

# PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF BANANA PEEL

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## ABSTRACT

*Banana (Musa paradisiaca), is grown worldwide and consumed as ripe fruit or used for culinary purposes. Peels form about 18-33% of the whole fruit and are a waste product. With a view to exploiting banana peel as a source of valuable components, the nutritional composition, antioxidant components were determined. The phytochemical analysis of aqueous and organic solvent extracts of banana peel (Musa Paradisiaca) reveal the presence of alkaloids, flavanoids, carbohydrates, proteins, Tannins, Terpenoid, Saponins, Glycosides and Anthroquiones. The phytochemicals alkaloids, flavanoids and tannins have a very good antioxidant property while saponins and terpenoids have antimicrobial activity. The qualitative analysis of Aqueous and organic solvent extracts of banana peel (Musa Paradisiaca) reveals the presence of reducing sugar (glucose and fructose). The qualitative analysis of Amino acids in banana peel (Musa Paradisiaca) reveals the presence of tryptophan, phenylalanine, tyrosine, arginine and cysteine. The quantitative analysis of starch, Proteins were done. The proteins and carbohydrates contents were higher in Musa paradisiaca peel extracts of ethanol. The qualitative analysis of tryptophan were carried out in Musa paradisiaca peel extract and the tryptophan was found to be higher in water and ethanol extracts. The amino acid typtophan is the precursor for the formation of serotonin which plays an important role as a neurotransmitter. In the central nervous system, serotonin plays an important role as a neurotransmitter in the modulation of anger, aggression, body temperature, mood, sleep, human sexuality, appetite, and metabolism. The qualitative analysis of minerals like Iron, Phosphorous, calcium, potassium, sodium were carried out in banana peel (Musa Paradisiaca). The mineral compound was higher in the aqueous extract then the organic extracts. Potassium ions plays an important role in the muscle construction and nerve impulse transmission. Sodium ions are necessary for the regulation of body fluids and blood. Phosphorous helps to maintain normal Acid-base balance by acting as one of the body's most important buffer. Iron is necessary to deliver oxygen through the body in the form of hemoglobin.*

**Keyword :** - Phytochemicals, aminoacid, Banana peel,serotonin, Musa paradisiaca

## 1.INTRODUCTION

Free radicals are continuously produced in our body either naturally or on exposure to environmental stress as well as other factors and can be implicated in many diseases like cancer, atherosclerosis, arthritis, Parkinson's disease, Alzheimer's disease, aging and other age related problems [1]. Mammalian cells possess elaborate defense mechanisms for radical detoxification. Antioxidants are agents, which scavenge the free radicals and prevent the damage caused by them. In spite of these in-built defense mechanisms, it seems more meaningful to utilize extra antioxidants available in diets, especially from fruits, vegetables and whole grains [2]. Due to their minimal side effects, there are growing interests in using natural products for preventive and therapeutic medicine [3]. The peels of a variety of fruits have gained attention as a natural source of antioxidants and phytochemical content which are rich in compounds with free radical scavenging activity.

Banana is one of the world's most important crops grown by small- and large-scale producers alike, with production occurring in more than 130 countries. The global production of banana is estimated to be around 72.5 million metric tonnes out of which 21.77 million metric tonnes is contributed by India. The peel which protects the banana fruit is discarded as waste after the inner flesh portion is eaten [4] and poses an environmental problem due to its nitrogen and phosphorus quantity. Banana and Plantain peels are major agricultural wastes which have been used as medicine, animal feeds, blacking of leathers, soap making, fillers in rubber and so on [5]. Fruit wastes are highly perishable and seasonal and are a problem to the processing industries and pollution monitoring agencies. This problem can be recovered by utilizing its high value compounds, including the dietary fibre fraction that has a great potential in the preparation of functional foods [6].

## **2. MATERIALS AND METHOD**

### **2.1 CHEMICALS:**

All the chemicals used were of analytical grade Methanol, Ethanol, Folin's phenol reagent, Anthrone, Bovine serum albumin, Alkaline copper solution, Silica gel-G, Acetic acid, DPPH, 2, 4 dinitrophenyl hydrazine (2,4DNPH), Trichloro acetic acid, Sulphuric acid (85%), Sodium carbonate.

### **2.2 PLANT SOURCE:**

The *Musa paradisiaca* (banana) were purchased from the local market. The fruit was washed thoroughly with tap water and then with distilled water. The peels of the fruits were air dried in the sunlight for three days. The dried peel was grounded into uniform powder using a milling machine. The powder was used for extraction preparation.

### **2.3 PREPARATION OF SAMPLE:**

#### **2.3.a. Ethanol Extraction :**

6gm of *Musa paradisiaca* peel powder was dissolved in 120ml of ethanol. The extract is prepared using a Soxhlet extractor. The supernatant was collected.

#### **2.3.b .Aqueous Extraction:**

6gm of *Musa paradisiaca* peel powder was dissolved in 120ml of distilled water and boiled on slow heat for two hours. It was then filtered through filter paper and the supernatant was collected.

#### **2.3.c . Chloroform Extraction:**

6gm of *Musa paradisiaca* peel powder was dissolved in 120ml of chloroform. The extract was prepared using a Soxhlet extractor. The supernatant was collected.

## **2.4 QUALITATIVE ANALYSIS OF PHYTOCHEMICALS ANALYSIS**

### **2.4. a. TEST FOR ALKALOIDS**

#### **(i) MAYERS TEST:**

To a few ml of filtrate, a drop of Mayer's reagent was added along the sides of the tube. A creamy white precipitate is formed, indicating the presence of Alkaloids.

#### **(ii) WAGNERS TEST:**

2ml of filtrate, 1% HCl and 6 drops of Wagner's reagent are added, a brownish red precipitate indicates the presence of Alkaloids.

#### **2.4.b. DRAGENDORFF TEST:**

To 2ml of filtrate 1% HCl and 6 drops of Dragendorff's reagent was added.

#### **2.4.c. TEST FOR FLAVANOIDS:**

To 5ml of dilute ammonia solution, extract was added, following by addition of concentrated sulphuric acid in side of the tube. Appearance of yellow coloration indicates the presence of Flavanoids.

#### **2.4.d .ALKALINE REAGENT TEST:**

To 1ml of extract, a few drops of dilute sodium hydroxide were added. Appearance of yellow colour indicates the presence of Flavoids.

#### **2.5 TEST FOR TANNINS:**

5g of dried powder was stirred with 10ml of distilled water. This was filtered and 0.1% ferric chloric reagent was added to the filtrate. A blue black precipitate indicates the presence of tannins.

#### **2.5.a. FERRIC CHLORIDE TEST:**

About 0.5g of dried powdered sample was boiled in 2ml of water in a test tube and then filtered. A few drops of 5% ferric chloride were added. A brownish green or a blue black colour indicates the presences of phenolic compound.

#### **2.6 TEST FOR TERPENOID**

#### **2.6.a. SALKOWSKI TEST:**

5ml of the extract was mixed with 2ml chloroform and concentrated sulphuric acid to form a layer. A reddish brown colour is formed, indicates the presence of Terpenoids.

#### **2.7 TEST FOR SAPONINS**

The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of shows the presence of saponins.

#### **2.8 TEST FOR CARBOHYDRATES**

#### **2.8.a. BENEDICTS TEST:**

To 0.5ml of filtrate, 0.5ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 minutes. A characteristic red coloured precipitate indicates the presence of sugar.

#### **2.9 TEST FOR PROTEIN**

#### **2.9.a. BIURET TEST**

To 1ml of extract equal volume of 5% NAOH solution and Copper sulphate solution were added. A pink colour indicates presence of proteins.

#### **2.9.b. TEST FOR ANTHRAQUINONES**

5ml of extract was hydrolysed with dilute Sulphuric acid and extracted with benzene. 1ml of dilute ammonia was added to the above mixture. Pink colorization suggested the positive response for anthraquinones.

#### **2.10 QUALITATIVE ANALYSIS OF AMINO ACID:**

#### **2.10.a. NINHYDRIN TEST:**

To 1ml of sample add 5 drop of nin hydrin reagent was added and heated in a boiling water bath for 2min. A Purple colour indicates the presence of amino acid .

**2.10.b. XANTHOPROTEIN TEST:**

To 3ml of sample add 1ml of concentrated nitric acid and heated for 3min. then cooled and 0.5ml of sodium hydroxide was added. the reddish orange colour indicates the presence of aromatic amino acid.

**2.10.c. FOLIN'S TEST:**

To 1ml of sample add 1ml of Folin's phenol reagent was added and followed by the addition of 1N sodium carbonate was added. The blue colour indicates the presence of Tyrosine and Tryptophan.

**2.10.d. MILLON'S TEST:**

To 1ml of sample add 1ml of Millon's reagent was added and heated for 3min. then the addition of 1% nitrite was added. The red colour indicates the presence of Tyrosine.

**2.10.e. PAULY'S TEST:**

To 1ml of sample add 1ml of 1% sulphosalicylic acid then cooled in ice and 1ml of 5% sodium nitrite was added. after 5min 2ml sodium carbonate was added cherry red colour indicates the presence of Histidine.

**2.10.f. MORNER'S TEST:**

To 1ml of sample add 1ml of Morner's reagent was added and heated for 3min. The green colour indicates the presence of Tyrosine.

**2.10.g. HOPKIN'S COLE TEST :**

To 1ml of solution add equal volume of glyoxylic acid add and followed by the addition of concentration sulphuric acid along the sides of test tube a violet colour ring formed at the junction of two layer. it indicates the presence of tryptophan.

**2.10.h. ALDEHYDE TEST:**

To 1ml of solution add equal volume of formaldehyde add and followed by the addition of concentration sulphuric acid along the sides of test tube. a violet colour ring formed at the junction of two layer. It indicates the presence of tryptophan.

**2.10.i. EHRLICH'S TEST:**

To 1ml of sample add 1ml of Ehrlich's reagent was added. The blue colour indicates the presence of Tryptophan.

**2.10.j. SAKAGUCHI'S TEST:**

To 1ml of sample add 5 drop of sodium hydroxide was added and 4 drop of  $\alpha$ -naphthol and shaken well to this 10 ml of bromine water was added the red colour indicates the presence of Arginine.

**2.10.k. SODIUM NITRO PROSSIDE TEST :**

To 1ml of sample add 1.5ml of sodium hydroxide was added and 0.5ml of nitro prosside and 1.5 ml of 1% glycine was added contents are boiled for 1min and cooled then add 3ml of 6N HCl allow to stand for 15min. the reddish purple colour indicates the presence of Methionine.

**2.10.l. SODIUM PLUMBATE TEST:**

To 1ml of sample add equal volume of 45% sodium hydroxide the contents are boiled for 2min and cooled then add 0.5ml of lead acetate was added. Dirty black colour indicates the presence of cystine.

## **2.11 QUANTITATIVE DETERMINATION OF PHYTOCHEMICALS :**

### **2.11.a.ALKALOID DETERMINATION:**

To 5g of the sample in 250ml beaker, 200 ml of 10% acetic acid ethanol was added, covered and allowed to stand for 4 h. this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtrate. The residue is the alkaloid, which was dried and weighted.

### **2.11.b.FLAVONOID DETERMINATION:**

10g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No 42 (125 mm). The later transferred into a crucible and evaporated into dryness over a water bath and weighted to a constant weight.

### **2.11.c. CAROTENE DETERMINATION :**

Weigh 2gm of sample and homogenise in mortar by adding 20ml of ether. Repeat the above procedure till the residues is forms pigment evaporation. The solution transferred into a chinadish and the ether to dryness over a boiling water bath dry and weights the residue as carotene.

## **2.12 FREE RADICAL SCAVINGING ACTIVITY:**

### **ANTIOXIDANT ACTIVITY DPPH:**

DPPH free radical scavenging activity of ethanolic extract can be determined by using **Kirby and Schmidt** method[14]. The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1- diphenyl-2-picrylhydrazyl. (DPPH)- free radical activity by modified method. The diluted working solutions of the test extract were prepared in ethanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in ethanol and 1 ml of this solution was mixed with 1 ml of sample and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. ethanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank.

## **2.13 QUATITATIVE DETERMINATIONS:**

### **2.13.a. ESTIMATION OF CARBOHYDRATE BY ANTHRONE METHOD:**

The amount of carbohydrate present in the plant extract was estimated by **E.W.Yemm and A.J.Willis** method[10].

### **2.13.b. ESTIMATION OF PROTEIN BY LOWRY'S METHOD:**

The amount of protein present in the plant extract was estimated by using **Lowrys** method[11].

### **2.13.c.ESTIMATION OF INORGANIC PHOSPHOROUS BY FISKY SUBBARROW METHOD:**

To estimate the amount of inorganic phosphorous present in the plant extract by **Fisky Subbarrow** method[13].

### **2.13.d. ESTIMATION OF IRON :**

The amount of iron present in the plant extract was estimated by Ramsay method.[12]

### **2.13. e. ESTIMATION OF SODIUM:**

The amount of sodium present in the plant extract by using the Systronic Flamephotometry Method[23].

**2.13.f. ESTIMATION OF POTASSIUM :**

The amount of potassium present in the plant extract was estimated by the Systronic Flamephotometry Method[23].

**2.14 DETERMINATIONS OF ANTIOXIDANT ACTIVITY :****2.14.a. ESTIMATION OF VITAMIN –C:**

The amount of vitamin-c present in the plant extract was estimated by Roe Joseph's and Carl DNPH colorimetric method[15].

**2.14.b .DETERMINATION OF VITAMIN-E BY DIPYRIDYL METHOD :**

The amount of Vitamin –E present in the plant extracts was estimated by Dipyrindyl method[16].

**2.15 THIN LAYER CHROMATOGRAPHY[17]:**

Thin layer chromatography (TLC) is one type of chromatography. The stationary phase is thin layer of adsorbent particles attached to a solid plate. A small amount of sample is applied (spotted) near the bottom of the plate and the plate is placed in the mobile phase. This solvents is drawn up by capillary action.

**3. RESULTS AND DISCUSSION****TABLE 1: QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN PEEL EXTRACTS**

S.NO	PHYTOCHEMICALS	WATER	ETHANOL	CHLOROFORM
1.	Test for Alkaloids	+	+	+
2.	Test for Flavonoids	+	+	-
3.	Test for Glycosides	+	+	+
4.	Test for Saponins	+	+	+
5.	Test for Tannins	+	+	-
6.	Test for Terpenoids	+	+	+
7.	Test for Proteins	+	+	+
8.	Test for Anthroquinones	+	-	-

The Phytochemical analysis were carried out in various solvent extracts of *Musa Paradisiaca* obtained using water, ethanol, chloroform in different concentrations. It shows the presence of alkaloids, flavonoids, saponins, Tannins, Terpenoids in all the 3 extracts. The phytochemical anthroquinone was found only in aqueous extract and not in organic solvents extracts. Alkaloids, flavanoids and tannins are responsible for the antioxidant property of the plant. Alkaloid have been documented to posses analgesic, antispasmodic and bactericidal effects[24]. Flavonoids are hydroxylated phenolic substance. They exhibit inhibitory effect against multiple viruses. It has been reported that flavonoids have antimicrobial activity[24]. Flavonoids also lower the risk of heart diseases[25]. Tannins is a general descriptive name for the group of polymeric phenolic substance. The consumption of tannin containing beverages

can cure or prevent a variety of ills. Many human physiological activities, such as stimulation of phagocytic cells, host mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins.[19] [26]. The non-sugar part of saponins also possess an antioxidant property. Saponins are known to have hypocholesterolemic activities[26]. Saponins and terpenoids are responsible for the antimicrobial property of the plant. The presence of these phytochemicals supports the medicinal use of *Musa paradisiaca*

**TABLE 2: QUALITATIVE ANALYSIS OF REDUCING SUGAR IN PEEL EXTRACTS**

S.NO	CARBOHYDRATES	WATER	ETHANOL	CHLOROFORM
1.	Benedict's Test	+	+	+
2.	Fellings Test	+	+	+
3.	Seliwanoff's Test	+	+	+

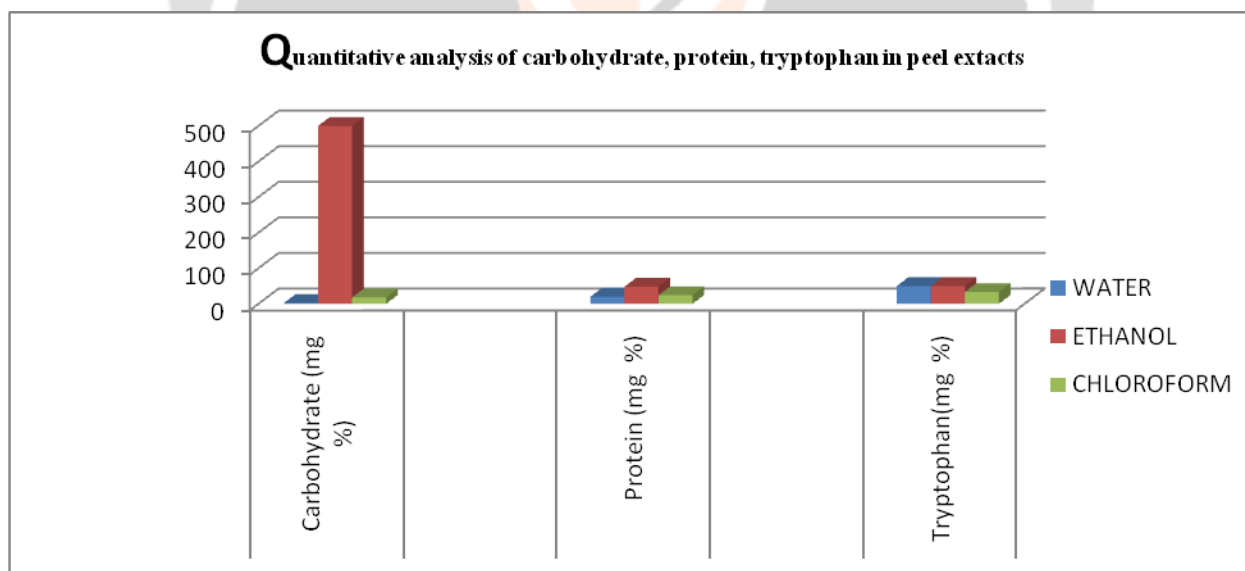
**TABLE 3: QUALITATIVE ANALYSIS OF AMINO ACIDS IN PEEL EXTRACTS**

S.NO	AMINOACIDS	WATER	ETHANOL	CHLOROFORM
1.	Nin Hydrin Test	+	+	+
2.	Xantho protein Test	+	+	+
3.	Folins Test	+	+	+
4.	Millons Test	+	-	-
5.	Pauly's Test	+	+	-
6.	Morner's Test	+	-	-
7.	Hopkins cole Test	+	+	+
8.	Aldehyde Test	+	+	+
9.	Ehrlich's Test	+	+	-
10.	Sakaguchi Test	+	-	-
11.	Sodium Nitroprusside Test	-	-	-
12.	Sodium Plumbate Test	+	-	-

The reducing monosaccharide glucose was present in all the three extracts of *Musa paradisiaca* peel. The keto sugar fructose showed its presence only in aqueous and ethanol extracts and not in the extract of chloroform[8]. It also exhibits the presence of aminoacids tryptophan, phenylalanine in all the 3 extracts of *Musa paradisiaca* peel. Tyrosine arginine and cysteine showed its presence only in aqueous extract. The amino acid methionine was absent in all the extracts[18]. The presence of essential aminoacids were seen in the peels of green, almost ripe and ripe banana. The sum of essential aminoacid of fruit peels ranged from 1.7% to 2.6% where as non-essential aminoacids ranged from 2.6% to 5.5%. It was reported that the sum of non-essential amino acids was higher than that of the essential amino acid [7]. In general total amino acid content of the peel did not show a marked change with stage of maturation[18]. The serotonin content of the peel were assayed 5 times and found to be in the range of 47 to 93  $\mu\text{g} / \text{g}$ [22].

**TABLE 4: QUANTITATIVE ANALYSIS OF STARCH, PROTEIN, TRYPTOPHAN IN PEEL EXTRACTS**

S.NO	SUBSTANCES	WATER	ETHANOL	CHLOROFORM
1.	Carbohydrate (mg %)	2.5	500	18
2.	Protein (mg %)	20	48	24
3.	Tryptophan(mg %)	50	50	33.3



**CHART1 : Quantitative analysis of carbohydrate, protein, tryptophan in peel extracts**

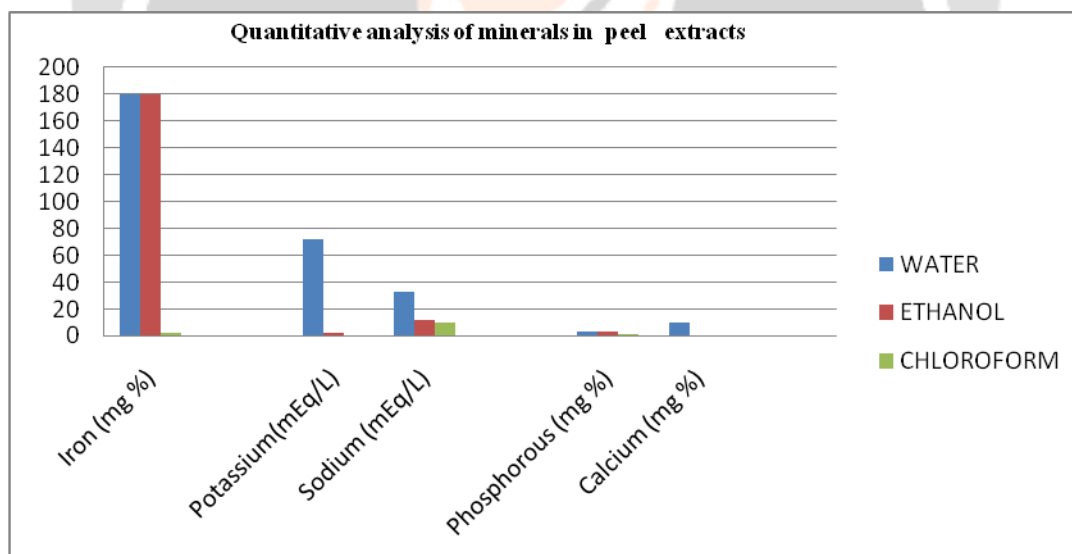
The starch content will be decreased considerably but the soluble sugar content increases in different concentrations during maturation in banana peel. The disappearance of the starch reserve during banana ripening appears to be relatively rapid because of the activities of several enzymes working together. The types of soluble sugars found in banana peels were glucose and fructose. The peel had highest fructose content than glucose. The carbohydrate content of banana peel was significant and could serve as a main carbon source for microbial protein [9]. The protein content of banana peel was higher than those of sweet potato. The high protein content of banana peel supports large scale cultivation of mould for the production of valuable microfungus mass and probably for enzyme and antibiotic production[20].



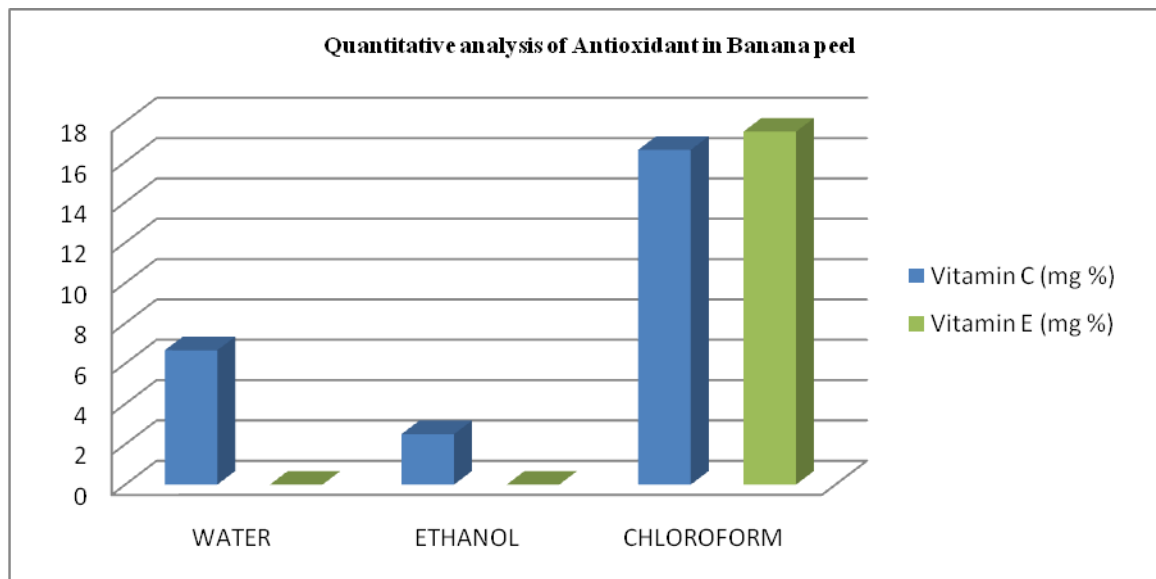
**TABLE 5: QUANTITATIVE ANALYSIS OF MINERALS IN PEEL EXTRACTS**

S.NO	SUBSTANCES	WATER	ETHANOL	CHLOROFORM
1.	Iron (mg %)	180	180	2
2.	Potassium(mEq/L)	71.9	2	0.2
3.	Sodium (mEq/L)	32.5	11.8	9.6
4.	Phosphorous (mg %)	3.2	2.4	1.6
5.	Calcium (mg %)	9.6	-	-

The quantitative analysis shows the presence of minerals like iron, potassium, sodium, phosphorous and calcium in the *Musa Paradisiaca* peel extract[18]. The banana peel had high amount of potassium, phosphorous, magnesium and sodium. While the iron and calcium was found to be low. Potassium has important roles in neuron function and calcium play a role in building stronger and denser bones [21].

**CHART2 : Quantitative analysis of Minerals in peel extracts****TABLE 6: QUANTITATIVE ANALYSIS OF ANTIOXIDANTS IN PEEL EXTRACTS**

S.NO	SUBSTANCES	WATER	ETHANOL	CHLOROFORM
1.	Vitamin C (mg %)	6.66	2.5	16.6
2.	Vitamin E (mg %)	0	0	17.5



### CHART3 : Quantitative analysis of Antioxidant in peel extracts

The banana peel contain the high amount of vitamin C in chloroform extract. The vitamin E was present only in chloroform extract[7].

### SEPARATION OF AMINO ACID BY TLC:

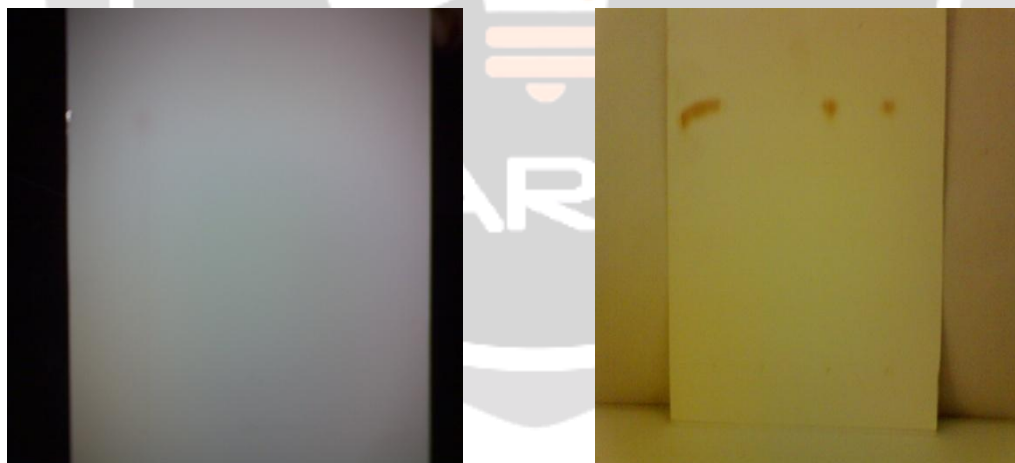


Fig. 1: SEPARATION OF AMINO ACID BY TLC

The TLC run by using aqueous and ethanol extract of *Musa paradisiaca* to confirm the presence of amino acid (tryptophan)[18]. Appearance of purple spot 1 represents standard tryptophan and the spot 2 and 3 represent the spot obtained by aqueous and ethanolic extract of *Musa paradisiaca*. The presence of tryptophan is further confirmed by its estimation.

#### 4. CONCLUSION

The present study highlighted that banana peel serves as a natural store of various health beneficial phytochemicals and there exist significant differences in the phytochemical composition, antioxidant properties. Taking into account the flavonoids content and metal chelating activity, Phenolics, free radical scavenging activity. Thus it is highly recommended to include proper combination of fruit peel in food products, whose phytochemicals synergistically act to reduce the risk of degenerative diseases like cardiovascular disease, cancer etc. Further studies based on Genetic engineering will increase the nutrition content of the peel.

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