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Suma VK
Assistant Professor, Karuna College of Pharmacy, Palakkad, Kerala, India

Revikumar KG
Director, Kids, Trivandrum, Kerala, India

Shan Shasidharan
Director, Pankajakasthuri Herbal Research, Trivandrum, Kerala, India

Corresponding Author:
Suma VK
Assistant Professor, Karuna College of Pharmacy, Palakkad, Kerala, India

Phytochemical analysis and antioxidant activity of seed extracts of *Artocarpus hirsutus*: An *in vitro* study

Suma VK, Revikumar KG and Shan Shasidharan

Abstract
The genus Artocarpus belongs to the family Moracea which comprises of about 60 genera and over 1000 species. Artocarpus species are known for its edible fruit with high nutritive values. *Artocarpus hirsutus* Lam. is one among the five available varities of jackfruits. The aim of the present study is to evaluate preliminary phytochemical analysis on roasted and non-roasted seeds of *Artocarpus hirsutus.* In the present study effort was aimed to evaluate the phytochemical components of *Artocarpus hirsutus* seed part with different organic solvent extracts by soxhlet extraction method with analytical grade solvents. The present study reveals the presence of phytochemical components like carbohydrates, phenolic compounds, tannins, phytosterols and proteins. In HPTLC finger prints of roasted and non-roasted ethanolic seed extract demonstrated the presence of phenolic compounds, whereas they were absent in all other seed extract of *Artocarpus hirsutus*. In present study an attempt has been carried out to evaluate antioxidant activity of seeds of *Artocarpus hirsutus* widely found in Western Ghats regions. The ethanolic seed extract of *Artocarpus hirsutus* was found to be effective in DPPH radical scavenging activity.

Keywords: *Artocarpus hirsutus*, photochemistry, HPTLC, DPPH, antioxidant activity

Introduction
Wild Jackfruit, also called Wild Jack is having the latin name *Artocarpus hirsutus Lam*. Belonging to the Moraceae family. It is common in Western Ghats form north Karnataka to Malabar Coast and Travancore [1]. *Artocarpus hirsutus* is a tropical evergreen tree species that is native to India, primarily in Kerala. It grows at an altitude ranging from sea level at an elevation of 1000 meters in places with an annual rainfall of 1500 mm or more [2]. *Artocarpus hirsutus* is an evergreen tree with a dense crown growing up to 50 metres tall. The straight, cylindrical bole can be 150cm or more in diameter. The heartwood is yellowish-brown; the sapwood white. The wood is moderately hard, durable; it lasts well in water and is not attacked by white ants. Fruits are edible, bright yellow, ovoid covered with spines, seeds ovoid and white. It required warm humid climate heavy rainfall and thrives week in any type of soil. Kerala's own fruit locally called 'Anjili Chakka' (*Artocarpus hirsutus*). Tree is propagated through seeds or by grafting, flowering season is from December to January and fruits get ripped in May and June.

Vernacular names of *Artocarpus hirsutus*
- English – Wild Jack
- Kannada- *Hebbalasu, hebbe-lasu*
- Malayalam- *Ayani, Anjili, Ayaniplavu, Annali, Annili, Aini, Ayari*
- Marathi- Pat-*phanas, Ranphanas*
- Tamil- *Kattuppala. Akkini, Anjili*
- Telagu- *Pejuta*

Taxonomy and Ethnobotany
Taxonomically *Artocarpus hirsutus* Lam. Belongs to angiosperms and the details are as follows:
- Kingdom– Plantae
- Division- Angiosperms (unranked)
- Phylum- Eudicots (unranked)
- Class- Rosids (unranked)
- Order- Rosales
- Family- Moraceae

- Tribe- Artocarpeae
- Genus- *Artocarpus*
- Species- *hirsutus*
- Specie authority- Lam

Fig 1: Fruits of *Artocarpus hirsutus*

The plant A. Hirsutus is notable for its valuable medicinal properties. Bark has the properties to cure ulcers, diarrhea and pimples. Roasted seeds powder mixed with honey is used for the treatment of asthma. Oil from the fruits are used for the treatment of skin diseases. Grinded bark of *Artocarpus hirsutus lam* is a constituent of many herbal medicines for piles, and Grinded bark is smeared on the affected part to cure piles. Latex of *Artocarpus hirsutus* is used for asthma and seeds are use as appetizer. Burnt leaves ash is taken internally to treat abdominal problems. Dry leaves are useful in treating buboes and hydrocele. Fruits are rich source of carbohydrates, ß-carotene and essential aminoacids. Unripe fruits are useful in vitiated conditions of *vata* and *pitta* and anorexia. The ripe fruits posses sour, sweet, cooling, appetizing, constipating and aphrodisiac properties. It causes flatulence, colic, tridosa and rakta vitiations. Studies on pylorus ligated rats demonstrates that the *A. hirsutus* stem bark extract reduces the gastric secretary volume, acidity and ulceration [3] An infusion of the bark is applied to cure small pimples and cracks on the skin, and the powdered bark is used to heal sores [4]. Bark ash mixed with coconut oil is used externally against 'dhobi's itch' and ringworm. Bark paste in coconut oil can be applied for snake bite [5]. Roots and bark decoctions are used to cure diarrhoea whereas leaves used along with white camphor and root of curcuma to treat venereal bubones and chronic haemorrhage. Juice of cooked fruits is potential for inducing appetite and applied to the anus to relieve the pains of haemorrhage. It's barks are used to cure diarrhea, pimples and ulcers [6]. Grinded bark of *Artocarpus hirsutus* is a constituent of the medicine for piles, and Grinded bark is smeared on the affected part to cure piles [7]. The timber of the plant is used for house and boat building and furniture manufacture.

Materials and Methods

Collection of samples

Fruits of *Artocarpus hirsutus* Lam. were collected from Mannarkkad area of Palakkad district, Kerala. Healthy plants with previous history of giving fruits were selected and identified. The fruits and seeds were authenticated by Dr. Usman Arerath, Young Scientist (SERB-DST), Department of Botany, MES Kalladi College, Mannarkkad, Palakkad, Kerala. Botanical characters were also compared with various floras.

Preparation of seed extract

Seeds were taken out from the fruit, washed properly and sun dried for further evaluation and studies. Half the seeds were roasted and half were non-roasted and taken separately for further studies. The roasted and non-roasted seeds were powdered separately. Then both will extracted separately by nine solvents with increasing polarity like Hexane, Toluene, Petroleum Ether, Chloroform, Ethylacetate, Acetone, Ethanol, Methanol And Water. The suspension was filtered using Whatman No: 1 filter paper. Then, the filtrates were centrifuged at 5000 rpm for 5 minutes. The extracts were stored at 4 °C for the further studies.

Phytochemical screening

All the roasted and non-roasted extracts were subjected to preliminary phytochemical qualitative and quantitative screening for the presence or absence of various primary and secondary metabolites such as Test for alkaloids - Mayer's, Wager's, Dragendroff's Test for Flavonoids- alkaline reagent, lead acetate, ferric chloride, Test for glycosides - Borntrager, Legal's, Killers Killani, Test for saponins - foam test, Test for tannins - 5% Fecl 3 gelatin, Test for phenols - ferric chloride, Test for Terpenoids and Phytosterols - Salkowski, libermann burchard and Test for carbohydrates - Molisch, Benedict, Barford's and Fehling's test [8, 9].

HPTLC fingerprinting

Sample preparation for HPTLC

Sample obtained in the procedure for the determination of un-saponifiable matter is dissolved in 10 ml of Methanol this was followed for the sample of *Artocarpus hirsutus oil*, and Methanol soluble portion was used for HPTLC.

HPTLC

HPTLC analysis was performed by CAMAG Linomat 5 HPTLC system (Switzerland). Chromatographic Conditions used are, stationary phase aluminum backed pre-coated silica gel plates Merck 60 F254 (0.2 mm thickness). 2, 4 µl of the above sample was applied on a precoated silica gel 60 F254 on aluminium plates to a band width of 6 mm using Linomat 5 TLC applicator in Ascending mode. The plate was developed in Toluene: Ethyl acetate: Formic acid (5: 3.5: 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 after derivatizing with 2% Ferric Chloride Solution In Ethanol as the reagent in CAMAG Visualizer

Determination of ash value

Total ash

- Weighed accurately about 3 gm of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red until free from carbon cooled and weighted and then calculated the percentage of total ash with reference to the air dried drug.

Acid insoluble ash

- The ash obtained as directed under total ash above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on ash less filter paper, washed with hot water ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Water soluble ash

- The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450ËšC. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash calculated with reference to the air dried drug.

Determination of antioxidant activity

DPPH free radical scavenging assay

0.1mM solution of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) in methanol was prepared and 1mL of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (50,100,200,400 & 800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid (1-100 µg/ml) was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity.

The capability of scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = {(A0 – A1)/A0)*100}

Results and Discussion

Extraction analysis

The weight and percentage yield of all nine extracts of roasted and nonroasted seeds of *Artocarpus hirsutus* is tabulated in Table 1.

Table 1: Percentage yield of various extracts

S. No.	Type of extraction	Percentage yield (%) roasted	Percentage yield (%) non roasted
1	Hexane	10.98	12.19
2	Toluene	2.107	1.903
3	Petroleum ether	0.884	0.227
4	Chloroform	1.390	1.02
5	Ethyl Acetate	1.14	2.856
6	Acetone	3.827	4.225
7	Ethanol	28.412	29.21
8	Methanol	2.908	3.354
9	Water	5.946	3.056

Phytochemical analysis

A. hirsutus plant seed is rich in major phytochemical compounds. The qualitative phytochemical analysis of seed extract of *A. hirsutus* is presented in Table 2 and Table 3

Table 2: Preliminary phytochemical evaluation (Non-roasted seeds)

Extracts	Reagents	Hexane	Toluene	Pet ether	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Water
Alkaloids	Mayers test	_	_	_	_	_	_	_	_	_
	Wagners test	_	_	_	_	_	_	_	_	_
	Hagers test	_	_	_	_	_	_	_	_	_
	Dragendorffs test	_	_	_	_	_	_	_	_	_
Carbohydrates	Molish	+	+	+	+	+	+	+	+	+
	Fehling	_	_	_	+++	_	++	+	++	_
	Barfoeds	_	_	_	_	+	_	++	++	_
	Benedicts	_	_	_	_	+	_	_	++	_
Glycosides	Borntragers test	_	_	_	_	_	_	_	_	_
	Legal test	_	_	_	_	_	_	_	_	_
Fixed oils and fats	Spot test	+++	+	_	_	_	_	_	_	_
	Saponification test	+	_	_	+	++	_	_	_	_
Phenolic compounds and tannins	Ferric chlorid test	_	_	_	_	_	+++	++	+	+++
	Gelatin test	_	_	_	_	_	+++	++	_	_
	Lead acetate		_	_	_	_	+++	++	_	+++
	Alkalin reagent	+	_	+	_	_	_	_	+	+
	Mg &hcl reduction	+	_	_	+	_	_	_	+	_
Phytosterols	Copper acetate	++	+	_	++	++	_	_	++	++
	Libermann-burchard test	++	+	_	++	++	++	++	++	++
Saponins	Froth test	+	++	+	_	_	_	_	_	+
Proteins	Millons	_	_	_	+	_	+	_	+++	+
	Biuret	_	_	_	_	++	+	++	++	+++
	Ninhydrin	_	_	_	_	_	_	_	+++	+

Qualitative analysis revealed the presence of Phytosterols, Fixed oils, Saponins, Tannins, Phenols, Terpenoids, Proteins and Carbohydrates.

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the plant extracts revealed the presence of phytochemicals, such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.

Table 3: Preliminary phytochemical evaluation (Roasted seeds)

Extracts		Hexane	Toluene	Pet ether	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Water
Alkaloids	Mayers test	_	_	_	_	_	_	_	_	_
	Wagners test	_	_	_	_	_	_	_	_	_
	Hagers test	_	_	_	_	_	_	_	_	_
	Dragendorffs test	_	_	_	_	_	_	_	_	_
Carbohydrates	Molish	+	+	+	+	+	++	+	+	+
	Fehling	_	_	_	+++	_	++	+	++	++
	Barfoeds	_	_	_	_	++	_	++	++	
	Benedicts	_	_	_	_	_	_	_	++	_
Glycosides	Borntragers test	_	_	_	_	_	_	_	_	_
	Legal test	_	_	_	_	_	_	_	_	_
Fixed oils and fats	Spot test	+++	+	_	_	_	_	_	_	_
	Saponification test	+	_	_	+	_	_	_	_	_
Phenolic compounds and tannins	Ferric chlorid test	_	_	_	_	_	+++	_	+++	+++
	Gelatin test	_	_	_	_	_	+++	_	+++	_
	Lead acetate		_	+	_	_	+++	_	+++	+++
	Alkalin reagent	+	_	+	_	+	_	_	+	+
	Mg &hcl reduction		_	_	+	_	_	_	_	_
Phytosterols	Copper acetate	+++	+	_	+	++	+	_	++	++
	Libermann-burchard test	+	++	_	++	+++	++	++	++	++
Saponins	Froth test	+	_	_	_	_	_	_	_	+
Proteins	Millons	_	_	_	_	++	_	_	++	+++
	Biuret	_	_	_	++	++	+++	+++	+++	+++
	Ninhydrin	_	_	_	_	_	_	_	+++	+++

HPTLC

HPTLC finger print profile of various extract of *Artocarpus hirsutus* Lam in the order RA1H, RG1ET, RH1M, RI1W, NA1H, NG1ET, NH1M, NI1W, RB1T, RC1P, RD1C, RE1E, RF1A, NB1T, NC1C, ND1C, NE1E, NFIA, GALLIC ACID has been obtained with suitable solvent system. The developed plates were visualized under UV light after derivatisa-tion with ferric chloride in ethanol reagent colour of the spots and densitometric scan at 254 was recorded. On photodocumentation, Densitometric scan at 254 nm showed Nonroasted And Roasted Ethanol Extract have given spots for Phenolic Compound at Rf Value 0.45 And 0.51(FIG I)

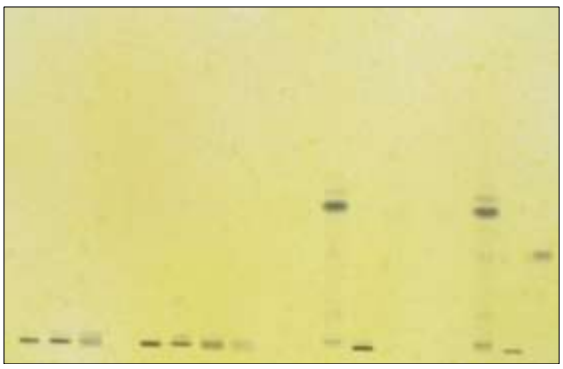

Fig 2: HPTLC photo documentation of various seed extract of *Artocarpus hirsutus*

Ash value

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. The total ash value, acid insoluble ash, water soluble ash was presented in Table 4.

Table 4: Ash values of roasted and non-roasted seed powder

	Total ash	Acid insoluble ash	Water soluble ash
Roasted	2.005%w/w	0.591%w/w	0.953%w/w
Non roasted	1.762%w/w	0.576%w/w	0.681%w/w

Antioxidant Activity (1, 1-Diphenyl-2-Picrylhydrazylradical) Scavenging assay

The Percentage of DPPH radical scavenging activity of nine extracts of non-roasted seeds of *Artocarpus hirsutus* presented in Table. 5. Although this seed extract shows lower scav-enging activity in comparison to ascorbic acid as statndard. Ethanolic, methanolic and water extract exhibited an-tioxidative potential.

Table 5: DPPH activity: OD and its % inhibition

S. No.	Extracts	OD at 515 nm	% inhibition
1	Hexane	1.06	45.06
2	Toluene	1.04	45.00
3	Petroleum ether	2.05	33.06
4	Chloroform	2.88	33.07
5	Ethyl Acetate	1.07	45.09
6	Acetone	1.08	44.67
7	Ethanol	0.98	90.06
8	Methanol	0.88	58.06
9	Water	0.87	72.40

Conclusion

The present study showed the significance of *Artocarpus hirsutus* seeds has a valuable source of secondary metabolites. Phytochemical analysis conducted on the various seed extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, phytosterols, fixed oils, carbohydrates and proteins. In HPTLC, solvent system (Toluene: Ethyl acetate: Formic acid) can be used during the isolation of phenolc compounds from all extracts. HPTLC finger prints of roasted and non-roasted ethanolic seed extract demonstrated the presence of phenolic compounds, whereas they were absent in all other seed extract. Hence the ethanolic seed extract cannot be replaced with all other seed extract for commercial purpose. In the present investigation, both various seed extract of *Artocarpus*

hirsutus showed potent antioxidant activity compared to reference standard ascorbic acid. In the present study revealed that ethanolic, methanolic and water extracts having potent antioxidant activity than other extracts. Further study need to be upgraded in the direction of isolation of pure secondary metabolites harbored in this plant. However, *Artocarpus hirsutus* can be used as a potent antioxidative agent and plants can be explored extensively for the future research.

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