this is due to the higher amount of phenolics present in unripe peel of banana. Research can be done on periodontal pathogens with different varieties of banana and its extract. Banana peels can be alternative source of pectin with potential applications as fat replacer in food industry. Banana peels can be used as potential source of natural antioxidant in the applications of food industry to suppress lipid oxidation

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