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Phytochemical screening and comparative bioefficacy assessment of *Artocarpus altilis* leaf extracts for antimicrobial activity

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Artocarpus altilis (breadfruit) leaf extracts in different solvent media (petroleum ether, methanol, and ethyl acetate) were assessed for antimicrobial activity. Breadfruit leaf extracts have been reported to have different phytoconstituents. The effect of leaf extracts in different solvent media on pathogenic organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Enterococcus faecalis* was studied by disc diffusion assay and MIC (minimal inhibitory concentrations) values were investigated. Phytochemical compounds like steroids, phytosterols, gums and resins were found to be present in leaf extracts with different extraction media. Phenols and terpenoids were detected in ethyl acetate and methanol leaf extracts. Flavonoids were present in the petroleum ether and ethyl acetate leaf extracts, whereas tannins were detected only in the methanol leaf extract. Maximum zone of inhibition was observed for *Strep. mutans*, *E. faecalis*, *S. aureus*, and *P. aeruginosa* by using 50 μl of ethyl acetate and methanol leaf extracts, 20 μl of petroleum ether leaf extract, 25 μl of petroleum ether leaf extract, and 50 μl of methanol leaf extract, respectively. The MIC values were reported in between 0.3 and 0.6 mg/ml corresponding to variations in different solvent media used for leaf extracts against four different pathogenic bacteria.

Keywords: *Artocarpus* leaf extract; antimicrobial activity; pathogenic bacteria; phytochemicals; minimal inhibitory concentrations (MIC)

Introduction

Medicinal plants play an important role in the modern world because they are the source of many imperative drugs that are used in allopathic and herbal medicines as well as in homoeopathy and aromatherapy. Plants that are used as therapeutic agents have lower incidence of adverse effects and are easily available to most people in the country of origin. To combat the detrimental side effects of conventional antibiotics, research on the medicinal values and antimicrobial properties of various plants has gained utmost attention throughout the world. It has been found that ethnic medicinal plants produce a variety of compounds of known therapeutic properties and these compounds can be used as an alternative to treatment of various diseases (Kumar et al. 2007; Ramappa & Mahadevan 2011). In the recent past, studies have focused on the use of plant extracts and their biologically active compounds (Suresh et al. 2010).

Artocarpus altilis (family Moraceae), commonly known as breadfruit, originated from New Guinea and now extensively grows in the southern parts of India. Breadfruit (*A. altilis* (Parkinson) Fosberg) is a multipurpose agroforestry tree crop that is primarily used for its nutritious, starchy fruit, which is a rich source of carbohydrates, calcium, and phosphorus (Ragone 1997). The multifarious importance of breadfruit includes food, medicine, clothing material, construction material, and animal feed. Other species of *Artocarpus* have also been studied for their antimicrobial activity (Consolacion et al. 2004; Shanmugapriya et al. 2011).

The medicinal value of *A. altilis* has gained immense importance in countries like Trinidad and Bahamas, where different parts of the plant are used for the treatment of various ailments such as tongue thrush, skin infections, sciatica, diarrhea, low blood pressure, and asthma. The juice of its leaves is used as eardrops. A powder of roasted leaves is used as a remedy for enlarged spleen (Morton 1987). Breadfruit has long been an important staple food in the Pacific Islands. Research has found that extracts from roots and stem barks show some antimicrobial activity against gram-positive bacteria and have potential use in treating tumors (Sundarrao et al. 1993). In some studies, the root and stem bark extracts were used against some bacteria (Seaforth et al. 1983; Ragone 1997). The chromatographic study of breadfruit has revealed high content of amino

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Figure 1. *Artocarpus altilis* plant with leaf.

acid, fatty acids, and carbohydrates (Golden & Williams 2001). Atrocarpin isolated from the heartwood extract of Thai breadfruit exhibits inhibitory effect on melanogenesis, showing high antioxidant activity. These effects indicate the potential use of heartwood of breadfruit in cosmetics (Donsing et al. 2008). The medicinal uses of breadfruit are being actively researched; however, there is still a huge dearth of information regarding the antimicrobial activity of different parts of this plant. Therefore, it is imperative to intensify investigations aimed at evaluating the potentiality of this plant against various microbial pathogens. This study might be the first one to investigate the potentiality of different leaf extracts of breadfruit for their antimicrobial properties.

This article determines the comparative effect of leaf extracts of breadfruit, using different solvents. Investigation was carried out to detect the inhibitory effect of leaf extracts of *A. altilis* on the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Enterococcus faecalis* with disc diffusion method by determining the minimal inhibitory concentrations (MIC) with different extraction media for their antimicrobial activity. The study emphasizes on the antimicrobial role of plant leaf extracts and their growth-inhibiting activity against multidrug-resistant organisms in different solvent media. To the best of our knowledge, this is the first study on the screening of phytochemical constituents and assessment of antimicrobial activity, using different solvent extracts of *A. altilis* leaves.

Materials and methods

Plant material

Leaves of *A. altilis* (Parkinson) Fosberg, growing in natural condition (Figure 1) were collected from Orissa University of Agriculture and Technology, Bhubaneswar, India. The plants were identified and authenticated at Herbarium Unit of Post Graduate Department of Botany, Utkal University, Bhubaneswar.

Microbial organisms

The pathogenic bacterial strains, viz. *S. aureus*, *P. aeruginosa*, *Strep. mutans*, and *E. faecalis*, were obtained from IMTECH, Chandigarh, India, and cultured on sterile Nutrient agar plates (HiMedia, Mumbai, India). After 18–24 hour broth culture of all the four types of microbial strains and after reaching absorbance values ranging between 0.129 and 0.134 at a wavelength of 625 nm (i.e. equivalent to 0.5 McFarland of culture), they were used for testing antimicrobial activity.

Preparation of stock and working solutions of the plant leaf extracts for antimicrobial studies

Fresh breadfruit leaves were thoroughly cleaned with deionized water, air dried at room temperature, powdered in a blender, and then stored in a clean glass container (Ajayi et al. 2011; Jamuna et al. 2011). Next, 100 g of finely macerated leaf powder was extracted separately in 750 ml of different solvents (methanol, petroleum ether, and ethyl acetate) individually in a Soxhlet apparatus for 3 days at room temperature (30–40°C) with intermittent shaking. This was followed by distillation process to concentrate the extract. The extracts were filtered using Whatman filter paper No. 42 (125 mm). The extracts obtained were further concentrated by evaporation, using water bath at 100°C. The stock solutions of the leaf extracts were prepared in 10% dimethyl sulfoxide to give a concentration of 30 mg/ml.

Antimicrobial activity testing by disc diffusion assay

The bacterial strains were cultured in brain heart infusion broth for 24 h at 37°C and the cultures were then swabbed on sterile Müller-Hinton agar plates. Sterilized Whatman filter paper No. 1 was used to prepare discs of diameter 6 mm. The sterile discs, which were dipped at various concentrations of the leaf extracts in different solvent media, were placed on culture plates. After 24 h of incubation at 37°C, the diameters of the inhibition zones produced by each discs were measured using the Hi-Antibiotic Zonescale (HiMedia, Mumbai, India). The inhibition zone diameters of less than 10 mm were considered as slightly effective and of more than 11 mm were considered as an effective concentration of the leaf extract. The extracts were tested for their antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. faecalis*, and *Strep. mutans*. The antimicrobial activity test of leaf extracts against different pathogens was conducted in triplicates. Different microorganisms were tested for their growth inhibition under different concentrations of leaf extracts. Inhibition of microbial growth was determined by measuring the diameter (in millimeters) of the clear zone around each disc. A control set of experiment was carried out with dimethyl sulfoxide along with the different solvents such as methanol and chloroform. The inhibition zone diameters were measured and recorded for each organism, and the MIC values was determined using different dilutions of extracts.

Determining MIC

The MIC value is considered as the lowest concentration of the sample extract, which inhibits the growth of a microbe. The MIC value of the leaf extracts of *A. altilis* was determined by disc diffusion assay (Bauer et al. 1959) and was evaluated by dilution method (Prescott et al. 2005) on plant extracts to observe the antimicrobial activity. The MIC study on leaf extracts in different solvents showed their high efficacy (inhibition zone >11 mm) against pathogenic microorganisms.

Phytochemical screening

Different phytochemicals, viz. alkaloids, flavonoids, phenolics, glycosides, phytosterols, steroids, tannins, terpenoids, fats, oils, and gums and resins, were screened in the laboratory as per the standard methods with little modification (Harbone 1998; Raaman 2006; Jamuna et al. 2011). The crude extracts were stored in desiccators for a maximum of 3 days and were later preserved in deep freezer (−20°C) for further use. The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in 3 different solvent extracts of leaves.

Statistical analysis

Each experiment was carried out in triplicates and the results were represented as the mean of the triplicates. SEM values were calculated for the data. A comparison was also made to check for the effectiveness of different solvent media against their antimicrobial activity.

Results

Qualitative screening of phytochemicals

The qualitative screening of phytochemicals showed the presence of a wide range of phytochemicals/secondary metabolites. All the necessary secondary metabolites such as steroids, phenols, tannins, phytosterols, gums and resins, and terpenoids (but except saponins, flavonoids, and alkaloids) were found in the methanol leaf extracts of *A. altilis*. Similar results were noted in the ethyl acetate leaf extracts with the exception of absence of tannins and presence of flavonoids. Petroleum ether leaf extracts were found of have only 4 metabolites, viz. steroids, flavonoids, phytosterols, and gums and resins, out of the 9 metabolites tested (Table 1).

Assessment of antimicrobial activity

The methanol leaf extracts of *A. altilis* exhibited maximum growth inhibition activity against *P. aeruginosa* (inhibition zone = 18 mm), followed by petroleum ether leaf extracts (inhibition zone = 15 mm) and ethyl acetate leaf extracts (inhibition zone = 13 mm) (Figure 2) at a concentration of 50 µl (≈1.5 mg dry leaf matter). The antimicrobial activity of methanol leaf extracts of *A. altilis* at a concentration of 50 µl was found highest, showing an inhibition zone of 16 mm, and most effective among all the different types of leaf extracts studied against *Strep. mutans* (Figure 2). Petroleum ether leaf extracts at a concentration of 25 µl showed maximum zone of inhibition with a diameter of 22 mm when tested against *E. faecalis*, followed

Table 1. Phytochemical screening of leaf extracts of *Artocarpus altilis*.

Phytochemical constituents	Leaf extracts in different solvent media		
	Petroleum ether	Ethyl acetate	Methanol
Alkaloid	−	−	−
Steroid	+	+	+
Phenol	−	+	+
Flavonoid	+	+	−
Saponin	−	−	−
Tannin	−	−	+
Phytosterol	+	+	+
Gums and resins	+	+	+
Terpenoid	−	+	+

Note: + refers to presence and − for absence of respective phytoconstituent.

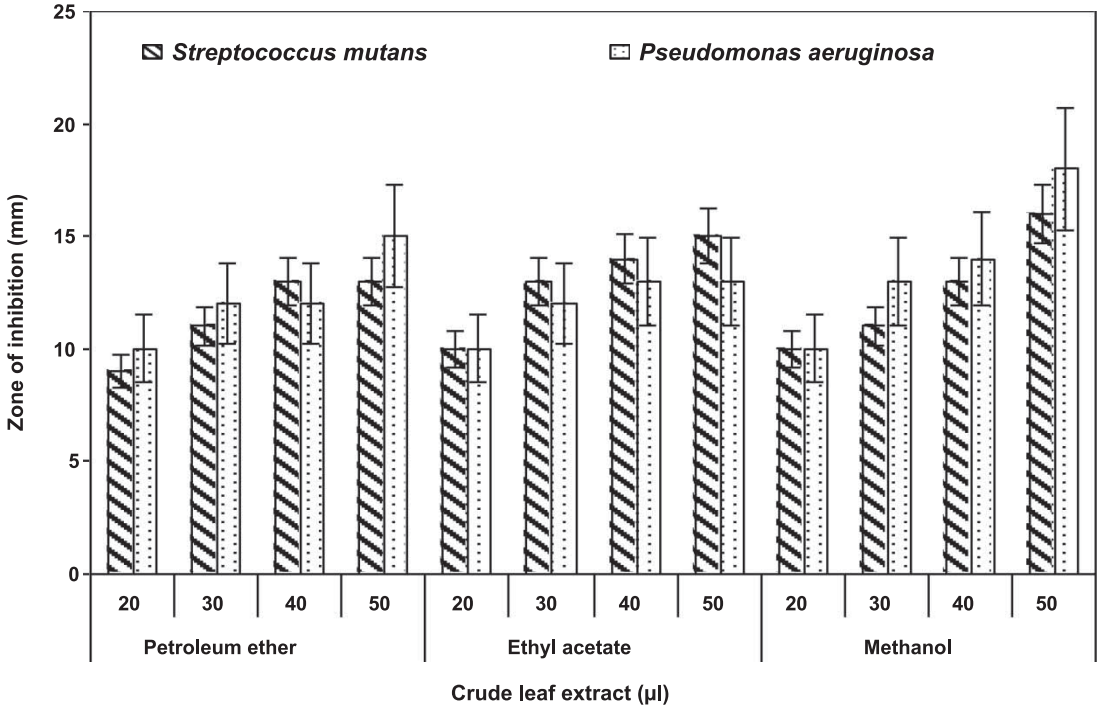

Figure 2. Antimicrobial activity of leaf extracts of *Artocarpus altilis* against *Streptococcus mutans* and *Pseudomonas aeruginosa*. (Values shown are mean ± SEM.)

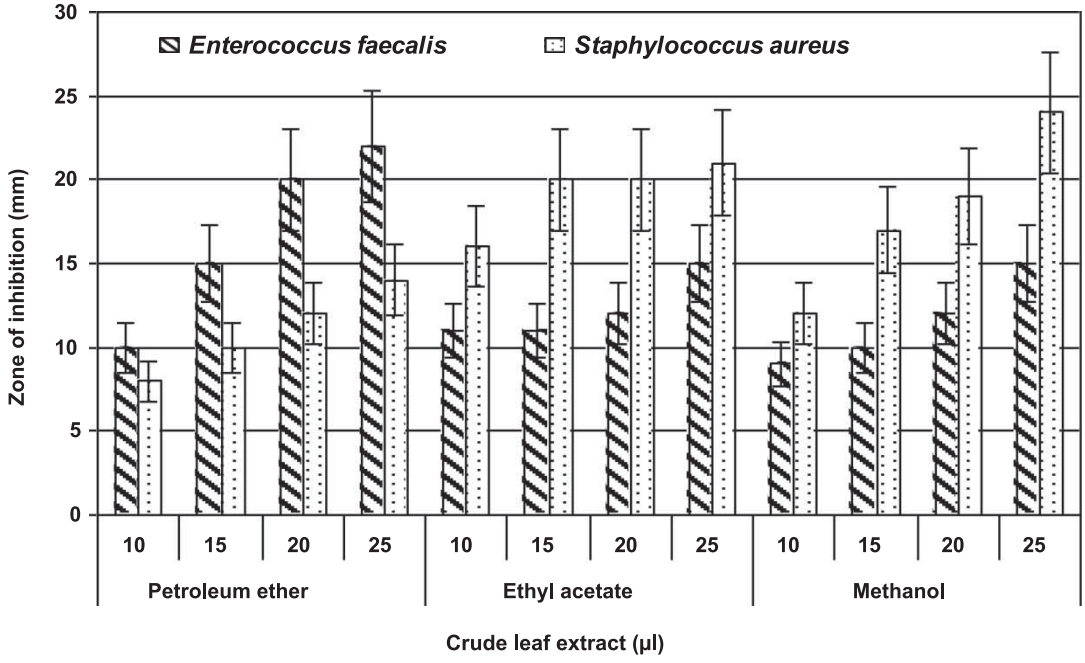

Figure 3. Antimicrobial activity of leaf extract of *Artocarpus altilis* against *Enterococcus faecalis* and *Staphylococcus aureus*. (Values shown are mean ± SEM.)

by methanol and ethyl acetate leaf extracts with an inhibition zone of 15 mm (Figure 3). Growth of *S. aureus* was highly inhibited by treatment with methanol leaf extracts at a concentration of 25 µl, as evident from its inhibition zone of 24 mm (Figure 3). This inhibition was highest among all the three types leaf extracts tested. Ethyl acetate leaf extract at a concentration of 10 µl was also found effective against *S. aureus*, showing an inhibition zone of 12 mm (Figure 3). Elevated growth inhibition of *Strep. mutans*, *S. aureus*, and *P. aeruginosa* was observed with increased dose of methanol leaf extracts, as evident from their diameters of the zone of inhibition. The MIC values of different

leaf extract of *A. altilis* against different pathogenic microorganisms also varied significantly. The MIC values of leaf extract of *A. altilis* were found to be 0.6 mg/ml against *Strep. mutans* (inhibition zone = 9 mm) and *P. aeruginosa* (inhibition zone = 10 mm) and to be ranging from 0.3 to 0.45 mg/ml against *E. faecalis* and *S. aureus*, with different solvent media used.

Discussion

The above comparative assessment of different leaf extracts of *A. altilis* for antimicrobial potential proves that the methanol leaf extracts were, at high concentrations, very much effective than others and showed highest antimicrobial activity, whereas petroleum ether and ethyl acetate leaf extracts showed better effectiveness at low concentrations. This means that the efficacy of methanol leaf extracts of *A. altilis* against various human pathogens is probably due to the presence of a wide range of phytochemical constituents (or secondary metabolites), especially tannins. These secondary metabolites play a significant role in inhibiting the growth of human pathogens and act against them by developing an effective defense mechanism (Daferera et al. 2003; Sahoo et al. 2011). Tannins, present in the methanol leaf extracts of *A. altilis*, have been found to form irreversible complexes with proline-rich proteins and these compounds are known to be biologically active in inhibiting the cell protein synthesis and as a result microbial growth is inhibited. Apart from their antimicrobial activity, tannins also react with proteins to provide the typical tanning effect. They act as stable and potent antioxidants and fight against various toxins released from the microbes (Trease & Evans 1983; Shanmugapriya et al. 2011). Tannins are potent inhibitors of proteolytic enzymes used by plant pathogens (Aboaba & Efuwape 2001; Sahoo et al. 2011). Many plants contain nontoxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens (Aboaba & Efuwape 2001). In recent years, screening of different phytochemical constituents of medicinally important plants, such as *A. altilis* for its multifarious antimicrobial activity, has gained utmost importance. The present study is a first kind of report on the medicinally important plant *A. altilis*, which has its great medicinal relevance in the recent years. The knowledge of various phytochemical constituents of *A. altilis* and their activity against tested pathogens may not only provide an insight into discovering therapeutic agents but may also be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, and essential oils as precursors for the synthesis of complex chemical substances (Akrout et al. 2010).

Natural products, either extracted or as pure compounds, contain diverse chemicals that provide unlimited prospects for the development of new drugs (Cos et al. 2006). Several plants have immeasurable ability to synthesize secondary metabolites of which at least 12,000 have been isolated and these substances serve as plant defense mechanism against predation by microorganisms, insects, and herbivores (Wink 1998; Sahoo et al. 2011). In this investigation, methanol, ethyl acetate, and petroleum ether leaf extracts, with different phytochemical constituents, were found to exhibit immense potential for antimicrobial activity. It may be inferred from the present study that the leaves of *A. altilis* have significant antibacterial activity against various pathogenic organisms. Purification of important secondary metabolites, their action on microbial activity, and subsequent structural studies can aid in isolation of active compounds from this medicinally important plant. Further investigations on purification and characterization of bioactive compounds responsible for antimicrobial activity are required for a clear understanding of the nature and property of the compound responsible for antimicrobial activity. The safety of using this phytochemical compound will be tested in animal models/cell lines in our further studies.

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