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Effect of extraction solvents on phenolic content and antioxidant activities of Indian gooseberry and guava

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Abstract

Solvents of various concentrations (50%, 70% and 100%) of ethanol, methanol, acetone and water were explored for the maximum extraction of antioxidants from Indian gooseberry and guava. The extracts were screened for their total phenolic compounds, reducing power, percent free radical scavenging activity and flavonoid content. 50% ethanol was found as best extracting solvent for extraction of phenolics and antioxidants for both the fruits. In Indian gooseberry and guava, 72.45 and 33.29 GAE mg/g total phenolics, 96.79 and 42.95 mM Fe (II)/g FRAP values were obtained respectively. It was observed that the flavonoid content of various Indian gooseberry extracts varied between 0.71 to 10.34 QE mg/g and for guava 2.03 to 16.34 QE mg/g which reveals that guava contains higher amount of flavonoids as compared to Indian gooseberry extracts. Percent free radical scavenging activity of Indian gooseberry and guava extracts in various solvents also showed the similar trend. Percent free radical scavenging activity of Indian gooseberry and guava ranged from 13.64% to 83.14% and 12.24 to 74.18% respectively, with maximum values in 50% ethanol

Introduction

The use of plants as antioxidants in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants. There is a growing interest in studies of plant extracts and essential oils for their potential antioxidant activity. Fruit antioxidants had synergistic effects and protective properties against various degenerative disorders. The protection mechanism generally functions at several different levels within cells in human body by inhibiting the formation of free radical species, intercepting radical-chained reactions, converting existing free radicals into less harmful molecules and repairing oxidative damage (Du *et al*., 2009).

Indian gooseberry and guava both provide significant health benefits because of their high antioxidants, vitamins, minerals and fibre content (Zhao, 2007). The high concentration of ascorbic acid, flavonoids and phenolic acids in guava and Indian gooseberry make these fruits attractive for consumers. Antioxidant activity of plant materials are well correlated with the content of their phenolic compounds (Velioglu *et al*., 1998). Both the berries are rich in antioxidants and help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, inflammation and brain dysfunction. In addition, antioxidants were reported to retard ageing (Rice-Evans *et al*., 2000; Ross and Kasum, 2002) besides preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species. Phenolic compounds are plant secondary metabolites commonly found in herbs and fruits such as berries, apples, citrus fruit, cocoa, grapes, vegetables like onions, olives, tomatoes, broccoli, lettuce, soybeans, grains and cereals, green and black teas, coffee beans, propolis, and red and white wines (Clifford, 1999; Brit *et al*., 2001; Rencher *et al*., 2001; Kris-Etherton *et al*., 2002).

Extracting antioxidants from plant material most often involves the method of solvent extraction. The choice of solvent has been shown to have a significant influence on the concentration of antioxidants extracted (Halliwell 1996; Feskanich *et al*., 2000). Phenolic compounds contribute to the overall antioxidant activities of the plant foods. However, owing to the differing antioxidant potential of compounds with different polarities in complex whole foods, all methods for assessing the antioxidant capacity of food samples are strongly affected by the solvents used during extraction (Sultana *et al*., 2009). The objective of present study was to optimise the extraction of total phenolics and antioxidants from Indian gooseberry and guava. The objective in extracting phytochemicals from these fruits is to liberate these compounds from the

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vacuolar structures where they are found, either through rupturing plant tissue or through a process of diffusion in various solvents and the further estimation of their antioxidant properties.

Material and Methods

Plant materials

Indian gooseberry *(chakaiya)* and guava *(Allahabad safeda)* were obtained from local market of Allahabad city and stored at 15±2°C with relative humidity of 90-95%. Fruit were selected for uniformity of size and color, and blemished and diseased fruit were discarded. Procured fruits were identified from Botanical survey of India, Allahabad. Ascorbic acid, quercetin, gallic acid standards were obtained from Sigma Aldrich Chemical Co. St. Louis, Missouri (USA).

Sample preparation

Fresh fruits (2.0 g) were extracted using 5 ml aqueous ethanol (ethanol: water; 70:30;50:50 v/v), methanol (methanol: water, 70:30;50:50 v/v), acetone (acetone: water70:30;50:50v/v) and absolute ethanol, methanol, acetone, water for 24 hours at room temperature in orbital shaker (REMI,C1S-24BL).The extracts were separated from the residues by filtering through Whatmann No.1 filter paper. The residues were extracted twice with the fresh solvent and extracts were combined. The extracts were stored in a refrigerator (5±2°C) until used for further analysis.

Total phenolic content

Total phenolic content was determined by the Folin Ciocalteau method using gallic acid monohydrate as standard. It was dissolved in various extraction solvents. An aliquot (0.05 ml) of sample or standard was placed in test tube and the volume was adjusted to 6 ml with deionised water. Then 0.3 ml of Folin Ciocalteau was added to all tubes. After 8 minutes 0.9 ml of 20% sodium carbonate was added to the mixture and then incubated for 30 minutes at 40°C. Absorbance of the resultant blue color was measured at 765 nm in spectrophotometer (Model: Evolution 600, Thermoscientific, Waltham MA, USA). Total phenolics were expressed as mg gallic acid equivalent/gm weight.

Ferric reducing antioxidant power assay

Ferric reducing power assay was used to determine antioxidant activity. The FRAP assay was done according to Benzie and Strain, (1996) with some modifications. The stock solutions included 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$. $6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3$. $6H_2O$ solution and then warmed at 37°C before using. Fruit extracts (150 mL) were allowed to react with 2850 µL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The standard curve was linear between 25 and 800 mM Gallic acid. Results are expressed in mg GAE/g. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

DPPH radical scavenging activity

Free radical scavenging activity of extracts was measured by the slightly modified method of (Moure *et al*., 2001; Alothman *et al.*, 2009) The antioxidant capacity of the fruit extracts was studied through the evaluation of the free radical scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot (100 µl) of fruit extract was mixed with 3.9 ml of 0.1 mM DPPH methanolic solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured at 515 nm. Results were expressed as percentage of inhibition of the DPPH radical. Ascorbic acid was used as standard.

Flavonoid content

Total flavonoid contents were measured with the aluminum chloride colorimetric assay. Hydroalcoholic extracts that has been adjusted to come under the linearity range and different dilution of standard solution of Quercetin (10-100 µg/ml) were added to 10ml volumetric flask containing 4ml of water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. After 6 min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Total flavonoid content of the extracts was expressed as mg of quercetin equivalent per gm weight of sample.

Statistical analysis

All analyses were carried out in triplicate and were reported as mean ± SD. An ANOVA test (SPSS 12.0, SPSS Inc., Chicago, IL, USA) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by using the Duncan test ($p < 0.05$).

Table 1. Effect of various extraction solvents on the total phenolics and antioxidant activities from Indian gooseberry (fwb)

Extraction solvent	Total phenolics (GAE, mg/g)	FRAP (GAE, mg/g)	Percent free radical scavenging activity (%)	Flavonoids (QE, mg/g)
Water 100 %	54.31 +2.48 ^{d}	42.57±11.54 ^{d}	27.51±3.12 ^{g}	1.0±0.01 ^{f,g}
Acetone 50%	70.29±2.95 ^{ab}	86.04±2.58^{b}	75.22±1.11^{b}	7.0±0.38 ^{b}
Acetone 70 %	58.34±2.21^{c}	70.10±3.23 ^{c}	46.58±3.14 ^{e}	2.15±0.31 ^{e}
Acetone 100%	49.73±1.49 ^{e}	31.99±1.32^{e}	15.45±0.77^{i}	0.75±0.06^{fg}
Methanol 50 %	69.93 +0.85 ^{ab}	77.53±2.25 ^{c}	71.44±1.51 ^{c}	5.0±0.66 ^{c}
Methanol 70%	54.58±1.04 ^{d}	48.76±3.06 ^{d}	37.98±1.83 ^{f}	1.53±0.35 ^{ef}
Methanol 100%	46.63±0.53^{e}	29.21±0.71^{e}	13.64±0.53^{i}	0.71±0.01^{g}
Ethanol 50%	72.46±1.09 ^{a}	96.79±2.08 ^{a}	83.14±1.79 ^{a}	10.34±0.66 ^{a}
Ethanol 70%	67.89±3.39^{b}	74.73 +2.95 ^{c}	61.75 +2.99 ^{d}	3.79 +0.78 ^{d}
Ethanol 100%	53.8±1.20^{d}	35.06±1.06^{e}	21.01±1.0^{h}	0.93±0.05^{fg}

All data are mean±SD of triplicate (n=3) analyses. Values with different superscript in the same column differ significantly ($p<0.05$).

Results and Discussion

Total phenolics

The values of total phenolics of Indian gooseberry in various solvents extracts varied from 46.63 to 72.46 of GAE mg/g whereas for guava it ranged from 5.99 to 33.29 GAE mg/g. The highest total phenolic was obtained with 50% ethanol for both the fruits. The difference in the extract yield from the Indian gooseberry and guava might be due to different availability of extractable components resulting from the varied chemical composition of gooseberry and guava. The amount of the antioxidant components that can be extracted is mainly affected by the vigour of the extraction procedure which probably may vary from sample to sample. Results of the present study showed that among all the solvent extracts the 50% ethanol had the maximum polyphenols extraction (Table 1 and 2). This might be due to the fact that phenolics are often extracted in more polar solvents such as aqueous ethanol, acetone and methanol.

The results of the total phenolics of selected fruits in this study are in agreement with Kumar *et al*. (2006) which revealed that total phenolics (free and bound) of Indian gooseberry was 126 mg/g dwb. Several other studies also revealed the efficiency of 50% hydroethanolic solution for extracting total phenolics from dried ground materials. Thaipong *et al*. (2006) reported a concentration of total phenolics 344.9 (GAE mg/100g) in *Allahabad safeda* guava which is lower than present results. Musa *et al*. (2011) found that pure solvents were inefficient extraction media for antioxidant. Luximon and Ramma *et al.* (2003) reported that white pulp guavas had higher ascorbic acid content and total phenolics than the pink pulp in which the total phenolics was 247.30 and 126.4 GAE mg/100g in white and pink pulp respectively. Patthamakanoporn *et al.* (2008) reported 148.0 GAE mg/100g total phenolics in guava. McCook-Russell *et al.* (2012) investigated that strawberry guavas were superior to common guavas in phenolic compounds and reported 4439 and 1952 GAE µg /g fwb respectively which is much lower amount than our results i.e. 33,290 GAE µg /g fwb. Alothman *et al*. (2009) reported phenolics in range 123.0 to 191.0 GAE mg/100g fwb in guava with maximum extraction observed in 90% acetone followed by 90% ethanol with no significant difference between them ($p<0.05$). Ethanol and water mixtures are commonly used for the extraction of phenols from plant materials (Bahorun *et al.*, 2004; Patthamakanokporn *et al*., 2008; Mc-Cook Russell *et al*., 2012). This is due to the wide range of phenols that the aqueous ethanol mixtures can dissolve. Gokmen, (2009) also suggested ethanol:water (50:50) as most appropriate solvent, based on results attained after performing thousands of food samples. Wide range of total phenolics has been reported by various authors in gooseberry which might be due to the difference in climacteric conditions, raw material composition and used solvents of different concentration for analysis (Prasad *et al*., 2009). Therefore, the selection of an appropriate solvent system is one of the most relevant steps in optimizing the recovery of total phenolic content and other antioxidant compounds from a sample (Gokmen *et al*., 2009).

Ferric reducing antioxidant power assay

Reducing power was based on the reaction with potassium ferricyanide and indicated electron transfer

Table 2. Effect of different extraction solvents on the total phenolics and antioxidant activities from guava (fwb)

Extraction solvent	Total phenolics (GAE, mg/g)	FRAP (GAE, mg/g)	Percent free radical scavenging activity (%)	Flavonoids (QE, mg/g)
Water 100 %	14.63±0.4^{d}	12.72±1.56^{c}	30.56±0.93^{g}	7.44±0.79^{e}
Acetone 50%	25.53±2.3^{b}	20.92±2.77^{b}	67.36±1.21^{b}	13.13±0.1^{b}
Acetone 70 %	19.25±1.17^{c}	13.21±2.77^{c}	44.53±1.21^{e}	9.10±0.1^{d}
Acetone 100%	8.77 ± 0.14 ^{f}	9.10±0.42^{d}	16.65±1.5^{i}	2.60±0.46^{g}
Methanol 50 %	21.19±2.07^{c}	18.92±1.01^{b}	63.55±2.18^{c}	11.88±0.51^{c}
Methanol 70 %	18.89±1.17^{c}	13.12±2.72^{c}	38.40±0.86^{f}	7.83±0.87^{e}
Methanol 100%	5.99±2.18^{g}	8.38±0.43^{d}	12.24±0.75^{j}	2.03±0.33^{g}
Ethanol 50%	33.29±1.51^{a}	42.95±2.16 ^{a}	74.18±1.16^{a}	16.34±0.72^{a}
Ethanol 70%	20.23±1.38^{c}	18.29±1.62^{b}	56.56±1.66^{d}	9.87±0.24^{d}
Ethanol 100%	11.76±0.22^{e}	9.95±0.95^{c,d}	21.22±2.82^{h}	5.66±0.65^{f}

All data are mean±SD of triplicate (n=3) analyses. Values with different superscript in the same column differ significantly ($p<0.05$).

ability to reduce ferric to ferrous. In contrast to other tests of total antioxidant power, the FRAP assay is simple, speedy, inexpensive, and highly reproducible. The results showed that FRAP values varies with the extraction solvents for both the fruits. FRAP values for 50% ethanol extraction was significantly higher ($p<0.05$) than other extraction solvents.

50% ethanol showed highest FRAP values for Indian gooseberry (96.79 mM Fe (II)/g) and guava (42.95 mM Fe (II)/g). In the present study the Ferric Reducing Power Assay (FRAP) values ranged from 31.99 mM Fe (II)/g to 96.79 mM Fe (II)/g for Indian gooseberry and 8.38 to 42.95 mM Fe (II)/g for guava, in various solvents (Table 1 and 2). Variations in the values of FRAP of different extracts might attribute to the change in relative polarity of different solvents used. The results of current study are in compliance with earlier studies, which reported that Indian gooseberry is a better source of phenolics which possess better antioxidant activity. Antioxidant activity of free and bound phenolic extracts of emblica was contributed predominantly by free phenolic acids which was constituted by gallic and tannic acid. kumaran and Karunakaran, (2007) studied the methanolic extracts of five plants from genus Phyllanthus (i.e. *Phyllanthus debilis, Phyllanthus urinaria, Phyllanthus virgatus, Phyllanthus maderaspatensis* and *Phyllanthus amarus*) and found that each of them possess high total antioxidant, reducing power and free radical scavenging activities. Among the plant phenolics responsible for antioxidant capacity, phenolic acids and flavonoids might play the major role (Zhao *et al*., 2006).

Percent free radical scavenging activity

It is generally recognized that free radicals produced in the body are partly associated with the etiology of cancers and other chronic diseases. Dietary antioxidants, capable of scavenging free radicals, are able to reduce the risk of the disease. Therefore, it is important to determine the radical scavenging effect of antioxidants in fruits. DPPH is a free radical and stable at room temperature, which produces a violet solution in ethanol. Reduction of DPPH by antioxidants results in a loss of absorbance. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. Percent free radical scavenging activity of Indian gooseberry and guava extracts in various solvents also showed the similar trend in results. Percent free radical scavenging activity of Indian gooseberry and guava ranged from 13.64% to 83.14% and 12.24 to 74.18% respectively, with maximum values in 50% ethanol (Table 1 and 2). Several earlier studies also showed that guava possess high amount of percent free radical scavenging activity (Kahkonen *et al.,* 1999; Vyas *et al.*, 2010). Liu *et al.* (2008) studied the antioxidative components of emblica fruit in methanol and then partitioned it by ethyl ether, ethyl acetate, butanol and water. The ethyl acetate fraction showed the strongest 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity among four fractions. Free (EOFP) and bound phenolics (EOBP) of emblica officinalis showed high values of free radical scavenging activity (Kumar *et al*., 2006).

Flavonoid content

Phenolic acids and flavonoids have been reported to be the main phytochemicals responsible for

Table 3. Pearson's correlation coefficients between total phenolics, flavonoids and DPPH, FRAP assay in various solvents in Indian gooseberry

	Total Phenolics	Flavonoids
DPPH	0.95	0.97
FRAP	0.89	0.86

Table 4. Pearson's correlation coefficients between total phenolics, flavonoids and DPPH, FRAP assay in various solvents in Guava

	Total Phenolics	Flavonoids
DPPH	0.92	0.89
FRAP	0.96	0.90

the antioxidant capacity of fruits and vegetables. Flavonoid content of the extracts was measured in terms of quercetin equivalents. Maximum extraction of flavonoids was found in 50% ethanol for both fruits. It was observed that the flavonoid content of various Indian gooseberry extracts varied between 0.71 to 10.34 QE mg/g and for guava 2.03 to 16.34 QE mg/g which reveals that guava contains higher amount of flavonoids as compared to Indian gooseberry extracts (Table 1 and 2).Total phenol and flavnonoid content of Allahabad safeda and Bhavanagar red varieties of guava was assessed by Viraj and Pillai, (2012) the results revealed that phenolics and total flavanoid contents were higher in Bhavnagar red variety compared to Allahabad safeda. Arima and Danno, (2002) reported two new flavonoid glycosides viz., morin-3-O-a-L-lyxopyranoside and morin-3-O-a-L-arabopyranoside and two known flavanoids viz., guaijavarin and quercetin in guava.

Correlation studies

The correlation coefficients between antioxidant activity, total phenolics and flavonoids are presented in Table 3 and 4. As shown in the tables there were positive significant linear correlations between antioxidant activity (expressed on the basis DPPH and FRAP) and contents of phenolics and flavonoids of Indian gooseberry and guava. In Indian gooseberry the correlation coefficient was higher (R =0.95) between total phenolics and DPPH activity than that of total phenolic and FRAP activity (R=0.89) where as in guava the correlation coefficient was found higher between total phenolics and FRAP activity (R=0.96). Positive correlation was found between flavonoids of Indian gooseberry and guava with antioxidant activity.

These correlations confirm that the phenolic compounds are the main micro-constituents contributing to the antioxidant activities of these fruits. The findings from the above correlation analyses indicated specific phenolic substances, which were extracted by the selected solvent systems, had different degrees of contributions to the overall antioxidant activities. These results are in accordance with many others where it is shown that higher total phenolic linearly correlates well with antioxidant activity (Demiray *et al.,* 2009; Viraj and Pillai, 2012).

Conclusion

50% ethanol was found as best extracting solvent for extraction of phenolic compounds and antioxidants for both the fruits. In Indian gooseberry and guava 72.45 and 33.29 GAE mg/g total phenolics, 96.79 and 42.95 mM Fe (II)/g FRAP values were obtained respectively. Consumer demand for health promoting products provides an opportunity to develop antioxidant rich functional foods, as well pharmaceutical grade or nutraceutical products. The antioxidant rich guava and Indian gooseberry can be explored for the above said properties which would be a great advantage to the health food industry.

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