

## PHYTOCHEMICAL SCREENING OF *ARTOCARPUS HIRSUTUS* AND ITS ANTIMICROBIAL POTENTIAL

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### ABSTRACT

**Objective:** In the present study, the effort was aimed to evaluate the phytochemical components, antioxidant activity, and antimicrobial activity of *Artocarpus hirsutus* fruit and leaf.

**Methods:** Each plant materials were extracted with two suitable solvents such as acetone and ethanol. Then, the extracts were tested for the presence of active phytoconstituents and antimicrobial activity. Then, it was subjected to antimicrobial activity against fungal and bacterial strains.

**Results:** The present study suggested that the selected plant extracts of *A. hirsutus* exhibit major phytoconstituents, such as alkaloids, glycosides, terpenoids, phenols, and saponins. Furthermore, the results showed that acetone fruit extract of *A. hirsutus* and ethanolic fruit extract of *A. hirsutus* exhibited significant antimicrobial activity with a maximum zone of inhibition against *Staphylococcus aureus* and *Aspergillus tamarii*.

**Conclusion:** The present study also exhibited remarkable antimicrobial activity against all the test microorganisms due to the presence of major bioactive phytochemicals.

**Keywords:** *Artocarpus hirsutus*, Antimicrobial, Alkaloids, Bacteria, Phytoconstituents.

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### INTRODUCTION

Phytochemicals, isolated from the medicinal plants exhibit toxicity to microorganisms by different mechanisms. Phytochemical screening refers to the extraction, screening, and identification of the medicinally active substances found in plants. Some of the bioactive substances that are derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants, and phenolic compounds [1]. Phytochemicals may have biological significance, for example, carotenoids or flavonoids but are not established as essential nutrients. It is a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection. Different phytochemicals possess a wide range of activities that may help in protection against various diseases [2].

Phenolic content act as antioxidants as free radical scavenging, oxygen radical absorbance, and chelating of metal ions. Several phenolic studies from plant sources have reported the antioxidant and antimicrobial properties [3] in medicinal plants due to their rich phenolic compounds for the herbal medicament. The secondary metabolites play an important role in protecting the plant body from the external biotic impacts and damaging as signaling components [4]. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. The mechanism underlying phenolic compound toxicity to microorganisms is thought to be mediated by enzyme inhibition possibly through interaction with sulfhydryl groups and with different proteins. Phenolic compounds present in plants have gained considerable importance due to their antioxidant activities.

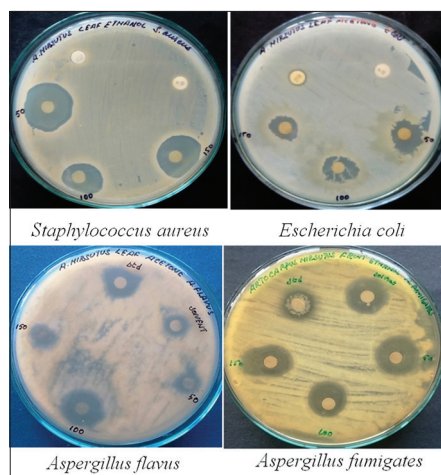
Flavonoids are the most diverse group of phytochemicals [5]. Flavonoids are hydroxylated phenolic substances known to be most widely distributed and synthesized natural products in plants [6]. Tannins are polymeric phenols which stimulate the immune system and are considered to have wide range of anti-infective activities [7]. They make complexes with proteins, and hence they have the ability to inactivate microbial enzymes, adhesion, and transport proteins [8].

Research on natural products with antimicrobial activity has increased significantly in recent years. Medicinal plants have been the subject of research in several countries such as Brazil [5]. This country holds a rich biodiversity and possessor of a diverse flora. In this way, the diversity of molecules found in plants makes them promising sources of new antimicrobials [9].

Diseases due to pathogenic fungi represent a critical problem to human health which is a major cause of morbidity and mortality worldwide. Widely distributed traditional medicinal plant-based antimicrobial drugs are cost effective in the treatment of infectious diseases having no side effects. Bioresources are primary (carbohydrates, proteins, and amino acids) and secondary metabolites (steroids, flavonoids, phenolics, glycosides, saponins, tannins, terpenoids, and coumarins) that impart medicinal properties to the plants [5].

Nowadays, these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, and cancer. Unlike pharmaceutical chemicals, these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings, these can also be considered as "man-friendly medicines" [10].

*Artocarpus hirsutus* belongs to the Moraceae family and consists of 50 different species. It is called Hebbalasu in Kannada; Vadahar in Hindi; Lakucha, Dahu, Adahu, Irvatam, and Panasah in Sanskrit; Ranpanas and Pachpanas in Konkani; Anhili, Ayani-maram, Ayani, Anjili, and Ayiniplavu in Malayalam; Kattupula, Aiyinipala, Kurangupala, and peipala in Tamil; Pejakai in Tulu; and Adavipanasa in Telugu. *A. hirsutus* is a tropical evergreen tree species that is native to India (Karnataka, Kerala, Maharashtra, and Tamil Nadu) [11]. They are widely acknowledged as a rich source of bioactive secondary metabolites such as flavonoids, stilbenes, triterpenoids, and xanthenes [12].



**Fig. 1: Antimicrobial activity of *Artocarpus hirsutus* Leaf and fruit extracts on different microbial strains**

The ripe fruit of *A. hirsutus* is eaten after removing the spiny outer skin. The structure of the fruit is similar to that of the much larger jackfruit. The seeds are also edible, usually fried as a snack [13]. Its bark cures diabetes, tapeworm infection, anemia, malarial fever, asthma, dermatitis, diarrhea, pimples, and ulcers. It has a long history of medicinal use in Chinese medicine, as a remedy for many kinds of diseases [14]. *A. hirsutus* have added a great deal in the field of phytochemistry with regard to their availability of complex phytochemical components, antibacterial activity, antihelminthic, anti-inflammatory, and antiviral activity [15].

Most of the modern researches on herbal medicine have hinged around traditional medicine. The modern medicine has brought with it an array of drugs, none of which is non-toxic and quite safer for human consumption. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very essential and needs urgent attention to know the value of the plant. Hence, this medicinal plant was validated with the main objectives to screening and analyses antimicrobial activity.

## METHODS

### Collection of samples

The fruits and leaves of *A. hirsutus* were collected from Kanyakumari district in Tamil Nadu and were authenticated in Botanical Survey of India, Tamil Nadu Agricultural University (TNAU), Coimbatore. Clinical isolates were collected from Aravind Eye Hospital, Coimbatore.

### Preparation of plant extract

The peel, whole fruit, and leaves of the selected plant were washed thoroughly and shade dried. The dried plant material was powdered. Then, each plant materials were extracted with two solvents such as acetone and ethanol. About 40 g of dried powder was suspended in 250 ml of ethanol and acetone as solvents. 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 extracts were subjected to preliminary phytochemical quantitative and quantitative screening for the presence or absence of various primary and secondary metabolites such as test for alkaloids - Mayer's, Wager'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 anthocyanins - Borntrager, test for tannins - 5% FeCl<sub>3</sub> gelatin, test for phenols - ferric chloride, test for terpenoids - Salkowski, test for carbohydrates - Molisch, Benedict, and test for leucoanthocyanin [16].

### Antimicrobial activity

Antimicrobial activity of *A. hirsutus* leaf and fruit were tested against the following bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Enterobacter* sp., and fungal strains such as *Aspergillus tamarii*, *Aspergillus fumigates*, *Aspergillus flavus*, and *Aspergillus niger*.

Antibacterial activity was carried out by standard disc diffusion method. Sterilized nutrient agar was poured aseptically into sterile petri plates and the plates were allowed to solidify at room temperature in a sterile condition. After solidification, the plates were evenly streaked with clinical sample onto the surface of the medium with a sterile cotton swab. The clinical isolates were *E. coli*, *S. aureus*, *Klebsiella* spp., and *Enterobacter* sp. The standard drug such as gentamycin was used for bacterial cultures. The antibiotic disc was placed in the agar plate using sterile forceps at one side then the paper was dipped with different concentration (50, 100, and 150 µl) of plant extracts was placed on another side. Then, the bacterial plates were incubated at 28°C for 24 hrs [17].

Antifungal activity was carried out by standard disc diffusion method. Sterilized potato dextrose agar was poured aseptically into sterile petri plates and the plates were allowed to solidify at room temperature in a sterile condition. After solidification, the plates were evenly streaked with clinical sample onto the surface of the medium with a sterile cotton swab. The clinical isolates were *A. tamarii*, *A. fumigates*, *A. flavus*, and *A. niger*. The paper was dipped with different concentration (50, 100, and 150 µl) of plant extract was placed in an agar plate. Nystatin antibiotic was used as a control. Then, the fungal plates were incubated at 37°C for 24 hrs [18].

## RESULTS AND DISCUSSION

### Phytochemical analysis

*A. hirsutus* plant is rich in major phytochemical compounds. The qualitative phytochemical analysis of fruit and leaf extract of *A. hirsutus* is presented in Table 1.

Qualitative analysis revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, phenols, terpenoids, and carbohydrates (Table 2).

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 [7]. 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. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, and tocopherols [19].

Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against a wide array of microorganisms *in vitro*. The plant extracts were also revealed to contain saponins which are known to produce an inhibitory effect on inflammation. They protect against hypercholesterolemia and antibiotic properties [6].

Tannins were known to possess general antimicrobial and antioxidant activities [20,21]. Saponins also have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include the formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties, and bitterness [22]. Steroids have been reported to have antibacterial properties and analgesic properties [23].

Table 1: Qualitative phytochemical screening of *A. hirsutus* leaf and fruit extract

Phytochemical	Test/reagent used	Leaf		Fruit	
		Acetone	Ethanol	Acetone	Ethanol
Alkaloids	Wagner's test	+	+	+	+
	Mayer's test	+	+	+	+
Flavonoids	Alkaline reagent	+	+	+	-
	Lead acetate test	+	-	-	-
	Ferric Chloride test	-	+	-	-
Glycosides	Borntrager's test	+	-	+	+
	Legal's test	+	+	+	+
	Killani test	+	-	+	+
	Liebermann's test	+	-	+	+
	Salkowski's test	+	+	-	+
Saponins	Foam test	+	+	+	+
Anthocyanins	Borntrager's test	-	-	-	-
Tannins	5% FeCl <sub>3</sub>	-	+	-	-
	Gelatin test	+	-	+	+
Phenols	Ferric chloride test	-	+	-	-
Terpenoids	Salkowski test	+	+	-	+
Carbohydrates	Molisch's test	-	-	-	-
	Benedict's test	+	+	-	+
Leucoanthocyanin	Borntrager's test	-	-	-	-

+: Presence, -: Absent, *A. hirsutus*: *Artocarpus hirsutus*

Table 2: Quantitative phytochemical screening in *A. hirsutus* leaf and fruit extract

Assay	Concentration (µg/ml)	Optical density (nm)			
		Leaf		Fruit	
		Ethanol	Acetone	Ethanol	Acetone
Total phenol content (mg/g)	100	1.91±0.02	1.58±0.01	1.19±0.01	0.63±0.01
	200	2.32±0.01	1.62±0.02	1.39±0.01	0.89±0.01
	300	2.61±0.01	2.05±0.01	1.76±0.01	1.43±0.01
Total tannin content (mg/g)	100	1.0±0.01	1.0±0.02	1.06±0.01	1.76±0.01
	200	1.3±0.01	1.87±0.01	1.49±0.01	1.98±0.01
	300	1.8±0.01	1.90±0.01	1.78±0.01	2.12±0.01
Total saponin content (mg/g)	100	0.65±0.01	0.24±0.01	0.26±0.01	0.48±0.01
	200	0.98±0.01	0.67±0.01	0.78±0.02	0.77±0.01
	300	1.45±0.01	1.23±0.01	1.14±0.01	1.27±0.01
Total flavonoid content (mg/g)	100	1.22±0.01	0.38±0.01	0.57±0.01	0.46±0.01
	200	1.30±0.01	0.46±0.01	0.60±0.01	0.51±0.02
	300	1.34±0.01	0.56±0.01	0.63±0.01	0.69±0.01
Total alkaloid content (mg/g)	100	0.03±0.01	0.98±0.01	1.40±0.01	0.64±0.01
	200	0.40±0.01	1.0±0.01	1.63±0.01	0.73±0.01
	300	0.55±0.01	1.11±0.01	1.84±0.01	0.99±0.02

All the values are means of three independent determinations n=3, analyzed in triplicate. Each value represents mean±SEM (n=3), one-way ANOVA was analyzed and followed by dunnett's multiple test for mean values showed differ significantly from each other (p<0.006), *A. hirsutus*: *Artocarpus hirsutus*

Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity and they protect against chronic disease [24]. The results obtained in this study thus suggest the identified phytochemical compounds are the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit [25]. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics [26]. Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria [27].

#### Determination of antimicrobial activity

Antimicrobial activity of fruit and leaf extract of *A. hirsutus* were tested against fungal and bacterial strains. Acetone extract isolated from both leaf and fruit of *A. hirsutus* have good active antibacterial activity. It exhibited good inhibition activity against *S. aureus*, *Klebsiella* spp., *Enterobacter* sp. Acetone extract showed the maximum zone of inhibition against *S. aureus* (55 mm). Ethanolic extract showed low inhibition activity against bacterial strains, respectively (Fig. 1 and

Table 3). Ethanolic extract isolated from both leaf and fruit of *A. hirsutus* have good active antifungal activity. It exhibited good inhibition activity against *A. tamarii*, *A. fumigates*, *A. flavus*, and *A. niger*. Ethanolic extract showed the maximum zone of inhibition against *A. tamarii* (20 mm) (Table 4).

The emergence of organisms resistant to nearly all classes of antimicrobial agents has become a serious public health concern in the past several years. It was considered as a potential source of natural or synthetic antimicrobial compounds with different molecular targets that control infections caused by microorganisms. Currently, out of eighty percentage of pharmaceuticals derived from plants, very few are being used as antimicrobials [27].

The potential in developing antimicrobials from plants lead to the development of phytomedicine to fight against pathogenic microorganisms [25]. Skin and nail infections caused by *Candida* sp. are one of the most common worldwide. Many researchers worked on crude plants extracts of folk medicinal plants and reported their effectiveness in controlling variety of microorganisms, worldwide. More than 10,000

Table 3: Antibacterial activity of *A. hirsutus* leaf and fruit extract

Bacterial strains	Concentration ( $\mu\text{g/ml}$ )	Standard drug	Antimicrobial activity (zone of inhibition, mm)			
			Leaf		Fruit	
			Ethanol	Acetone	Ethanol	Acetone
<i>Escherichia coli</i>	100	17	23	24	10	10
	200		25	20	13	12
	300		27	18	30	27
<i>Staphylococcus aureus</i>	100	28	28	35	30	31
	200		20	32	22	50
	300		25	30	27	55
<i>Klebsiella</i> spp.	100	19	18	18	13	18
	200		12	17	17	13
	300		29	25	25	26
<i>Enterobacter</i> sp.	100	12	17	10	7	15
	200		5	25	20	20
	300		15	15	22	24

*A. hirsutus*: *Artocarpus hirsutus*

Table 4: Antifungal activity of *A. hirsutus* leaf and fruit extract

Fungal strains	Concentration ( $\mu\text{g/ml}$ )	Standard drug	Inhibition zone diameter in mm			
			Leaf		Fruit	
			Ethanol	Acetone	Ethanol	Acetone
<i>A. tamarii</i>	100	7	10	8	25	15
	200		10	5	14	13
	300		16	15	21	20
<i>A. fumigates</i>	100	8	15	17	18	15
	200		10	5	14	13
	300		16	15	21	20
<i>A. flavus</i>	100	8	15	15	15	13
	200		13	10	17	12
	300		17	12	15	15
<i>A. niger</i>	100	9	15	12	15	15
	200		10	14	13	14
	300		14	10	15	13

*A. tamarii*: *Aspergillus tamarii*, *A. fumigates*: *Aspergillus fumigates*, *A. flavus*: *Aspergillus flavus*, *A. niger*: *Aspergillus niger*, *A. hirsutus*: *Artocarpus hirsutus*

active secondary metabolites have been isolated and identified among Indian medicinal plants. Many phytochemical preparations with high flavonoid and saponins contents have been reported to exhibit potential antimicrobial activity against pathogenic microorganisms [17].

The present study showed the significance of *A. hirsutus* plant has a valuable source of secondary metabolites [17]. Further, it revealed the antimicrobial activity against the fungal and bacterial strains.

## CONCLUSION

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. Antimicrobial activity of fruit and leaf extract of *A. hirsutus* were tested against fungal and bacterial strains. Acetone extract isolated from both leaf and fruit of *A. hirsutus* have good active antibacterial activity.

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