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International	Antimicrobial Activity and Phytochemical Ev Aqueous Extract of Artocarpus Hirsutus La	aluation of am. Bark	
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ABSTRACT The herbal based medicines are a part of Indian culture. Many plants are widely used based on our ethano-botanical knowledge in curing many disorders. The key advantage of herbal formulations is its low toxicity and side effects.			

Hence, there is a great demand for phyto-medicines in modern world. The current study prospects the antimicrobial efficacy of the aqueous extract of bark of Artocarpus hirsutus against selected microorganisms namely against E.coli, Pseudomonas aeruginosa and Bacillus subtilis. The aqueous extract was found very significantly inhibiting the E.coli and Pseudomonas even at low concentrations. The phytochemical analysis revealed that the extract possess alkaloids, tannins, Saponins etc which may be attributed to the antimicrobial effect of the extract. The bark of the Artocarpus hirsutus is less explored for its bio-potential and hence the work is a preliminary report with significant findings of its bio-potential.

KEYWORDS : Artocarpus hirsutus, antimicrobial, bark, aqueous extract, phytochemicals

INTRODUCTION

The modern medicines are formulated with high potential to cure many emerging diseases. However, these medicines possess many side-effects and harm the health of humans. There is a high demand for the herbal based formulation for curing these diseases. The major advantage of herbal formulations is that they are free from any side effects. India being a country with rich bioresource has plenty of herbal formulations. Trees and its parts are being widely used for curing many diseases in different forms. *Artocarpus* species are known for its large edible fruit with high nutritive values. Apart from the consumable fruits, extracts of aerial and underground parts have been used traditionally for curing diabetes, dermatitis, malarial fever, asthma, diarrhoea, anaemia, tapeworm infection, and many other diseases [1].

Among the Artocarpus sp., Artocarpus hirsutus Lam. an endemic species of southern Western Ghats commonly called as 'wild jack' is less explored for its medicinal potentials. It forms one of the major keystone species of Western Ghats [2,3].However, there is documentation of its medicinal properties in the third volume of Hortus Malabaricus, a book on the natural plant wealth of Asia. 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, relieve the pains of haemorrhage [1]. Its barks are used to cure diarrhea, pimples and ulcers [4].

In spite of new medicines, the demand for phyto-medicines is ever increasing. There is an increase in phytochemistry research in prospecting new drug molecules from many unexplored plants. In this research paper, *Artocarpus hirsutus* Lam. bark is being used for its antimicrobial evaluation. Phytochemical screening of the extract is also being carried out.



Figure 1: Artocarpus hirsutus Lam. tree bearing bright yellow fruits a) tree b) bark and c) fruits

Materials and Methods

Collection of Plant Material Bark samples of Artocarpus hirsutus Lam

Bark samples of *Artocarpus hirsutus* Lam. were collected from Ernakulam District of Kerala, India. The samples were shade dried and made into powder form using blenders.

Preparation of aqueous extract

10 g powder of *Artocarpus hirsutus* bark was subjected to Soxhlet extraction method. 10g of powder material was uniformly packed into the Soxhlet extractor and the extraction was performed overnight to obtain concentrated aqueous extract of the bark. Later the extract was filtered with the help of filter paper and water was evaporated from extract using a rotary evaporator and was stored at 4°C for further use.

Phytochemical Analysis of Extract

Phytochemical evaluation of the aqueous extract was performed using the standard procedures. Different phytochemicals, namely alkaloids, flavonoids, phenolics, glycosides, saponins, steroids, tannins and terpenoids were screened [5].

Agar diffusion assay

The modified agar well diffusion method was employed. 24hrs old bacterial cultures of *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were inoculated onto Muller Hinton agar media (HiMedia, Mumbai) by spread plate technique. 6mm diameter wells were made using sterile gel puncher. Aqueous bark extract of *Artocarpus hirsutus* was dissolved in DMSO (Dimethyl Sulfoxide) from which 50 and 75 µl of extract were added into agar wells. The plates were sealed and incubated at 32° C for 24 hr. The inhibition zone diameter was recorded after the incubation period. All the experiments were conducted in triplicates.

Determining MIC

The MIC value is defined as the lowest concentration of the sample extract, which inhibits the growth of a microorganism. The MIC value of the bark extract of *A. hirsutus* was determined by microtiter plate dilution method to observe the antimicrobial activity. Different concentration of extract was added into microtiter plate well along with fixed volume of culture. After 24 hrs incubation, the absorbance was read at 600 nm in a microtiter plate reader. The MIC study on aqueous bark extract showed its high efficacy against pathogenic microorganisms tested.

Results and Discussion

Phytochemical Analysis of Artocarpus hirsutus Bark Extract

Phytochemical analysis of the aqueous extract was performed (Table 1). The results revealed the presence of carbohydrates, proteins, amino acids, Saponins, terpenoids, alkaloids, cardiac glycosides and flavanoids. The phytochemical analysis of other parts of *A. hirsutus* was reported with many other solvents [**5**]. However, there were no reports on the phytochemical analysis of aqueous extract of the bark.

Table 1. Phytochemical	analysis	of Aque	ous barl	< extract
of Artocarpus hirsutus Lan	n.			

Phytochemicals	Status
Carbohydrates	+
Proteins	+
Amino acids	+
Saponins	+
Terpenoid	+
Alkaloids	+
Cardiac glycosides	+
Flavanoids	+

Antimicrobial Activity of Artocarpus hirsutus Bark Extract using Well Diffusion Method

Antimicrobial activity of Artocarpus hirsutus bark extract was evaluated using well diffusion assay. Pathogenic cultures namely *E.coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were used in the experiment. The spread plated Muller Hinton media were punched and added 2 different concentrations of aqueous bark extract and incubatedfor 24 hrs. The zone of inhibition was measured following incubation time (Figure 2).



The zone of inhibition was measured in mm and tabulated in Table 2. A higher inhibition was in case of *E.coli* and *Pseudomonas* at higher concentration of 75µg tested. This was compared with standard antibiotics Chloramphenicol. All the three cultures were resistant to erythromycin.

Antimicrobial activity of other parts of *Artocarpus hirsutus* were evaluated previously. However, this is the pioneer work on bark extract showing antimicrobial activity even at low concentration.

Antimicrobial activity of edible part of fruit was carried out against *Staphylococcus aureus* and *Klebsiella pneumonia*. Maximum zone of inhibition was observed compared with standard drug tetracycline [6].

MIC of Artocarpus hirsutus Bark Extract



Figure 3: MIC of Artocarpus hirsutus bark extract against selected microorganisms [Values represent Mean ±S.D of triplicate experiments

Table 2. Antimicrobial Activity of Aqueous bark extract of Artocarpus hirsutus Lam. compared with standard antibiotics

Microorgan- isms	Zone of Inhibition (mm)				
	Aqueous Extract (µg/ml) Chloramphenicol		Standard Antibiotics (µg)		
			Erythromycin		
	50	75	50	10	
E.coli	12 ± 0.17	14 ± 0.28	13 ± 0.54	NID	
B.subtilis	12 ± 0.31	13 ± 0.19	18 ± 0.12	NID	
P.aeroginosa	15 ± 0.21	17 ± 0.23	16 ± 0.26	NID	

[NID- Not Inhibition Detected; Values represent Mean ±S.D of triplicate experiments]

Figure 3 depicts that MIC of *Artocarpus hirsutus* bark extract against selected microorganisms. It shows that there was a good inhibition of the tested pathogens from 25 μ g to 50 μ g/ml concentrations. At maximum concentration of 150 μ g/ml there was almost a complete inhibition of the microbial growth and was found suitable as a antimicrobial agent.

Conclusion

This study concluded that the aqueous extract of bark of Artocarpus hirsutus is a suitable antimicrobial agent against E.coli, Pseudomonas and Bacillus sp. In case of E.coli and Pseudomonas there was significant inhibition observed even at low concentrations. The phytochemical analysis revealed that the extract possess alkaloids, tannins, Saponins etc which may be attributed to the antimicrobial effect of the extract. This is a first report on Biopotential activity of aqueous bark extract of Artocarpus hirsutus.

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