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COMPARATIVE ACCOUNT OF SECONDARY METABOLITES AND ANTIOXIDANT ACTIVITIES OF TWO VARIETIES OF GUAVA FRUIT *(PSIDIUM GUAJAVA* LINN) SOLD IN CENTRAL INDIA

Biological Science

Rajni Nigam*

Department of Botany and Microbiology, Govt. M. H. College of Home Science and Science for Women, Jabalpur 482001 (MP), India *Corresponding Author

ABSTRACT

The present work was taken up to compare the phytochemical and antioxidant properties of two guava cultivars being sold in the local markets of Central India, the local cultivar and the Thai cultivar, which is produced by grafting method. The results showed that the Thai cultivar has lower amountys of phenolic compounds, i.e. flavonoids, anthocyanins, tannins and triterpenes as compared to the local fruit cultivar. Further, the amount of antioxidant activity against reactive oxygen species and DPPH was also lowered in Thai cultivar, stating that this grafted varieties has less potential to scavnege free radicals and combating diseases.

KEYWORDS

Psidium guajava, Thai guava, Phytochemicals, Free radicals, Antioxidants

INTRODUCTION

It is now a well established fact that antioxidants are able to reduce the risk of cardiovascular diseases, strokes as well as cancer in human body[1]. Fruits are the natural sources of antioxidants and are widely used and recommended worldwide. Local and seasonal fruits are often preferred[2].

Guava (*Psidium guajava* Linn.) is native to tropical countries, including India, where it is grown as garden tree in most of the parts of India, including Central India. The guava fruit is eaten as a fruit or used in culiniary purposes such as drinks, smoothies and desserts. Guava fruits are known for various medicinal properties, including antioxidant activity primarily[3]. Guava is a winter fruit available in abundance in Central India, and the local cultivars are usually inexpensive and easily available. The Central Indian market is now flooded with a Thai cultivar for the last few years. This Thai guava is prepred by the grafting technique. These guava cultivars are larger in size and weight, have soft flesh and less seeds, with better taste than the local cultivar, which has smaller size, hard flesh with abundance of seeds.

There are scientific evidences that different cultivars and varieties of guava have variations in their phytochemical profile as well as their potency to scavnege reactive oxygen species and free radicals[4]. Since, fruits are prime sources of antioxidants, the present study investigated the two cultivars for their phytochemical profile as well as their comparative antioxidant activities against reactive oxygen species and free radicals.

MATERIALS AND METHODS

Collection of fruits

The Thai cultivars of guava were purchased from a local marketplace. The local cultivar of Central India was obtained from a kitchen garden of local area. The fresh fruits were taken to the laboratory, washed thououghly with running tap water, cut into small pieaces are shade dried for a week or longer to achieve constant weights. The dried fruits are milled, sieved through 100 no. seive and the powder obtained was stored in airtight containers till use.

Extraction of phytochemicals

The phytochemicals from dried fruit powder (10 g) were extracted sequentially with solvents of varying polarity, starting from polar (aqueous) to non polar solvents (methanol, ethyl acetate and petroleum ether). Aprt from aqueous extraction, which was done using cold percolation method, other extractions were performed using a Soxhlet extractor[5]. The extracts were concentrated *in vacuuo* upto 10 ml (1 g ml^{-1} concentration).

Phytochemical screening

The secondary metabolites from two cultivars of guava were qualitatively screened as per the methods given by Harborne[6], in all four solvent extracts separately.

Determination of antioxidant activities

The antixodiant activities of two guava cultivars were assessed by the potential to scavenge reactive oxygen species as well as artificial free radicals. For sample preparation, all four solvent extract were dried under vacuum, and redissolved in ethaol to get 4 mg ml^{-1} concentration as per dry weight. Ascorbic acid in same concentration was used as a positive control, while ethanol served as negative control. The antioxidant activity (%) was calculated as below:

% antioxidant activity = (A-Ax)/A x 100
Where
A- Absorbance of reaction mixture with ethanol (blank).
Ax- Absorbance of reaction mixture with test solution

a. Hydrogen peroxide radical scavenging activity

Scavenging of hydrogen peroxide (H_2O_2) by the extracts was estimated as per the protocol described by Bozin *et al*.[7]. For this, 0.6 ml of 40 mM H_2O_2 in phosphate buffer (pH 7.4) solution was added to the test tubes containing 3.4 ml of the guava extracts. After thoroughly mixing the reaction mixture, absorbance was noted on spectrophotometer (EI, India) at 230 nm.

b. Superoxide radical scavenging activity

The superoxide radical scavenging activity was determined as described by Noda et al.[8]. The antioxidant activity of guava extracts is monitored as reduction of nitroblue tetrazolium chloride (NBT) by superoxide radicals. Superoxide radicals were generated by the phenazine methosulfate (PMS)/ β -nicotinamide adenine dinucleotide (NADH) system. The reaction mixtures in the sample wells consisted of NADH (166 µM), NBT (43 µM), guava extract (100 µl) in a final volume of 300 µl. The reaction was started by adding and PMS (2.7 µM, 100 µl), continued for 2 minutes and the decrease in absorbance of the reaction mixture was recorded at 560 nm using a spectrophotometer.

c. Nitric oxide scavenging activity

Nitric oxide radical scavenging was determined as per the Hazra et al.[9]. The reaction mixture contained 10 mM sodium nitroprusside, phosphate buffered saline (pH 7.4) and 1 ml of guava extract in a final volume of 3 ml. The reaction mixture is incubated at room temperature for 2.5 h, before adding of 1 ml sulfanilamide (0.33% in 20% glacial acetic acid) to 0.5 ml of the incubated reaction mixture. The reaction mixture is allowed to stand for 5 min again before addition of 1 ml of napthylethylenediamine dihydrochloride (NED) (0.1% w/v). The mixture is again incubated for 30 min at RT. The absorbance of the reaction mixture was measured spectrophotometrically at 540 nm against a blank sample.

d. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The free radical scavenging activity of guava extracts against a synthetic free radical; 2,2, diphenyl-1-picrylhydrazyl (DPPH) was measured as the modified method of Kumar et al.[10]. The reaction mixture consisted 500 µl of guava extract with 500 µl DPPH solution (60 µM DPPH in ethanol) and incubated for 60 min at RT in dark. The absorbance was then measured at 517 nm using a spectrophotometer against blank.

e. Ferric ion reducing antixodant power assay (FRAP)

The FRAP assay was performed as per the procedure given by Aparadh

et al.[11]. For this, 2.5ml of guava extract was mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixture was incubated at 50˚C for 20 minutes followed by quick chilling, before the addition of 2.5ml of 10% trichloroacetic acid. The reaction mixture is and centrifuged at 3000 rpm for 10 minutes. Afterwards, to the supernatant (2.5 ml), added 2.5ml of distilled water and 0.5 ml of 0.1% ferric chloride.The mixture was mixed well and allowed to stand for 10 minutes before the absorbance was measured at 700 nm.

RESULTS

The guava fruits of local and Thai cultivar were extracted with solvants of varying polarity, in order to extract all the important secondary metabolites. Table 1 shows the phytochemical screening results of both the guava samples. It was shown that amounts of alkaloids, flavonoids and triterpenes in aqueous extracts in two cultivars varied, as identified with their qualitative results. Similarly, local cultivar showed presence of anthraquinone, coumarins, resins and tannins in aquesous extract, while the Thai cultivar failed to show the presence of these in aqueous extracts, indicating the amount present is too low. Similar observations were noted in methanolic extracts of the two fruit extracts, where local cultivar clearly has the edge for presence of flavonoids, tannins and triterpenes in good amount, as compared to the Thai cultivar. Not much variation could be observed with ethyl acetate and petroleum ether extracts of the two guava cultivars.

Table 1: Comparison of Phytochemical profile of fruit of two varieties of guava (*Psidium guajava*), when extracted with different solvents.

#	Phytochemical	Extraction solvents							
		Aqueous		Methanol		Ethyl Acetate		Petroleum ether	
		L	T	L	T	L	T	L	T
1.	Alkaloids	++	+	+	ND	ND	ND	ND	ND
2.	Anthraquinone	+	ND	ND	ND	ND	ND	ND	ND
3.	Cardiac glycosides	ND	ND	ND	+	ND	ND	ND	ND
4.	Coumerins	+	ND	ND	ND	ND	ND	ND	ND
5.	Flavonoids	++	+	++	ND	ND	ND	ND	ND
6.	Resins	+	ND	ND	ND	ND	ND	ND	ND
7.	Saponins	+	+	ND	ND	ND	ND	ND	ND
8.	Sterols	ND	ND	ND	ND	+	+	+	+
9.	Tannins	++	ND	+	ND	++	+	ND	ND
10.	Triterpenes	++	+	++	ND	+	ND	ND	ND

L=Local cultivar, T=Thai cultivar, + positive, ++ strongly positive, ND= not detected

When the antioxidant capabilities for different reactive oxygen species and free radicals were studied. The positive control, ascorbic acid produced more than 80% antioxidant activity in all cases, with highest (96.2%) with hydron peroxide scavenging system (Table 2). For the two guava cultivars, it was noted that hydrogen peroxide scavenging activity was primarily associated with methanol extracts, followed by ethyl acetate extracts. The local guava cultivar has shown more antioxidant capabilities in both the extracts (66.8 and 54.3% respectively) as compared to its Thai counterpart (61.2 and 39.2% respectively). Similar observations were recorded with superoxide and nitric oxide scavenging activities, which were found in aqueous and methanolic extracts primarily. Major reduction in antioxidant capability of Thai cultivar was observed with nitric oxide scavenging activity, where it was reduced by approximately 70% in comparison to the activity shown by local cultivar (Fig 1).

Table 2: Antioxidant activity of fruit of two varieties of guava (*Psidium guajava*), when extracted with different solvents, as the capability to scavenge DPPH free radical, as compared to the control, ascorbic acid (1 mg ml^{-1}).

#	Antioxidant activity	% Antioxidant activity in different extracts								
		Ascorbic acid (Control)	Aqueous		Methanol		Ethyl Acetate		Petroleum Ether	
			L	T	L	T	L	T	L	T
1.	H2O2 Scavenging activity	96.2	12.4	10.2	66.8	61.2	54.3	39.2	10.2	9.7
2.	Superoxide scavenging activity	87.1	58.9	42.3	63.2	60.1	12.3	11.1	5.2	5.6
3.	Nitric oxide scavenging activity	83.2	74.2	52.3	87.1	56.2	7.4	8.2	11.6	12.1
4.	DPPH scavenging activity	86.9	77.6	52.1	66.2	51.0	11.2	10.2	7.4	6.6
5.	FRAP activity	81.4	28.5	22.3	77.2	61.3	66.2	42.3	18.1	16.2

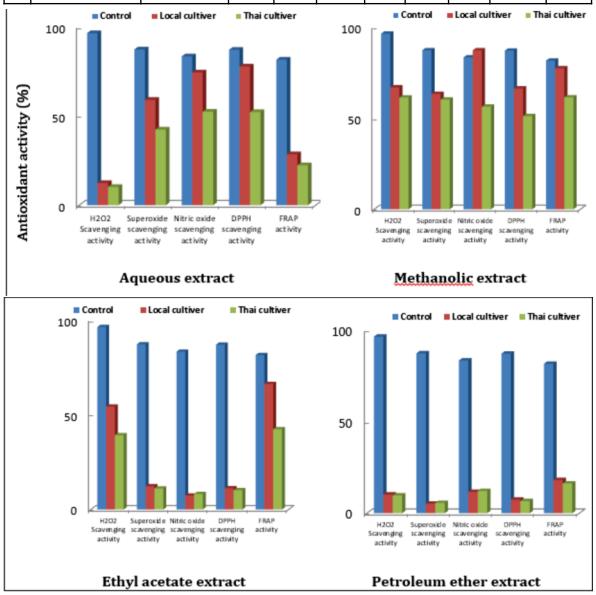

Fig 2: Comparison of various antioxidant activities of local and Thai cultivar of guava fruit with reference to the positive control; ascorbic acid, when phytochemicals were extracted with solvents of varying polarity.

DISCUSSION

Guava fruits are known to be good sources of phytochemicals, especially those have antioxidant capabilities. These phytochemicals include ascorbic acid, carotenoids, and polyphenolics, i.e. flavonoids, tannins and triterpenes[12]. Among major phytochemicals, guava fruits contain proanthocyanidins and ellagitannins. Tannins, especially, ellagitannins are stable and more prominent antioxidants[13]. Our study showed that the Thai cultivar failed to show presence of anthocyanins as well as tannins in extracts in comparison to the local cultivar of guava fruit, which may be the reason why the Thai cultivar had lower antioxidant capacities.

The antioxidant capacity is measured as the potential to scavenge reactive oxygen species, including superoxide anion (O2.-), hydrogen peroxide (H2O2), and nitric oxide radical (NO.)[14]. These reactive oxygen species are produced as byproducts of normal aerobic metabolism in human body, and because of their highly reactive nature, they interefere with other biological processes resulting in various diseases i.e. loss of energy, fatigue, early aeging to more serious like cardiovascular and nuerological diseases[15]. Since, fruits are the main sources to scavenge these reactive molecules, they are primarily consumed for antioxidative properties, rather then nutrition. Our study shows that Thai guava cultivar, although better in taste, is inferior to the local cultivar of guava, when their phytochemical profile and antioxidant capabilities are compared. Based on the results, we strongly recommend to use the local guava cultivars for human use.

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