Antioxidant activity of selected lesser known edible fruits from Western Ghats of India

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Six species of lesser known edible fruits from Western Ghats of India were analysed for their anthocyanin, ascorbic acid, total phenolics and flavonoid levels and their antioxidant activities using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The data obtained shown the anthocyanin, ascorbic acid, total phenolics and flavonoid levels were significantly higher in the methanol extract of *Mahonia leschenaultii* (Wall. ex Wight & Arn.) Takeda fruits. *Gaultheria fragrantissima* Wall. and *Rubus ellipticus* Sm. fruits were also found to have significant amount of phytochemicals analyzed. *M. leschenaultii* fruits had highest antioxidant activity when compared to all other fruit extracts. There was a strong correlation between the content of phytochemicals and antioxidant activities in all fruit extracts in the following decreasing order: *M. leschenaultii* > *G. fragrantissima* > *R. ellipticus* > *Grewia tiliaefolia* Vahl > *Ziziphus rugosa* Lam.> *Flueggea leucopyrus* Willd.

**Keywords**: Anthocyanin, Antioxidant, Ascorbic acid, Flavonoids, Edible fruits, Phenolics, Western Ghats.

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**Introduction**

Wild edible plants contribute significantly to the nutrition of inhabitants of rural areas of Western Ghats. Although these foods are consumed by people throughout the year in fresh and dried forms, reliance on these foods increases during periods of cereal shortages. Now-a-days, however, a nutritional transition is occurring in the poorest countries of the world resulting in the replacement of traditional plant-based diets that are rich in fruits and vegetables with diets that are rich in calories provided by animal fats and sugars, and low in complex carbohydrates. Wild edible foods include fruits, leaves, flowers and seeds from spontaneous trees and shrubs. Of these, fruits are receiving increased interest from researchers working on wild edible plant species because of their nutritional value, vitamin and mineral contents. These fruits are being investigated as potential food supplements in the Western Ghats region so as to increase quality of daily food for the rural population.

Currently, increasing attention has been paid by consumers to the lesser known fruits which have unusual flavours and qualities, and many of which are rich in antioxidants and anthocyanins. Detailed information about the health promoting components of lesser known fruit species could lead to a better understanding of the beneficial effects and an increased consumption of these fruits, including their utilization in functional foods and as ingredients in nutraceuticals, medicine and pharmaceuticals.

Research on wild fruits and other wild edible plants is also intended to promote the preservation of these species, presently under threat by human activities. In addition to their nutritional value, the preservation of these fruits also has economical advantages, as there is a significant trade with some of these wild edible and medicinal fruits. Any scientific evidence for the health benefits of such wild fruits in addition to their nutritional value would be a value addition to the plants producing such fruits. Concerning their medicinal properties, the most commonly studied benefit is their antioxidant effects. Antioxidants play a crucial role in the prevention of chronic ailments such as heart disease, cancer, diabetes, hypertension, stroke and Alzheimer’s disease by combating oxidative stress.

Numerous studies have shown that fruit and vegetables are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant properties. In addition to vitamin C, a great number of other phenolics (especially the flavonoids) have strong antioxidant activity in vitro. In fact, the vast majority of the activity seen in various fruit juice

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samples is associated with molecules other than vitamin C. Anthocyanins are a group of widespread natural phenol compounds in plants. As a major sub-group, flavonoids and anthocyanins are water-soluble plant pigments responsible for the blue, purple and red colour of many plant tissues. Anthocyanins have been reported to be strong antioxidants and may exert a wide range of health benefits through antioxidant or other mechanisms. The potential for these compounds to act as antioxidants in vivo is dependent upon their bioavailability, an area currently receiving much attention.

Dietary supplements such as vitamin C have become popular for their perceived ability to enhance the body’s antioxidant defenses. Reactive oxygen species (ROS) have been shown to cause a broad spectrum of damage to biological systems. Scavenging of ROS is part of a healthy, well-balanced, antioxidant defense system. A high scavenging activity of berry extracts towards chemically generated ROS has been described in several studies. In this study, the content of anthocyanins, ascorbic acid, phenolics and flavonoids and the antiradical activity of selected lesser known edible fruit species were evaluated.

Materials and Methods

Fruit samples

Fresh fruits samples of *Flueggea leucopyrus* Willdl. (Euphorbiaceae), *Grewia tiliaefolia* Vahl (Tiliaceae), *Mahonia leschenaultii* (Wall. ex Wight & Arn.) Takeda (Berberidaceae), *Gaultheria fragrantissima* Wall. (Ericaceae), *Ziziphus rugosa* Lam. (Rhamnaceae) and *Rubus ellipticus* Sm. (Rosaceae) were collected (Plate 1) from Palni hills of Western Ghats during the year 2008-2009. Fruits were botanically identified with the help of local Flora and the herbarium specimens were compared with authentic sheets deposited in the Botanical Survey of India, Southern Circle, Coimbatore, India. The fresh fruits were washed with running tap water and kept at 20 ± 2°C until extraction.

Sample extraction

For each fruit sample, extraction was performed using 70% aqueous methanol. Crushed fruits were homogenized and 1 g of the homogenate was extracted three times with 10 ml of solvent by shaking vigorously for 10 min. The samples with solvent were centrifuged (4000 rpm for 15 min in a bench centrifuge). The three supernatants collected after centrifugation were combined and filtered through a Whatman no.1 filter paper, after which the filtrate was evaporated to dryness at 40°C under vacuum. The extracted phenolics were dissolved in methanol water (4:1, 10 ml).

Determination of anthocyanin content

Total anthocyanins were estimated by a pH-differential method. Two dilutions of fruit extracts were prepared, one with potassium chloride buffer (pH 1.0) (1.86g KCl in 1 litre of distilled water, pH value adjusted to 1.0 with concentrated HCl), and the other with sodium acetate buffer (pH 4.5) (54.43 g CH₃CO₂Na·3H₂O in 1 litre of distilled water, pH value adjusted to 4.5 with concentrated HCl), diluting each by the previously determined dilution factor [fruits extracts 1:5 (v/v)]. Absorbance was measured simultaneously at 510 and 700 nm after 15 min incubation at room temperature. The content of total anthocyanins was expressed in mg of cyanidin-3-glucoside equivalents (CGE) per 100g of berries using a molar extinction coefficient of cyanidin-3-glucoside and molar weight (MW) (449.2 g/mol).

Determination of ascorbic acid

The reduction properties of ascorbic acid are used in the measurement. A basic solution is made from the chopped fruit sample with phosphoric acid, which is filtered. Then Fe³⁺ ions are added, which are reduced to Fe²⁺ ions by ascorbic acid. Thereinafter dipridyl reagent is added to the Fe²⁺ ions, which results a red complex. Commercial ascorbic acid was used as standard for the comparison. The content of this complex is measured by spectrophotometer (a method by Paulovicsova et al).

Determination of total phenolics

The total phenolics of each fruit extract were determined by the Folin-Ciocalteu method. The diluted aqueous solution of each extract (0.5 ml) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 ml). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g/l in water, 2 ml) was added. After 2 h of incubation, the absorbance was measured at 760 nm against water blank. A standard calibration curve was plotted using gallic acid (0-200 mg/l). The results were expressed as mg of gallic acid equivalents (GAE)/100 g of fruit weight.

Determination of total flavonoids

The total flavonoids were estimated according to Arvouet-Grand et al. A diluted methanolic solution
(2 ml) of each fruit extract was mixed with a solution (2 ml) of aluminium trichloride (AlCl₃) in methanol (2 %). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a methanol (2 ml) and plant extract (2 ml) without AlCl₃. Quercetin was used as reference compound to produce the standard curve, and the results were expressed as mg of quercetin equivalents (QE)/100 g of fruit weight.

**DPPH radical scavenging activity**

The hydrogen donating ability of extracts was examined in the presence of DPPH (2,2-diphenyl-1-
picrylhydrazyl) stable radical\textsuperscript{16}. One milliliter of 0.3 mM DPPH ethanol solution was added to 2.5 ml sample of different fruit extracts and allowed to react at room temperature. After 30 min, the absorbance values were measured at 517 nm. Methanol extract of each sample (1.0 mL) with water (2.5 ml) was used as a blank for respective samples. DPPH solution (1.0 ml, 0.3 mM) with methanol (2.5 ml) served as negative control. The antioxidant content was determined using a standard curve of ascorbic acid (0-10 \( \mu \)g/ml). The results were expressed as mg of ascorbic acid equivalent antioxidant content (AEAC) per 100 g of fruit weight.

**Statistical analysis**

All data presented are means of six determinations along with standard deviations. Statistical analysis used the MS Excel software (CORREL Statistical function) to calculate quercetin, ascorbic acid and gallic acid equivalents, to determine inhibition percentage and to establish linear regression equations. The SigmaStat2.0 was used to determine correlation coefficients (R).

**Results and Discussion**

Present study established the determination of anthocyanin, ascorbic acid, total phenolics and flavoid level of six lesser known wild edible fruits from Western Ghats (Table 1). \textit{M. leschenaultii} fruits showed highest level of anthocyanin, ascorbic acid, total phenolics and flavonoids in methanol extracts. The reduction of all four phytochemicals analysed were in the order: \textit{M. leschenaultii} \( > \) \textit{G. fragrantissima} \( > \) \textit{R. ellipticus} \( > \) \textit{G. tiliaeolia} \( > \) \textit{Z. rugosa} \( > \) \textit{F. leucopyrus}. Highest antioxidant activity was established for \textit{M. leschenaultii} fruits (361.2 \( \pm \) 3.69 AAE/g). The antioxidant activities of other fruit extracts decreased almost in the order of phytochemical contents (Table 1). A high degree of positive correlation between the phytochemical contents analyzed and the antioxidant activity was established \((R = 0.962)\) in all fruit extracts. Highest phytochemical contents corresponded to highest antioxidant activity. A correlation between the phenolics and flavonoid content and the antioxidant activity has been established for many herbs\textsuperscript{17,18}. A direct correlation with total polyphenol content has been established in surveys of commercial fruits analyzed for antioxidant potential\textsuperscript{19}.

Different \textit{Rubus} species were recognized as berries with high antioxidant activity\textsuperscript{20-22}, for that reason \textit{R. ellipticus} was chosen as a species to which the antioxidant activity and the polyphenol content of the other fruits was compared. There are data about a moderate antioxidant activity of \textit{R. ellipticus} and high antioxidant activity of \textit{M. leschenaultii} fresh fruits, while data about the antioxidant properties of \textit{G. tiliaeolia}, \textit{Z. rugosa} and \textit{F. leucopyrus} are limited.

Our study demonstrates highest antioxidant activity for two fruit species of \textit{M. leschenaultii} and \textit{G. fragrantissima}, which are 2.5 times higher than the antioxidant activity of the \textit{Z. rugosa} and \textit{F. leucopyrus} extracts. Medium antioxidant activity of fruits extracts was observed from \textit{R. ellipticus} and \textit{G. tiliaeolia}. Different antioxidant and radical scavenging activity may partly be due to wide variety of antioxidant constituents such as anthocyanins, vitamins, phenolics, flavonoids and carotenoids. Also types of antioxidants, inhibitors of free radicals which initiate oxidation and inhibitors of free radical chain propagation reactions are known. Different mechanisms of action and kinetics of the inhibitory effect of these antioxidants using different procedures resulted in the discrepancy of these findings\textsuperscript{23}. Owing to the complexity of the antioxidant materials and their mechanism of actions, it is obvious that no single testing method is capable of providing a

<table>
<thead>
<tr>
<th>Fruit species</th>
<th>Anthocyanin (CGE/100g)</th>
<th>Ascorbic acid (AAE/100g)</th>
<th>Total phenolics (GAE/100g)</th>
<th>Total flavonoids (QE/100g)</th>
<th>Antioxidant activity (DPPH (( \mu )g/ml))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flueggea leucopyrus</td>
<td>0.34 ( \pm ) 0.09</td>
<td>39.4 ( \pm ) 4.93</td>
<td>31.7 ( \pm ) 4.92</td>
<td>42.7 ( \pm ) 0.46</td>
<td>76.8 ( \pm ) 1.22\textsuperscript{c}</td>
</tr>
<tr>
<td>Gaultheria fragrantissima</td>
<td>2.47 ( \pm ) 0.07</td>
<td>67.6 ( \pm ) 8.41</td>
<td>80.4 ( \pm ) 3.18</td>
<td>94.3 ( \pm ) 1.35</td>
<td>240.2 ( \pm ) 1.28\textsuperscript{b}</td>
</tr>
<tr>
<td>Grewia tiliaeolia</td>
<td>2.60 ( \pm ) 0.09</td>
<td>70.5 ( \pm ) 3.07</td>
<td>44.1 ( \pm ) 1.81</td>
<td>47.1 ( \pm ) 0.92</td>
<td>120.2 ( \pm ) 1.86\textsuperscript{d}</td>
</tr>
<tr>
<td>Mahonia leschenaultii</td>
<td>8.58 ( \pm ) 0.02</td>
<td>69.9 ( \pm ) 9.76</td>
<td>86.8 ( \pm ) 0.30</td>
<td>95.5 ( \pm ) 1.76</td>
<td>361.2 ( \pm ) 3.69\textsuperscript{a}</td>
</tr>
<tr>
<td>Rubus ellipticus</td>
<td>1.71 ( \pm ) 0.08</td>
<td>44.0 ( \pm ) 4.95</td>
<td>72.0 ( \pm ) 1.25</td>
<td>86.4 ( \pm ) 2.04</td>
<td>196.4 ( \pm ) 1.84\textsuperscript{c}</td>
</tr>
<tr>
<td>Ziziphus rugosa</td>
<td>0.34 ( \pm ) 0.02</td>
<td>35.0 ( \pm ) 3.21</td>
<td>41.8 ( \pm ) 0.20</td>
<td>41.8 ( \pm ) 1.20</td>
<td>116.9 ( \pm ) 1.02\textsuperscript{d}</td>
</tr>
</tbody>
</table>

(Results are mean \( \pm \) SD; n=6).

*Values with the same letter are not significantly different \((P > 0.05)\).*
comprehensive picture of the antioxidant profile of a studied samples and a combination of different methods is necessary. Despite such limitations, DPPH method can be helpful for primary screening and finding of novel antioxidants. The present study suggested that the tested lesser known wild edible fruits have moderate to potent antioxidant activity. More detailed studies on chemical composition of the fruit extracts, as well as other in vivo assays are essential to characterize them as biological antioxidants. It should also be kept in mind that antioxidant activity measured by in vitro methods may not reflect in vivo effects of antioxidants. Many other factors such as absorption/metabolism are also important.

Conclusion
The study confirmed a high correlation between phytochemical contents in wild fruits and antioxidant activity. The findings of this study support this view that some wild fruits are promising sources of potential antioxidants and may be efficient as preventive agents in some diseases. The providing data can enrich the existing comprehensive data of antioxidant activity of wild fruits and its biological value.

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