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Research Article

COMPARISION OF TOTAL PHENOLIC CONTENT OF SOME SELECTED INDIGENOUS GARCINIA SPECIES FOUND IN ASSAM

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ABSTRACT

Objective: Phenolics are thought be an important part of the plants defense system against pests and diseases including root parasitic nematodes and are reported to have great antioxidant activity. The purpose of the present study was to analyze the total phenolic contents, their comparative analysis and their properties in the aqueous extracts of some of the local species of *Garcinia* commonly available in the North-Eastern part of India as they exhibit high antioxidant potentials according to various studies.

Methods: Total phenolic content *in vitro* were determined for water, ethanol, methanol, acetone, ethyl acetate and petroleum ether extracts of leaves, flowers and stems, and fruits and seeds for the five Garcinia species- *G.pedunculata* Roxb ex. Bach Ham, *G. morella* Gaertn. Desr, *G. lancifolia* Roxb, *G. xanthochymus* and *G. acuminata* Plancho and Triana. The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method. The data were calculated by comparison between a standard curve of gallic acid and the absorbance of each sample. The total phenolic content is here expressed as mg Gallic acid equivalents (GAE) per 100 g of dry weight (dw). The measurement was conducted in triplicates and the reported value for each sample was calculated as the mean and SD of three independent experiments.

Results: Based on the solvent used, high concentrations of phenolic compounds were found in water, methanolic and dicholoromethane extracts, among which methanol stem extract of *G.pedunculata* Roxb ex. Bach Ham (76.30 g GA/g) contained the highest phenolic content. Ethanolic and petroleum ether extracts in all the tested samples had low concentrations of phenolic compounds. In water extracts samples, phenol concentrations were seen to be higher in leaves than in other plant parts. This result was seen to be similar in the methanolic extracts samples. In other groups of extracts values are higher in stem parts in opposite to the value of other parts of plant extract.

Conclusion: Solvents with relatively lower polarity except ethanol and chloroform were more efficient in general for extracting phenolic compounds in *Garcinia* species. Pure solvents with higher polarity and no acid extracted significantly higher amounts of phenolic compounds than non-polar solvents. The solvent most suitable for the extraction of phenolic compounds appeared to be methanol in all species. Although the methanolic extract of *G. pedunculata* had the highest total phenol content, the ethanol extract of *Garcinia* species recorded the least total phenol content within the five *Garcinia* species.

Keywords: Garcinia, phenol content, Folin Ciocalteu, pedunculata, morella, xanthochymus, lancifolia, accuminata

INTRODUCTION

Plants produce a large variety of compounds that contain a hydroxyl functional group on an aromatic ring called phenol, a chemically heterogeneous group which is one of the largest groups of secondary metabolites. They range from simple structures with one aromatic ring to complex polymers that scavenge the free radicals activity thus inhibiting the oxidative mechanisms that lead to emergence of various diseases as these molecules are electron rich. They donate electrons to ROS and neutralize these chemical species [1, 2]. Polyphenols are generally found in both edible and inedible plants and have been reported to have various biological effects including antioxidant activity [3]. They have been known to act as antioxidants due to their capability to donate electrons as well as the effectiveness of stabilizing radical intermediates in the prevention of oxidation at cellular and physiological level [4]. Therefore, the phenolic content of plants may contribute directly to their antioxidant action contribute significantly to the total antioxidant activity of medicinal and aromatic samples. The Folin-Ciocalteu procedure of Singleton [5] has been used as a measure of total phenolics in natural products for many years [6] which have also been used in this study. Other functions such as antioxidants, antimutagenics, on plant growth regulation and on resistance to plant diseases have also been attributed to the group of natural polyphenols. They could be an important part of the plants defense system against pests and diseases including root parasitic nematodes [7]. The phenolic–protein interactions are also thought to be, in part, responsible for the putative function phenolics as plant defense compounds [8and 9]. Likewise, epidemiological studies also have shown that consumption of food and beverages with ample phenolic compounds can minimize the risk of heart disease.

These compounds diminish the development of atherosclerosis through acting as antioxidants towards low-density lipoprotein [10]. Thus the extraction of plant constituents is essential to isolate biologically active compounds and in understanding their role in disease prevention and treatment and in knowing their toxic effects as well. However, very less information is available about the medicinal and pharmacological properties and biological activities of phytochemicals derived from the indigenous *Garcinia* species of Assam commonly known to have been used as culinary items by the local population. These species include *G.pedunculata* Roxb ex. Bach Ham, *G. morella* Gaertn. Desr, *G.lancifolia* Roxb, *G. xanthochymus* and *G. acuminata* Plancho and Triana. The information available on these important plants indicates that apart from some recent findings, not much attention has been paid towards the presence of phenol content and their medicinal potentials. Keeping this information in view, an endeavour has been made in this study for a comparative analysis of the total phenolic constituents and their properties in the aqueous extracts of aforesaid medicinally important plants commonly available in the North-Eastern part of India. *Garcinia,* belonging to the family Clusiaceae, is a large genus of polygamous trees or shrubs, distributed in the tropical Asia, Africa and Polynesia and is a rich source of bioactive molecules including xanthones, flavonoids, benzophenones, lactones and phenolic acids [11and 12]. The research on antioxidant compounds in the Clusiaceae family has been focused on phenolic diterpenes, flavonoids and phenolic acids. Several studies have shown that *Garcinia* species exhibit high antioxidant potentials, which are tightly connected with the total phenolic content [13, 14 and 15].

MATERIALS AND METHODS

Preparation of plant extracts

Total phenolic content *in vitro* were determined for water, ethanol, methanol, acetone, ethyl acetate and petroleum ether extracts of

leaves, flowers and stems, and fruits and seeds for the five aforementioned *Garcinia* species separately. For the leaves, flowers and stems ,the air-dried plant material (10 g) was coarsely crushed in small pieces of 2-6 mm using the cylindrical crusher and extracted with organic solvents (water, methanol, acetone, ethyl acetate and petroleum ether) in a ratio of 1:10 (w/v) and kept on a rotary shaker for 24 h at 20°C. The extract was filtered through a paper filter (Whatman No. 1) and evaporated under reduced pressure by the rotary evaporator. In this manner twenty-five different extracts were obtained. After extraction of 10 g of dried plant material, the largest volume of crude extract was obtained using polar solvents.

The fruits rinds of *G. pedunculata, G.morella* and *G. xanthochymus* were separated from the seeds, sliced and dried in the sun. They were macerated into small pieces and mixed with various extracting solvents following the above procedure .The whole fruit of *G. accuminata* were dried, mixed with extracting solvents and extracts were prepared from them. The fruits of *G. lancifolia* are juicy and pulpy in nature. For extract preparations, about 20 g of the fruit of *G. lancifolia* were sliced, homogenized, and squeezed in two-layered muslin cloth, to extract the complete juice. The juice was centrifuged at 3000 rpm for 5 min and used for determination of total phenolics content while the pulp was homogenized with methanol and chloroform (50:50 v/v). The extraction was repeated until it became colorless. The extract was filtered and a final volume was made up to 10 ml with methanol.

The Folin-Ciocalteu Method

The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method [16]. Extracts were diluted to the concentration of 1 mg/mL in the various solvents and 0.5 mL of the soluted extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 mL of NaHCO3 (7.5%). The phenolic compounds were determined using the Folin-Ciocalteu method, based on the reduction of phosphor-wolframate-phosphomolybdate complex by phenolics to a blue reaction product. After 15 min at 45°C, the absorbance was measured at 765 nm versus blank sample on spectrophotometer (ISKRA, MA9523-SPEKOL 211) after cooling at room temperature. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Gallic acid was used as standard for the calibration curve and was plotted at 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml Gallic acid that was prepared in 80% (v/v) methanol. The data were calculated by comparison between a standard curve of Gallic acid and the absorbance of each sample. The total phenolic content is here expressed as mg Gallic acid equivalents (GAE) per 100 g of dry weight (dw).

Statistical analysis

All determinations measurements of total phenolic content for the five *Garcinia* species in various plant extracts were conducted in triplicates. The reported value for each sample was calculated as the mean and SD of three independent experiments.

RESULTS AND DISCUSSION

The results of the total phenolic content determination of the examined plant extracts, using Folin-Ciocalteu method, are presented in table 1 and table 2. The total phenolic content (TPC) values summarized in Table 2 were quantified based on the linear equation obtained from Gallic acid standard calibration curve. Thus, TPC values were expressed as Gallic acid equivalent (g GAE/100 g samples). The content of total phenols in extracts, expressed as Gallic acid equivalents (GA) per gram of dry extract, ranged between 0.27 to 77 g GA/g

Table 1: Analysis of phenol content in various extracting solvents of selected *Garcinia* species

Extract	Water	80% Ethanol	70% Methanol	Chloroform	Acetone	Dichloro Methane	Petroleum Ether
G.pedunculata Roxb ex. Bach Ham							
Stem	++	+	+++	-	+	++	-
Leaf	++	+	+++	-	+	++	-
Flower	++	+	+++	-	+	++	-
Fruit	++	+	+++	-	++	++	-
Seed	++	+	+++	-	+	+	-
G. morella Gaertn. Desr,							
Stem	++	+	+++	-	++	++	-
Leaf	++	+	+++	-	+	++	-
Flower	+++	+	+++	-	+	++	-
Fruit	+++	+	+++	-	+	++	-
Seed	++	+	++	-	+	++	-
G.lancifolia Roxb							
Stem	+	+	+++	-	+	+	-
Leaf	+	+	+++	-	+	+	-
Flower	+	+	+++	-	+	+	-
Fruit	+	+	++	-	+	+	-
G. xanthochymus							
Stem	++	+	+++	-	+	++	-
Leaf	++	+	+++	-	+	++	-
Flower	++	++	+++	-	+	+	-
Fruit	++	++	+++	-	+	+	-
Seed	+	+	++	-	+	+	-
G. accuminata Plancho and Triana.							
Stem	+	++	+++	-	+	+	-
Leaf	+	++	+++	-	+	+	-
Flower	+	++	++	-	+	+	-
Fruit	+	++	+++	-	+	+	-

Table 2: Total phenol contents of *Garcinia* species using different solvents [in terms of g GA/g].

Extract	Water	80% Ethanol	70% Methanol	Acetone	Dichloro Methane
G.pedunculata Roxb ex. Bach Ham					
Stem	11.09±0.26	1.97±0.07	76.30± 0.69	2.21±1.35	13.02±0.92
Leaf	15.12±0.22	4.18±0.27	47.09± 0.34	1.97±0.07	9.65±0.64
Flower	11.26±0.03	1.66±0.05	26.49± 0.51	1.00±0.09	8.44±0.56
Fruit	9.09±1.35	1.00±0.26	32.23± 0.98	8.44±0.56	10.66±0.34
Seed	11.24±0.07	1.07±0.13	53.71± 0.93	1.56±0.33	1.06±0.04
G. morella Gaertn. Desr,					
Stem	11.00±0.12	8.44±0.56	47.09±0.73	4.18±0.28	12.07±0.08
Leaf	24.63±0. 55	9.65±0.64	55.91±0.93	1.66±0.05	13.01±0.92
Flower	15.48±0.03	13.95±0.55	27.09±0.88	1.89±0.63	10.77±0.12
Fruit	22.01±1.35	13.07±0.90	45.54±1.19	1.76±0.02	11.44±0.43
Seed	16.93±0.02	13.02±0.92	17.07±0.55	1.97±0.07	8.89±0.45
G. xanthochymus					
Stem	11.65±0.	5.17±0.05	47.03±11.22	4.98±0.24	13.08±0.24
Leaf	5513.97±0.70	7.04±1.15	23.63±4.34	1.83±0.62	10.68±0.77
Flower	11.00±0.12	12.21±1.15	42.86±20±14	1.67±0.62	7.03±11.08
Fruit	9.65±0.64	13.48±0.68	33.21±1.15	8.60±2.37	8.82±2.19
Seed	5.67±0.67	3.13±0.63	15.09±2.19	3.21±1.15	3.23±0.55
G.lancifolia Roxb					
Stem	0.86±0.24	1.97±0.07	32.6±0.54	5.80±0.14	1.23±0.08
Leaf	1.88±0.50	1.76±0.02	19.6±0.64	4.70±0.44	1.26±0.09
Flower	0.35±0.25	1.00±0.17	19.2±2.04	2.50±0.78	1.59±0.08
Fruit	1.55±1.25	1.26±0.05	11.2±1.13	3.10±0.66	1.41±0.37
G. accuminata Plancho and Triana					
Stem	3.09±5.13	6.26±0.81	25.83±1.24	2.89±0.25	0.41±0.08
Leaf	7.5±1.26	4.25±5.13	40.53±1.26	4.7±0.20	0.55±0.17
Flower	4.25±0.59	3.42±7.59	19.2±0.64	2.89±0.25	0.43±0.37
Fruit	1.45±1.3	3.8±1.26	41.2±1.66	6.4±0.80	0.26±0.06

*Mean of three determinations ± SD (standard deviation).

In relation to the solvent used, high concentrations of phenolic compounds were found in water, methanolic and dicholoromethane extracts, among which methanol stem extract of *G.pedunculata* Roxb ex. Bach Ham (76.30 g GA/g) contained the highest phenolic content. Ethanolic and petroleum ether extracts had low concentrations of phenolic compounds.

In the group of water extracts phenol concentrations in the leaves was higher than the concentrations in the tested plant parts. Between the tested methanolic extracts, the highest concentration of phenolic compounds was measured in the extract of leaves in relation to the all of tested methanolic extracts. In other groups of extracts values are higher in stem parts in opposite to the value of other parts of plant extract.

CONCLUSION

Total phenol contents were determined in the *Garcinia* species using pure solvents of water, methanol, ethanol, acetone, petroleum ether and chloroform by Folin-Ciocalteu method and significant differences were found in terms of type of extracts and type of solvents. Solvents with relatively lower polarity except ethanol and chloroform were more efficient in general for extracting phenolic compounds in *Garcinia* species (Table 1). On the other hand, pure solvents with higher polarity and no acid extracted significantly higher amounts of phenolic compounds than non-polar solvents. The solvent most suitable for the extraction of phenolic compounds appeared to be methanol in all species. Although the methanolic extract of *G. pedunculata* had the highest total phenol content (table 2), the ethanol extract of *Garcinia* species recorded the least total phenol content within the five *Garcinia* species. The values obtained by applying the Folin method conclude that the order of solvent efficiency is methanol > dichloromethane > water > acetone > ethanol> ethyl acetate > petroleum ether. The presence of phenol in other *Garcinia* species had also been reported by previously by several authors [14, 17, 18, 19 and 20]. There is no record of investigation on the phenolic content in terms of different plant parts of *G. accuminata* and *G.lancifolia* species in detail in the literature. In the future, investigation of the specific activity associated with further purification, identification and quantification of each phenolic compound are necessary to provide useful comparative information on the antioxidant level and activities in the studied *Garcinia* species.

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