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Research Article

Determination of Antioxidant Activities and Total Phenolic Contents Along with HPTLC Fingerprinting Analysis of Different Extracts of 11 Fruits

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Abstract

The antioxidant capacity and Total Phenolic Content (TPC) of different extracts of 11 fruits were studied. The extraction of fruit powder samples was done using solvents such as methanol, ethanol, water and hot water. The antioxidant capacity was evaluated using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical-scavenging assay while the Total Phenolic Content (TPC) was determined using Folin-Ciocalteu reagent and the absorbance were measured on UV-Visible spectrophotometer at 765 nm. The Total Phenolic Content (TPC) was expressed as gallic acid equivalent (mg of GAE/g sample) in accordance to the standard curve of gallic acid. HPTLC method was developed for fingerprinting analysis of 11 different fruit extracts on precoated silica plates using ethyl acetate: glacial acetic acid: formic acid: water (5:1:1:1 v/v/v/v) as mobile phase. The developed plate was sprayed with Anisaldehyde Reagent (ANS) and then scanned at wavelength of 450 nm. The results exhibited the high TPC content in the different samples extracted from ethanol and hot water as compared to samples extracted using methanol and water. The water and hot water extracts of watermelon provided highest antioxidant activity. The TPC of the samples extracted with ethanol and hot water were much higher than those extracted with methanol and water.

Keywords: Antioxidant capacity; Fruit powders; Total phenolic content; HPTLC

Introduction

Nature has gifted mankind with tremendous medicinal plants to create a disease free and healthy life [1]. Most of the medicinal fruit plants are presented in the Indian traditional systems of medicine like Ayurveda, Unani, and Siddha for the treatment of many diseases and disorders. Indeed, various researchers concluded that the medicinal property of the fruit and other plants belonged to the secondary metabolites and the antioxidant potentials [2]. The antioxidative properties of those fruits are predominantly due to the low molecular weight phenolic compounds, which are known as antioxidants [3]. As Antioxidant Traditional Medicine has been used as an alternative medicine for treating complex pathophysiological conditions. Like various fruits Banana, Amla, Orange peel, Lemon, Pineapple, Mango, Watermelon, Papaya, Apple, Guava and Sapota has a long history for use in medicines as antioxidants because they are rich in vitamin C (ascorbic acid) and polyphenol contents [4]. The strong antioxidant activity of the phenolics and their ability to protect cells against oxidative damage caused by free radicals are well established [5]. Due to the presence of conjugated ring structures the hydroxyl group of many phenolic compounds has the potential to function as singlet oxygen and as antioxidants by scavenging superoxide anions and lipid peroxy radicals. Phenolic compounds are the major chemical constituents of Banana, Amla, Orange peel, Lemon, Pineapple, Mango, Watermelon, Papaya, Apple, Guava and Sapota having strong antioxidant property [6,7]. The intake of dietary antioxidants may help in the prevention of free radical damage in human body. Antioxidants can scavenge free radicals through the inhibition of the initiation process or interruption of the propagation process of lipid oxidation and provide preventive function by several actions.

The objective of this study was to determinate the antioxidative properties and the antioxidant related composition of phenolics in Banana, Amla, Orange peel, Lemon, Pineapple, Mango, Watermelon, Papaya, Apple, Guava and Sapota fruit extracts.

Materials and Methods

Fruit powder samples

The fruit powder samples for Banana, Amla, Orange peel, Lemon, Pineapple, Mango, Watermelon, Papaya, Apple, Guava and Sapota were purchased from local market. The information of fruit powders was listed in Table 1.

Chemicals and reagents

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was supplied by Sigma, Bangalore. Trolox was purchased from Aldrich Co. Gallic acid, Folin-Ciocalteu reagent and other analytical grade solvents and chemicals were purchased from SD Fine Chemicals, India.

Extraction of fruit samples

Each of the fruit powder samples were prepared by pulverization method. 5.0 g of each fruit sample was accurately weighed into a 4 set of beakers. 80 mL of ethanol, methanol, water and hot water were added into each beaker separately for the preparation of extracts.

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Table 1: List of fruit powder extracts.

Sr. no.	Common name	Scientific name	Mode of production	Place of origin
1	Banana	*Musa acuminata*	Agriculture	Pune
2	Amla	*Phyllanthus emblica*	Agriculture	Pune
3	Orange peel	*Citrus sinensis*	Agriculture	Pune
4	Lemon	*Citrus limon*	Agriculture	Pune
5	Pineapple	*Ananas comosus*	Agriculture	Pune
6	Mango	*Mangifera indica*	Agriculture	Pune
7	Watermelon	*Citrullus lanatus*	Agriculture	Pune
8	Papaya	*Carica papaya*	Agriculture	Pune
9	Apple	*Malus pumila*	Agriculture	Pune
10	Guava	*Psidium guajava*	Agriculture	Pune
11	Sapota	*Manilkara zapota*	Agriculture	Pune

Table 2: Antioxidant properties of various extracts of different fruit powder extracted by different solvents.

Sr. No.	Common name	DPPH free radical scavenging capacities (µmole TE/g)			
		Methanol extracts	Ethanol extracts	Water extracts	Hot water extracts
1	Banana	0.5	0.59	0.35	0.37
2	Amla	1.16	1.17	0.91	0.56
3	Orange peel	2.52	2.6	2.02	1.59
4	Lemon	2.63	2.78	2.45	1.96
5	Pineapple	0.89	0.78	0.73	0.25
6	Mango	0.46	0.42	0.36	0.23
7	Watermelon	2.01	2.18	2.73	2.92
8	Papaya	0.18	0.21	0.25	0.19
9	Apple	0.16	0.26	0.22	0.36
10	Guava	0.64	0.68	0.43	0.63
11	Sapota	0.16	0.27	0.28	0.23

Note: Data were expressed as mean ± (n=3).
Abbreviations: DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; µmole TE/g- Micromol Trolox Equivalents per gram.

The samples were extracted for 24 hours and then centrifuged by a centrifuge at 8000 rpm for 10 minutes. Then each supernant layers were transferred into new centrifuge tubes and stored at -20°C. The resulting extracts were taken for further analysis.

Determine antioxidant properties of various extracts of different fruit powder

For determination of anti-oxidant activity, 1 mL of each extract and standard solutions were added into a pre-labeled test tube and mixed with 3 mL of DPPH solution. For determination of antioxidant activity, ethanol, methanol, water and hot water extracts along with blank and standard solutions were added into a pre-labeled centrifuge tube and mixed with 3.8 mL of DPPH solution. The mixtures were mixed evenly vortex and stood in the dark at room temperature for 20 minutes. Then the mixtures were centrifuged at 3000 rpm for 10 minutes. The absorbance of each mixture was measured by an UV-Visible spectrophotometer at 517 nm against solvent blank. The results were expressed as Trolox equivalents (µmole of TE/g sample) in accordance to the standard curve of Trolox.

Determination of Total Phenolic Content (TPC) of various extracts of different fruit powder

For determination of total phenolic content 50 µL of each extract, blank and standard solution was mixed with 3 mL of distilled water, 250 µL of Folin-Ciocalteu reagent and 750 µL of 7% Na_2CO_3 solution and the mixtures were vortexed. After standing at room temperature for 8 min, 950 µL of distilled water was added into each mixture and the mixtures were allowed to stand at room temperature for 1 hour. The absorbance of each mixture was measured by the UV-Visible spectrophotometer at 765 nm by using distilled water as the blank. The Total Phenolic Content (TPC) was expressed as gallic acid equivalent (mg of GAE/g sample) in accordance to the standard curve of gallic acid.

Sample preparation for HPTLC fingerprinting

The sample solutions were prepared by dissolving 1 gm of each fruit powder in 20.0 mL mixture of water and methanol (10:10) in separate volumetric flasks. The resultant solutions were sonicated in ultrasonic water bath for 30 min and then the solutions were filtered using Whatman No. 41 filter paper. The resulting solutions were used as sample solutions.

HPTLC fingerprinting

The HPTLC fingerprinting was performed using CAMAG HPTLC with Linomat 5 applicator, Scanner 3, Camag TLC visualizer

Table 3: Total Phenolic Content (TPC) of various extracts of different fruit powder extracted by different solvents.

Sr. No.	Common name	Total phenolic content (%)			
		Methanol extracts	Ethanol extracts	Water extracts	Hot water extracts
1	Banana	5.23	6.12	4.72	5.26
2	Amla	10.56	11.72	9.16	10.2
3	Orange peel	23.75	25.06	24.53	25.36
4	Lemon	29.91	30.12	27.02	28.4
5	Pineapple	7.86	7.96	6.13	6.79
6	Mango	4.09	4.78	3.45	3.07
7	Watermelon	21.45	22.06	21.36	21.45
8	Papaya	2.03	2.08	2.06	2.96
9	Apple	1.74	1.89	1.06	2.75
10	Guava	6.18	6.78	5.62	6.7
11	Sapota	3.75	3.99	3.37	4.37

Note: Data were expressed as mean ± (n=3).
Abbreviation: %: Percent.

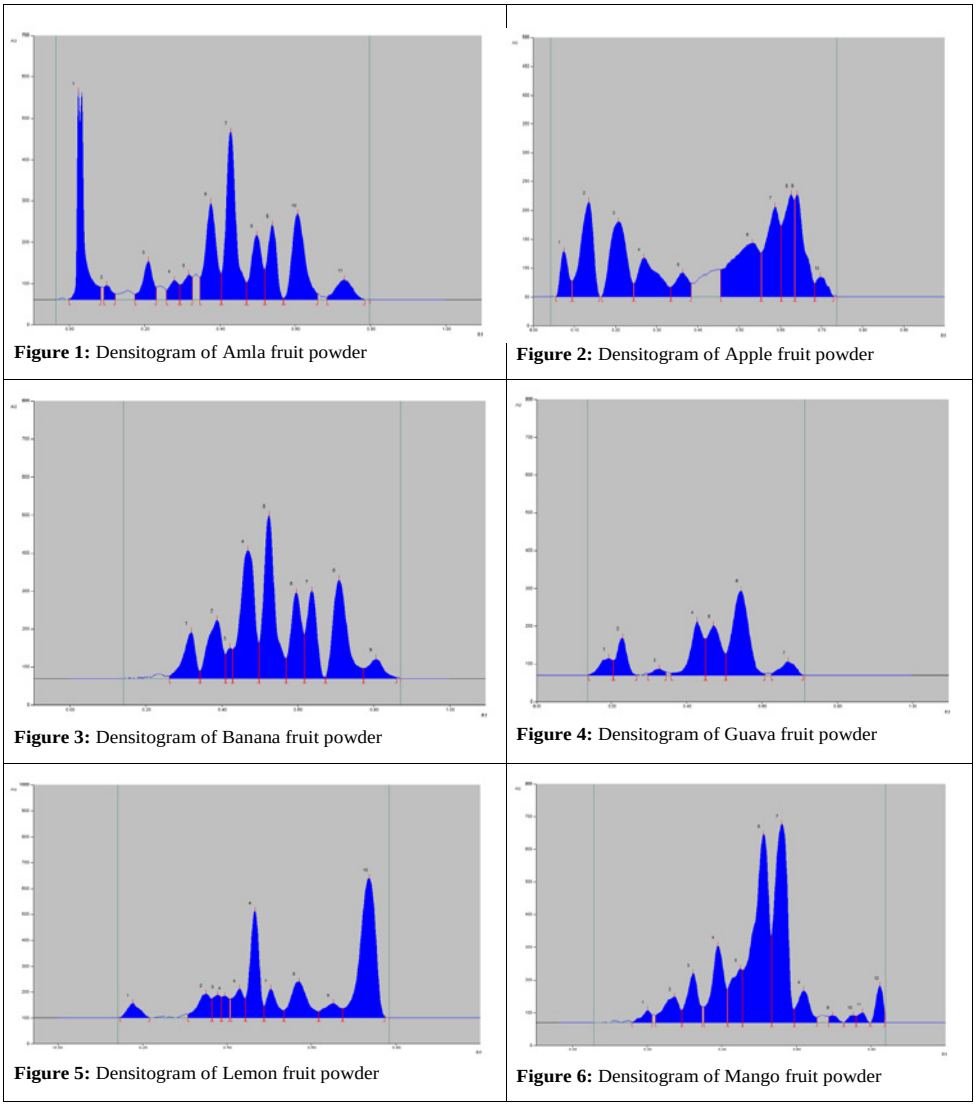

Figure 1: Densitogram of Amla fruit powder

Figure 2: Densitogram of Apple fruit powder

Figure 3: Densitogram of Banana fruit powder

Figure 4: Densitogram of Guava fruit powder

Figure 5: Densitogram of Lemon fruit powder

Figure 6: Densitogram of Mango fruit powder

Figure 1-6:

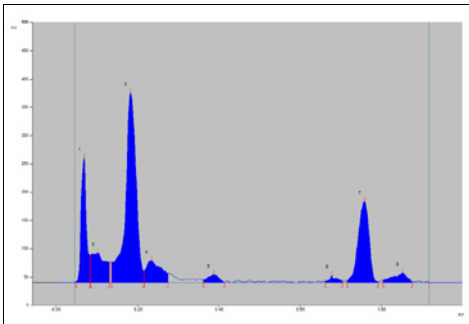

Figure 7: Densitogram of Orange fruit powder

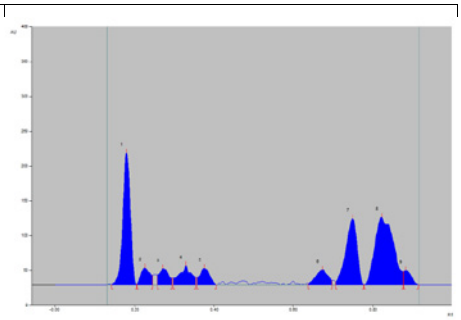

Figure 8: Densitogram of Papaya fruit powder

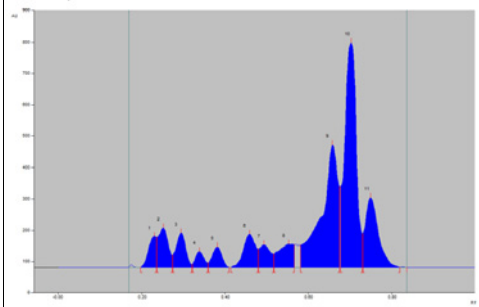

Figure 9: Densitogram of Pineapple fruit powder

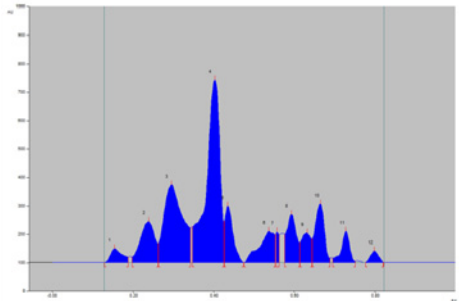

Figure 10: Densitogram of Sapota fruit powder

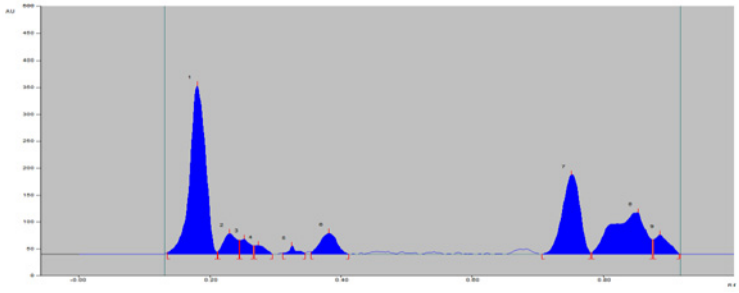

Figure 11: Densitogram of Watermelon fruit

Figure 7-11:

and WinCATS software. HPTLC was performed on pre-coated plate silica 60F_{254} and sample application was done using HPTLC syringe 100µL. The optimized mobile phase for HPTLC development was ethyl acetate: glacial acetic acid: formic acid: water (5:1:1:1 v/v/v/v). The samples were applied as bands of 10µl each, with the help of Linomat 5 applicator. The plate was developed in twin trough chamber (20×10) which was saturated for 30 min. After development, the plate was activated at 105°C for 5 min in oven. After activation, the plate was sprayed with Anisaldehyde Reagent (ANS), then the plate was scanned at wavelength of 450 nm.

Statistical analysis

The data obtained were analyzed by ANOVA with the use of SPSS Statistics (Version 17.0, SPSS).

Results and Discussion

Antioxidant properties of various extracts of different fruit powder

Free radical scavenging is one of the mechanism involved in inhibiting lipid oxidation; therefore it is normally used for determination of antioxidant activity. The DPPH free radical scavenging activity of extracts from 11 different fruit powder were tested. DPPH gives violet color when dissolves in ethanol and the discoloration occurs when antioxidants donate protons to DPPH.

Antioxidant capacities of the extracts from 11 different fruit powder samples with four different extraction solvents were presented in Table 2. Among all the extracts, the water and hot water extracts of watermelon provided highest antioxidant activity with 2.73 and 2.92 µmole of TE/g sample, followed by the ethanol extracts of lemon, which provided the antioxidant activity with 2.78 µmole of TE/g sample.

Total Phenolic Content (TPC) of various extracts of different fruit powder

Phenols are known as important botanical constituents due to their scavenging ability provided by the hydroxyl groups. Phenolic compounds may have direct contribution to antioxidant action. Phenolic compounds were reported to be associated with antioxidant

activity and play important roles in the stabilizing of lipid peroxidation. Natural phenolics are able to provide antioxidant function through various ways, such as intercepting singlet oxygen, decomposing primary products of oxidation, preventing continue hydrogen abstraction from substances etc. In addition, total polyphenols were considered as the major naturally occurring antioxidant compounds in the fruit powders.

The results of TPC values of fruit powder samples were shown in Table 3. The TPC of the samples extracted with ethanol and hot water were much higher than those extracted with methanol and water. Among the four different solvents, ethanol extraction provided the highest TPC. There were no significant difference in TPC content between methanol extracts and water extracts; they presented in low amounts.

According to the results, the overall trend was that the antioxidant capacities were positively related to the phenolic levels.

HPTLC fingerprinting

A well-discriminated HPTLC method was developed for fingerprinting analysis of extracts of fruit powder samples using the mobile phase (ethyl acetate: glacial acetic acid: formic acid: water in the ratio 5:1:1:1 v/v/v/v). The densitogram for extract of fruit powder were obtained by scanning the developed plate at 450nm, as shown in Figures 1-11.

Conclusion

The current study compared the antioxidant activities among extracts of different fruit powder and the effects of different extraction solvents on distribution of antioxidants and phenolics. The fruit powder extracts was studied for the antioxidant activity and total phenolic compounds. The antioxidant activity was studied by using DPPH free radical scavenging activity of extracts from 11 different fruit powder were tested. DPPH gives violet color when dissolves in ethanol and the discoloration occurs when antioxidants donate protons to DPPH. The water and hot water extracts of watermelon provided highest antioxidant activity. The TPC of the samples extracted with ethanol and hot water were much higher than those extracted with methanol and water. Also, a simple and effective HPTLC method has been developed for fingerprinting analysis of 11 different fruit powder extracts.

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