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PRELIMINARY PHYTOCHEMICAL, VITAMIN A, VITAMIN C, ANTIOXIDANT AND ANTI NUTRITIONAL ANALYSIS OF AVERRHOA BILIMBI L. AND AVERRHOA CARAMBOLA L.

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Abstract
Averrhoa bilimbi and *Averrhoa carambola* belongs to the family Oxalidaceae. Fruits of both plants were used for various treatments. The phytochemical screening in different solvents such as hexane and ethanol showed the presences of certain compounds. Vitamin A and Vitamin C content was high in ripened *Averrhoa bilimbi* and *Averrhoa carambola*. Cooking reduces vitamin A and vitamin C content. The radical scavenging properties that are unique to ripe and unripe fruit extract were elucidated with the help of DPPH assay. In the present study antinutritional factors like phenolics, tannins, saponin, phytic acid and oxalate were tested. Oxalate content is found to be high in raw *Averrhoa bilimbi*. In the present study an attempt has been made to highlight the preliminary phytochemical analysis, analysis of vitamin A, vitamin C, antioxidant (DPPH) and anti- nutritional property of *Averrhoa bilimbi* and *Averrhoa carambola.*

Key words: *Averrhoa bilimbi,* oxalate,, phenolics,

Introduction

Medicinal plants contribute a very important national resource to India. Medicinal plants have been considered as a valuable source of natural products and thus they were explored continuously for therapeutics for human wellbeing (Duke James, 2009). Oxalidaceae or wood sorrel family, are a small family of five genera of herbaceous plants, shrubs and small trees, with the great majority of the 570 species in the genus oxalis (wood sorrels). Members of this family typically have divided leaves, the leaflets showing "sleep movements", spreading open in light and closing in darkness. *Averrhoa bilimbi* (commonly known as bilimbi, cucumber tree, or tree sorrel) is a fruit-bearing tree of the genus *Averrhoa*, family Oxalidaceae. It is a close relative of carambola tree. Its trunk is short and quickly divides up into ramifications. *Averrhoa carambola* is a species of tree in the family Oxalidaceae; it has a number of common names, including carambola and starfruit. *A. carambola* is a small tree or shrub that grows 5–12 metres tall, with rose to red-purple flowers. The flowers are small and bell-shaped, with five petals that have whitish edges. The flowers are often produced year round under tropical conditions. The tree is cultivated in tropical and semitropical regions for its edible fruits and for its medicinal uses.

Materials and Methods

Fruits of *Averrhoa bilimbi* and *Averrhoa carambola* (raw, half mature, ripened) were collected from different localities like Kuttiyani and Kattakada respectively. The materials were collected, washed and stored for the present investigations.

Preliminary Phytochemical Analysis

The collected fruits of *Averrhoa bilimbi* and *Averrhoa carambola* were washed with tap water to remove the impurities. The unwanted parts of the fruits, removed with the help of a sharp blade. Slice the fruits (remove the seeds) and dried in shade for 3 -4 weeks. Then, the dried fruits were powdered separately. The powdered material is extracted with hexane and ethanol solvent to investigate the phytochemical compounds present in them. Preliminary test done for the detection of Alkaloids, Terpenoids, Flavonoids, Phenols, Saponins, Tannins, Proteins, Amino Acids, Carbohydrates and Steroids.

Estimation of Vitamin A

The method of Arnon (1949) was employed for the quantitative estimation of carotenoids in

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a. Raw
b. Half mature/Half Ripened
c. Ripened
d. Cooked for 10 minutes
e. Cooked for 30 minutes

From the optical densities, carotenoid content was calculated using the formula.

Carotenoid content (mg/gm) = 7.6(D.480) – 1.49 (D.510) x V x W/ 1000

Where D = Optical densities
V = Final volume of 80% acetone (10 mL)
W = Weight of sample taken (0.05 g)

Estimation of Vitamin C
The method followed was of Harris and Ray (1935) to determine Vitamin C
Calculations: Amount of ascorbic acid in mg/100g of sample =

(0.5mg/V_1 mL) x (V_2/5mL) x (100mL /Weight of sample) x 100.

Antioxidant Activity
(2, 2-Diphenyls -1– Picryldydrazyl)
The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al [2001].The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ ml DMSO) was used as reference.
Calculation: % inhibition =

$$\frac{control-test}{control} X100$$

Antinutritional Assays
Determination of Total Phenolics
Total phenols can be detected spectrophotometrically (Malik, & Singh, 1980).
Calculations**:** Amount of phenol present in 1g of the sample = mg of phenol / volume of sample x 100

Determination of Tannin
To determine the tannin content Vanillin Hydrochloride method was followed (Burns R E, 1971).
Calculations: Amount of tannin present in 1g of the sample = mg of tannin / volume of sample x 100
Determination of Saponin
Samples were analyzed for saponin content according to the method of Yoko, Keiko, and Kazuo (2000).

Calculations:
Amount of saponin present in 1g of the sample = mg of saponin / volume of sample x 100

Determination of Phytic Acid
Samples were analyzed with the help of TCA.

Calculations:

Amount of phytic acid present in 1g of the sample =mg of phytic acid / volume of sample x 100

Determination of Oxalate
This method was proposed by Shimadzu, UV-1900.

Calculations:

Amount of oxalate present in 1g of the sample = mg of oxalate /volume of sample x 100

Results and Discussion
Phytochemical Screening
The preliminary phytochemical investigation of fruits samples was carried out using solvents such as hexane and ethanol. The results of qualitative phytochemical observations are tabulated in the Table 1. From among the phytochemicals studied such as Alkaloids, Terpenoids, Flavonoids, Phenolic Compounds, Saponins, Carbohydrates, Tannins, Proteins, Amino Acids and Steroids, their presence were mostly were found in ethanol extracts. Alkaloids were found to be present only in ethanolic extract of *bilimbi* fruit. Steroids was completely absent in all the samples of hexane. Phenolic compounds being the largest and unique constituent of plant phytochemicals special attention was needed for such metabolites (Singh and Singh, 2007). They are responsible for the inhibition of specific enzymes causing inflammation. In the present study, phenolic content was detected in ethanolic extracts of two samples of fruit. Flavonoids belong to a family of polyphenolic compounds with low molecular weights. Flavonoids along with phenolic constitute a major group of biomolecules that have several biological effects including antioxidant free radical scavenging abilities, anticarcinogenic properties (Hodek *et.al.,*2002 and Devi

and Ganjewala, (2011).). Flavonoids enhance the effect of Vitamin C and are also known to be biologically active against liver toxins, virus, tumours, and are sometimes antimicrobial (Korkina and Afanas'ev, 1997). Alkaloids are used medicinally and they provide information to determine the structures for novel synthetic drug. In this study alkaloids occurred in ethanolic extract of *bilimbi* fruit. Terpenoids are very essential to plants and are life saviours. Medicinally potent terpenoids are having therapeutic properties including anti-malarial, antimicrobial activities (Pichersky and Gershezon, 2002, Deganhardt, 2003). The study conducted showed the presence of terpenoids in fruits of both.

Parekh and Chanda (2008), noted that tannins are capable of reacting with proteins which is important for the amelioration of conditions like inflammation and ulcers. These bioactive compounds are potential toxic agents to fight fungi, bacteria and virus infection and also humans, thereby help to reduce the risk of coronary heart disease (Ranjithkumar, 2010). In the present study proteins and bioactive tannins are present in all extracts of fruit. Saponins have hypotensive and cardio depressant properties (Olaleye, 2007). Saponins present in the plant have been suggested as possible anti-carcinogens. Presence of saponins in extracts has supported the usefulness of this plant in managing inflammation. Carbohydrates were found to be present in all samples. Steroids were completely present in all the ethanolic extracts of fruits.

Estimation of Vitamin A

Vitamin A has multiple functions; it is important for growth and development, for the maintenance of immune system and good vision (Tanumihardjo, 2011). Vitamin A needed by the retina of the eye in the form of retinal. Vitamin A also functions in a very different role as an irreversibly oxidized form of retinol known as retinoic acid, which is an important hormone like growth factor for epithelial and other cells. Carotenoid content of the two species were shown in Table 2.

Estimation of Vitamin C

Ascorbic acid is a sugar acid with anti oxidant properties. The major function of ascorbate is the formation of tissue collagen and intracellular cementing substances. Ascorbic acid also plays a key role in tyrosine metabolism and electron transport in the microsomal fraction. It is a mild reducing agent and anti oxidant. Ascorbic acid can be used as an anti oxidant to increase fluorescent signal and chemically retard dye photo bleaching. Table 3 shows vitamin C content.

Antioxidant Activity

The radical scavenging properties that are unique to ripe, unripe and cooked fruit extract were elucidated with the help of DPPH assay. Influence of crude extracts on scavenging of free radical effect of the fruits was assessed by the decolouration of 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) solution. Degree of colour discrepancies was used to study the potential of bioactive molecules to remove free radical species. The concentrations were taken as 12.5 µg, 25µg, 50 µg, 100 µg and 200 µg. The absorbance values are 0.0724, 0.0549, 0.0356, 0.0260, 0.0149 and percentage of inhibition is 46.92, 59.75, 73.90, 80.94, 89.08 respectively. Ascorbic acid is taken as standard.

Antnutritional Assays

Dietary antinutritional factors have been reported to adversely affect the digestibility of protein, bioavailability of amino acids and protein quality of foods. The use of plant-derived materials such as legume seeds, different types of oilseed cake, leaf meals, leaf protein concentrates, and root tuber meals as fish feed ingredients is limited by the presence of a wide variety of antinutritional substances. In this work antinutritional properties are tested for Phenolics, Tannin, Saponins, Phytic acid and Oxalate for *A.bilimbi* and *A.carambola* in raw, half ripened/half mature and ripened fruits.

Phenolics showed varied values in *A.bilimbi* raw, *A.bilimbi* half ripened/half mature and *A.bilimbi* ripened such as 3.12, 2.15 and 2.07 mg/g respectively. In *A.carambola* raw, *A.carambola* half ripened and *A.carambola* ripened showed 3.40, 2.56 and 2.23 mg/g respectively. Results were shown in Table 4.

Tannins are heat stable and they decreased protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen. Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption.

Tannins showed varied values. Table 5 shows the content of tannin.

Saponin reduces the nutritive value of pulses, but the saponins appear to be beneficial as they are responsible for lowering the cholesterol in body and may be important in human nutrition in reducing the risk of heart diseases and also inhibited colon cancer. Saponin content was shown in Table 6.

Phytic acid (known as inositol hexakisphosphate (IP6), or phytate or its salts) forms insoluble complexes with calcium, zinc, iron and copper. Phytic acid is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytate is not digestible to humans or no ruminant animals, however, so it is not a source of either inositol or phosphate if eaten directly. Phytic acid is absent in *A.carambola* ripened (Table 7).

Oxalate, also called oxalic acid, is an organic acid found in many plants. These include leafy greens, vegetables, fruits, cocoa, nuts and seeds. Human body can produce oxalate on its own or obtain it from food. Vitamin C can also be converted into oxalate when it's metabolized. Once consumed, oxalate can bind to minerals to form compounds, including calcium oxalate and iron oxalate. This mostly occurs in the colon, but can also take place in the kidneys and other parts of the urinary tract. Oxalate content are high in *A.bilimbi* raw. Antinutritional factors in foods are responsible for the deleterious effects that are related to the absorption of nutrients and micronutrients which may interfere with the function of certain organs. The results were shown in Table 8.

Conclusion

In the present investigation, ripened *A.bilimbi* and *A.carambola* shows high carotenoid content and *A. bilimbi* and *A. carambola*, cooked 30 minutes showed low carotenoid content. In the present study, ascorbic acid content in all samples show varied results. Ripened *A. bilimbi* and *A. carambola* shows high ascorbic acid content and *A.bilimbi* and *A. carambola,* cooked 30 minutes shows low ascorbic acid content. Hence it can be concluded that it is better to consume fruits in ripened stage than in the cooked stage. Cooking reduces the carotenoid and ascorbic acid content in the present investigation.The radical scavenging properties that are unique to ripe, unripe and cooked fruit extract were elucidated with the help of DPPH assay. The concentrations were taken as 12.5 µg, 25µg, 50 µg, 100 µg and 200 µg. In the present study, percentage of inhibition is found to be high in *A.bilmbi* cooked 30 minutes and *A.carambola* ripened. In this work antinutritional properties are tested for Phenolics, Tannin, Saponins, Phytic acid and Oxalate for *A.bilimbi* and *A.carambola* in raw, half ripened/half mature and ripened fruits. Antinutritional factors in foods are responsible for the deleterious effects that are related to the absorption of nutrients and micronutrients which may interfere with the function of certain organs. In general, plants may provide potential alternatives to currently used insect/microbe control agents because they constitute a rich source of bioactive chemicals. The present study warrants further detailed investigation on to the isolation, characterisation, purification of the active principle present in the *A. bilimbi* and *A. carambola* for its effective utilization as a natural herbal remedy for therapeutic purposes.

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References

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris.* Plant Physiol., 24: 1-15.

Deganhardt, J. (2003). Attracting friends to feast on foes: Engineering terpene emission to make crop plant more attractive to herbivore enemies. Curr. opin.Biotechnol. 14: 169-176.

Devi, S. A and Ganjewala, D. (2011). Antioxidant activities of methanolic extracts of sweet-flag (*Acorus calamus*) leaves and Rhizomes. Journal of herbs, spices & medicinal plants. 17(1): 1 -11.

Duke James A. (2009). Dukes handbook of Medicinal Plants of Latin America. Vol.2: 309-320.

Harris, L. J and Ray, S. N. (1935). Diagnosis of vitamin C subnutrition by urine analysis. Lancet, 228, 71.

Hodek, P, Trefil, P and Stiborova, A. (2002). Flavanoid potent and versatile biologically active compounds interacting with cytochrome P450. Chemco-Biological interactions. 139: 1-21.

Korkina, L.G and Afanas'ev, I.B. (1997). Antioxidant and chelating properties of flavanoids. ADv.Pharmacol. 38: 151-163.

Malik E.P and Singh M.B. (1980). Plant Enzymology and Hittoenzymology (1st Edn.) Kalyani Publishers: New Delhi; 286.

Olaley, M.T. (2007). Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa.* Journal of Medicinal plants Research. 1: 9-13.

Parekh, J and Chanda, S. (2008). *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. Afr.J.Biotech. 7: 4349-4353.

Pichersky, E and Gershezon, J. (2002). The formation and function of plant volatiles, perfumes for pollinator attraction and defence. Curr.Opin.Plant Biol. 5: 237-243.

Ranjithkumar, J. (2010). Secondary metabolites investigations and its derivatives on *Cassia occidentalis*. Journal of Chemical and Pharmaceutical Research. 2(4): 371-377.

Robert E. Burns. (1971). Method for Estimation of Tannin in Grain Sorghum. Agronomy Journal, vol 63(3):511-512

Shang-Tzen Chang, Jyh-Horng Wu, Sheng-Yang Wang, Pei-Ling Kang, Ning-Sun Yang and Lie-Fen Shyur. (2001). Antioxidant Activity of Extracts from *Acacia confusa* Bark and Heartwood. *Agric. Food Chem.* 49 (7):3420-3424

Singh AK and Singh J. (2010). Evaluation of anti-diabetic potential of leaves and stem of *Flacourtia jangomas* in streptozotocininduced diabetic rats. Indian J Pharmacol; 42:301-305.

Tanumihardjo S A. (2011). Vit A: biomarkers of nutrition for development. American Journal of Clinical Nutrition .94(2): 658-665.

Yoko Uematsu, Keiko Hirata, and Kazuo Saito. (2000). Spectrophotometric Determination of Saponin in *Yucca* Extract Used as Food Additive. Journal of AOAC International vol. 83 (6):1451-1454.

Table 1. Qualitative Detection of Phytochemicals

Sl. No.	Sample	Carotenoid Content (mg/
1.	*A.bilimbi* raw	1.757
2.	*A.bilimbi* half mature	2.039
3.	*A.bilimbi* ripened	2.451
4.	*A.bilimbi* cooked 10 minutes	1.537
5.	*A.bilimbi* cooked 30 minutes	1.407
6.	*A.carambola* raw	1.536
7.	*A.carambola* half mature	1.894
8.	*A.carambola* ripened	2.237
9.	*A. carambola* cooked 10 minutes	1.377
10.	*A. carambola* cooked 30 minutes	1.247

'+' indicates presence '-'indicates absence

Table 2. Carotenoid (Vitamin A) Content

Sl.No.	Phytochemicals	Hexane Ex-tract	Ethanol Ex-tract	Hexane Ex-tract	Ethanol Ex-tract
		Averrhoa bilimbi		***Averrhoa carambola***	
1.	Alkaloids	-	+	-	-
2.	Terpenoids	-	+	-	+
3.	Flavonoids	-	+	-	-
4.	Phenolic Compounds	-	+	-	+
5.	Saponins	+	-	-	+
6.	Carbohydrates	-	+	-	+
7.	Tannins	-	+	-	+
8.	Proteins	+	-	-	+
9.	Amino Acids	-	+	-	+
10.	Steroids	-	+	-	+

Table 3. Ascorbic Acid (Vitamin C) Content

Sl. No.	Sample	Vitamin C mg/g
1	*A.bilimbi* raw	14.82
2	*A.bilimbi* half mature	15.55
3	*A.bilimbi* ripened	15.75
4	*A.bilimbi* cooked 10 min	12.32
5	*A.bilimbi* cooked 30 min	11.00
6	A.*carambola* raw	9.05
7	A.*carambola* half mature	11.08
8	A.*carambola* ripened	11.39
9	A.*carambola* cooked 10 min	8.96
10	A.*carambola* cooked 30 min	7.35

Table 4. Estimation of Phenolics

Sl. No.	*A.bilimbi*	Phenolics mg/g	*A.carambola*	Phenolics mg/g
1	raw	3.12±0.46	raw	3.40±0.19
2	half ripened	2.15±0.39	half ripened	2.56±0.28
3	ripened	2.07±0.28	ripened	2.23±0.27

Table 5. Estimation of Tannin

Sl. No.	*A.bilimbi*	Tannin mg/g	*A.carambola*	Tannin mg/g
1	raw	0.45± 0.12	raw	0.64±0.04
2	half ripened	0.33±0.01	half ripened	0.54±0.03
3	ripened	0.13±0.11	ripened	0.31±0.08

Table 6. Estimation of Saponin

Sl. No.	*A.bilimbi*	Saponin mg/g	*A.carambola*	Saponin mg/g
1	Raw	1.23±0.08	raw	1.90±0.18
2	half ripened	1.65±0.18	half ripened	1.51±0.43
3	ripened	1.44±0.17	ripened	1.09±0.19

Table 7. Estimation of Phytic acid

Sl. No.	*A.bilimbi*	Phytic acid mg/g	*A.carambola*	Phytic acid mg/g
1	Raw	0.12±0.06	raw	0.05±0.03
2	half ripened	0.09±0.05	half ripened	0.04±0.03
3	ripened	0.04±0.19	ripened	Absent

Table 8. Estimation of oxalate

Sl. No.	*A.bilimbi*	Oxalate mg/g	*A.carambola*	Oxalate mg/g
1	Raw	4.98±0.61	raw	4.92±0.75
2	half ripened	3.87±0.51	half ripened	2.44±0.91
3	ripened	3.11±0.62	ripened	2.09±0.48