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Varietal Impact on Phytochemical Contents and Antioxidant Properties of *Musa acuminata* (Banana)

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Abstract-The present study highlighted that banana serves as a natural store of various health beneficial phytochemicals and there exist significant differences in the phytochemical composition and antioxidant properties among four different varieties of banana rasthali, karpooravalli, manjal vazhapazham (yellow) and pachai vazhapazham (green). Taking into account the flavonoid content and, metal chelating activity the cultivar rasthali stood superior in comparison to other three varieties under investigation where as green variety hold the highest concentration of total phenolics, free radical scavenging activity and reducing power activity. Thus the cultivars rasthali and green was considered to be more beneficial to health in terms of antioxidant potential in comparison to other two cultivars.

Keywords: Banana, antioxidant, flavonoid, phenol, metal chelating power, reducing power

1. INTRODUCTION

Fruits and vegetables are rich sources of various health beneficial phytochemicals such as flavonoids, phenols, vitamins, minerals, carbohydrates etc.[1] Extensive research by various groups has revealed the role played by these phytochemicals in the reduction of incidence of certain degenerative diseases such as cardiovascular diseases, cancers, arthritis. [2][3] Banana is one of the most widely distributed and consumed fruit in the tropical and subtropical countries. [4][5] Considering the nutritional aspects, it is one of the world's leading food crops with a great source of minerals, vitamins, carbohydrates, flavonoids, phenolic compounds etc.[5] It is both economical and easily accessible to people of all sections of the society, thus addressing food insecurity problems in many countries. It can be consumed both as cooked and uncooked form. As oxidative damage of lipids, proteins, and nucleic acids is implicated in the pathology of many chronic diseases, a great interest was developed by many research groups in exploring the major phytochemicals with antioxidant properties in banana. Though many groups have explored the phytochemical composition and the antioxidant properties of banana fruit, to date a comparative evaluation of various phytochemicals and its antioxidant properties in banana varieties mostly consumed in Tamil Nadu such as rasthali, karpooravalli, manjal vazhapazham (yellow) and pachai vazhapazham (green) is not reported.

This study is of paramount importance as the nutritional quality of fruits and vegetable is highly variable with its varieties, climatic conditions, soil type, temperature, light intensity and many more factors. The phytochemical profiles of fruits is dependent on maturity, cultivars , geographical origin, growing season, post harvest storage condition and processing techniques. [6][7][8][9][10]The fact that banana is used in the preparation of many commercial dietary supplements and processed food products highlights the importance of studies on the phytochemical and antioxidant studies of the most popular and widely consumed varieties of banana in a region. [5] A wide variability in the local availability of different varieties of a particular fruit, along with an inter alia cultural and socio economic difference influence the dietary pattern of people living in different parts of the world. In this context analysis of potent health protective phytochemicals of most widely consumed varieties of banana locally available is of vital importance as it provides information on phytochemical and antioxidant intake of Tamil Nadu population largely depending on the fruits like banana. Besides, it provides an idea regarding the type and variety of banana that has to be included in the daily diet for best possible health benefits. This article quantifies the total phenolics, flavonoids, and antioxidant property of 4 varieties of banana such as rasthali, karpuravalli, manjal vazhapazham (yellow) and pachai vazhapazham (green).

2. RESULTS AND DISCUSSION

In this study besides the quantitative estimation of the levels of phenolics and flavonoids, a comparative evaluation of antioxidant activity via free radical scavenging activity, reducing power activity, metal chelating activity, have been carried out in four different varieties of banana widely consumed in Tamil Nadu such as rasthali, karpooravalli, manjal vazhapazham (yellow) and pachai vazhapazham (green). The disease protective role played by fruits and vegetables in the human diet has tremendously increased the research on fruits and vegetables recent years. Phytochemical composition and antioxidant potential of commonly consumed fruits in several regions were studied.[11][12][13][14] Some groups focused on the analysis of the phytochemicals in different parts of a fruit such as peel, pulp, seed etc.[15][16][17] Analysis of phytochemicals, antioxidant potential in different varieties of a particular fruit was also carried out. [6][18][19] In the year 2011 correlation between total phenolics and mineral contents with antioxidant activity was studied in of 8 varieties of Malaysian banana. [20]

2.1. Phenolics and Flavonoids

Phenolics and flavonoids, the bioactive health beneficial factors implicated in the prevention of a variety of diseases like cardiovascular diseases, cancers and neurodegenerative disorders has gained considerable interest since years. [2][21] Previously many research groups have focused on the quantification of these phytochemicals in fruits and vegetables mostly consumed in their region of interest. This gave an understanding on nutritional value and human health protective potency of selected fruits or vegetables or both in those areas. [22] Some others have paid attention towards the quantification of specific forms of flavonoids and phenolic acids in their study as it gives an idea on the most abundant form of specific phytochemical present in those samples. [23][24][25] Apart from quantification of total phenolic acid composition some groups focused on the seeing whether the phenolic acids were in free form or as conjugated form as this strongly influence the bioavailability of those phytochemicals. [26] In this study we have determined total phenolics and flavonoids levels in these 4 selected varieties of banana locally available and mostly consumed by population in Tamil Nadu. It was seen that total phenolic and flavonoids contents varied significantly between the 4 varieties under study. The total phenolic level in each fruit is presented in Table 1 from which it is understood that green variety possessed the highest amount with 180 mg/g of fruit extract followed by yellow variety containing 154 mg/g of fruit extract. Karpooravalli variety with 113 mg/g was the candidate with least amount of total phenolics. The phenolics level can be represented as green > yellow > rasthali > karpooravalli. When flavonoids was quantitatively determined it was observed that the flavonoids levels in rasthali > green > karpooravalli > yellow as shown in Table 1. The total flavonoids in rasthali and yellow were 4.78 mg/g and 3.06 mg/g respectively. Using one way ANOVA it was seen that the flavonoids and total phenolic levels varied significantly among different varieties with $p < 0.05$.

Table 1: Total phenolic and flavonoid content in different varieties of Banana

Variety	Total Phenolic content (µg GAE /mg)	Total flavonoid content (µg QE /mg)
Green	180±8.220	3.58±0.162
Yellow	154±2.217	3.06±0.025
Rasthali	125±4.203	4.78±0.334
Karpuravalli	113±1.707	3.32±0.136

2.2. Chromatographic determination of polyphenols

Polyphenols such as quercetin, naringenin, chlorogenic acid were quantitatively determined through high performance liquid chromatography to find whether there exist any correlation between the antioxidant activity displayed and the levels of flavonoids such as quercetin, naringenin, chlorogenic acid (Figure 1-7). It was observed that rasthali possessed highest concentration of chlorogenic acid and naringenin where as karpooravalli possessed highest concentration of quercetin (Table 2). There existed no strong correlation between the concentration of chlorogenic acid, quercetin, naringenin and the antioxidant potential displayed with r value 0.234. Thus it is evident that the antioxidant properties can be attributed to the synergistic action of many other phytochemicals with antioxidant potential possessed by the fruits.

Table 2: Chromatographic determination of polyphenols in methanolic extracts of four varieties

Standards	Concentration of polyphenols (µg/g)			
	Yellow	*Green*	*Rasthali*	*Karpooravalli*
Chlorogenic acid	34.6	104	782	49.12
Quercetin	32.3	65	50.84	125.09
Naringenin	8.9	10.7	12.2	-

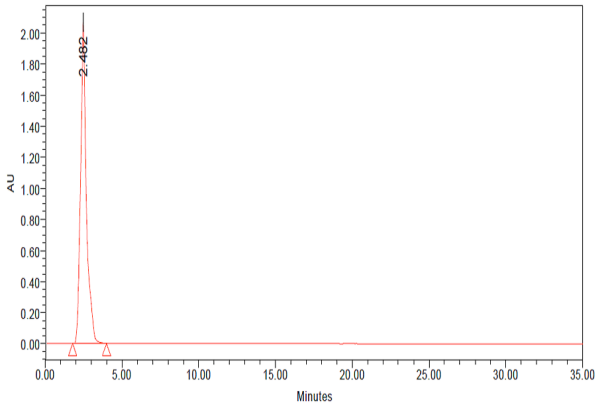

Figure 1: HPLC chromatogram of Chlorogenic acid

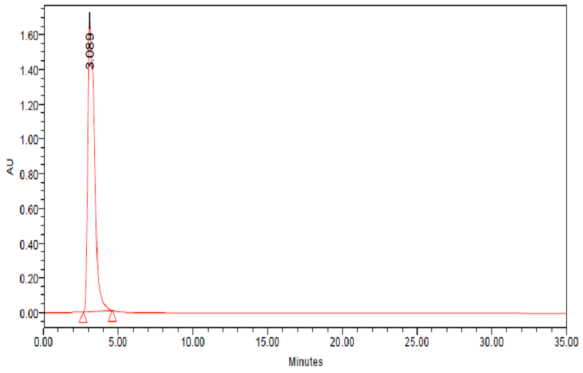

Figure 2: HPLC chromatogram of quercetin

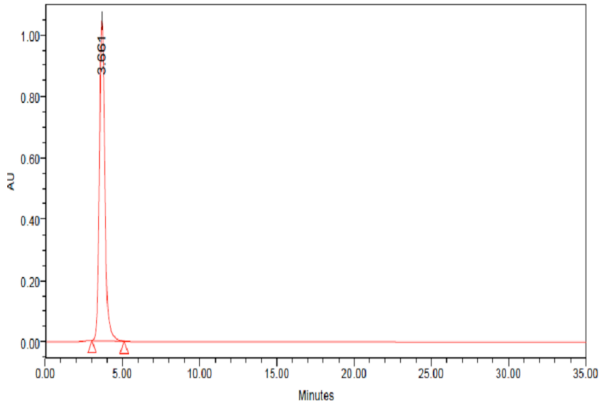

Figure 3: HPLC chromatogram of naringenin

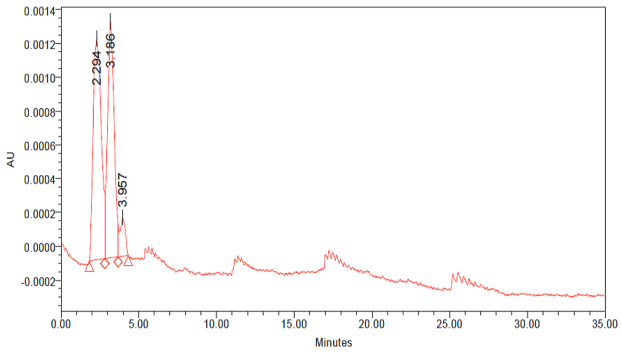

Figure 4: HPLC chromatogram of polyphenols of Yellow variety (Retention time of Chlorogenic acid, quercetin and naringenin are 2.294 min, 3.186 min and 3.957 respectively)

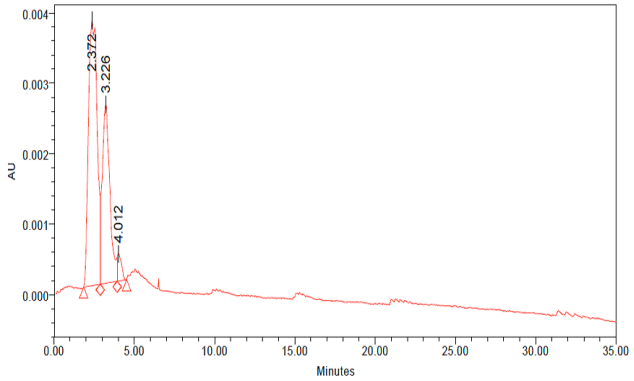

Figure 5: HPLC chromatogram of polyphenols of Green variety (Retention time of Chlorogenic acid, quercetin and naringenin are 2.372 min, 3.226 min and 4.0 min respectively)

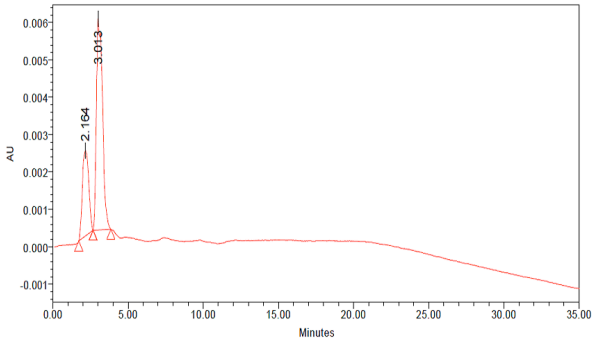

Figure 6: HPLC chromatogram of polyphenols of Rasthali variety (Retention time of Chlorogenic acid, quercetin and naringenin are 2.292 min, 3.180 min and 3.990 respectively)

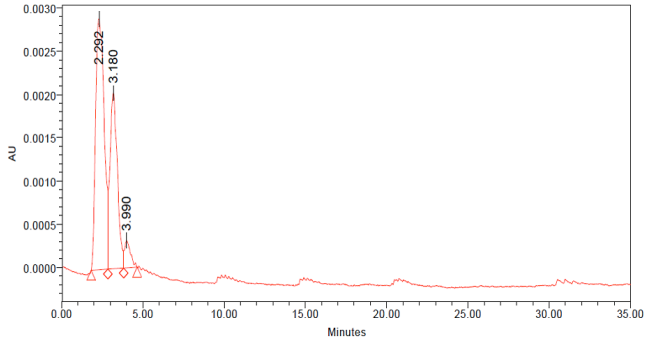

Figure 7: HPLC chromatogram of polyphenols of Karpooravalli variety (Retention time of Chlorogenic acid and quercetin are 2.164 min, 3.013 min respectively)

2.3. Reducing power assay

Through reducing power assay antioxidant potential of the banana extracts were indirectly determined by measuring the capacity of the extract to reduce the transition metal iron (III) by electron transfer. An increase in the absorbance read at 700 nm indicates the reducing power of the extract. For a given weight of fruit extract, higher the absorbance read, higher the reducing power. In this study the fruit extracts in the concentrations 5, 20, 100, 500 µg/ml were used for the assay. As the concentration increased, the absorbance also increased which gave highest absorbance value at a concentration of 500 µg/ml which corresponds to increased reducing power. Upon comparing the absorbance value at 500 µg/ml it was seen that variety green exhibited the highest reducing power as shown in Table 3. The reducing power of the four varieties were in the order green > rasthali > yellow > karpooravalli (Figure 8). Many studies determining antioxidant potential of fruit extracts by assessing the reducing power have been reported. In a previous study the comparative analysis of reducing power of different fruits were carried out including banana with an absorbance value of 0.08 at 20 mg/ml. [27]

Table 3: Reducing power activity of four varieties under various concentration

Banana Variety	Absorbance at 700 nm for different concentration of extracts			
	5 (µg/ml)	*20 (µg/ml)*	*100 (µg/ml)*	*500 (µg/ml)*
Rasthali	0.06	0.177	0.936	1.309
Yellow	0.064	0.14	0.635	1.312
Green	0.174	0.827	1.085	1.366
Karpooravalli	0.06	0.121	0.362	1.226

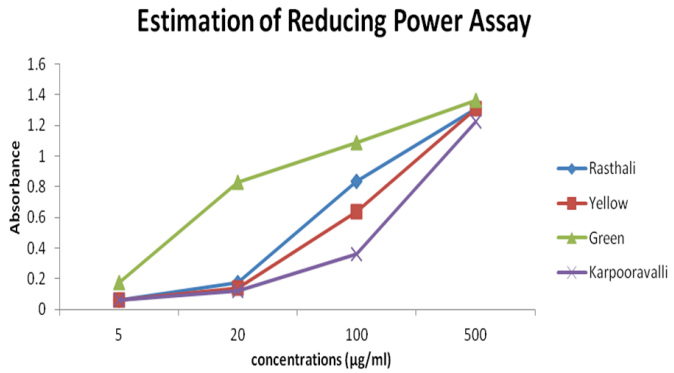

Figure 8: Graphical representation of reducing power capability of four different varieties

2.4. Metal chelating activity

Measuring the capacity of the fruit extracts to chelate ferrous ions is an important method of assessing its antioxidant potential as ferrous ions are reported to be the most effective prooxidants in the food system.[28] Ferrous ions are implicated in the pathology of many diseases as they could stimulate lipid peroxidation by Fenton reaction and also decompose lipid hydroperoxies into peroxyl and alkoxyl radicals that

stimulate the chain reaction of lipid peroxidation. [29] Here the ability of different banana varieties to chelate ferrous ion was studied. From the plot of chelating % Vs concentration of fruit extract in ug/ml it was seen that highest chelating power was shown by rasthali variety with a 78 chelation % at 500 mg/ml as seen in Table 4. The chelating power was represented as rasthali (78%) > green (69%) > karpooravalli (65.21%) > yellow (60.86%) as shown in Figure 9. The chelating ability of banana was previously reported by Lim and group, in which they proved that banana possess excellent chelating power, thereby act as good secondary antioxidants compared to many fruits under study. [27] The variation in the chelating potential reported by several groups can be attributed to differences in many factors such cultivars, extraction procedures, geographical location and prevailing conditions such as soil, temperature, sunlight, horticultural practices and so on.[6][7][8][9][10]

Table 4: Metal chelation percentage of four varieties at various concentrations of the fruit extract

Banana Variety	Percentage of chelation at different concentrations of extract			
	5 (µg/ml)	*20 (µg/ml)*	*100 (µg/ml)*	*500 (µg/ml)*
Rasthali	8.69	26.08	47.82	78.26
Yellow	4.34	13	21.73	60.86
Green	13	17.39	30.43	69.56
Karpooravalli	4.34	21.7	47.82	65.21

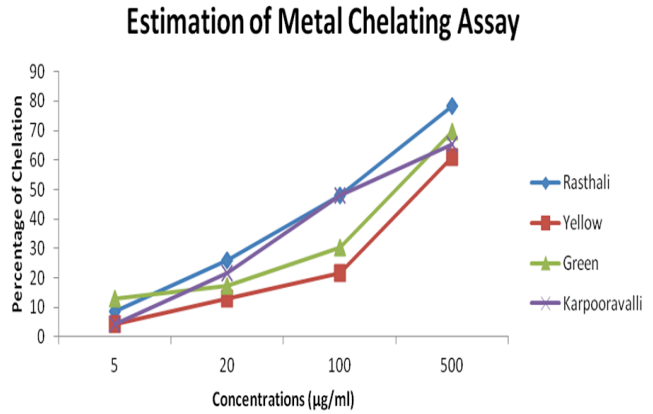

Figure 9: Graphical representation of metal chelating activity of four different varieties

2.5. Antioxidant property

The antioxidant property of an extract can be determined based on its capacity to inhibit lipid peroxidation, to scavenge free radicals, to reduce a transition metal, to chelate a ferrous ion, to interact with secondary oxidation products and so on to represent various stages of an oxidation reaction. Here the ability of the methanolic fruit extract of each variety to scavenge free radicals was determined based on its hydrogen donating ability. The decolorization of a stable radical DPPH in methanol upon introduction of the fruit extract which gives an absorbance maximum at 517 nm was measured. It was observed that the highest antioxidant activity (least IC50) was shown by Green variety (Table 5). The free radical scavenging activity can be represented with their respective IC values as green (IC50-620µg/ml) > rasthali (IC50-620µg/ml) > yellow (IC50-620µg/ml) > karpooravalli (IC50-620µg/ml) as shown in Figure 10. Therefore it was understood that among the four different cultivars under investigation, Green variety has got highest radical scavenging activity which can be attributed to its high phenolic and flavonoid contents. Previously several groups have studied the antioxidant potential of extracts of banana, banana peel etc.[30][31] Rafaela and group revealed that extraction temperature, time and number of steps are critical factors in the determination of antioxidant capacity of banana. [30]. In the year 2004 Maria and group reported the antioxidant potential of banana with IC50 value of 1281 µg .[31] The antioxidant activity of 8 different varieties of banana in Malaysia was studied by Shaida and group. They have clearly shown that there exist difference in the phytochemical composition and antioxidant potential of different cultivars which are in line with our findings. [32] Besides, the antioxidant activity of banana flavonoids in rats showed that concentrations of peroxidation products namely malondialdehyde, hydroperoxides and conjugated diens were significantly decreased. [33] The variation in the antioxidant potential reported by several groups can be attributed to differences in cultivars, extraction procedures, geographical location and prevailing conditions such as soil, temperature, sunlight, horticultural practices and so on.[6][7][8][9][10]

Table 5: DPPH inhibition percentage of methanolic extracts of four varieties at various concentrations

Estimation of DPPH	Concentrations (µg/ml)				
Banana Variety	**5**	**20**	**100**	**500**	**1000**
Rasthali	4.52	14.56	23.34	36.77	62.87
Yellow	3.45	11.56	20.89	32.53	59.89
Green	5.78	15.53	25.95	39.65	65.96
Karpooravalli	2.98	10.59	19.46	30.56	60.73

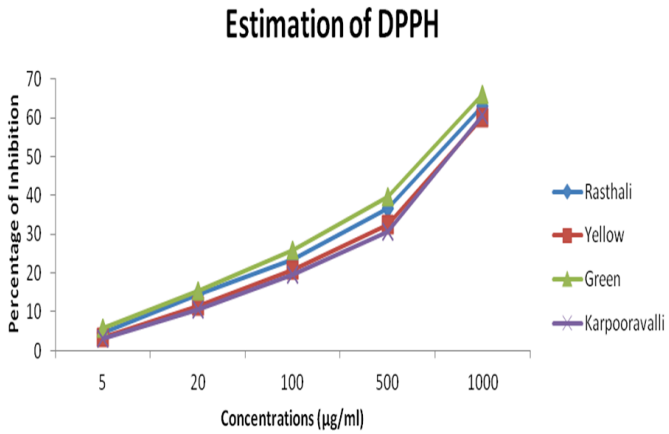

Figure 10: Graphical representation of DPPH inhibition percentage of four different varieties

3. EXPERIMENTAL

3.1. Sample collection

The four different cultivars of banana at mature ripened stage were obtained from local dealers in Vellore, Tamil Nadu. They were cleaned, peeled and the flesh part was used for the further experimental analysis.

3.2. Extraction and quantitative analysis of total phenolics

For the extraction of phenolics from the fruits under study, methanol and 1% hydrochloric acid in the ratio 8:2 was used as a solvent system. The total phenolic content was measured using Folin-Ciocalteau method. [34] About 0.5 ml of methanolic extracts of fruits was taken into test tubes to which 0.5 ml of Folin-Ciocalteau reagent was added. 1 ml of 20 % Na2CO3 was added and incubated at room temperature for 30 minutes. Using ascorbic acid as standard total phenolics was estimated by reading the absorbance at 725 nm. The total phenolic content was expressed in µg of gallic acid equivalents (GAE) / mg of fruit.

3.3. Extraction and quantitative determination of total flavonoids

Extraction of flavonoids from the five different fruits was performed using methanol as the solvent. 1 g of fruit sample was weighed to which 10 ml of methanol was added. The extraction was carried out for 1 hour in an ultrasonic bath at 50˚C. Later the sample was cooled to room temperature and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and the residue was subjected to extraction till it became colorless. The extract obtained was filtered using Whatman filter paper and evaporated to dryness using rotary evaporator. The dried residue was dissolved in methanol and stored in air tight vials under -20˚C.

The Dowd method was followed to determine the total flavonoids content in the fruits.[35] Aluminium chloride colorimetry was used for the total flavonoids estimation. About 100 µl of methanolic extract of the fruits were taken in test tubes to which 1.0 ml of ethanol, 0.1 ml of 1 M potassium acetate and 0.1 ml of aluminium chloride was added. After 40 minutes incubation at room temperature, the absorbance was read at 415 nm. The amount of total flavonoids was determined from the calibration plot prepared from quercetin as standard. Total flavonoids content was expressed as µg of quercetin equivalents (QE) / mg of fruit sample

3.4. High Performance Liquid Chromatography

Reverse phase (RP)-HPLC system for the analysis of phenolic compounds was used, comprising of Shimadzu LC-20AC pumps and a LUNA C-18 column with a UV detector at 342 nm. The polyphenols such as quercetin, naringenin and chlorogenic acid was separated and quantitatively determined. The mobile phase consisted of solvent A (0.05% trifluoroacetic acid) and solvent B (0.038% trifluoroacetic acid in 83% acetonitrile v/v) with the following gradient: 0-15 min, 15% B in A; 5-10 min, 15-70% B in A; 10-15 min, 70% B in A. The flow rate of 1 ml/min was maintained with the UV detector at 342 nm. The phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds.

3.5. Reducing Power Assay

The ferric reducing power of the fruit extracts was determined by potassium ferricyanide–ferric chloride method. [36] Varying concentrations of extracts 5, 20, 100, 500 µg/ml amounting to 1ml were taken in a test tube into which 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%) was added. The mixtures were incubated at 50˚C for 20 minutes and 2.5 ml of trichloroacetic acid (10%) was added. The absorbance was read at 700 nm. A graph of absorbance vs. fruit extracts concentration was plotted to observe the reducing power.

3.6. Metal Chelating activity

The metal chelating activity was measured by the decrease in the absorbance at 562 nm of the iron (II)-ferrozine complex. [27] Varying concentrations of extracts 5, 20, 100, 500µg/ml amounting to 1ml was taken in a test tube into which 1 ml 0.125 mM FeSO4 and 1.0 ml 0.3125 mM ferrozine were added. The mixture was allowed to stand for 10 min and the absorbance was read at 562 nm. The decrease in the absorbance read at 562 nm was calculated relative to the control (consisting of iron and ferrozine only) using the formula

Chelating effect % = [(AC-AS)/AC] ×100

Where AC = absorbance of control and AS= absorbance of sample. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC^{50} value.

3.7. Free radical scavenging activity

In this assay, 200 µl of extract solution (concentrations ranging from 5– 1000 µg/ml) was mixed with 3 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH - 0.1 mM) in methanol solution followed by incubation in dark for 30 minutes. The absorbance of reaction mixture was read at 517 nm. The radical scavenging activities were expressed as percentage of inhibition and calculated according to the following equation.

Percentage of DPPH inhibition= [(AC-AS)/AC] ×100

Where AC = absorbance of control and AS= absorbance of sample. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC^{50} value.

3.8. Statistical analysis

Data were reported as mean of three determinations. The results obtained were statistically analyzed with the one way anova test using a significance level of $P < 0.05$. Graph Pad Instat version 3 was used for the statistical analysis.

4. CONCLUSION

The present study highlighted that banana serves as a natural store of various health beneficial phytochemicals and there exist significant differences in the phytochemical composition, antioxidant properties among four different varieties of banana rasthali, karpooravalli, manjal vazhapazham (yellow) and pachai vazhapazham (green). Taking into account the flavonoids content and, metal chelating activity the cultivar rasthali stood superior in comparison to other three varieties under investigation where as green variety hold the highest concentration of total

phenolics, free radical scavenging activity and reducing power activity. Thus the cultivars rasthali and green was considered to be more beneficial to health in terms of antioxidant potential in comparison to other two cultivars. Further studies are required to determine the levels of several other phytochemicals present in these varieties, thereby drawing a complete picture of the nutritional profile of each variety and thus procure the best possible benefits from them. Thus it is highly recommended to include proper combination of fruits in your daily diet, whose phytochemicals synergistically act to reduce the risk of degenerative diseases like cardiovascular disease, cancer etc. In future, genetic engineering of these fruits can be adopted in a view to elevate the phytochemical levels, thus incorporating relevant amounts through our daily diet.

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