

Original Article

PHYTOCHEMICAL EVALUATION OF *ARTOCARPUS HIRSUTUS* LAM. FRUIT EXTRACT: A POTENTIAL WILD ENDEMIC PLANT

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ABSTRACT

Objective: The present study includes the qualitative and quantitative estimation of phytochemical constituents and HPTLC (High-performance thin layer chromatography) analysis of phenolic acids in the fruit extract of *Artocarpus hirsutus*.

Methods: Qualitative and quantitative analysis of *Artocarpus hirsutus* methanol fruit extract using standard methods. HPTLC analysis was performed by the CAMAG HPTLC system (Switzerland) for gallic acid, caffeic acid and ferulic acid determination.

Results: The Preliminary phytochemical analysis revealed the presence of phytoconstituents such as flavonoids, phenols, tannins, steroids, glycosides, alkaloids, carbohydrate and protein. The methanol fruit extract has high carbohydrate content (267 ± 0.02 mg/g FW). And good amount of protein, phenol, flavanoid were also found. The results of mineral studies shows that elements such as potassium (1.601%) and nitrogen (1.4%) were present in higher quantity. The result showed the presence of caffeic acid within peak 7 with an Rf (Retention factor) value 0.49, ferulic acid within peak 8 with an Rf value 0.60, and gallic acid within peak 5 with an Rf value 0.25.

Conclusion: The results indicated that the *Artocarpus hirsutus* fruit contains an appreciable amount of bioactive compounds.

Keywords: *Artocarpus hirsutus*, Bioactive compounds, Ethnomedicine, Wild, Mineral, HPTLC, Endemic, Moraceae, Phenolic acid, Phytochemicals

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INTRODUCTION

In India, the indigenous fruits collected from the wild play a significant role in the food and nutrient security of tribals and rural poor. Wild edible plants are the precious gift of nature of the country and most of the ethnic communities strongly depend on it for their day -to-day life [1].

However, there is no precise information about the nutritional potential of many wild edible plants. Nowadays many exotic and introduced fruits are used by modern people and indigenous species are discarded from dining tables. Food plants serve as alternatives to staple food during periods of food deficit and are the valuable supplements for a nutritional balanced diet, one of the primary alternative source of income for many resource-poor communities, and the source of species for domestication [2]. Apart from their traditional use of food, potentially these wild plants have many advantages. They are used as medicine, fodder, and for rituals and other functions. They are immune to many diseases and often used in different formulations of traditional medicine in India. It is considered that special attention should be paid to maintain and improve this important source of food supply [3].

The *Artocarpus hirsutus* fruit locally called Anjili Chakka belongs to the Moraceae family. Aini (Anjili) is a tall evergreen tree, generally 20-25 m in height and up to 5 m in girth, fruits are edible, bright yellow (Fig.1-2), ovoid or globose covered with spines, seeds ovoid and white. *Artocarpus hirsutus* is an endemic tree species of the southern Western Ghats of peninsular India and Maharashtra Sahyadris [4].



Fig. 1: Mature fruits of *Artocarpus hirsutus*



Fig. 2: Ripened fruit without pericarp

Natural products from plants called secondary metabolites are the end products of primary metabolites such as carbohydrates, amino acid, chlorophyll, lipids and so on. They are synthesis a large variety of chemical substances known as secondary metabolites which include alkaloids, steroids, flavonoids, terpenoids, glycoside, saponin, tannins, and phenolic compounds. The secondary metabolites are very good antioxidant compounds. The richness of phytochemical bioactive compounds possess antioxidant, antitumor, antimutagenic, anticarcinogenic, and antiparasitic activities [5].

High-performance thin-layer chromatography (HPTLC) is becoming a routine analytical technique because of the advantages that include the small amount of mobile phase required, the speed of the method and the possibility of analysing several samples simultaneously (i.e. on the same plate), unlike HPLC. It thus reduces analysis time and cost per analysis. Cloudy samples and suspensions can also be analyzed directly by HPTLC. Automatic sample application is possible and repeated scanning can be performed on the same plate, so scanning conditions can be changed [6].

Plants have developed efficient protective defense mechanisms against various diseases where pre-existing and induced secondary metabolites play a significant role to protect them against various adverse climatic conditions. The biosynthetic pathway of phenolic compounds is derived from the shikimate pathway and the backbone of phenolic compounds are aromatic amino acids. Role of plant phenolics in inducing resistance in plants is well documented in the literature. Results of the present experiments reveal the presence of some phenolic acids in the fruit pulp of *Artocarpus hirsutus* [1].

MATERIALS AND METHODS

Preparation of fruit pulp extract

Mature fruits of *Artocarpus hirsutus* were collected from various localities of Kannur and Kozhikode district, Kerala. The dried material was powdered. From this powder, 10g was weighed and dissolved in methanol and kept in a shaker for 24 h. The slurry was filtered. Methanol extract was screened for the presence of secondary metabolites, Quantitative analysis of primary metabolites and minerals.

Qualitative analysis

Fruit extract was screened for the presence of secondary metabolites such as alkaloids, saponins, tannins, steroids, flavonoids,

terpenoids, phlobotannins, coumarins, cycloglycosides, phenols and quinines. The method of analysis employed were those described by Kokate [7] for the presence of various active components.

Quantitative analysis

Estimation of total carbohydrate was done by using the method of Sdasivam and manickam [8]. And Estimation of total protein by Lowry *et. al.* [9]. Makkar method was followed for phenolics [10]. The acid digestion method of Toth *et al.* [11] has been followed for the analysis of inorganic constituents.

HPTLC analysis

HPTLC analysis was performed by CAMAG HPTLC system (Switzerland). Chromatographic Conditions used are, stationary phase aluminum backed pre-coated silica gel plates Merck 60 F254 (0.2 mm thickness). Samples were applied to the plate as bands at 10 mm from the bottom of the plate by using CAMAG ATS 4. The plate was developed up to 80 mm in ascending mode with solvent system Toluene: ethyl acetate: formic acid (5:5:0.5) at room temperature (28±2 °C) in a Twin Trough Chamber (Camag, Switzerland) which previously saturated with the mobile phase. After development, the air-dried plate scanned at 254 nm 366 nm, and 550 nm after derivatizing with anisaldehyde sulphuric acid reagent in CAMAG TLC SCANNER 3 with winCATS software.

Statistical analysis

Data were analyzed using MS-Excel. Each set of data is an average of triplicates. The data represents mean±SE.

RESULTS

The Preliminary phytochemical analysis revealed the presence of phytoconstituents such as flavonoids, phenols, tannins, steroids, glycosides, alkaloids, carbohydrate and protein. Saponin was absent in the extract (table 1).

Table 1: Preliminary qualitative analysis of fruit pulp of *Artocarpus hirsutus*

S. No.	Phytochemical compound	<i>Artocarpus hirsutus</i>
1	Saponin	-
2	Tannin	+
3	Steroid	+
4	Flavanoid	+
5	Terpenoid	+
6	phlobotannin	+
7	Coumarin	+
8	Cycloglycoside	+
9	Total phenol	+
10	Quinine	+

The methanol fruit extract has high carbohydrate content (267±0.02 mg/g FW). And a good amount of protein, phenol, flavanoid was also found (table 2).

Table 2: Quantitative analysis of total phenol, carbohydrate and protein contents in methanol extract of fruit pulp

Parameters	Results
Total carbohydrate	267±0.02 mg/g FW
Total protein	5.71±0.01 mg/g FW
Total phenolic content	1.91±0.02 mg GAE/g DW
Total flavanoid content	1.22±0.01 mg/g FW

The results of mineral studies shows that elements such as potassium (1.601%) and nitrogen (1.4%) were present in higher quantity (table 3).

Table 3: Mineral analysis of *Artocarpus hirsutus* fruit

Minerals	Content (%)
Sodium	0.067
Potassium	1.601
Calcium	0.61
Magnesium	0.20
Iron	0.0002
Zinc	0.016
Copper	0.0024
Nitrogen	1.4
Carbon	41.18

Phenolic compounds are considered as secondary metabolites that are synthesized by plants during normal development. HPTLC

phenolic acid profile of methanolic extract of *Artocarpus hirsutus* fruit was recorded in fig. 3 and table 4.

Table 4: Peak table with R_f values, height, and area of phenolic acids

Peak	Start position	Start height	Max position	Max Height	Max %	End Position	End height	Area	Area %
5	0.25Rf	0.5AU	0.25Rf	39.4AU	12.79	0.27Rf	9.0AU	1088.4AU	13.35
7	0.49Rf	7.4AU	0.47Rf	84.1AU	27.33	0.50Rf	5.8AU	2735.2AU	33.56
8	0.58Rf	8.8AU	0.60Rf	15.3AU	4.96	0.62Rf	6.9AU	397.4AU	4.88

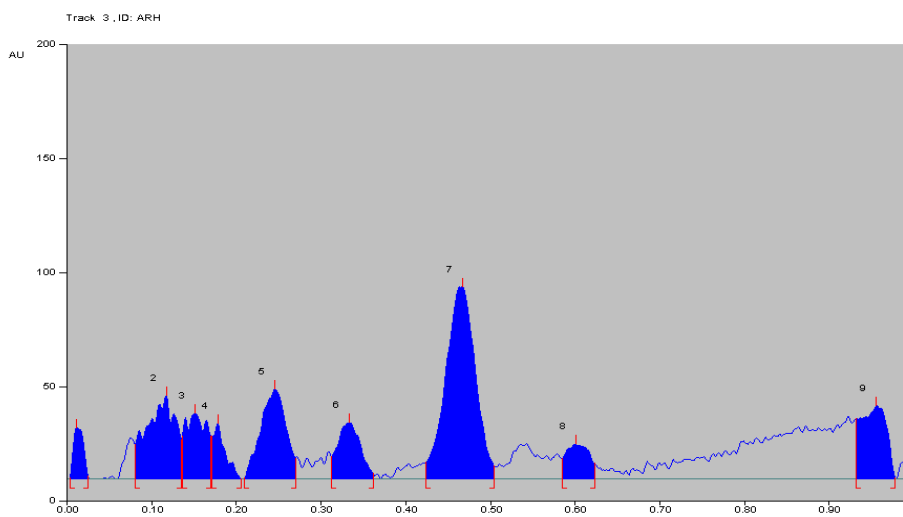


Fig. 3: Densitogram display for gallic acid, caffeic acid and ferulic acid of methanolic fruit extract of *Artocarpus hirsutus*

The extract was run along with the standard phenols compounds such as caffeic acid, ferulic acid and gallic acid. The result showed the presence of caffeic acid within peak 7 with an R_f value 0.49, ferulic acid with in peak 8 with an R_f value 0.60, and gallic acid within peak 5 with an R_f value 0.25.

DISCUSSION

These results were supported by Johnson *et al.* [12] who reported that saponins, alkaloids, tannins, phytates, oxalate, phenols, HCN and flavonoids known phytochemicals were present in the fruit, seed and rind of *C. lanatus*. The results obtained with the hexane, ethyl acetate and methanol extracts showed that hexane was more efficient solvent with a yield of 10% followed by methanol, ethyl acetate with 8% and 7% respectively. This result may be due to the high polarity of methanolic solvent which can draw a higher variety of plant constituents than the other solvents [13].

The presence of phenolic compounds (total phenols and flavonoids) provides pharmacological activities like anticancer [14], antioxidant [6], antimicrobial [16], wound healing [17] and anti-inflammatory activities [18].

Potassium is one of the important nutrients for controlling human blood pressure, therefore fig and mulberry fruits were recommended for hypertension in previous studies. Toth *et al.* [11] were studied vitamins and mineral substances in fruit and nuts of wild siberian plants. Potassium content is found to be higher in cranberry (2760). Bilberry is rich in Sodium (794 mg/l), Magnesium (736 mg/l), and manganese (393 mg/l) and low in copper (not detected). Ayensu (1985) reports that the amounts of calcium, iron, sodium, and potassium in *A. officinalis* were 22 mg/100 g, 1 mg/100 g, 2 mg/100 g, 278 mg/100 g, respectively. They also reported the amounts of ash, calcium, iron, potassium, and sodium in *P. oleracea* to be 20 mg/100 g, 1,500 mg/100 g, 29 mg/100 g, 1,800 mg/100 g and 55 mg/100 g respectively. Potassium is essential in the maintenance of cellular water balance, pH regulation in the

body and also associated with protein and carbohydrate metabolism [20].

Sarma and Singh [19], Maurya *et al.* [20] reported that ferulic acid has potent antimicrobial properties. Strong antioxidant activities of jackfruit pulp extracts were reported earlier and the same is correlated with the total phenolic and flavonoids content in the samples.

Soong and Barlow [21] also reported that the seeds of jackfruit are rich in total phenolic acid content and have strong antioxidant properties. Presence of phenolic compounds in food enhances food quality as they provide various benefits to human health.

CONCLUSION

The results indicated that the *Artocarpus hirsutus* fruit contain an appreciable amount of bioactive compounds. Fruit is a good source of carbohydrate, protein, phenol, and alkaloid. The presence of phenolic acids was confirmed by HPTLC analysis. Medically the presence of these phytochemicals especially the phenols and flavonoids explains the use of this plant in ethnomedicine for the management of various ailments.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the above article.

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