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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, anthocyanin and antioxidant capacity such as ABTS, DPPH and FRAP assay. Total flavonoid content and total monomeric anthocyanin were found only in jambhul i.e., 227.38 mg/g CE/g DW, 27.40 mg/g as cyn-3-glucoside 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: Anthocyanin, 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 chutneys 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, anthocyanin,

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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. These 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-Ciocalteu reagent, Sodium carbonate anhydrous, Sodium hydroxide was purchased from FINAR Chemicals, Mumbai, India. Vanillin, 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, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Trolox, catechin were obtained from Sigma-Aldrich. Methanol (HPLC grade) purchased from Merck and Ethanol from SD Fine Chem., Mumbai, India. All other chemicals and reagents used in the present study were of analytical grade.

Proximate analysis: Jambhul, wood apple, ambadi and ambat chukka were subjected to proximate analysis such as moisture, fat, protein, ash by AOAC (1995).

Extraction of phenolics: The edible portion of jambhul and wood apple was separated and homogenized using laboratory blender. This homogenized edible fruit part (1 g) was extracted for 3 h with 10 mL of methanol solvent on an orbital shaker set at 180 rpm (30±1°C). The extract obtained after shaking was further centrifuged at 10,000 rpm at 37°C. The supernatant was collected and stored at 4±1°C in amber colored bottles until further analysis. The analysis was carried out within 3 days. The waste part of jambhul and wood apple viz., seeds and skin respectively were extracted for phenolics. Further ambadi ambat chukka leaves were also processed for extraction of phenolics. The storage conditions (time and temperature) were same for all types of fruits.

Determination of total phenolic content: The total phenolic content was measured by Folin-ciocalteu method explained by Shingh *et al.* (2002). For phenolics gallic acid was used as a standard. Methanol extracts of phenolics (0.2 mL) from jambhul, wood apple and ambadi and ambat chukka was added with 1 mL of Folin-ciocalteu reagent and was diluted with

distilled water in a ratio of 1:10. Sodium bicarbonate (7.5%) 0.8 mL was added to this mixture. This mixture was allowed to stand for 30 min at room temperature in dark and absorbance was measured at 765 nm. The standard curve was linear between 0 and 100 µg/mL gallic acid. Results were represented as mg of GAE/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 dispensed into test tube and 2.5 mL of vanillin reagent (8% HCl in methanol/4% vanillin in methanol, 1:1, v/v) was added to the sample and 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 anthocyanin pigment content: The samples were analyzed for its total monomeric anthocyanin 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 anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, were calculated and expressed as follows:

$$\text{Anthocyanin pigment (Cyanidin-3-glucoside equivalent, mg/L)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l}$$

where

A = (A_{520 nm} - A_{700 nm}) pH 1.0 - (A_{520 nm} - A_{700 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

ϵ = 26 900 molar extinction coefficient, in L·mol/cm, for cyd-3-glu
 10^3 = Factor for conversion from g to mg

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 7mM 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 \pm 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 μ M Trolox. Results are expressed in μ M 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 μ M Trolox. Results are expressed in μ M 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 μ M Trolox. Results were expressed in μ M 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%.

Ambadi leaves showed highest protein content i.e., 36.24% as compared to other fruits and vegetables whereas wood apple protein content was 10.43%. Highest total ash content was observed in case of ambat chukka leaves as compared to other fruits and vegetables i.e., 13.29% whereas for ambadi leaves, wood apple jambhul and jambhul seeds the ash content observed was 8.33, 2.66, 2.13 and 1.47%, respectively. The fat content was highest in wood apple (12.33%) as compared to others. From the above results it was observed that wood apple, ambadi and ambat chukka were rich sources of protein and ambadi and ambat chukka were found rich in mineral content.

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)

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

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

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

Samples	TPC (mgGAE/g)	TFC (mg of CE/g)	AA (mg of cynidin3-glucoside equivalent/g)	Ascorbic acid content (mg of L-AAE/g)
Jambhul pulp (WB)	5.53±0.53	14.40±0.93	1.74±0.09	1.40±0.03
Jambhul pulp (DB)	87.37±0.53	227.38±0.93	27.40±0.09	22.04±0.03
Wood apple pulp (WB)	1.48±0.08	nd	nd	0.22±0.02
Wood apple pulp (DB)	38.61±0.08	-	-	3.41±0.02
Ambadi leaves (WB)	3.99±0.05	nd	nd	0.93±0.07
Ambadi leaves (DB)	46.85±0.05	-	-	10.95±0.07
Ambat chukka leaves (WB)	2.23±0.05	nd	nd	0.83±0.05
Ambat chukka leaves (DB)	20.98±0.05	-	-	7.89±0.05
Jambhul seed (dry basis)	78.29±7.56	6.00±0.48	-	-
Wood apple skin (DB)	2.34±0.09	-	-	-

TPC: Total phenolic content; TFC: Total flavonoid content; AA: Anthocyanin 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 anthocyanin content in the jambhul pulp: Total monomeric anthocyanin content in the jambhul pulp was (27.40±0.09) mg/g as cyn-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 anthocyanin pigments due to anthocyanin is associated with non-green colored fruits and vegetables (Dewanto *et al.*, 2002).

Benherlal and Arunmughan (2007) observed monomeric anthocyanin content of 134 mg cyd 3-glu/100 g (fresh weight) in jambhul fruits. Veigas *et al.*

(2007) observed 230 mg of cyd 3-glu/100 g (dry basis). Anthocyanin content of jambhul was observed 0.14 g/100 g on fresh weight of jambhul (Chowdhury and Ray, 2007). The variation in the anthocyanin content observed as compared to previous study could be due the varieties, variation in soil and environmental conditions.

Total ascorbic acid content in jambhul, wood apple, ambadi and ambat chukka: Ascorbic acid is abundantly present in all plant cells and has many biological functions. As an antioxidant, it prevents browning of tissue, which is an oxidation reaction, directly and indirectly (Smirnoff, 1996). It efficiently scavenges reactive oxygen species such as O₂, OH, peroxy radicals and singlet oxygen. It is important to consider the contribution of vitamin C in addition to that of phenolic compounds with antioxidant activity in chemical systems. From the Table 2 it can be seen that ascorbic acid content in jambhul pulp was 22.04, wood apple pulp 3.41, ambadi 10.95 and ambat chukka 7.89 mg of L-AAE/g equivalents DW of sample. The order for ascorbic acid content in the above fruits and vegetable is: jambhul>ambadi>ambat chukka>wood apple. Chowdhury and Ray (2007) found 0.25 g of ascorbic acid in 100 g of fresh weight of Indian varieties of jambhul. It is reported that ascorbic acid content in wood apple were 8.52 mg/100 g Fw (Chowdhury *et al.*, 2008). Gupta and Prakash (2011) reported ambat chukka contained ascorbic acid 7.2 mg/100 g of fresh weight.

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

Table 3: Antioxidant capacity of jambhul, wood apple, ambadi and ambat chukka

Sample	ABTS (μM of TE/g)	DPPH (μM of TE/g)	FRAP (μM of TE/g)
Jambhul pulp (WB)	8.94 \pm 0.47	25.09 \pm 0.77	12.42 \pm 0.54
Jambhul pulp (DB)	141.20 \pm 0.47	396.09 \pm 0.77	196.06 \pm 0.54
Wood apple pulp (WB)	0.77 \pm 0.07	3.04 \pm 0.03	1.83 \pm 0.40
Wood apple pulp (DB)	20.02 \pm 0.07	78.99 \pm 0.03	47.55 \pm 0.40
Ambadi leaves (WB)	2.16 \pm 0.03	3.73 \pm 0.13	1.30 \pm 0.10
Ambadi leaves (DB)	25.38 \pm 0.03	43.71 \pm 0.13	15.23 \pm 0.10
Ambat chukka leaves(WB)	1.02 \pm 0.05	3.51 \pm 0.04	1.54 \pm 0.10
Ambat chukka leaves (DB)	9.60 \pm 0.05	32.97 \pm 0.04	14.51 \pm 0.10
Jambhul seed (dry basis)	118.61 \pm 2.96	360.02 \pm 21.07	194.08 \pm 8.33
Wood apple skin (DB)	1.20 \pm 0.01	3.23 \pm 0.20	1.26 \pm 0.07

All the values are Mean \pm S.D. of three determinations

contrast, the Ferric Reducing Antioxidant Power (FRAP) assay measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants, which are reductants with half-reaction reduction potentials above $\text{Fe}^{3+}/\text{Fe}^{2+}$. These methods are widely used to evaluate antioxidant activity in foods and biological systems.

The antioxidant capacity of jambhul, wood apple, ambadi and ambat chukka was determined by ABTS, DPPH and FRAP assay which is presented in Table 3. The free radical scavenging activity determined by DPPH jambhul was 396.09 whereas for wood apple it was 78.99 μM of TE/g of dry weight of sample. Ambadi showed antioxidant activity of 43.71 μM of TE/g of dry weight of sample and for ambat chukka antioxidant activity observed was 32.97 μM of TE/g of dry weight of sample. Moreover waste of jambhul fruit i.e., jambhul seed showed an antioxidant activity of 360.02 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. Antioxidant activity observed by ABTS test for jambhul was 141.20 μM of TE/g of dry weight of sample whereas for wood apple it was found to be 20.02 and for ambadi it was 25.38 and for ambat chukka it was 9.60 μM of TE/g of dry weight of sample. From Table 3 it can be seen that in waste part of jambhul seed an antioxidant activity of 118.61 was observed, wood apple skin showed an activity of 1.20 μM of TE/g of dry weight of sample. The differences in the results were also observed by Du *et al.* (2009) between the antioxidant capacity obtained by ABTS and DPPH for the different variety of Actinidia fruits. In the FRAP assay the ability of jambhul, wood apple, ambadi and ambat chukka to reduce Fe^{3+} to Fe^{2+} were 196.06 \pm 0.54, 47.55 \pm 0.40, 15.23 \pm 0.10 and 14.51 \pm 0.10 respectively and the waste jambhul seed and wood apple skin showed 194.08 \pm 8.33 and 1.26 \pm 0.07 μM of TE/g of dry weight of sample. The antioxidant potential of commonly consumed above fruit has been rated in the order of jambhul>wood apple>ambadi>ambat chukka by DPPH and FRAP assay and by ABTS assay Jambhul>ambadi>wood apple>ambat chukka.

CONCLUSION

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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