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Total Polyphenols and Phytochemical Constituents in A Few neglected and Underutilized Tropical Minor Fruits

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Abstract

Many of the plant species that are cultivated for food are neglected and underutilized while they play a crucial role in the food security, nutrition, and income generation of the rural poor. While these crops continue to be maintained by cultural preferences and traditional practices, they remain inadequately characterized and neglected by research and conservation. Four such tropical sour fruits which are neglected and underutilized when compared to other fruits were used for the present study. The fruits were tested for their total polyphenols and other phytochemical contents. Highest concentration of total phenolics (65 mg GAE/g) was detected in *Averrhoa bilimbi* L. This was followed by *Averrhoa carambola* L. (60 mg GAE/g), *Phyllanthus acidus* Skeels (53 mg GAE/g) and *Flacourtia jangomas* (Lour.) Raeusch.(48 mg GAE/g) respectively. This can be considered as a very good indicator of their nutritional and medicinal values.

Key words : Averrhoa bilimbi L., Averrhoa carambola L., Phyllanthus acidus Skeels, Flacourtia jangomas, phytochemicals, polyphenols.

Introduction

Modern agricultural systems that promote cultivation of a very limited number of crop species have relegated indigenous crops to the status of neglected and underutilized crop species (NUCS). While these crops continue to be maintained by cultural preferences and traditional practices, they remain inadequately characterized and neglected by research and conservation. Underutilized, minor, orphan or neglected crops are labels often applied to plant species that are indigenous, rather than non-native or adapted introductions, and which often form a complex part of the culture and diets of the people who grow them (Padulosi *et al.,* 2002).These species were immensely constructive by surviving harsh agro-climatic conditions, and can be established on degraded lands, which are presently being underutilized either due to poor soil fertility or moisture scarcity. Due to unsustainable market pressures and rapid urbanization, majority of these species have come to near extinction (Hellin and Higman 2009). Though minor fruits are popularly known as 'less known fruits' these fruits have great values as nutraceuticals. Polyphenols form a large group of chemicals and most commonly occurred polyphenols in food include flavonoids and phenolic acids. Dietary polyphenols are of current interest because substantial evidence *in vitro* have suggested that they can affect numerous cellular processes like, gene expression, apoptosis, platelet aggregation, intercellular signaling, that can have anti-carcinogenic and anti-antherogenic implications (Duthie *et al* 2003).

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Materials and Methods

Plant Material Used

Four species of the underutilised minor tropical sour fruits (*Averrhoa bilimbi* L. (Oxalidaceae), *Averrhoa carambola* L.(Oxalidaceae), *Phyllanthus acidus* Skeels (Phyllanthaceae), and *Flacourtia jangomas* (Lour.) Raeusch.(Salicaceae) were selected for the present study. The plant materials were collected from different parts of Kozhikode district.

Phytochemical Analysis

A. Phytochemical Screening for Secondary Metabolites

Chemical tests were carried out qualitatively on the extract following standard procedures to identify the phytochemical constituents

Test for Alkaloids

Dragendorff's test: In a test tube containing 1 ml of extract, few drops of Dragendorff's reagent was added and the colour developed was noticed. Orange colour indicated the presence of alkaloids.

Test for Flavonoids

Alkaline reagent test: To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

Test for phenolic compounds

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Test for tannins

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

Test for Terpenoids

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicated the presence of steroids.

In the above filtrate, formation of reddish brown colour of interface after addition of concentrated sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids.

Test for Saponins

Foam test: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously then some drops of olive oil were added. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Coumarins

2 ml of the extract was mixed with 3 ml of 10% NaOH. Colour was noted.

Test for Glycosides

5 ml of the plant extract was and added 2 ml of the glacial acetic acid and 2 ml of 2% ferric chloride solution was added and mixed well. 2 ml of conc. Sulphuric acid was added along the sides. Presence of brown ring at the interphase was noted.

B. Quantitative Estimation of Total Phenol Content

Total Phenolic assay

The total phenolic content was determined by using the Folin-Ciocalteu assay. An aliquot (1 ml) of extracts or standard solution of Gallic acid (100, 200, 300, 400, and 500ìg/ml) was added to 25 ml of volumetric flask, containing 9 ml of distilled water. Reagent blank using distilled water was prepared. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 10 ml of 7% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer. Total phenolics content was expressed as mg Gallic acid Equivalents (GAE) (Singleton *et al*., 1965).

Results and Discussion

Phytochemical Screening for Secondary Metabolites

Presence of secondary metabolites in the methanolic extract was tested and the results are summarized in table 1. All the analyzed samples contained phenolics, terpenoids and coumarins. Other constituents showed variations among the samples.

Sl no	Secondary metabolites	*Averrhoa carambola L.*	*Averrhoa bilimbi L.*	*Phyllanthus acidus Skeels.*	*Flacourtia jangomas*(Lour) Raeusch.
1	Alkaloids	+	+	-	-
2	Flavonoids	-	+	-	+
3	Phenolics	+	+	+	+
4	Tannins	+	-	-	-
5	Terpenoids	+	+	+	+
6	Saponins	-	+	-	+
7	Coumarins	+	+	+	+
8	Glycosides	+	+	+	-
9	Steroids	-	+	+	-

(+) implies presence; (-) implies absence

A. Quantitative Estimation of Total Phenolics

Concentrations of phenolic substances in three of the samples studied were detected from the standard graph of gallic acid. Highest concentration (65 mg GAE/g) was detected in *Averrhoa bilimbi*L. This was followed by *Averrhoa carambola* L. (60 mg GAE/g), *Phyllanthus acidus* Skeels (53 mg GAE/g) and *Flacourtia jangomas* (Lour.) Raeusch.(48 mg GAE/g) respectively (Table 2).

Table 2. Concentration of total phenolics in the samples

Sl. No.	Plants	Concentration of total phenolics *(mg GAE/g)
1	*Averrhoa bilimbi L.*	65 ± 0.5
2	*Averrhoa carambola L.*	60 ± 0.8
3	*Phyllanthus acidus*Skeels.	53 ± 0.6
4	*Flacourtia jangomas*(Lour)Raeusch.	48 ± 0.4

* Values are the mean of triplicate experiments, represented as Mean ± Standard Deviation, GAE= Gallic Acid Equivalents

Polyphenolic compounds, like flavonoids, tannins and phenolic acids, usually found in plants have been reported to have multiple biological effects, including antioxidant activity. Flavonoids and tannins present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action in the tested models. Phenolic compounds being part of the total phytochemical composition of plants are acclaimed for their high free radical scavenging ability in addition to having other disease prevention potential such as being antimicrobial, antiviral and anti-inflammatory agents (Gui-Fang *et al*., 2012; Oskana *et al*., 2012).

In recent years, fruits and vegetables played an important role in the prevention and treatment of chronic nutritional diseases. The beneficial role of fruits and vegetables is linked to their secondary metabolites content, including antioxidants (WHO, 2004). Phenolic compounds commonly found in plants, have been reported to have multiple biological effects, including antioxidant activity (Thaipong *et al.,* 2006). Many studies had revealed that phenolic content in plants could be correlated to their antioxidant activities. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (Parke, 1994).

In the present study it is revealed that some neglected crops like *Averrhoa bilimbi*L., *Averrhoa carambola*L., *Phyllanthus acidus* Skeels and *Flacourtia jangomas* (Lour.) Raeusch.) possess considerable amounts of polyphenols and other secondary metabolites in their fruits. This can be considered as a very good indicator of their nutritional and medicinal values.

Conclusion

All the species of the neglected and underutilized minor fruits category showed the presence of considerable amounts of phytochemicals. This can be considered as a very good indicator of their nutritional and medicinal values. Study on neutraceutical compounds, comparatively a newer aspect in the food science research, is gaining much importance today. In this context, food sources that provides extra health benefits, i.e., able to decrease the risk of disease, in addition to the basic nutritional value found in food become more valuable. These kind of natural products and antioxidant substances are capable of scavenging free superoxide radicals and have a very crucial role in maintenance of human health.

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