Phytochemical and antibacterial activity of *Artocarpus heterophyllus* Lam. and *Artocarpus communis* Forst. on *Bacillus subtilis* and *Pseudomonas fluorescens*

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Abstract

Antibacterial effect of *Artocarpus heterophyllus and Artocarpus communis* in leaf and bark were studied against *Bacillus subtilis* and *pseudomonas fluorescens* and its effect was then compared with the standard. Phytochemical screening was done by using water and methanol extracts of leaf and bark of both the plants. methanol extracts of *A.heterophyllus* bark and *A.communis* leaf and bark extracts showed the best antibacterial activity; and hence they can be further subjected to isolation of the therapeutic antimicrobials and for the further phytochemical and pharmacological studies.

Key words

Artocarpus species, Phyto chemicalanalysis, Anti bacterial activity

INTRODUCTION

India is endowed with a rich wealth of medicinal plants. These plants have made a good contribution to the development of ancient Indian Materia Medica. India is one of the 12 mega diversity centers of the world and the richest country in plant wealth as well as in medicinal plants heritage. Human beings have been utilizing plants for their basic preventive and creative health care since time immemorial. A recent estimate suggests that over 9,000 plants have been known to medicinal applications in various cultures and countries, and this is without having conducted compressive research amongst several indigenous and other communities.

Traditional medicines are used by about 60 percent of the world's population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. Herbal medicines are derived from plants or some other natural sources. Plant kingdom is the unlimited resource of extra ordinary variety of compounds which are commonly called as primary and secondary metabolites. The organic compounds such as carbohydrates, proteins, fats, membrane lipids, nucleic acids, chlorophylls etc are found throughout the plant kingdom and are central to metabolisms of plants. These compounds are known as primary metabolites. Apart from these substances many plants particularly of certain genera and families synthesis a number of organic compounds in them, which are not in the main stream of metabolism. These are chemically diverse compounds as secondary metabolites (Kordono et al., 1990) and which include such well known substances such as alkaloids, glycosides, terpenes, sterols, tannis, flavanoids, phenols and resins etc. At present people have realized the efficacy of herbal remedies and their valuable contributions in the treatment of various diseases. Plants compounds of historical importance are still using include atropine, reserpine, colchicines etc. (Kapoor 1990).Their importance in making medicinal drugs, flavoursand industrial materials on commercial scale is well established.

The present study deals with the phytochemical and

antimicrobial studies on *Artocarpus heterophyllus and Artocarpus communis* belongs to the family Moraceae, using gram +ve and gram -ve bacteria *Bacillus subtilis and pseudomonas fluorescens* respectively.

MATERIALS AND METHODS

Plant materials selected for the present study were tree members belonging to the family moraceae namely *Artocarpus heterophyllus Lam.* and *Artocarpus communis Forst* . Microorganisms selected were *Bacillus subtilis* a gram positive bacteria and *pseudomonas fluorescens* a gram negative bacteria.

Plant collection and extraction

Artocarpus heterophyllus and Artocarpus communis were collected from the regions of puthenchira,Thrissur district,Kerala .After that the plant parts such as leaf and bark were coarsely powdered and subjected to successive solvent extraction using soxhlet apparatus.

Phytochemical screening

Qualitative phytochemical screening with the extract of both the plants *Artocarpus heterophyllus* and *Artocarpus communis* was determined as follows: Carbohydrates(Anthrone method),Alkaloids(200 mg plant material in 10 ml methanol ,filtered); a 2ml filtrate + 1%HCL + steam,1 ml filtrate+6 drops of Mayor's reagent/Wagner's reagent/Dragendroff reagent,creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Flavanoids (200 mg plant material in 10 ml ethanol,

filtered) ; a 2 ml filtrate + conc. HCL+ magnesium ribbon pink-tomato red colour indicated the presence of falvanoids.Tannins, (200 mg plant material in 10 ml distilled water , filtered): a 2ml filtrate + 2 ml $FeCl_3$, blue black precipitate indicated the presence of tannins. Glycosides(Keller-Killani test: 2 ml filtrate+ 1 ml glacial acetic acid + $FeCl_3$ + conc.H_2SO_4); green - blue colour indicted the presence of glycosides. steroids(Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered);a 2ml filtrate +2 ml acetic anhydride +conc.H_2SO_4. blue ring indicated the presence of terpenoids, Saponins(frothing test: 0.5 ml filtrate+ 5 ml distilled water); frothing persistence indicated presence of saponins. Anthraquinones- 2 ml of plant extracts were treated with 1 ml of dilute ammonia and shaken vigorously. Pink red colour in the ammonical layer indicates the presence of anthraquinones. Cardiac glycosides (Keller-Killani test) were analysed. Anti microbial screening were carried out in nutrient agar media.

Standard used as ampicillin is β-lactum antibiotic that has been used extensively to treat bacterial infectons since 1961. It belongs to the penicillin group of β-lactum antibiotics and acts as a competitive inhibitor of the enzyme transpeptidase, which is needed by bacteria to make their cell wall..

Antisensitivity tests were performed by agar - well diffusion method Cole ,1994 ; Espinol-Ingroff et al ,1995; Okeke et al ,2001]. Different plant extracts were prepared and reconstituted in specific solvent systems used

and 200 micro liter extracts was dispensed in to each of the wells with the aid of a Pasteur pipette .The zone of inhibition was calculated by measuring the diameter of their inhibition zone around the well (in mm) including the well diameter. The readings were taken in two different fixed directions and the average values were calculated.

RESULTS AND DISCUSSION

Antibacterial effect of *Artocarpus heterophyllus* and *Artocarpus communis* in (Leaf and bark) were studied against *Bacillus subtilis* and *Pseudomonas fluorescence*. Antimicrobial effect was then compared with standard as antibiotic Ampicillin.

Phytochemical screening was done by using water and methanol extracts of leaf and bark of both the plants. The water extract of *Artocarpus heterophyllus* leaf and bark showed the presence of glycosides, terpenoids and in addition alkaloids, saponins were also found in the bark extract. While the methanol extract of leaf showed flavanoids, phenols, glycosides, and terpenoids and its bark showed above all these compounds alkaloids, tannins, steroids, saponins and anthraquinone except cardiac glycosides.

In *Artocarpus communis* water extract of leaf contains phenols, glycosides, terpenoids, saponins and its bark shows the presence of tannins, steroids, anthraquinones, glycosides and terpenoids. Methanol extract of leaf shows tannins, steroids , phenols, glycosides, terpenoids and anthraquinones while bark showed the presence of these some compounds except phenols .

Accordingto E.S.Karthy et al.,(2009)

ethanol,methanol,acetone,chloroform and petroleum ether seed extracts of four different plants were assed for antibacterial activity against Multidrug Resistant-Methicillin Resistant *S.aureus* (MDR-MRSA).. However it is interesting to note that *A.heterophyllus* which have traditionally been used for antibacterial activity, indicates that the active compounds are mainly distributed in aerial parts, roots and rhizomes but not in seeds. In the present study leaf and bark exacts were tested against *B.subtilis* and *P.fluorescens.*

Antibacterial activity of water extract of *A.heterophyllus* leaf showed inhibition zone of 8.5mm in *B.subtilis* and 6.5mm in *P.fluorescens*. Methanol extract showed inhibition zone of 9.5mm in *B.subtiis* and 6.5mm in *P.fluorescens*.(Table-3). In the case of water extract of bark shows the inhibition zone of 7mm in both *B.subtilis* and *P.fluorescens.* Methanol extract shows the inhibition zone 20mm in *B.subtilis* and 10mm in *P. fluorescens.*T he above results indicate that the water extract of *A.heterophyllus* leaf and bark shows very little effect on both the bacteria, *B.subtilis* and *P.fluorescens*. While its methanol extract of leaf shows little effect on both bacteria but its bark extract was more effective towards *B.subtilis* than any other extracts used. Jigna parekh and Sumithra V. Chand (2008) conducted antibacterial activity of aqueous and alcoholic extracts of 34 Indian medicinal plants belonging to 28 different families including *Artocarpus communis* against three *staphylococcus species*, namely s*taphylococcus aureus* ,*staphylococcus*

epidermidis and *staphylococcus subflava.*

Water extract of *A.communis* leaf shows the inhibition zone of 6.5mm in both *B. subtilis* and *P.fluorescens*. Methanol extract of leaf have inhibition zone 15mm in *B.subtilis* and 9.5mm inhibition zone in *P.fluorescens*. (Table-5), Water extract of bark shows the inhibition zone 8mm in *B.subtilis* and 7.5mm in *P.fluorescens*. Its methanol extract shows the inhibition zone 13mm in *B.subtilis* and 9mm zone of inhibition in *P.fluorescens*.(Table-6).

A.communis the leaf and bark extract in water shows very little effect towards both bacteria but Its methanol extract of leaf and bark showed more effectiveness towards both the bacteria . Effective gradient sensitivity was noted for *B.subtilis* in all methanol extracts of both the medicinal plants. The standard antibiotic Ampicillin showed comparatively higher sensitivity than plant extracts on both Gram positive *B. subtilis* and Gram negative *P.fluorescens*. M. R. Khan *et al.*, (2003) conducted antibacterial activity of *Artocarpus heterophyllus* in methanolic extracts of stem, root barks, stem and root -heart wood, leaves, fruits and seeds and their subsequent partitioning with petrol, dichloromethane, ethyl acetate and butanol gave fraction that exhibited a broad spectrum of antibacterial activity.

The bacterial activity of 34 Indian plants against seven members of Enterobacteriaceae were noticed, none of the aqeous extracts(except one or two) produced zones of inhibition(Parekh and Chanda S,2007).This might have resulted from the lack of solubility of the

active constituents in aqueous solutions. In this study also water extract of leaf and bark of *A. heterophyllus* and *A. Communis* shows less antibacterial activity against both the bacteria *B. subtilis* and *P. fluorescens.* Alternatively, in insufficient quantities in the crude extracts to show activity with the dose levels employed (Taylor et al., 2001). Methanolic extracts, on the other hand, showed some activity. Maximum antibacterial activity was shown by bark extracts of *A. heterophyllus* (20mm) and *A. communis* (13mm) towards Gram positive *B. subtilis*.

From the screening experiment, methanol extracts of *A. heterophyllus* bark and *A. communis* leaf and bark extracts showed the best antibacterial activity; and hence they can be further subjected to isolation of the therapeutic antimicrobials and for the further pytochemical and pharmacological studies that may open the possibility of finding new clinically effective antimicrobial compounds. The important of the traditional medicine which involves the use of plant extract is very significant for the common ailments which are of uncomplicated nature, herbal medicine is the best answer.

TABLES

The data of the preliminary phytochemical screening were shown in tables 1&2.The results of antimicrobial activities are given in table 3, 4, 5,6 & 7.

Table 1- preliminary phytochemical screening of *Atrocarpus heterophyllus*.

SL.NO.	PHYTOCHEMICAL TEST	EXTRACTS			
				LEAF	
		WATER	METHANOL	WATER	METHANOL
1	ALAKALOIDS	–	–	+	+
2	TANNINS	–	–	–	+
3	FLAVANOIDS	–	–	–	+
4	STEROIDS	–	–	–	+
5	PHENOLS	–	+	–	+
6	GLYCOSIDS	+	+	+	+
7	TERPENOIDS	+	+	+	+
8	ANTHRAQUINONES	–	–	–	+
9	SAPONINS	–	–	+	+
10	CARDIAC GLYCOSIDES	–	–	–	–

Table 2- preliminary phytochemical screening of *Atrocarpus communis*.

		EXTRACTS		
				LEAF

SL. NO.	PHYTOCHEMICAL TEST	WATER	METHANOL	WATER	METHANOL
1	ALAKALOIDS	–	–	–	–
2	TANNINS	–	+	+	+
3	FLAVANOIDS	–	–	–	–
4	STEROIDS	–	+	+	+
5	PHENOLS	+	+	–	–
6	GLYCOSIDS	+	+	+	+
7	TERPENOIDS	+	+	+	+
8	ANTHRAQUINONES	–	+	+	–
9	SAPONINS	+	–	–	–
10	CARDIAC GLYCOSIDES	–	–	–	–

Table 3- shows zone of inhibition (in mm) in different extracts of *A. heterophyllus* leaf against two bacterial pathogens.

EXTRACTS	ORGANISMS					
	Bacillus subtilis			*Pseudomonas fluroscence*		
		Zone diameter in mm.			Zone diameter in mm.	
WATER	Well 1	Average		Well 1	Average	
		8 8	8	8.5	6 7	6.5
	Well 2 9 9	9			Well 2 7 6	6.5
METHANOL	Well 1 10 9	9.5	9.5	Well 1 6 7	6.5	6.5
		Wel l 2 10		9.5		

		9				
CONTROL Distilled Water	Nil	Nil	Nil	Nil	Nil	Nil
CONTROL Methanol	Nil	Nil	Nil	Nil	Nil	Nil

Table 4- shows zone of inhibition (in mm) in different extracts of *A.heterophyllus* bark against two bacterial pathogens.

EXTRACTS	ORGANISMS					
WATER	*Bacillus subtilis*			*Pseudomonas fluroscence*		
		Zone diameter in mm.			Zone diameter in mm.	
	Well 1	Average		Well 1	Average	
		7 7	7	7	7 7	7
	Well 2 7	7			Well 2 7	7

	7				7	
METHANOL	Well 1 20 20	20	20	Well 1 11 11	11	10
		Wel 1 2 20 20		20		
CONTROL Distilled Water	Nil	Nil	Nil	Nil	Nil	Nil
CONTROL Methanol	Nil	Nil	Nil	Nil	Nil	Nil

Table 5- shows zone of inhibition (in mm) in different extracts of *A. communis* leaf against two bacterial pathogens.

EXTRACTS	ORGANISMS	
	Bacillus subtilis	*Pseudomonas fluroscence*

WATER		Zone diameter in mm.			Zone diameter in mm.	
	Well 1	Average		Well 1	Average	
		6 7	6.5		7 7	7
	Well 2 7 6	6.5		6.5	Well 2 6 6	6
METHANOL	Well 1 15 15	15	15	Well 1 12 10	11	9.5
		Wel l 2 16 14		15		
CONTROL Distilled Water	Nil	Nil	Nil	Nil	Nil	Nil
CONTROL Methanol	Nil	Nil	Nil	Nil	Nil	Nil

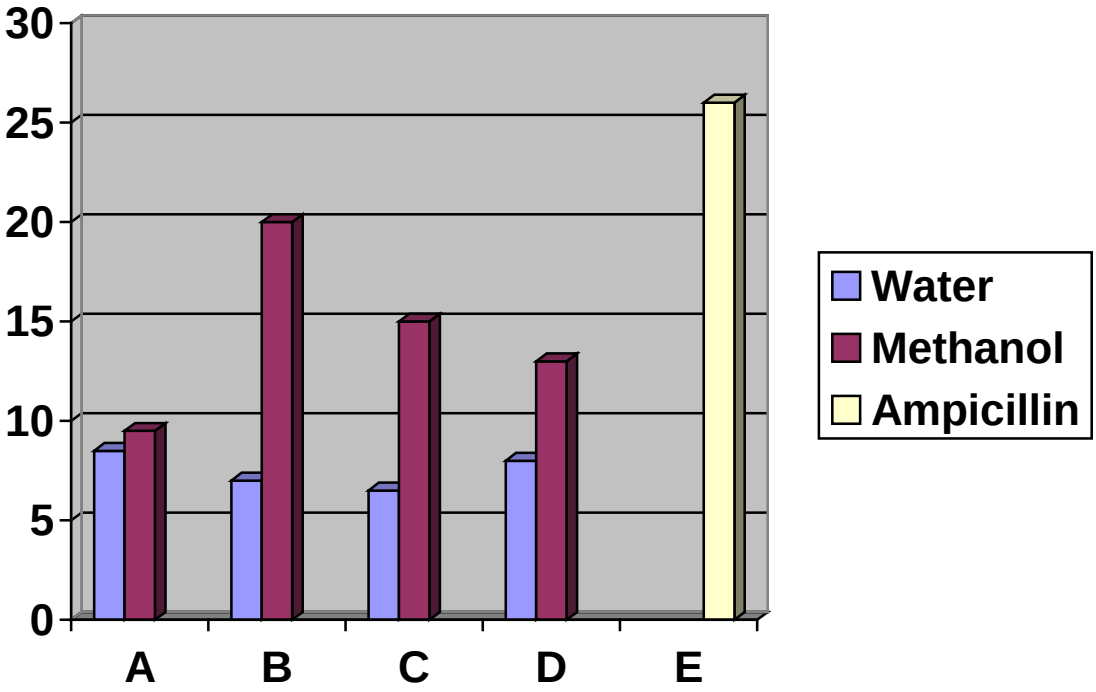
Table 6- shows zone of inhibition (in mm) in different extracts of *A. communis* bark against two bacterial pathogens.

EXTRACTS	ORGANISMS					
	Bacillus subtilis			*Pseudomonas fluroscens*		
		Zone diameter in mm.			Zone diameter in mm.	
WATER	Well 1	Average		Well 1	Average	
		8 8	8		8 6	7
	Well 2 8 8	8		8	Well 2 8 8	8
METHANOL	Well 1 13 11	12	13	Well 1 8 9	8.5	9
		Wel 1 2 16 12		14		
CONTROL Distilled Water	Nil	Nil	Nil	Nil	Nil	Nil
CONTROL Methanol	Nil	Nil	Nil	Nil	Nil	Nil

Table 7- The data showing sensitivity of the test organism against Anti biotic Ampicillin.

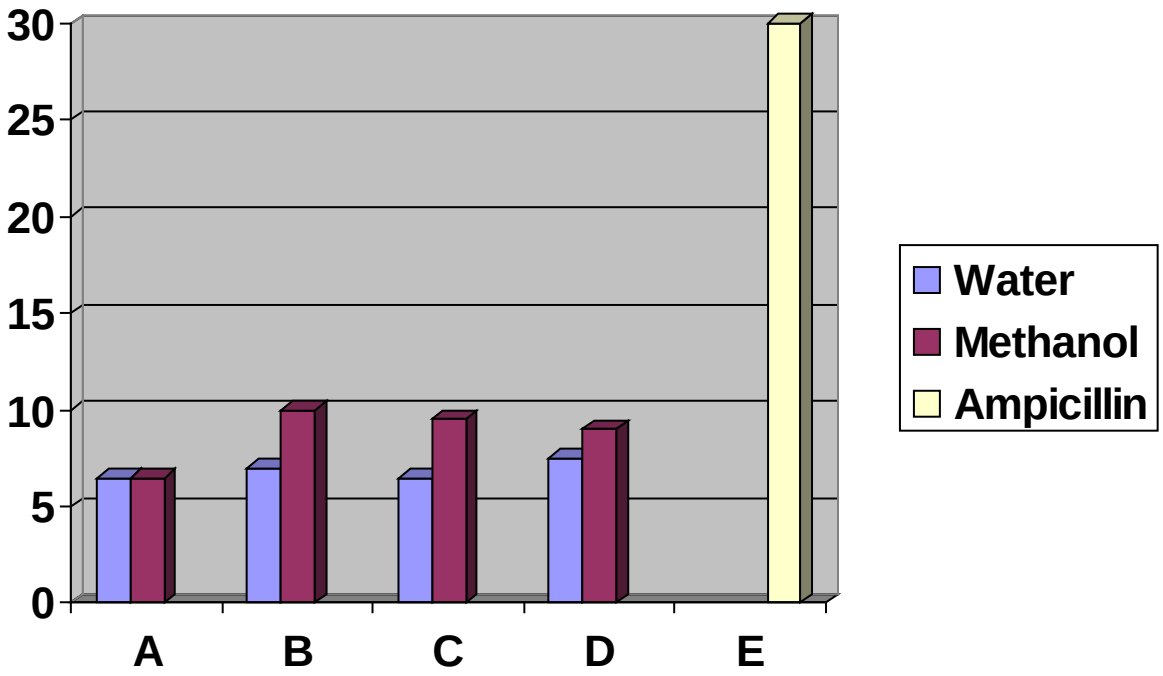
Name of the antibiotic	Organisms					
		Bacillus subtilis(zone of inhibition in mm.)			*Pseudomoas fluorescens* zone of inhibition in mm.)	
Ampicillin	Well 1 26 26	26	Avg. 26	Well 1 30 30	30	Avg. 30
		Well 2 26 26		26		
CONTROL Distilled Water	Nil	Nil	Nil	Nil	Nil	Nil
CONTROL Methanol	Nil	Nil	Nil	Nil	Nil	Nil

Data showing antimicrobial activity of Plant extracts and Antibiotic towards *Bacillus subtilis*.



A - *A. heterophyllus* Leaf
B - *A. heterophyllus* Bark
C - *A. communis* Leaf
D - *A. communis*
E - Ampicillin

Data showing antimicrobial activity of Plant extracts and Antibiotic towards *Pseudomonas fluorescens*.



A – *A. heterophyllus* Leaf
B – *A. heterophyllus* Bark
C – *A. communis* Leaf
D – *A. communis* Bark
E – Ampicillin

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