African Journal of Pharmacy and Pharmacology Vol. 5(18), pp. 2067-2072, 15 November, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.475
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Phytochemical constituents and antioxidant potential of some underused fruits

Firdose R. Kolar, Vaishali S. Kamble and Ghansham B. Dixit*

Laboratory of Cytogenetics and Plant Breeding, Department of Botany, Shivaji University Kolhapur-416 004 (MS), India.

Accepted 20 October, 2011

Phenols, a major group of antioxidant phytochemicals, have profound importance due to their biological and free radical scavenging activities. To identify their potential sources, extracts of some underused fruits (*Muntingia calabura*, *Averrhoea bilimbi* and *Artocarpus altilis*) were assessed with regard to their total phenolics and flavonoid content, as well as antioxidant activity in different solvent systems (methanol, ethanol and acetone) and distilled water. The phenolics and flavonoid content of *M. calabura* was 1.356 to 3.872 mg tannic acid equivalents/g fresh weight (TAE/g fw) and 0.026 to 0.068 mg rutin equivalents/g fresh weight (RE/g fw), respectively. That of *A. bilimbi* was 0.581 to 1.334 mg TAE/g fw and 0.021 to 0.037 mg RE/g fw, and that of *A. altilis* was 0.689 to 1.723 mg TAE/g fw and 0.013 to 0.043 mg RE/g fw. The extracts were also found to have significantly different levels of antioxidant activities. A correlation between antioxidant activity and phenolic content was observed, which shows that the tested plant extracts are having potential antioxidants of natural origin.

Key words: Phenolics, flavonoids, antioxidants, *Artocarpus altilis, Averrhoea bilimbi, Muntingia calabura.*

INTRODUCTION

Plants being a major source of natural therapeutic remedies are known to possess high amounts of polyphenols and potent antioxidant capacity or free radical scavenging activity. Numerous epidemiological studies suggest that diets rich in phytochemicals and antioxidants execute a protective role in health and disease (Lampe, 1999; Guo and Yang, 2001). Fruits are diverse in antioxidant composition and those with high antioxidant activity generally contain more antioxidants (Guo et al., 1997). The major group of phytochemicals that may contribute to antioxidant capacity of fruits includes polyphenols, carotenoids and the traditional antioxidant vitamins, such as vitamin C and E. The fruit polyphenols are the most important group of natural antioxidants, because of their diversity and extensive distribution. They have been reported to possess antioxidant activity which allows them to scavenge both active oxygen species and electrophiles (Rice-Evans et al., 1995). The majority of the antioxidant activity of fruits and vegetables may be from phenolic compounds rather than vitamin C and E, or β-carotene since some phenolic compounds have much stronger antioxidant activities against peroxyl radicals (Cao et al., 1997). Thus, polyphenols have become an intense focus of research interest, because of their perceived beneficial effects for health, including anticarcinogenic, antiatherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory and antioxidant activity.

Despite many reports of commonly consumed fruits on their phenolic content (PC) and antioxidant capacity (AC), little information is available for underused fruits. These underused fruits may contain a significant amount of phytochemicals or even unique compounds that are health promoting. Their antioxidant capacity may be comparable or even superior to that of the more extensively studied fruits. Thus, the present study evaluates three different types of underused fruits namely: *Artocarpus altilis* (Parkinson) Fosberg (Moraceae),

*Corresponding author. E-mail: g_b_dixit@yahoo.co.in. Tel: +91-0231-2609365. Fax: +91-0231-2691533 or +91-0231-2692333.

Averrhoea bilimbi L. (Oxalidaceae) and *Muntingia calabura* L. (Elaeocarpaceae) as new potential sources of natural antioxidants and phenolic compounds.

MATERIALS AND METHODS

Plant

Fresh fruits of the selected species, namely: *A. altilis, A. bilimbi* and *M. calabura* were collected separately, from the botanical garden of the Department of Botany, Shivaji University, Kolhapur. The herbaria for voucher specimens of the following plants (*M. calabura*: SUK-3104, *A. bilimbi*: SUK-3105, *A. altilis*: SUK-3106) were prepared and deposited in the Department of Botany, Shivaji University Kolhapur (M.S.) India.

Preparation of fruit extracts

Fruit extracts were prepared using three different solvent systems (methanol, ethanol and acetone) and distilled water (H_2O).

Determination of total phenolic content

Total phenolic contents (TPC) of the fruit extracts were determined using Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of the extracts (0.125 ml was mixed with Folin-Ciocalteu reagent. Then 1.25 ml of saturated Na_2CO_3 solution was added and allowed to stand for 90 min at room temperature. Then, the absorbance was measured at 760 nm. A calibration curve was prepared using a standard solution of tannic acid (10 to 100 µg/ml, r^2 = 0.998). Results were expressed on fresh weight (fw) basis as mg tannic acid equivalents (TAE)/g of sample.

Determination of total flavonoids

Total flavonoid contents (TFC) of the fruit extracts were analyzed according to the colorimetric method (Luximon-Ramma et al., 2002). In brief, 1.5 ml of fruit extract was mixed with 1.5 ml of $AlCl_3$ (2% w/v). It remained at room temperature for 10 min, the absorbance was measured at 368 nm. A calibration curve was prepared using a standard solution of rutin (10 to 100 µg/ml, r^2 = 0.964). The results were also expressed on a fresh weight basis as mg rutin equivalents (RE)/g of sample.

DPPH free radical-scavenging assay

The free radical scavenging activity of the extracts was measured *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Aquino et al., 2001). An aliquot (25 µl) of fruit extract was mixed with 3 ml of 25 mM DPPH ethanolic solution, the reaction mixture was left in the dark at room temperature for 20 min and the absorbance was measured at 515 nm, against a blank of ethanol without DPPH. Results were expressed as percentage of inhibition of the DPPH radical.

Ferric reducing antioxidant power assay

The ability to reduce ferric ions was measured using a modified version of the method described by Pulido et al. (2000). An aliquot (90 µl of extract was added to 2.7 ml of FRAP reagent) 10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution and 1 part of 20 mM $FeCl_3$. $6H_2O$ solution and the reaction mixture were incubated at 37°C for 15 min. After that, the absorbance was measured at 593 nm. A calibration curve was prepared, using an aqueous solution of ascorbic acid (100 to 1000 µM, r^2 = 0.998). FRAP values were expressed on a fresh weight basis as micromoles of ascorbic acid equivalent per gram of sample.

Ferrous ion chelating activity

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to Dinis et al. (1994) method. To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2 mM) was added. After 30 s, 0.1 ml ferrozine (5 Mm) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. The percentage of chelating activity of the extract was determined.

Statistical analysis

Experimental results are expressed as means ± SE. All measurements were replicated three times. Data were subjected to different statistical analysis using MS Excel and Graphpad instat software.

RESULTS AND DISCUSSION

Polyphenol content

Phenolics and flavonoids are ubiquitously found in many plant sources including different vegetables, fruits and medicinal plants. Recently, the role of phenolic compounds in the prevention of free radical mediated diseases has become more important due to the discovery of the link between lipid peroxidation of low density lipoprotein (LDL) and arteosclerosis. They possess different antioxidant properties, which can be ascribed to a broad range of pharmacological activities. These compounds in general, act by quenching free radicals, inhibiting the activation of procarcinogens, or by binding carcinogens to macromolecules (Krishnaswamy, 1996). The level of the phenolic compounds in the fruits is as shown in Table 1. The total phenolic content (TPC) of the fruit extracts ranged from 0.689 to 1.723 mg TAE/g (fw) for *A. altilis*, while it ranged from 0.581 to 1.356 mg TAE/g (fw) for *A. bilimbi* and from 1.334 to 3.872 mg TAE/g (fw) for *M. calabura*. The fruit extracts of *M. calabura* had higher phenolic content when compared with the other two fruits.

The total flavonoid content (TFC) of these fruits was also determined (Table 1). *M. calabura* fruits also had highest TFC (0.026 to 0.068 mg RE/g fw) followed by *A. altilis* (0.013 to 0.043 mg RE/g fw) and *A. bilimbi* (0.021

Table 1. Total phenolic and flavonoid content of fruits extracts obtained from different solvent extraction systems.

Solvent	*A. altilis*		*A. bilimbi*		*M. calabura*	
	Total phenolics (mg TAE/g fw)	Total flavonoids (mg RE/g fw)	Total phenolics (mg TAE/g fw)	Total flavonoids (mg RE/g fw)	Total phenolics (mg TAE/g fw)	Total flavonoids (mg RE/g fw)
Aqueous	0.689 ± 0.01	0.043 ± 0.13	1.334 ± 0.16	0.021 ± 0.08	1.356 ± 0.06	0.050 ± 0.02
Methanol	1.723 ± 0.11	0.042 ± 0.02	0.581 ± 0.09	0.031 ± 0.01	2.256 ± 0.14	0.026 ± 0.12
Ethanol	0.889 ± 0.16	0.013 ± 0.18	0.607 ± 0.23	0.029 ± 0.11	1.790 ± 0.06	0.027 ± 0.01
Acetone	0.718 ± 0.03	0.022 ± 0.05	0.933 ± 0.16	0.037 ± 0.06	3.872 ± 0.13	0.068 ± 0.09

Values expressed are means ± S.E. of three parallel measurements.

to 0.037 mg RE/g fw). Correlation analysis was performed on the polyphenolic content analysis methods for the three fruits. The correlations between TPC and TFC assays were 0.162, 0.304 and 0.384 for *A. altilis*, *A. bilimbi* and *M. calabura*, respectively, which were not significantly correlated. These results indicate that flavonoids are not the major phenolic compounds in the fruits. Similar correlations were also found by other researchers (Meda et al., 2005). This could be explained by the fact that flavonoids could be related to other antioxidant compounds contained in fruits, such as amino acids and proteins that can also react with Folin-Ciocalteu reagent.

Antioxidant capacity

The antioxidant capacities of the plant extracts largely depend upon the compositions of the extracts and conditions of the test system. The antioxidant capacities are influenced by many factors that cannot be fully described with one single method. It is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (Wong et al., 2006). Therefore, in this study, three different methods have been used to evaluate the antioxidant capacity of the fruit extracts; they are DPPH free radical-scavenging, ferric reducing antioxidant power (FRAP) assay and ferrous ion chelating activity assay.

DPPH radical scavenging activity

DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity. The essence of DPPH radical scavenging assay is that antioxidants react with DPPH (deep violet color) and convert it to yellow colored 1, 1-diphenyl-2-picrylhydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant (Blois, 1958; Huang et al., 2005). In the present study, the fruits analyzed were able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decolorization. The DPPH free radical scavenging activity of the plant extracts are shown in Table 2. *M. calabura* showed highest DPPH free radical scavenging activity followed by *A. bilimbi* and *A. altilis.* It appears that *M. calabura* had a strong hydrogen-donating capacity and can efficiently scavenge DPPH radicals. This high scavenging property of *M. calabura,* may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger (Gyamfi et al., 1999).

Ferric reducing antioxidant power

FRAP assay measures the reducing potential of antioxidant. Antioxidant compound which act as a reducing agent exert its effects by donating hydrogen atom to ferric complex and thus, break the radical chain reaction (Singh and Rajini, 2004). The ability of the plant extracts to reduce ferric ions was depicted in Table 2. *M. calabura* exhibited relatively strong ferric ion reducing activities as compared to *A. bilimbi* and *A. altilis* (Table 2). The highest reducing power of *M. calabura* is probably due to the action of hydroxyl group of the phenolic compounds which might act as an electron donor.

Ferrous ion chelating activity

Ferrous ion, commonly found in food systems,

Table 2. Antioxidant capacity of fruits extracts obtained from different solvent extraction systems.

Solvent	*A. altilis*			*A. bilimbi*			*M. calabura*		
	DPPH inhibition (%)	FRAP (µM AAE/g fw)	Ferrous ion chelating activity (%)	DPPH inhibition (%)	FRAP (µM AAE/g fw)	Ferrous ion chelating activity (%)	DPPH inhibition (%)	FRAP(µM AAE/g fw)	Ferrous ion chelating activity (%)
Aqueous	50.15	10333.29 ± 0.37	42.83	57.00	19309.91 ± 0.05	13.90	57.69	19400.35 ± 0.11	59.94
Methanol	59.29	16280.62 ± 0.02	57.69	50.72	2419.39 ± 0.03	21.97	66.73	26635.92 ± 0.02	59.62
Ethanol	56.81	9587.12 ± 0.07	50.54	55.86	3075.11 ± 0.03	48.29	57.20	26703.75 ± 0.07	61.75
Acetone	54.34	12413.51 ± 0.02	44.27	58.53	6218.06 ± 0.13	36.88	72.24	65301.72 ± 0.08	68.70

Values expressed are means ± S.E. of three parallel measurements.

is well known as an effective pro-oxidant (Hsu et al., 2004). The purpose of the test of ferrous ion chelating activity was to determine the capacity to bind the ferrous ion catalyzing oxidation. Ferrozine can quantitatively form complexes with Fe^{2+} but in the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of color reduction therefore, allows estimation of chelating activity of the co-existing chelator (Yamaguchi et al., 2000). In this assay, the extracts of fruits interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and can capture ferrous ion before ferrozine. The fruit extracts of *A. bilimbi* chelated the least amount of ferrous ions, while *M. calabura* chelated the most, followed by *A. altilis*. Interestingly, the data reveals that *M. calabura* exhibited potent antioxidant properties.

Comparatively, among all the plants tested, *M. calabura* extracts exhibited highest FRAP, DPPH and ferrous ion chelating activities which can be interpreted as the highest antioxidant capacity as compared to *A. altilis* and *A. bilimbi*. This might be due to polyphenols present in this fruit, which have contributed to high antioxidant capacity. As reported by Guo et al. (1997) that high antioxidant capacity of fruits is most probably due to highpolyphenol compounds, such as phenolic acids and flavonoids.

Effect of solvent system

Extraction is critical to the recovery of antioxidant phytochemicals. Many factors can affect the extract recovery of antioxidant phytochemicals from natural materials. Solvents, such as water, ethanol, methanol, acetone or their mixtures are commonly used to extract phytochemicals from plants, and they are another important factor affecting both extraction yield and antioxidant activity of extracts. Due to the complexities of both the chemical characteristics of solvent and the diverse structure and composition of the plant materials, the behavior of material-solvent systems were different from each other and can hardly be predicted. No single solvent could extract all the antioxidants of different polarity and solubility in a single plant (Dongmei et al., 2007).

From the results shown in Table 1, it is evident that the recovery of phenolic compounds was dependent on the solvent used and its polarity (for the three fruits). For *A. altilis* fruit extracts, methanol gave the highest yield of TPC (1.723 mg TAE/g fw) and aqueous extract has highest TFC (0.043 mg RE/g fw). In *A. bilimbi*, aqueous extract could recover the highest yield of TPC (1.334 mg TAE/g fw) and TFC (0.037 mg RE/g fw) was highest in acetone with significant difference when compared with all other solvent systems. The highest yield of *M. calabura* TPC (3.872 mg TAE/g fw) and TFC (0.068 mg RE/g fw) was obtained using acetone.

The results presented in Table 2 shows the effect of solvents on the antioxidant activity. In *A. altilis* methanolic extract exhibited highest free radical scavenging (59.29%), ferric reducing (16280.62 mg AAE/g fw) and ferrous ion chelating (57.69%) activities. In *A. bilimbi*, the acetone extract showed higher radical inhibition (58.53%), while the highest ferric reducing (19309.91 mg AAE/g fw) and ferrous ion chelating (48.29%) activity was found for aqueous and ethanolic extracts, respectively. Whereas in the case of *M. calabura*, acetone extract exhibited highest radical scavenging (71.53%), ferric reducing (65301.72 mg AAE/g fw) and ferrous ion chelating activities (68.70%). It should be noted that in *M. calabura*, acetone extract having the highest amount of TPC

Table 3. Regression coefficients (r^2 value) for antioxidant activity analyzed by different methods and the relative influence of antioxidant components.

Methods of analysis of antioxidant activity	*A. altilis*		*A. bilimbi*		*M. calabura*	
	Total phenolics	Total flavonoids	Total phenolics	Total flavonoids	Total phenolics	Total flavonoids
Free radical scavenging activity (DPPH)	0.619	0.833*	0.354	0.007	0.918*	0.358
Ferric reducing antioxidant property (FRAP)	0.736	0.252	0.939*	0.545	0.952*	0.537
Ferrous ion chelating activity	0.651	0.198	0.352	0.316	0.856*	0.597**

Data were statistically analyzed using Pearson correlation coefficient test. *Indicates a significant difference at the level of $P < 0.05$. **Indicates a significant difference at the level of $P < 0.01$.

and TFC, also exhibited strong antioxidant activities in all the assays.

Relationship between antioxidant capacity of TPC and TFC

The data was subjected to multiple regression analysis to test the relationship between antioxidant activity in different systems and the antioxidant components (phenolics and flavonoids). The results are presented in Table 3. The correlations of TPC against the antioxidant activity based on the DPPH, FRAP and ferrous ion chelating assays in all the three plant species were satisfactory. In *A. altilis*, a good correlation was found between TPC and against DPPH and FRAP; however, it is significantly correlated with ferrous ion chelating activities with coefficient of correlation 0.892. In the case of *A. bilimbi*, TPC correlated significantly with FRAP, but was not significantly correlated with DPPH and ferrous ion chelating activities. For *M. calabura*, the correlations of TPC with DPPH, FRAP and ferrous ion chelating activities was found to be highly significant.

These correlations confirm that the phenolic compounds are the main micro constituents contributing to the antioxidant activities in *A. altilis* and *M. calabura* but not for *A. bilimbi*, where it could be related to other antioxidant compounds contained in *A. bilimbi* fruits.

Similarly, when total flavonoid content was correlated with DPPH, FRAP and ferrous ion chelating activities (Table 3), *A. altilis* showed a significant relationship of TFC with DPPH. However, a poor correlation was found with FRAP, but no significant correlation was found with ferrous ion chelating activities with coefficient of correlation > 0.2. The coefficient of correlation in *A. bilimbi* for TFC and antioxidant activities showed poor correlation with FRAP and ferrous ion chelating activities but no significant correlation was observed with DPPH. In the case of *M. calabura*, when total flavonoid content was correlated with the antioxidant activities, showed significant correlation with FRAP and metal chelating activities but was not significantly correlated with DPPH. Such negative correlation of flavonoids with antioxidant activity were also observed by Liu et al. (2008), who reported that flavonoids could be related to other antioxidant compounds contained in the fruits.

Conclusion

Based on these studies, it was concluded that various fruit extracts of *A. altilis, A. bilimbi* and *M. calabura* exhibited a wide range of total phenolic, total flavonoid content and antioxidant activity making them valuable sources of natural antioxidants, both for preparation of crude extracts and for further isolation and purification of antioxidant components. Thus, the study has brought to light the potential antioxidant capacity of these underused fruits and their consumption which may contribute substantial amounts of antioxidants to the diet.

ACKNOWLEDGEMENTS

Authors are grateful to the University Grant commission for financial support and Department of Botany, Shivaji University Kolhapur, for laboratory facilities provided to carry out this study. We are also indebted to Mr. Niwas Desai for his help in statistical analysis.

REFERENCES

Aquino R, Morelli S, Lauro MR, Abdo S, Saija A, Tomaino A (2001). Phenolic constituents and antioxidant activity of an extract of *Anthurium versicolor* leaves. J. Natl. Prod., 64: 1019-1023.

Blois MS (1958). "Antioxidant determinations by the use of a stable free radical," Nature, 4617(181): 1199-1200.

Cao G, Sofic E, Prior RL (1997). Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. Free Rad. Biol. Med., 22: 749-760.

Hsu CL, Chen W, Weng YM, Tseng CY (2004). Chemical

composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chem., 83: 85-92.
Huang D., Boxin OU, Prior RL (2005). The chemistry behind antioxidant capacity assays. J. Agri. Food Chem., 53(6): 1841-1856.
Krishnaswamy K (1996). Indian functional food: role in prevention of cancer. Nutri. Rev., 54: S127–S131.
Lampe JW (1999). Health effects of vegetables and fruits: Assessing mechanisms of action in human experimental studies. Am. J. Clin. Nutr., 70: 475S-490S.
Liu HY, Qiu NX, Ding HH, Yao RQ (2008). Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res. Int., 41(4): 363-370.
Luximon-Ramma A, Bahorun T, Soobrattee MA, Aruoma OI (2002). Antioxidant activities of phenolic, Proanthocyanidin and flavonoid components in extracts of *Cassia fistula*. J. Agric. Food Chem., 50: 5042-5047.
Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem., 91: 571-577.
Pulido R, Bravo L, Saura-Calixto F (2000). Antioxidant of dietary polyphenols as determined by a modified Ferric Reducing Antioxidant Power assay. J. Agric. Food. Chem., 46: 3396-3402.
Rice-Evans CA, Miller NJ, Bolwell, PG, Gramley PM, Pradham JB (1995). The relative antioxidant activities of plant derived polyphenolic flavonoids. Free. Radical. Res., 22: 375-383.
Singh N, Rajini PS (2004). “Free radical scavenging activity of an aqueous extract of potato peel,” Food Chem., 85(4): 611-616.
Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. J Agric Food Chem., 51: 609-614.
Wong SP, Leong LP, Koh JHW (2006). Antioxidant activities of aqueous extracts of selected plants. Food Chem., 99(4): 775-783.
Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa K (2000). Antioxidative and antiglycation activity of garcinol from *Garcinia indica* fruit rind. J. Agr. Food Chem., 48: 180-185.