Research Article

Antioxidant Activity of Jambhul, Wood Apple, Ambadi and Ambat Chukka: An Indigenous Lesser Known Fruits and Vegetables of India

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Abstract: Indigenous fruits such as jambhul, wood apple and vegetables viz., ambadi, ambat chukka were extracted with methanol and analyzed for Total Phenolic Content (TPC), total flavonoid content, ascorbic acid, anthocynin and antioxidant capacity such as ABTS, DPPH and FRAP assay. Total flavonoid content and total monomeric anthocynin were found only in jambhul i.e., 227.38 mg/g CE/g DW, 27.40 mg/g as cyn838glucoside as equivalent DW respectively. Increased TPC was observed in the order: jambhul>ambadi>wood apple>ambat chukka. Higher ascorbic acid was observed in order of: jambhul>ambadi>ambat chukka>wood apple. The antioxidant potential of above fruits has been rated in the order: jambhul>wood apple>ambadi>ambat chukka by DPPH and FRAP assay and for ABTS assay Jambhul>ambadi>wood apple>ambat chukka.

Keywords: Anthocynin, antioxidant capacity, ascorbic acid, flavonoids, phenolics

INTRODUCTION

Jambhul (Syzygium cumini L) is an indigenous minor fruit of India. It is especially available in summer season. Jambhul fruit is universally accepted to be very good source for medicinal purpose especially for curing diabetes because of its beneficial effect on pancreas. Jambhul fruit and seeds are sweet, acrid and sour. The fruit and seed contain glucoside jamboline and ellagic acids which are reported to have the ability to convert starch into sugar in case of excess production of sugar (Bopp et al., 2009). Jambhul has prophylactic anti-septic effect that is associated with recruitment of activated neutrophils to the infectious site and to a diminished systemic inflammatory response (Maciel et al., 2008). The other constituents of fruit are resin, albumen, gallic acid, essential oil and tannic acid. Seed is used in various alternative system of medicine like Ayurveda, Unani and Chinese system of medicine. The fruit concentrate of jambhul has medicinal importance and has a large market for the treatment of chronic diarrhea and other enteric disorders, including its uses as an antimicrobial (Migliato, 2005). It has been utilized in the preparation of juices, squash, Ready to Serve (RTS) beverages, jam and jelly.

The wood apple (Limonia acidissima) is native to and commonly found in dry plains of India and Ceylon. Wood apple is used in the preparation of chutney and for making jelly and jam (Morton, 1987). Wood apple has got high medicinal value. Every part of the fruit posse’s medicinal property. Fruits, leaves and stem bark of wood apple have been studied for anti-tumor (Saima et al., 2000) and antimicrobial activity (Rahman and Gray, 2002). Fruit pulp has anti-inflammatory, antipyretic and analgesic activity (Ahamed et al., 2008). Wood apple has anti-diabetic and antioxidant potential by reducing the level of blood glucose and malondialdehyde (Patel et al., 2012). Fruit is much used in India as a liver and cardiac tonic and when unripe, as a means of halting diarrhea and dysentery and for effective treatment for high cough, sore throat and disease of the gums. In addition to this, wood apples also have hypoglycemic activity, antitumor, larvicidal and antimicrobial activity and hepatoprotective activity (Vidhya and Narain, 2011).

Ambadi (Hibiscus sabdariffa Linn) and ambat chukka (Rumex vesicarius Linn.) are green leafy vegetables typically provides low calories, high dietary fiber and phytochemicals and micronutrients such as iron, vitamins and carotenoids (Mahadevan et al., 2009). Though these vegetables are abundantly available in Maharashtra region of India; these are under utilized for the preparation of various food products.

Jambhul, wood apple, ambadi and ambat chukka posse’s very good nutrient content and they have been known traditionally for the medicinal value however, the technical and detailed scientific study is lacking. Hence, in the present research efforts are made to study systematically the physicochemical parameters such as fat, protein and carbohydrates and biochemical parameters such as phenolics, flavonoids, anthocynin,
ascorbic acid and antioxidant activity of jambhul, wood apple and leafy green vegetables such as ambadi and ambat chukka in detail.

MATERIALS AND METHODS

Materials: Jambhul, wood apple, ambadi, ambat chukka were purchased in large quantity from local market. Theses fruits were stored at -20°C until their final usage to prevent it from damage and spoilage and to maintain uniformity in the quality throughout the entire project.

Chemicals: Folin-Ciocalteau reagent, Sodium carbonate anhydrous, Sodium hydroxide, 2,4,6-Tripyridyl-S-Triazine (TPTZ), FeCl₃, Pet ether (60-80°C) was purchased from Hi-Media, Mumbai, India. HCl, gallic acid, L-ascorbic acid, 2,6 dichloroindophenol, meta-phosphoric acid, Sodium bicarbonate, sodium acetate, potassium chloride, potassium per sulphate, glacial acetic acid were purchased from SD Fine Chemicals, Mumbai, India. 2,4-dichlorophenol-indophenol solution (containing 2, 6-dichlorophenol-indophenol and sodium bicarbonate) was added to the reaction and red color was measured at 518 nm within 10 to 15 sec. The standard curve was linear between 0 and 100 µg/mL, L-AAE. The ascorbic acid was represented as mg of L-AAE/g FW and DW of fruit.

Determination of total flavonoid content: The total flavonoid content was measured by Vanillin-HCl method as explained by Rebecca et al. (2010). Methanol extracts of phenolics (0.5 mL) from jambhul, wood apple and ambadi and ambat chukka was added with 1 mL of Vanillin-HCl reagent which was incubated in water bath for 20 min at 30°C. The absorbance was taken at 500 nm. The standard curve was linear between 0 and 250 µg/mL catechin. For flavonoid catechin was used as a standard. The flavonoids were represented as mg of CE/g FW and DW of fruit.

Determination of ascorbic acid content: The total ascorbic acid content was measured by direct colorimetric method as explained by Ranganna (1999). Initially sample was extracted with 2% of meta-phosphoric acid (1:10, w/v). This extracted sample was used in the determination of ascorbic acid. 0.5 mL of extract was dispensed into the test tube and 1 mL of dye solution (containing 2, 6-dichlorophenol-indophenol and sodium bicarbonate) was added to the reaction and red color was measured at 518 nm within 10 to 15 sec. The standard curve was linear between 0 and 100 µg/mL, L-AAE. The ascorbic acid was represented as mg of L-AAE/g FW and DW of fruit.

Total monomeric anthocynin pigment content: The samples were analyzed for its total monomeric anthocynin pigment content by pH differential method (Lee et al., 2005). Test portion was diluted with buffer of pH 1.0 and 4.5 at 520 and 700 nm until absorption within linear range of spectrophotometer which was measured within 20-50 min of preparation. Results of anthocynin pigment concentration, expressed as cyanidin-3-glucoside equivalents, were calculated and expressed as follows:

\[
\text{Anthocynin pigment (Cyanidin-3-glucoside equivalent, mg/L) } = \frac{A \times MW \times DF \times 10^3}{\varepsilon} \times \frac{1}{l}
\]

where
- \( A = (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \) pH 1.0 - (A_{520 \text{ nm}} - A_{700 \text{ nm}}) pH 4.5
- \( MW \) (Molecular Weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu)
- \( DF \) = Dilution factor established in D
- \( l \) = Path length in cm

Antioxidant capacity determined by ABTS: Antioxidant activity was measured using Hitachi Spectrophotometer using the improved ABTS methods by Re et al. (1999). The ABTS reagent was prepared freshly and used within two days. The reagent was made by mixing 7 mM ABTS and 2.45 mM potassium persulfate and incubated for 16 h at 37°C. The ABTS cations diluted with ethanol to set O.D at 0.7 (±0.02) at 734 nm (1:30, v/v). 3.9 mL (absorbance of 0.700±0.02) was added to the 0.1 mL of tasted sample and mixed thoroughly and absorbance was measured at 734 nm immediately after 6 min. The standard curve was linear between 0 and 20 µM Trolox. Results are expressed in µM Trolox Equivalents (TE) /g FW and DW.

Antioxidant capacity determined by DPPH: The ability to scavenge DPPH free radicals was determined based on the method by Sahreen et al. (2010) with little modification in the mixture of test sample concentration and DPPH concentration. (0.1 mM) of DPPH prepared in ethanol was diluted to set the absorbance below 1.2 (±0.02) at 517 nm and added to the 1 mL of test sample in test tube and it was vigorously shaken and kept for 15 min for incubation in dark room. The absorbance was measured at 517 nm. The standard curve was linear between 0 and 30 µM Trolox. Results are expressed in µM Trolox Equivalents (TE) /g FW and DW.

Antioxidant capacity determined by Ferric Reducing Antioxidant Power (FRAP): Ferric Reducing Antioxidant Power (FRAP) assay was performed by Benzie and Strain (1996) methods with slight modifications in the mixture of test sample concentration and FRAP reagent concentration. Firstly, FRAP reagent was prepared by mixing the following solutions: 10 fold 300 mM acetate buffer + 1 fold TPTZ (10 mM in 40 mM HCl) + 1 fold FeCl₃ (20 mM) which was further diluted with methanol (1:3 v/v). This diluted 3 mL of FRAP reagent was added to the 0.1 mL of sample extract which was then vigorously shaken and the absorbance was measured at 593 nm after incubation of 30 min. The standard curve was linear between 0 and 500 µM Trolox. Results were expressed in µM Trolox Equivalents (TE) /g FW and DW.

RESULTS AND DISCUSSION

Proximate composition of jambhul, wood apple, ambadi and ambat chukka: The proximate composition of jambhul, wood apple, ambadi, ambat chukka and jambhul seed is presented in the Table 1. Jambhul pulp showed protein content of 3.15% whereas jambhul seed had protein content 4.49%.

Total phenolic content in jambhul, wood apple, ambadi and ambat chukka: Total Phenol Content (TPC) was determined in comparison with standard gallic acid and the results are expressed in terms of mg GAE/g dry sample. From Table 2 it can be seen that Total Phenolic Content (TPC) of fruits of jambhul and wood apple was 83.97, 38.67 mg (GAE) /g DW respectively. These amounts were comparable with results shown by Benherlal and Arumughan (2007) who also observed TPC of jambhul pulp i.e., 39 mg (GAE) /g of FW. Further these results are in accordance with the results of Veigas et al. (2007) who also observed TPC in jambhul i.e., 560 mg GAE/100 mL with including anthocynin. Wood apple showed 38.61 mg (GAE) /g DW sample respectively TPC content. Jambhul showed highest phenolic content followed by ambadi, wood apple and ambat chukka. This difference could be because variation in the assay procedures for solvent extractions and variety of jambhul and wood apple used by the authors. Whereas green leafy vegetables such as ambadi and ambat chukka showed a phenolic content of 46.85 and 20.98 mg (GAE) /g DW sample respectively. There are no reports available as per our knowledge on ambadi and ambat chukka phenolic content.

Further efforts were made to analyze the waste portion of jambhul and wood apple i.e., jambhul seed and wood apple skin. Highest total phenolics of 78.29 mg Gallic Acid Equivalents (GAE) /g DW were observed for jambhul seed whereas wood apple skin showed TPC of 2.34 mg Gallic Acid Equivalents (GAE) /g DW. According to the findings of Arun et al. (2011) phenolic content in jambhul seed observed was 471.67 mg Gallic Acid Equivalents (GAE) /g. Thus these waste portions can also be considered for utilization in value added nutraceutical foods.

The total phenolic content in jambhul, wood apple, ambadi, ambat chukka, jambhul seed and wood apple skin were different. Fu et al. (2010) observed that differences in total phenolic content in different fruits and vegetables are due to some non-phenolic reducing compounds, such as organic acids and sugars, which interferes the determination of total phenolic contents.
Table 1: Proximate composition of jambhul (pulp and seed), wood apple, ambadi and ambat chukka leaves (on dry weight basis)

<table>
<thead>
<tr>
<th>Composition</th>
<th>Jambhul pulp (%)</th>
<th>Jambhul seed (%)</th>
<th>Wood apple pulp (%)</th>
<th>Ambadi leaves (%)</th>
<th>Ambat chukka leaves (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>86.19±0.14</td>
<td>57.33±0.14</td>
<td>73.97±2.70</td>
<td>88.27±0.32</td>
<td>90.60±0.75</td>
</tr>
<tr>
<td>Fat</td>
<td>2.00±0.12</td>
<td>4.54±0.24</td>
<td>12.33±0.58</td>
<td>0.50±0.03</td>
<td>0.40±0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>3.15±0.18</td>
<td>4.49±0.10</td>
<td>10.43±0.25</td>
<td>36.24±1.54</td>
<td>24.43±1.54</td>
</tr>
<tr>
<td>Ash</td>
<td>2.13±0.11</td>
<td>1.47±0.08</td>
<td>2.66±0.11</td>
<td>8.33±0.58</td>
<td>13.29±0.62</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>92.72±4.12</td>
<td>89.50±3.95</td>
<td>74.58±3.35</td>
<td>54.93±1.78</td>
<td>61.88±2.74</td>
</tr>
</tbody>
</table>

*: All the values are Mean±S.D. of three determinations

Table 2: Content of total phenolics, flavonoids, anthocynin content and ascorbic acid in jambhul, wood apple, ambadi and ambat chukka leaves

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC (mg GAE/g)</th>
<th>TFC (mg of CE/g)</th>
<th>AA (mg of cyanidin-3-glucoside equivalent/g)</th>
<th>Ascorbic acid content (mg of L-AAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jambhul pulp (WB)</td>
<td>5.53±0.53</td>
<td>14.40±0.93</td>
<td>1.74±0.09</td>
<td>1.40±0.03</td>
</tr>
<tr>
<td>Jambhul pulp (DB)</td>
<td>87.37±0.53</td>
<td>227.38±0.93</td>
<td>27.40±0.09</td>
<td>22.04±0.03</td>
</tr>
<tr>
<td>Wood apple pulp (WB)</td>
<td>1.48±0.08</td>
<td>nd</td>
<td>nd</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>Wood apple pulp (DB)</td>
<td>3.61±0.08</td>
<td>nd</td>
<td>nd</td>
<td>3.41±0.02</td>
</tr>
<tr>
<td>Ambadi leaves (WB)</td>
<td>3.99±0.05</td>
<td>nd</td>
<td>nd</td>
<td>0.93±0.07</td>
</tr>
<tr>
<td>Ambadi leaves (DB)</td>
<td>46.85±0.05</td>
<td>-</td>
<td>-</td>
<td>10.95±0.07</td>
</tr>
<tr>
<td>Ambat chukka leaves (WB)</td>
<td>2.23±0.05</td>
<td>nd</td>
<td>nd</td>
<td>0.83±0.05</td>
</tr>
<tr>
<td>Ambat chukka leaves (DB)</td>
<td>20.98±0.05</td>
<td>-</td>
<td>-</td>
<td>7.89±0.05</td>
</tr>
<tr>
<td>Jambhul seed (dry basis)</td>
<td>78.29±7.56</td>
<td>6.00±0.48</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wood apple skin (DB)</td>
<td>2.34±0.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TPC: Total phenolic content; TFC: Total flavonoid content; AA: Anthocynin content; WB: Wet basis; DB: Dry basis; nd: Not detected; All the values are Mean±S.D. of three determinations

by the Folin-Ciocalteu method, which leads to an overvaluation of the phenolic content. Furthermore, different phenolics might present different responses with the Folin Ciocalteu reagent.

Total flavonoid content in jambhul, wood apple, ambadi and ambat chukka: From Table 2 it can be seen that content of flavonoids found in jambhul pulp was 227.38 and for Jambhul seed it was 6.0 mg Catechin Equivalents (CE)/g DW sample. Kim et al. (2003) found TFC content of different cultivars of java plum in the range of 118-237 mg CE/100 g fresh sample. In the previous study reported by Luximon-Ramma et al. (2003) TFC of 13.5 mg/g was observed in jambhul, whereas 7 mg/100 g TFC was observed in jambhul by Benherlal and Arumughan (2007). These differences in the TFC could be attributed to the inherent variability of the raw material, as well as to the differences in methodology or standard used. The vanillin test for the detection of flavonoid in wood apple, ambadi and ambat chukka showed negative TFC which indicates that there is absence of condensed tannin in these fruits and vegetables.

Total monomeric anthocynin content in the jambhul pulp: Total monomeric anthocynin content in the jambhul pulp was (27.40±0.09) mg/g as cynidin-3-glucoside as equivalent DW of sample (Table 2). In case of other fruits and vegetables TMAC content was not determined as they were deficient in anthocynin pigments due to anthocynin is associated with non-green colored fruits and vegetables (Dewanto et al., 2002).

Benherlal and Arumughan (2007) observed monomeric anthocynin content of 134 mg cyanidin-3-glucoside/g dry weight in jambhul fruits. Veigas et al. (2003) TFC of 13.5 mg/g was observed in jambhul, whereas 7 mg/100 g TFC was observed in jambhul by Benherlal and Arumughan (2007). These differences in the TFC could be attributed to the inherent variability of the raw material, as well as to the differences in methodology or standard used. The vanillin test for the detection of flavonoid in wood apple, ambadi and ambat chukka showed negative TFC which indicates that there is absence of condensed tannin in these fruits and vegetables.

Antioxidant capacity of jambhul, wood apple, ambadi and ambat chukka: ABTS and DPPH assay is based on the antioxidant ability to react with ABTS and DPPH radical action generated in the assay system. In
The antioxidant capacity of jambhul, wood apple, ambadi and ambat chukka was compared using ABTS, DPPH and FRAP assay. The antioxidant activity of jambhul seed was 118.61 ± 2.96 µM of TE/g of dry weight of sample, whereas for wood apple it was found to be 20.02 ± 0.07 µM of TE/g of dry weight of sample. Ambadi leaves showed an antioxidant activity of 43.71 µM of TE/g of dry weight of sample, and for Ambat chukka, the activity was 32.97 ± 0.04 µM of TE/g of dry weight of sample. Moreover, waste of jambhul fruit i.e., jambhul seed showed an antioxidant activity of 32.97 µM of TE/g of dry weight of sample.

The antioxidant activity of wood apple skin was observed with an antioxidant activity of 3.23 µM of TE/g of dry weight of sample. Further wood apple skin was observed with an antioxidant activity of 3.23 µM of TE/g of dry weight of sample. The finding of this study indicates that each type of fruits and vegetable had a different antioxidant activity which was contributed by different antioxidant components. Jambhul showed highest phenolic content amongst all fruits and vegetables under study i.e., wood apple, ambadi and ambat chukka. Further jambhul was found with highest antioxidant capacity against ABTS, DPPH (free radical scavenging) and FRAP compared to wood apple, ambadi and ambat chukka. Waste portion of jambhul i.e., seed could be used as good source of natural antioxidants. Ambadi leaves showed highest nutritional components compared to ambat chukka. Ambadi and Ambat chukka could be utilized in protein and mineral rich food preparations such as papad. This fruits such as jambhul, wood apple and vegetable such as ambadi and ambat chukka can be consumed as novel fruits and vegetable outside of India and would be utilized for preparation of various products.

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REFERENCES


