

Phytochemical Screening and Antimicrobial Activity of *Artocarpus heterophyllus* and *Artocarpus altilis* Leaf and Stem Bark Extracts

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Abstract

Increasing drug resistance of pathogens and negative consequences of antibiotic usage has led to the search for alternative medicines from nature. Many plants have been exploited to cure infectious diseases from time immemorial. The present investigation evaluated the antimicrobial and phytochemical properties of *Artocarpus heterophyllus* i.e. Jack fruit (Kos in Sinhala) and *Artocarpus altilis* i.e. Bread fruit (Dhel in Sinhala) leaf and stem bark extracts. Hexane, dichloromethane and ethanol were used as extraction solvents and test organisms were *Escherichia coli*, *Micrococcus luteus*, *Aspergillus niger* and *Trichoderma* sp. A disc diffusion test was adopted to test the susceptibility of the selected microbes to the extracts while Minimum inhibitory concentration (MIC) was determined using serial dilution of extracts. Phytochemical screening was carried out by specific chemical identification tests. Bioassay data were statistically analyzed using two-way ANOVA (SPSS 20 at 95% confidence level). Ethanolic stem bark extracts (30mg/ml) of *A.heterophyllus* and *A.altilis* possessed significant antibacterial activity against *Escherichia coli* with 9.50 ± 0.44 mm and 7.49 ± 0.28 mm inhibition zone radii respectively. Dichloromethane extracts of leaf and stem bark showed lesser antibacterial activity against both of the bacteria with inhibition zones of 3.00 ± 0.34 mm to 5.66 ± 0.16 mm while hexane extracts did not show any antibacterial activity. Antifungal activity on the other hand was not detected in any of the extracts. Bacterial antibiotic tetracycline and fungal antibiotic ketoconazole which were used as positive controls were more effective even at 1/10 concentration compared to all the plant extracts tested. Phytochemical screening confirmed the presence of phytosterols, anthraquinone, terpenoids, phenols, glycosides, flavonoids and diterpenes in both of the trees. These results confirm the potential antibacterial activity of *A.heterophyllus* and *A.altilis*

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stem barks and the presence of medicinally important phytochemicals in aerial parts of the plants.

Keywords: Artocarpus heterophyllus, Artocarpus altilis, Antimicrobial, Phytochemical, Plant extracts, ethnomedicinal

Introduction

According to the World health organization (2012) the evolving public health threat of antimicrobial resistance is driven by both appropriate and inappropriate use of anti-infective medicines. Kumaraswamy *et al.* (2008) state that the biochemical molecules of plant origin appear to be a suitable alternative to overcome the problems caused by antibiotic resistant pathogens.

A wide range of plants express complex mixtures of secondary metabolites within each species (Arguedas & Coley, 2005). Phenols, terpenoids, flavonoids, glycosides, tannins, alkaloids, steroids, saponins and resins are some important phytochemicals found in plants (Tiwari *et al.* 2011). An estimated 74% of pharmacologically active plant derived components were discovered after following up on ethnomedicinal use. Thus, medicinal plants can be regarded as the richest bio-resource of drugs of modern medicine, folk medicine and chemical entities or templates for synthetic drugs (Joshua & Takudzwa, 2013). Further, the discovery of medically important metabolites in common and abundant plants would minimize over exploitation of well known, rare medicinal plants.

Sri Lanka is recognized as a biodiversity hotspot as a great variety of flora and fauna inhabit the wide range of natural ecosystems found within the country (Pethiyagoda, 2005). In Sri Lanka, *Artocarpus heterophyllus* (Jack) and *Artocarpus altilis* (Dhél) fruits are a popular food among the locals. *A. heterophyllus* bark and fruit are medicinally used to treat sprains, fractures, diabetes and are also used for laxative effect of abdomen and to increase the breast milk production in nursing mothers (Fernando, 2003). Almost all the parts of *A. heterophyllus* and *A. altilis* are utilized to treat several infections and other ailments in the Caribbean islands, Polynesian islands, Taiwan, Malaya, Java, and Borneo (Deivanai and Bhore 2010; Jitendra *et al.* (2014). However, the usage of *A. heterophyllus* and *A. altilis* to treat infections has not been reported in Sri Lanka.

Satish *et al.* (2008) conducted an antimicrobial assay to test the efficacy of leaf extracts of 46 plants including *A. heterophyllus*

against 14 pathogenic bacteria including *E.coli*. Aqueous extract of *A.heterophyllus* leaf didn't inhibit any of the test pathogens. Another study showed that the silver nanoparticles synthesized using an aqueous leaf extract of *A.heterophyllus* as a reducing agent, increased the inhibitory effect of six antibiotics against *Salmonella paratyphi* and *Klebsiella pneumonia*. In addition, it was reported that, among the 54 chemical components of the aqueous leaf extract, 37.4% were alcohols and 32.2% were carboxylic acids (Thirumurugan *et al.* 2010).

Kuete *et al.* (2011) tested the effectiveness of crude methanolic extract of *Artocarpus communis* (Synonym for *A.altilis*) stem bark and terpenoids and flavonoids isolated from the bark against selected bacteria and fungi. Lowest MIC value recorded for crude extract against *E.coli* was 64 µg/ml. Lowest MIC value of 32 µg/ml was recorded for one flavonoid compound against *P.aeruginosa* which was more effective than the antibiotic chloramphenicol (64 µg/ml). Raman *et al.* (2012) and Mohanty and Pradhan (2014) reported that the methanolic and ethanolic leaf extracts of *A.altilis* were highly effective against some selected gram negative and gram positive bacteria including *E.coli* and *M.luteus* while the aqueous extracts (100 mg/ml, 10 µl per disc) were found to be the least effective.

Binumol & Sajitha (2013) state that the methanolic extracts of *A.heterophyllus* bark and *Artocarpus communis* leaf and bark extracts showed effectiveness against *Bacillus subtilis* and *Pseudomonas fluorescens* while aqueous extracts did not show considerable activity against test organisms. Mbaeyi Nwaoha & Onwuka (2014) investigated the antimicrobial properties and phytochemical composition of *A.altilis* leaf extracts using ethanol, n hexane and water. The ethanol extracts (at MIC 5 mg/ml) were more effective against bacteria including *E.coli* and fungi including *A.niger* compared to the other solvents. n hexane extracts inhibited all the test organisms (at MIC 8 mg/ml) while water extracts didn't inhibit any of the test organisms.

In addition to the antimicrobial activity of *A.heterophyllus*, anti inflammatory, anti oxidant, anti cholinergic, anti diabetic, immune modulatory effect, inhibition of protease, oestrogen regulation and inhibition of melanin biosynthesis have also been reported through several pharmacological research investigations of the plant parts (Jitendra *et al.* 2014). *A.altilis* has been found to exhibit antitubercular, antiplasmodial, antiatherogenic, antioxidant, antiausteric, antihypertensive, antihelmintic and skin lightening effects (Sikarwar *et al.* 2014).

Although many studies have focused on the antibacterial activity of *A.heterophyllus* and *A.altilis* extracts, little attention has been given to antifungal activity. Further, while *A.heterophyllus* (Jak fruit) and *A.altilis* (Bread fruit) (Moraceae) are very common trees in Sri Lanka and popular as a source of food, their antimicrobial potential has not yet been locally utilized.

Therefore, the present study was conducted to examine the antifungal activity of *A. heterophyllus* and *A.altilis* in addition to its antibacterial activity, and to ascertain the phytochemical constitution of these two trees which are extensively grown throughout Sri Lanka.

Methodology

Aseptic techniques and universal lab safety protocols were followed throughout all experiments.

Plant Collection

Healthy fresh leaves and bark of both *A.heterophyllus* and *A.altilis* were collected from Colombo and Kalutara districts of Sri Lanka, cleaned, surface sterilized using 70% ethanol, washed with distilled water and allowed to shade dry for seven days at room temperature. Dried plant material was cut into small pieces and ground using an electric grinder (JENCONS-Osterizer) to obtain small sized material (approximately 2-4 mm in size) as reported by Karthy *et al.* (2009).

Plant Extraction

Extracts were prepared by maceration of the powdered samples (10 g each) in 100 ml of 95% ethanol, 99% dichloromethane or 99% hexane (A/R Grade) with intermittent shaking (mrc, TS-400 orbital shaker) for three days at room temperature. Extracts were filtered using Whatman grade 1 filter paper and were concentrated by rotary evaporator (IKA® RV 05 basic) at 45°C. Concentrated extracts were weighed to find the extraction efficiency on dry weight basis. All the twelve extracts were stored at 4°C until further use as described by Karthy *et al.* (2009).

Extraction efficiency was calculated as follows;

Extraction efficiency % = (Final dry weight of extract / Initial weight of dried plant material) x 100 (Shilpakar *et al.* 2011).

Microbial Cultures

Escherichia coli, *Micrococcus luteus* and fungal strains *Aspergillus niger* and *Trichoderma sp* were obtained as stock cultures from the Department of Botany, The Open University of Sri Lanka. Streaking of bacterial cultures was done and pure cultures were obtained from single colony isolation and were stored in Nutrient agar (NA) while pure cultures of fungi were obtained from a single spore isolate obtained from a spread plate. These were stored in Potato Dextrose Agar (PDA) and were stored at 4°C until further use (Kuetze *et al.* 2011). Sub culturing of bacteria and fungi was done at two weeks intervals.

Antimicrobial Assay

Agar disc diffusion test was adopted to test the antimicrobial activity of extracts as described by Hasan *et al.* (2009). Stock solutions of extracts were prepared in 30 mg/ml concentration by dissolving dried plant extract in its respective extraction solvent. 3 mg/ml stock solutions of antibacterial antibiotic tetracycline and antifungal antibiotic ketoconazole were prepared using sterile distilled water and served as positive controls.

Sterile discs (Whatman grade 1 filter paper, each 6mm diameter discs) were separately loaded with 50 µl of plant extracts, positive control (Antibiotics Tetracycline against bacteria and Ketoconazole against fungi) and extraction solvents which served as negative control (95% Ethanol, 99% dichloromethane and 99% hexane). All discs treated as above were left to dry overnight under sterile conditions and then placed in spread plates prepared from bacterial cell or fungal spore suspensions.

Bacterial cell suspensions were prepared from 24 hour cultures of bacteria by diluting a small amount of inocula in sterile distilled water. Turbidity was compared to match the 0.5 Mcfarland standard (approximately 1.5×10^8 cfu/ml) ensuring that the cell concentration of the microbial suspension used to prepare each spread plate would be approximately 1.5×10^8 cfu/ml (Tiga, 2011).

Fungal spore suspensions were prepared by flooding 7 day old fungal culture plates with sterile distilled water dislodging the spores with a sterile glass rod and filtering through muslin cloth. Total number of fungal spores in the suspension was counted using a

haemocytometer (NEUBAUER improved HBG) and was adjusted to 1×10^6 spores/ml by diluting the suspension (Rana *et al.* 2011).

Preparation of Culture Plates

NA and PDA were used to culture bacteria and fungi respectively. Spread plates of the microorganisms were prepared using 100 μ l of microbial suspension prepared as indicated above. Discs treated as described above were transferred to the agar plates after the microbial suspension had been absorbed onto the agar. Plates were incubated at 37°C, 24 h and 25°C, 72 h for bacteria and fungi respectively. After the incubation period, radii of the clear zones were measured as described by Hasan *et al.* (2009). The Bio assay was repeated three times and mean values were obtained.

Determination of MIC

MIC was determined using the serial agar dilution method (Hasan *et al.* 2009). The extracts which showed good activity in the antimicrobial assay were selected to determine the MIC. Serial dilutions (0.5, 1, 2 and 3 mg/ml) of each selected extract were prepared using sterile distilled water. Appropriate amounts of Nutrient broth (5 ml) and selected 24 hours old standardized bacterial suspensions (10 μ l) were added to each of the extract dilutions and the whole set up was incubated at 37°C, 24 h. The tube with the lowest concentration which showed no visible growth was considered as the MIC for that particular microbe.

Statistical Analysis

Results of the bio assay were represented as mean \pm SD of three replicates in each test. An analysis of variance (Two – way ANOVA) with type III sums of squares was performed using SPSS 20 software at 95% confidence interval.

Phytochemical Screening

Presence or absence of selected phytochemicals: phytosterols, anthraquinones, terpenoids, phenols, glycosides, flavanoids, diterpenes, tannins and alkaloids in each extract was determined by performing qualitative chemical tests as described by Tiwari *et al.* (2011), Shilpakar *et al.* (2011) and Sasidharan *et al.* (2011).

Results

Extraction Efficiency (on Dry Weight Basis)

Extraction efficiency was higher in leaf extracts compared to the bark extracts except the hexane extract of *A.heteophyllus* in which bark extraction efficiency was higher than that of the leaf. Highest extraction efficiency of 22.88% was obtained in ethanolic leaf extract of *A.heterophyllus* whereas least extraction efficiency of 0.84% was obtained in ethanolic bark extract of *A.altilis* (Table 1).

Table 1. Percentage (%) Extraction efficiency of *A.heterophyllus* and *A.altilis* leaf and stem bark by different extraction solvents (on dry weight basis)

	95% Ethanol	99% Dichloromethane	99% Hexane
<i>A.heterophyllus</i>			
Leaf	22.88	12.17	5.29
Bark	5.17	3.98	9.54
<i>A.altilis</i>			
Leaf	7.98	8.89	18.62
Bark	0.84	8.30	15.04

Antimicrobial Assay

Highest inhibition zone radius of 9.50 ± 0.44 mm was found in the ethanol extract of *A.heterophyllus* bark against *E.coli* followed by the ethanolic extract of *A.altilis* bark (7.49 ± 0.28 mm) against *E.coli*. Dichloromethane extracts showed moderate activity against test bacteria, the exception being the dichloromethane leaf extract of *A.heterophyllus* against *M.luteus* where no inhibition was noted. None of the hexane extracts of both trees showed any activity against tested bacteria. Tetracycline was generally more effective (except ethanolic extract of *A. altilis* bark against *E. coli*) against both *E.coli* and *M.luteus* than the plant extracts even at 1/10th concentration (Table 2).

Table 2. Mean inhibition zone radius \pm SD in mm of *A.heterophyllus* and *A.altilis* leaf and stem bark extracts against *E.coli* and *M.luteus*

Treatment		<i>E.coli</i>			<i>M.luteus</i>		
		95% ETOH	99% DM	99% HEX	95% ETOH	99% DM	99% HEX
<i>A.heterophyllus</i> (Each disc was loaded with 50 μ l of 30 mg/ml concentrated extract)	Leaf	0.00	3.89 \pm 0.20	0.00	0.00	0.00	0.00
	Bark	9.50 \pm 0.44	4.33 \pm 0.29	0.00	0.00	3.00 \pm 0.34	0.00
<i>A.altilis</i> (Each disc was loaded with 50 μ l of 30 mg/ml concentrated extract)	Leaf	0.00	3.33 \pm 0.33	0.00	4.16 \pm 0.16	4.49 \pm 0.28	0.00
	Bark	7.49 \pm 0.28	5.66 \pm 0.16	0.00	5.44 \pm 0.53	4.16 \pm 0.16	0.00
Solvent (50 μ l) (Negative control)		0.00	0.00	0.00	0.00	0.00	0.00
Tetracycline (3 mg/ml, 50 μ l) (Positive control)		6.78 \pm 0.25	7.00 \pm 0.00	6.95 \pm 0.21	6.72 \pm 0.35	6.89 \pm 0.10	6.72 \pm 0.26

ETOH = Ethanol, DM = Dichloromethane, HEX = Hexane, SD = Standard deviation

None of the extracts of *A.heterophyllus* and *A.altilis* showed any antifungal activity against *A.niger* or *Trichoderma* sp. Antifungal antibiotic ketoconazole was effective against both of the fungi, while negative control solvents did not inhibit fungi.

Minimum Inhibitory Concentration (MIC)

The four extracts which showed the highest inhibition zone radius in the disc diffusion test were selected for the MIC determination test. Ethanolic bark extracts of *A.heterophyllus* and *A.altilis* inhibited *E.coli* in all concentrations tested. Dichloromethane bark extract of *A.altilis* inhibited *E.coli* in 2 mg/ml and 3 mg/ml concentrations.

Ethanolic bark extract of *A.altilis* inhibited *M.luteus* in all concentrations except 0.5 mg/ml (Table 3).

Table 3. Presence or absence of visible microbial growth in treatment tubes as an indication of the MIC of selected extracts

Concentrations Extracts	0.5 mg/ml	1 mg/ml	2 mg/ml	3 mg/ml
Ethanol – <i>A.heterophyllus</i> bark against <i>E.coli</i>	----	-	-	-
Ethanol – <i>A.altilis</i> bark against <i>E.coli</i>	----	-	-	-
Dichloromethane – <i>A.altilis</i> bark against <i>E.coli</i>	+	+	----	-
Ethanol – <i>A.altilis</i> bark against <i>M.luteus</i>	+	----	-	-
Positive control – Tetracycline	----	-	-	-

(-) – Growth absent, (+) – Growth present, (----) – Growth absent (Minimum inhibitory concentration of particular extract according to tested concentrations in present study)

Phytochemical Analysis

All the extracts of *A.heterophyllus* showed the presence of phytosterols and terpenoids. Anthraquinone was present in all the bark extracts. Flavonoids were present only in ethanolic extracts of leaves and bark. Phenols and glycosides were present only in ethanolic leaf extract and ethanol and dichloromethane extracts of bark. Diterpenes were present only in ethanolic leaf extract while tannins and alkaloids were absent in all the extracts of *A.heterophyllus* (Table 4).

In *A.altilis* all the bark extracts and ethanolic leaf extract showed the presence of phytosterols. Anthraquinone was present in all the leaf extracts and absent in bark extracts. Terpenoids were present in hexane extracts of leaves and bark and dichloromethane extract of leaf. Phenols were present only in ethanol and dichloromethane extracts of leaves. Flavonoids and diterpenes were present only in ethanolic extracts of leaves and bark. Glycosides were present in

ethanolic leaf and bark extract and dichloromethane leaf extract. Tannins and alkaloids were absent in all the extracts (Table 4).

Table 4. Phytochemical screening of *A.heterophyllus* and *A.altilis* leaves and stem bark extracted using different solvents

Treatment Phytochemicals	Leaves						Barks					
	95% ETOH		99% DM		99% HEX		95% ETOH		99% DM		99% HEX	
	A.h	A.a	A.h	A.a	A.h	A.a	A.h	A.a	A.h	A.a	A.h	A.a
Phytosterols	+	+	+	-	+	-	+	+	+	+	+	+
Anthraquinone	-	+	-	+	-	+	+	-	+	-	+	-
Terpenoids	+	-	+	+	+	+	+	-	+	-	+	+
Phenols	+	+	-	+	-	-	+	-	+	-	-	-
Glycosides	+	+	-	+	-	-	+	+	+	-	-	-
Flavonoids	+	+	-	-	-	-	+	+	-	-	-	-
Diterpenes	+	+	-	-	-	-	-	+	-	-	-	-
Tannins	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-

A.h – *A.heterophyllus*, A.a – *A.altilis*, (+) – Present, (-) – Absent, ETOH – Ethanol, DM – Dichloromethane, HEX – Hexane

Discussion

Extraction efficiency was high in leaf extracts compared to bark extracts except in *A.heterophyllus* hexane extracts, in which the extraction efficiency of bark was 9.54% and leaf was only 5.29% (Table 1). Leaves are thinner and less complex than the bark. Therefore, compared to bark extraction, solvents could penetrate more easily and dissolve more of the phytochemicals in leaves. The highest extraction efficiency was found in the ethanolic extract of *A.heterophyllus* leaf (22.88%) (Table 1). Franco *et al.* (2008) report that ethanol is preferred by many researchers since it poses less toxicity and higher extraction yield. Rahmoun *et al.* (2014) state that extraction yield increases with the increasing polarity of the solvent which is in agreement with the present results.

Although investigations on antimicrobial activity of *A.heterophyllus* and *A.altilis* aqueous extracts were carried out by Binumol & Sajitha (2013), Mbaeyi Nwaoha & Onwuka, (2014), Raman *et al.* (2012) and Satish *et al.* (2008), none of the reports recommended the use of water as an extraction solvent as little or no antimicrobial activity was seen in any of the investigations. Since the literature available up to date reiterate about very little or no antimicrobial activity of aqueous extracts of both the plants, using distilled water as an extraction solvent and thereby investigating the antimicrobial potential of aqueous extracts were omitted in the present study even though working with the aqueous extracts is less expensive and more environmentally friendly than the use of other extraction solvents.

Both *A.heterophyllus* and *A.alilis* bark showed significant antimicrobial activity against the gram negative bacterium tested. Ethanolic bark extracts of *A.heterophyllus* (9.50 ± 0.44 mm) and *A.altilis* (7.49 ± 0.28 mm) strongly exerted significant antimicrobial activity against *E.coli* which is a gram negative bacterium. But ethanolic leaf extracts of both plants failed to inhibit *E.coli* (Table 2). Similar finding related to bark extract has been reported by Kuete *et al.* (2011) and Binumol & Sajitha (2013), in which methanolic extract of *Artocarpus communis* (now known as *A.altilis*) stem bark and some isolated compounds (Terpenoids and Flavonoids) were effective against *E.coli* and methanolic bark extracts of both plants were effective against *P.fluorescens* (gram negative) and *B.subtilis* (gram positive) respectively. Efficient extraction by high polar ethanol and presence of bio active phytochemicals in the stem bark being stated as the reasons for this notable higher antimicrobial activity.

Contrary to the present findings, Raman *et al.* (2012), Mbaeyi Nwaoha & Onwuka, (2014) and Binumol & Sajitha (2013) reported the antimicrobial activity of *A.heterophyllus* and *A.altilis* methanolic and ethanolic leaf extracts against certain gram positive and gram negative bacteria including *E.coli*. This difference between literature and the present study regardless of the phytochemistry of leaf extracts, may be due to the result of inadequate usage of extracts in the bioassay (≈ 1.5 mg/disc).

Among all the extracts tested, only the dichloromethane bark extract of *A.heterophyllus* was effective against *M.luteus*. However in *A.altilis*, ethanol and dichloromethane leaf and bark extracts showed considerable activity against *M.luteus* (Table 2). Similar findings have been reported by Raman *et al.* (2012). All the dichloromethane

extracts except *A.heterophyllus* leaf extract have inhibited *E.coli* and *M.luteus* at a considerable level (Table 2). Omar *et al.*, 2013 reported that stigmaterol isolated from the dichloromethane twig extract of *A.altilis* was effective against *S.aureus*. Leaf extracts of *A.altilis* obtained using some other medium polar solvents such as chloroform and ethyl acetate have also shown good antimicrobial activity against bacteria including *E.coli* and *M.luteus* (Mohanty & Pradhan, 2014; Raman *et al.* 2012). As such it seems probable that the medium polar solvents effectively extract bioactive phytochemicals.

All the n-hexane extracts (≈ 1.5 mg/disc) of both of the plants did not show any antimicrobial activity against *E.coli* or *M.luteus* (Table 2). Results indicate that, hexane is significantly ineffective compared to the other medium and high polar extraction solvents in extracting bioactive compounds. It appears that hexane failed to extract bioactive phytochemicals although hexane showed good extraction efficiency (Table 1). However, one report by Mbaeyi Nwaoha & Onwuka (2014) state that n hexane extract of *A.altilis* leaf inhibited *E.coli* and further Omar *et al.* (2013) stated cycloartenyl acetate isolated from hexane twig extract of *A.altilis* inhibited *S.aureus*. So it is possible that higher concentrations of crude hexane extracts could inhibit bacteria.

None of the extracts showed antifungal activity against *A.niger* and *Trichoderma* sp at the concentrations tested in the present study. Mbaeyi Nwaoha & Onwuka (2014) reported that ethanolic leaf extract of *A.altilis* inhibited *A.niger* at 6 mg/ml MIC while n hexane inhibited at 8 mg/ml MIC. Kuete *et al.* (2011) and Raman *et al.* (2012) found that methanolic and ethanolic leaf extracts of *A.altilis* were effective against *Candida* sp and *Mucor* sp. The reason behind the absence of antifungal activity may be due to inefficient extraction of antifungal phytochemicals or inadequate dosage of the extracts. Since all the replicates showed no antifungal activity and only one publication (Mbaeyi Nwaoha & Onwuka (2014) states antifungal activity of *A.altilis* leaves at higher dosage (MIC of ethanolic extract was 5 mg/ml and MIC of n hexane extract was 8 mg/ml against *A.niger*), it is not possible to conclude on the antifungal activity of *A.heterophyllus* and *A.altilis*.

Antibiotics tetracycline and ketoconazole (≈ 0.15 mg/ disc) were more effective than any of the tested plant extracts against bacteria and fungi respectively. Use of an equal amount of antibiotics as plant extracts resulted in very large zones of inhibition in the very first trial of this present study. As the main objective of this study

was to investigate and compare the antimicrobial activity of *A.heterophyllus* and *A.altilis* trees, the concentration of antibiotics was reduced to 1/10th of the concentration of plant extracts and considered only as a positive control. This reduction in concentration further provided the plant extracts sufficient space to show their inhibitory effect.

Kuete *et al.* (2011) reported that the phytochemicals are classified as antimicrobials on the basis of MIC in the range of 100-1000 mg/ml. According to Rios & Recio (2005) quantities lower than 1 mg/ml for extracts and quantities lower than 0.1 mg/ml for isolated compounds are considered noteworthy. In the present study ethanolic bark extracts of *A.heterophyllus* and *A.altilis* inhibited *E.coli* at MIC 0.5 mg/ml (Table 4). Kuete *et al.* (2011) reported that the lowest MIC of methanolic bark extract of *A.communis* against *E.coli* was 64 µg/ml.

According to Mbaeyi Nwaoha & Onwuka (2014) ethanolic leaf extract of *A.altilis* showed inhibition of *E.coli* and *A.niger* at MIC 6 mg/ml. This further supports the statement that, stem barks of *A.heterophyllus* and *A.altilis* (MIC at 0.5-1 mg/ml, Table 3) are more effective than leaves.

Previous reports on phytochemical screening of *A.heterophyllus* leaf showed the presence of flavanoids, terpenoids, steroids, phenols, glycosides and saponins while stem bark showed all the above stated phytochemicals and in addition alkaloids and tannins. Meanwhile *A.altilis* leaves showed the presence of alkaloids, flavanoids, glycosides, steroids, saponins, tannins, and stem barks showed the presence of flavanoids, glycosides, terpenoids, tannins, steroids and anthraquinones (Binumol & Sajitha, 2013; Kute *et al.* 2011; Mbaeyi Nwaoha & Onwuka, 2014; Raaman *et al.* 2014; Raman *et al.* 2012).

When comparing the plants *A.heterophyllus* and *A.altilis*, all the tested phytochemicals were present in both of the plants. But interestingly anthraquinones were present only in *A.heterophyllus* bark but in *A.altilis* those were present only in the leaves. Further, the present results are in agreement with the findings of Mohanty & Pradhan (2014) and Amponsah *et al.* (2014) hence proves the phytochemical diversity of secondary metabolites in both of the tested plants.

Although the presence of tannins and alkaloids have been reported in both of the plants (Amponsah *et al.* 2014; Binumol & Sajitha, 2013; Elevitch & Manner, 2006; Mbaeyi Nwaoha & Onwuka, 2014;

Mohanty & Pradhan, 2013; Raman *et al.* 2012) tests for tannin and alkaloids gave negative results in all the extracts in the present study (Table 4).

Conclusion

Results of the present *in vitro* antimicrobial assay and MIC test revealed the antibacterial potentiality of stem barks of *A.heterophyllus* and *A.altilis* which reiterate the broad spectrum of antibacterial activity of both of the plant extracts against gram negative and gram positive bacteria. The most effective of the extraction solvents proved to be ethanol, followed by dichloromethane, and hexane was found to be the least effective of the tested solvents. The antimicrobial activity of extracts was further supported by the presence of phenols, terpenoids, flavonoids, anthraquinone, phytosterols, glycosides and diterpenes in the extracts which are known to possess antimicrobial properties. Therefore, the present investigation draws attention to the phytochemistry and the antimicrobial properties of both *A.heterophyllus* and *A.altilis* by providing supportive data to indicate that the isolation, characterization and ethnomedicinal *in vivo* investigations of phytochemicals from *A.heterophyllus* and *A.altilis* could act as a platform to formulate promising antimicrobials against pathogens.

References

- Amponsah, I. K., Annan, K., Koffuor, G. A., Sarkodie, J. A., Umerie, I. J., & Osei wusu, S. (2014). Anti-inflammatory and antioxidant properties of the ethanolic stem bark extract of *Artocarpus altilis* (Parkinson) Fosberg (Moraceae). *Der Pharmacia Lettre*, 6(3), 211-217.
- Arguedas, T. B., & Coley, P. D. (2005). Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species. *OIKOS*, 108, 410-420.
- Binumol, M. & Sajitha, T. (2013). Phytochemical and antibacterial activity of *Artocarpus heterophyllus* Lam. and *Artocarpus communis* Forst. on *Bacillus subtilis* and *Pseudomonas fluorescens*. *International Journal of Scientific & Engineering Research*, 4, 1766-1784.

- Deivanai, S., & Bhore, S. J. (2010). Bread fruit (*Artocarpus altilis* Fosb.) – An underutilized and neglected fruit plant species. *Middle East journal of Scientific research*, 6(5), 418-428.
- Elevitch, C. R. & Manner, H. I. (2006). *Artocarpus heterophyllus* (Jackfruit). Retrieved from <https://retirenicaragua.files.wordpress.com/2012/05/a-heterophyllus-jackfruit1.pdf> (Accessed 26 on Nov.2014).
- Fernando, S. (2003). Herbal food and medicines in Sri Lanka. New Delhi. (Original work published in 1982).
- Franco, D., Sineiro, J., Rubilar, M., Sanchez, M., Jerez, M., Pinel, M., Nunez, M. J. (2008). Polyphenols from plant materials: extraction and antioxidant power. *Electronic journal of environmental, agricultural and food Chemistry*, 7(8), 3210-3216.
- Hasan, M. F., Das, R., Khan, A., Hossain, M. S., & Rahman, M. (2009). The Determination of Antibacterial and Antifungal Activities of *Polygonum hydropiper* (L.) Root Extract. *Advances in Biological Research*, 3, 53-56.
- Jitendra, R., Kalpana, S., Shweta, S., Kumar, M. S., & Manish, B. (2014). *Artocarpus heterophyllus* (jackfruit) potential unexplored in dentistry- an overview. *Universal journal of Pharmacy*, 3(1), 50-55.
- Joshual, M. & Takudzwa, M. (2013). Antibacterial properties of *Mangifera indica* on *Staphylococcus aureus*. *African journal of clinical and experimental microbiology*, 14, 62-74.
- Karthy, E. S., Ranjitha, P., Mohankumar, A. (2009). Antimicrobial potential of plant seed extracts against multidrug resistant methicillin resistant *Staphylococcus aureus* (MDR-MRSA). *International Journal of Biology*, 1, 34-40.
- Kuete, V., Ango, P. Y., Fotso, G. W., Kapche, G. D. W. F., Dzoym, J. P., Wouking, & Abeghas, B. M. (2011). Antimicrobial activities of the methanolic extract and compounds from *Artocarpus communis* (Moraceae). *BMC Complementary and Alternative Medicine*, 11, 1-5.
- Kumaraswamy, M. V., Kavitha, H. U., & Satish, S. (2008). antibacterial evaluation and phytochemical analysis of *Betula*

utilis D. Don against some human pathogenic bacteria. World Journal of Agricultural Sciences, 4(5), 661-664.

Nwaoha, I. E. M., & Onwuka, C. P. (2014). Comparative evaluation of antimicrobial properties and phytochemical composition of *A.altilis* leaves using ethanol, n hexane and water. African journal of Microbiology research, 8(37), 3409-3421.

Mohanty, M. & Pradhan, C. (2014). Phytoconstituent analysis and comparative bioefficacy assessment of breadfruit fruit and leaf extracts for antipathogenic potentiality. American journal of phytomedicine and clinical therapeutics, 2(1), 77-87.

Omar, M. N., Muzammil, A., Khan, N. T., Zahid, M. & Kamal, T. (2013). Isolation and identification of bioactive compounds from twigs of *Artocarpus altilis*. Merit Research Journal of Environmental Science and Toxicology, 1, 147-155.

Pethiyagoda, R. (2005). Exploring Sri Lanka's biodiversity. The raffles bulletin of Zoology, 12, 1-4.

Rahmoun, N. M., Ziane, H. & Boucherit Otmani, Z. (2014). Antibacterial and antifungal screening of four medicinal plants. Journal of Coastal Life Medicine, 2(12), 975-979.

Raaman, N., Sivaraj, C. & Tenzing. (2014). Antioxidant activities and phytochemical analysis of methanol extract of leaves of *Artocarpus heterophyllus* Lam. International Journal of Pharmacy and Pharmaceutical Sciences, 6, 289-293.

Raman, V., Sudhahar, D. & Anandarajagopal, K. (2012). Preliminary phytochemical investigation and screening of antimicrobial activity of leaf extracts of *Artocarpus altilis*. Asian journal of Biological and Life Sciences, 1(2), 104-107.

Rana, I. S., Rana, A. S. & Rajak, R. C. (2011). Evaluation of antifungal activity in essential oil of the *Syzygium aromaticum* (L.) by extraction, purification and analysis of its main component eugenol. Brazilian Journal of Microbiology, 42, 1269-1277.

Rios, J. L. & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100, 80-84.

- Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M. & Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative medicines*, 8, 1.
- Satish, S., Raghavendra, M. P. & Raveesha, K. A. (2008). Evaluation of the antimicrobial potential of some plants against human pathogenic bacteria. *Advances in Biological Research*, 2, 44.
- Shilpakar, A., Gaire, B. P., Sunar, C. B., Lamichhane, R. & Neupane, S. (2009). Phytochemical screening and analysis of antibacterial and antioxidant activity of *Ficus auriculata*, Lour. stem bark. (Bachelor's thesis). Retrieved from http://www.researchgate.net/profile/Bhakta_Gaire/publication/221677460Phytochemical_screening_and_analysis_of_antibacterial_and_antioxidant_activity_of_Ficus_auriculata_%28Lour.%29_Stem_Bark/links/0922b4f5ed8b5bf060000000 (Accessed on 25 Nov. 2014).
- Sikarwar, S. M., Hui, B. J., Subramaniam, K., Valeisamy, B., Yean, L. K. & Balaji, K. (2014). A Review on *Artocarpus altilis* (Parkinson) Fosberg (breadfruit). *Journal of Applied Pharmaceutical Science*, 4 (08), 091-097.
- Thirumurugan, A., Tomy, N. A., Jai ganesh, R. & Gobikrishnan, S. (2010). Biological reduction of silver nanoparticles using plant leaf extracts and its effect on increased antimicrobial activity against clinically isolated organism. *Der Pharma Chemica*, 2, 279-284.
- Tiga, S. (2011). Characterization and identification of some medically important bacterial isolates. (Master's dissertation). Retrieved, http://ethesis.nitrkl.ac.in/2435/1/Suman_Thesis_final.pdf. (Accessed on 12 Nov. 2014).
- Tiwari, P., Kumar, B., Kaur, M., Kaur, G. & Kaur, H. (2011). Phytochemical screening and Extraction: A Review. *International Pharmaceutica Scientia*, 1, 98-106.
- World health organization. (2012). The evolving threat of antimicrobial resistance options for action. Retrieved from http://whqlibdoc.who.int/publications/2012/9789241503181_eng.pdf (Accessed 29 on Nov.2014).

