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Screening of three wild edible fruits for their antioxidant potential

Sujata R. Valvi, V.S. Rathod and D. P. Yesane

Department of Botany, Shivaji University, Kolhapur-416004 (M.S.), India

KEYWORDS

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CORRESPONDENCE

Sujata Valvi, Department of Botany, Shivaji University, Kolhapur-416004 (M.S.), India

E-mail: sujata.valvi@gmail.com

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Gadgile D.P.

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ABSTRACT

The antioxidant properties of three wild edible fruits, viz. *Mimusops elengi* L.Sp., (Sapotaceae), *Cipadessa baccifera* (Roth) Miq. (Meliaceae), *Bridelia scandens* (Roxb.) willd.(Euphorbiaceae) were determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical-scavenging activity, ferric reducing antioxidant property (FRAP), reducing power ability and chelating activity on ferrous ions .The solvent systems used were Acetone, ethanol, methanol and 100% distill water. The different levels of antioxidant activities were found in the solvent systems used.

Introduction

Antioxidants are the substance that reduce oxidation and so counteract the reactive species. Reactive oxygen species (ROS) are major free radicals generated in many redox processes, which may induce oxidative damage to biomolecules, including carbohydrates, proteins, lipids, and DNA. Reactive oxygen species affect living cells, which mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers, and other degenerative diseases (McDermott, 2000). The action of ROS is opposed by a balanced system of antioxidant compounds produced in vivo (Halliwell and Gutteridge, 1999). Endogenous antioxidants are insufficient, and dietary antioxidants are required to countermeasure excess ROS (Lim and Murtijaya, 2007).

Fruits can be rich sources of various vitamins, minerals and fibers required by human body for optimal health. Epidemiological studies have shown that high fruit intake can be associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer one possible mechanism is attributed to the antioxidant activity (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 classic antioxidants are vitamin C, E and ß-carotene. Other antioxidants include phenolic compounds which have been identified as important antioxidants found in fruits. Some of these compounds have been shown to have even more antioxidant activity than vitamin C and E in vitro and significant bioavailability has been demonstrated by animal and human studies (Bravo, 1998; Rice-Su et al., 2003, Ader et al., 2000; Cao et al., 1998).

Among the physiological function, the antioxidant or radical scavenging property is especially important because of its potential to provide health protection against reactive oxygen species and free radicals, which have been implicated in more than 100 diseases (Halliwell, 1992). Therefore in present investigation we have selected three wild edible fruits viz, *Mimusops elengi, Cipadessa baccifera, Bridelia scandens.* Thus the results from this preliminary study will provide a better understanding of the antioxidant properties of these fruits.

Materials and Methods

Plant material

Fresh and healthy fruits of the selected species viz. *Mimusops elengi* L.Sp., (Sapotaceae), *Cipadessa baccifera* (Roth) Miq. (Meliaceae), *Bridelia scandens* (Roxb.) willd. (Euphorbiaceae) were collected from various localities of Kolhapur district.

Chemicals and reagents

All chemicals and reagents used in the study were of analytical grade. Ferric chloride, 2,4,6-Tris (1-pyridyl)-5-triazine (TPTZ), and 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Glacial acetic acid, Potassium ferricyanide, Trichloro acetic acid, Ferrous chloride, Ferrozine and solvents like methanol, ethanol, and acetone.

Extraction

First, fruits were washed with clean sterile water. After that, 1 g of fruits were diced into small cubes and blended for 3 min. and then extracted with 10 ml of organic solvent. The fruit extracts were then filtered using a clean muslin cloth and centrifuged at 10,000 rpm for 15min. Three different solvent extraction systems were used methanol, ethanol and acetone at 70% concentrations in distilled water and 100% distilled water (H_2O).

DPPH free radical-scavenging assay

The antioxidant capacity of the fruit extracts was also studied through the evaluation of the free radical-scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by Aquino *et al.* (2001). An aliquot (25µl) of fruit extract was mixed with 3ml of 25mM DPPH ethanolic solution, the reaction mixture was left in

the dark at room temperature for 20mins.The absorbance was measured later, at 515 nm, against a blank of ethanol without DPPH. Results were expressed as percentage of inhibition of the DPPH radical. The Percentage of inhibition of the DPPH radical was calculated according to the following equation:

% inhibition of DPPH = 1- (Abs sample /Abs control) × 100.

Where Abs of control is the absorbance of DPPH solution without extracts.

Ferric reducing/antioxidant power assay (frap assay)

FRAP assay was performed according to a modified method described by Pulido *et al.* (2000). Briefly, a 90µl aliquot of properly diluted fruit extract was mixed with 2.7 ml of FRAP reagent. Then, the reaction mixture was incubated at 37°C for 15 min. After that, the absorbance was determined at 593 nm against a blank that was prepared using distilled water. FRAP reagent should be pre-warmed at 37°C and should always be freshly prepared by mixing 5 ml of a 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with 5 ml of 20 mM $FeCl_3$. $6H_2O$ and 50 ml of 0.3 M acetate buffer, pH 3.6. A calibration curve was prepared, using an aqueous solution of ascorbic acid (100 µM 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.

Reducing power assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986). The extract (0.5 mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 1mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 rpm for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) and incubated for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

Metal chelating activity assay

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis et al. (1994). 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 sec, 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 chelating activity of the extract for Fe^{2+} was calculated as

Chelating rate (%) = (Abs control – Abs sample) / Abs control × 100.

Where Abs control is the blank,without extract.

Results and Discussion

There are a huge varieties of antioxidants contained in fruits. Therefore, measuring the antioxidant capacity of each compound separately becomes very difficult. Several methods have been developed to estimate the antioxidant capacity of different plant materials (Guo et al., 2003). Usually, those methods measure the ability of antioxidants, in a particular plant material, to scavenge specific radicals, by inhibiting lipid peroxidation or chelating metal ions. In this study, four different methods have been used to evaluate the antioxidant capacity of the extracts of the three fruit extracts; they are DPPH free radical-scavenging, ferric reducing/antioxidant power assay (FRAP assay), Reducing power assay and Metal chelating activity-assay.

Table No. 1. Antioxidant capacity of fruits extracts obtained from different solvent extraction systems using DPPH and FRAP assay

Solvent	*Cipadessa baccifera*		*Bridelia scandens*		*Mimusops elengi*	
	(%) DPPH inhibition	FRAP (µM AAE/ g fresh weight)	(%) DPPH inhibition	FRAP(µM AAE/ g fresh weight)	(%) DPPH inhibition	FRAP(µM AAE/ g fresh weight)
Aqueous	40.21%	9873.56±0.351	58.86%	19102.67±0.152	57.00%	19303.53±0.404
Methanol	44.24%	14181.03±0.702	51.72%	2118.4±0.1	62.41%	25634.57±0.416
Ethanol	53.19%	9381.26±0.208	55.71%	2956.73±0.152	53.86%	25851.5±0.308
Acetone	51.20%	12234.63±0.251	57.43%	5834.43±0.305	85.29%	65221.6±0.3

Data are presented as the mean □□SD of each triplicate test

Table No. 2. Antioxidant capacity of fruits extracts obtained from different solvent extraction systems using Reducing power and Metal chelating assay

Solvent	*Cipadessa baccifera*		*Bridelia scandens*		*Mimusops elengi*	
	Reducing Power	Metal Chelating (%)	Reducing Power	Metal Chelating (%)	Reducing Power	Metal chelating (%)
Aqueous	0.085±0.002	48.45%	0.034±0.003	19.92%	0.115±0.002	61.78%
Methanol	0.123±0.003	43.21%	0.166±0.000	27.87%	0.156±0.002	60.62%
Ethanol	0.054±0.001	55.59%	0.155±0.003	47.19%	0.228±0.001	64.25%
Acetone	0.087±0.002	42.38%	0.192±0.001	35.82%	0.286±0.002	67.88%

Data are presented as the mean □□SD of each triplicate test

The assay of the scavenging of DPPH radical is widely used to evaluate the antioxidant capacity of extracts from different plant materials. Unlike other free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition (Amarowicz et al., 2004). The essence of DPPH assay is that the antioxidant react with the stable free radical 1,1-Diphenyl-2-picrylhydrazyl (deep violet

color) and converts it to 1,1-Diphenyl-2-picrylhydrazine with a yellow color. The degree of discoloration indicates the scavenging potential of the sample antioxidant (Tianpech et al., 2008) resulting in a decrease in absorbance at 517nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. In present study, the fruits analyzed were able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decrease in absorbance. The DPPH free radical scavenging activity of the plant extracts are shown in table 1 *Mimusops elengi* showed highest DPPH free radical scavenging activity followed by *Bridelia scandens and Cipadessa bacccifera.*

Alothman et.al (2009) were studied the antioxidant capacity and phenolis content of selected tropical fruits from Malaysia, using different solvent system. Total three fruits were studied viz. *Ananas comosus, Musa paradasiaca, Psidium guajava.* The DPPH results, obtained by them(12.7 - 93.7 %) were somewhat similar to the results obtained in present study (40.21-85.29%). Peteros and Uy (2010) studied the antioxidant screening of four medicinal plants, using methanolic extract of different concentration. The values of DPPH (10.8-98.3%) were somewhat similar to the present work (40.21-85.29%). Oki, et. al (2006) worked radical scavenging activity of eight cultivar of mulberry fruits at different stage, the DPPH results (0.27-29.56%) obtained by them were lesser than the present studied fruits(40.21-85.29%). Yilmaz et al (2009) worked on phytochemical analysis of nine cultivated and sixteen wild blackberry fruits. The results of DPPH (5.8- 8.58 EC_{50} in blackberry cultivar and 6.4 - 9.8 EC_{50} in blackberry genotype) were lesser than present studied fruits (40.21-85.29%).

Ferric Reducing Antioxidant Power (FRAP) is a simple inexpensive assay and may offer putative index of antioxidant activity. Principally, FRAP assay treats the antioxidants in the sample as reductant in a redoxlinked colorimetric reaction (Huang et al., 2005). The FRAP assay measures the reducing potential of antioxidant to react on ferric tripyridyltriazine (Fe 3+ -TPTZ) complex and produce blue color of ferrous form which can be detected at absorbance 593 nm (Benzie and Strain, 1996). *Mimusops elengi* showed relatively strong ferric ion reducing activities, followed by *Cipadessa bacccifera and Bridelia scandens.* The FRAP results obtained by Alothman et.al (2009) were lesser (0.59 $\pm$ 0.15 - 31.9 $\pm$ 0.95µ mol Fe (II)g FW) than present studied fruits (9381.26 $\pm$ 0.208 – 65221.6 $\pm$ 0.3µ mol AAE/g FW). Wong et al (2005) worked on the antioxidant activities of the twenty five tropical edible plants, the FRAP activity analyzed by them (25-300 mol trolox/g) were lesser than present studied fruits (9381.26 $\pm$ 0.208 – 65221.6 $\pm$ 0.3µ mol AAE/g FW). Jablonska, et al (2009) worked on the seven wild fruits for their antioxidant capacity, the somewhat similar (10.75 $\pm$ 0.07 – 12.778 $\pm$ 1.85 m Mol Fe/100g) results were found in present study (9381.26 $\pm$ 0.208 – 65221.6 $\pm$ 0.3µ mol AAE/g FW).

Reducing power assay measures the electron-donating capacity of an antioxidant (Yen, 1995). In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+} /ferricyanide complex used in this method to the ferrous form may serve as a significant indicator of its antioxidant capacity (Yildirim et al., 2000). The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom (Singh and Rajini, 2004). The reduction of the Fe^{3+} /ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution. Absorbance of Fe^{2+} can be measured at 700 nm (Zou et al., 2004). Table 2 shows the reductive effect of three wild edible fruits. The result shows that *Mimusops elengi* shows highest reducing power than *Bridelia scandens and Cipadessa bacccifera.* The reducing power activity was studied by Hazra et al. (2008) in *Spondias pinnata.* The reported values of reducing power (0.2 – 0.4 mg/ml) were higher than the present study (0.034-0.286). Li et al. (2010) analyzed the comparative study of antioxidant activity of *Crataegus pinnatifida var.Typica schneider* and *C.pinnatifida.* The results of reducing power obtained by these authors were higher (0.29 – 0.55) than values obtained in present study (0.034 -0.286).

Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion (Hsu et al., 2003). The iron- chelating capacity measures the ability of antioxidants to compete with ferrozine in chelating ferrous ion (Elmastas et al., 2006). In this assay ferrozine can make complexes with ferrous ions and in the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Measurement of color reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals (Aboul-Enein et al., 2003). The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. The ability to chelate ferrous ions gives an indication whether compounds found in a particular extract contain potential secondary antioxidants. Table no.2 shows the metal chelating activity of studied fruits. *Mimusops elengi* shows highest activity than *Cipadessa bacccifera and Bridelia scandens.* Metal chelating activity reported by Hazra et al. (2008), were somewhat similar (47-100%) to the present studied fruits (19.92-67.88%).

The antioxidant capacities of the fruit extracts tested varied. Among all the plants tested *Mimusops* extracts exhibited high FRAP, DPPH, Reducing power and Metal chelating activities which can be interpreted as the highest antioxidant capacity among the three fruits studied. These differences may be due to their different antioxidant mechanisms.

Effect of solvent system

The solvent extraction has been widely used to extract bioactive components from plants. Solvent extraction is a process designed to separate soluble antioxidant compounds by diffusion from a solid matrix (plant tissue) using a liquid matrix (solvent). The commonly used solvents for extracting antioxidants were methanol, ethanol, and acetone either singly or in combination with aqueous (Lim et al., 2007; Thaipong et al., 2006; Tachakittirungrod et al., 2007; Kahkonen et al., 1999; Velioglu et al., 1998; Zielinski and Kozlowska, 2000). The polarities of the different organic solvent greatly influence the selection of a specific solvent for the extraction of a specific group of bioactive compounds. Acetone–water mixtures are good solvent systems for the extraction of polar antioxidants (Luximon-Ramma et al., 2003).

Mimusops elengi showed the highest DPPH scavenging activity in methanol (62.41%) and acetone extract (85.29%).while ethanolic extract (55.71%) and aqueous extract (58.86%) of *Bridelia scandens* showed the highest DPPH activity. Among these fruits *Cipadessa baccifera* shows least DPPH activity. The highest ferric reducing capacity was found for methanolic extract in *M. elengi* (65221.6±0.3), followed by methanolic (25634.57±0.41) and aqueous extract (19303.53±0.40), while in ethanolic extract, FRAP activity was higher in *Cipadessa baccifera* (9381.26$\pm$0.208). In reducing power assay methanol showed highest reducing capability for *A.altilis* (0.123±0.003) while Acetone has greater reducing power in *B.scandens* and *M.elengi* (0.192±0.001& 0.286±0.002). The metal chelating activity were higher in *Mimusops elengi* fruits (acetone (67.88%), aqueous (61.78%), methanol (60.62%), ethanol (64.25%)The ethanol extract of *C.baccifera* (55.59%) and *B. scandens* (47.19%) showed the higher metal chelating activity. From these results, the efficiency of solvents to extract the antioxidant compounds differ among different fruits and among different assays performed. Therefore it is very hard to develop a standards extraction solvent suitable for the extraction of all plant antioxidant compounds. This allows more scope in the choice of solvent to be used in an extraction process possibly leading to an economic process and improved environmental, health, and safety considerations.

Conclusion

Relative antioxidant activities of Mimusops elengi, Cipadessa baccifera, Bridelia scandens were determined. Based on these studies it is concluded that the antioxidant activity of any extract varies with its ability to react with the biologically harmful free radicals. Among all these fruits, Mimusops elengi showed the highest antioxidant capacity. From our research we found that acetone is the better solvent extraction system than other systems used.

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