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ASSESSMENT OF THE ANTIBACTERIAL POTENTIAL OF BREADFRUIT LEAF EXTRACTS AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Artocarpus altilis **(**breadfruit) leaf extracts in different solvent media (petroleum ether, methanol and ethyl acetate) were examined for the antimicrobial activity against some pathogenic bacterial species like *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans* and *Enterococcus faecalis* following the MIC (minimal inhibitory concentrations). Steroids, phytosterols, gums and resins were detected in methanolic, ethyl acetate and petroleum ether leaf extracts. Phenols and terpenoids were detected in both the ethyl acetate and methanolic leaf extracts. Flavonoids were present in the petroleum ether and ethyl acetate leaf extracts whereas tannins were detected only in the methanolic leaf extract of *Artocarpus altilis*. The MIC values ranges from 0.3 mg/ml to 0.6 mg/ml which correspond to variations in different solvent media used for leaf extracts against four different pathogenic microbes.

Keywords:- Antimicrobial activity; Breadfruit; MIC; Phytochemicals; Solvents.

INTRODUCTION

Now-a-days medicinal plants occupy an important position in allopathic medicine, herbal medicine, homoeopathy and aromatherapy, as being the sources of many imperative drugs of the modern world [1]. The use of plants as therapeutic agents is cheaper and easily available to most people in the developing countries. Currently utmost attention has been given to researches on the medicinal values and antimicrobial properties of plants for overcoming the detrimental side effects of conventional antibiotics. The ethnic medicinal plants are used as an alternative treatment of diseases by producing a variety of biologically active compounds of known therapeutic properties [2,3,4]. *Artocarpus altilis* (Family-Moraceae) commonly known as breadfruit is originated from New Guinea and extensively grows in the Southern parts of India. Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg.) is a multipurpose agroforestry tree crop which is primarily used for its nutritious, starchy fruit with rich source of carbohydrates, calcium and phosphorus [5]. The multifarious importance of breadfruit includes food, medicine, clothing material, construction materials and animal feed. The other species of *Artocarpus* has been studied for its antimicrobial activity by several researchers [6, 7].

Different plant parts of *Artocarpus* accounts for a number of medicinal values viz. the treatment of tongue thrush, skin infections, sciatica, diarrhoea, low blood pressure and asthma have gained immense importance in countries like Trinidad and Bahamas. A powder of roasted leaves is used as a remedy for enlarged spleen [8]. In the Pacific Islands the Breadfruit was used as an important staple food. Root and stem bark extracts showed antimicrobial activity

against Gram-positive bacteria with potential use in treating tumors [5, 9, 10]. High content of amino acid, fatty acids and carbohydrates were recorded by the chromatographic study of breadfruit leaf and fruit extracts [11]. Thai Breadfruit heartwood extract rich in Atrocarpin exhibits high antioxidant activity and inhibitory effect on melanogenesis and their potential use in cosmetics [12].

Though breadfruit is being actively researched for its medicinal use still there is a huge dearth of information regarding its antimicrobial activity. Intensified researches on its potentiality against various microbial pathogens become very much essential. The present investigation on antimicrobial potentiality of breadfruit leaf extracts and different phytochemicals might be reported to be the first and original one.

In this study a comparative effect of breadfruit leaf extracts using different solvents and different isolated phytochemical constituents against growth of microbes were conducted. The inhibitory effect of isolated phytochemicals of leaf extracts of *A. altilis* on the growth of *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans* and *Enterococcus faecalis* by disc diffusion method with different extraction media for its antimicrobial activity were detected. The antimicrobial role along with growth inhibiting activity of plant leaf extracts and isolated phytochemicals against various pathogenic bacterial species in different solvent media were given importance in this study. To the best of our knowledge this is the first report on assessment of different phytochemical constituents of *A. altilis* leaf extract against antimicrobial activity.

MATERIALS AND METHODS

Plant material: The leaves of naturally grown breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) were collected from the campus of Orissa university of Agricultural Technology, Bhubaneswar, India. The identification and authentication of the plant (Accession No. CP-001) were done at Herbarium Unit of Post Graduate Department of Botany, Utkal University, India.

Microbial organisms: The Bacterial cultures of various human pathogens viz. *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans* and *Enterococcus faecalis* obtained from IMTECH, Chandigarh, India, were cultured on sterile Nutrient agar (Hi Media, India) plates.

Preparation of stock and working solutions of the plant leaf extracts for antimicrobial studies: The extracts of the collected fresh leaves were performed as per the method prescribed by Pradhan et al. [1] with a little modification [13, 14]. The stock solutions of the leaf extract was prepared in 10% dimethylsulphoxide (DMSO) to give a concentration of 30 mg/ml[1].

Antimicrobial activity testing by disc diffusion assay: The extracts were tested for its antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis and Streptococcus mutans* as prescribed in Pradhan et al.[1]. The experiments were carried out in triplicates. The diameter of the inhibition zone was measured and recorded for each organism. Minimal Inhibitory Concentration (MIC) was determined using different dilutions of extracts[1].

Determination of minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of the leaf extracts of *Artocarpus altilis was determined* by disc diffusion assay [1]. The MIC value is considered as the lowest concentration of the sample extract which inhibits the growth of a microbe. It was determined by the microbroth dilution method according to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines [15]. The MIC method was performed as described above on extracts of leaf that showed their high efficacy against pathogenic microorganisms by the disc diffusion method (inhibition zone higher than 11mm).

Phytochemical screening: The phytochemical screening for alkaloids, flavonoids, phenolics, glycosides, phytosterol, steroid, tannin, terpenoids, fats, oils, gums and resins were conducted qualitatively in the laboratory as per the standard methods with little modification [14,16,17]. The crude extracts were preserved in deep freezer (-20^0C) for further use.

Thin Layer Chromatography (TLC): The TLC was performed for the crude extracts showing R_f values of different phytochemicals and their subsequent isolation using solvent system described in Table 1 and the results were recorded.

Statistical analysis: Experiments were conducted in triplicates and data were recorded. The results of different experiments were represented as the mean of the triplicate data.

RESULTS

Qualitative screening of phytochemicals: A wide range of phytochemicals / secondary metabolites were observed through qualitative phytochemical screening study[1]. Methanolic leaf extracts of *Artocarpus altilis* contains all the necessary secondary metabolites such as steroids, phenols, tannins, phytosterols, gums & resins and terpenoids except saponins, flavonoids and alkaloids. Ethyl acetate leaf extracts showed the absence of tannins and presence of flavonoids. Only four metabolites viz. steroids, flavonoids, phytosterols, gums & resins were detected in petroleum ether leaf extracts (Table.2).

Assessment of antimicrobial activity: At a concentration of 50µl (≈1.5 mg dry leaf matter) the methanolic leaf extracts of *Artocarpus altilis* exhibited maximum growth inhibition (Zone of Inhibition: 18mm) activity against *Pseudomonas aeruginosa* followed by petroleum ether (Zone of Inhibition: 15mm) and ethyl acetate extracts (Zone of Inhibition: 13mm) (Table 3.)[1]. Highest growth inhibition zone of 16mm was recorded for *Streptococcus mutans* using methanolic leaf extract of *Artocarpus altilis* at a concentration of 50µl (Table 3.). The growth of *Enterococcus faecalis* was greatly inhibited by using Petroleum ether leaf extracts at a concentration of 25µl as evident from its maximum zone of inhibition with a diameter of 22mm followed by methanol and ethyl acetate leaf extracts with an inhibition zone of 15mm. (Table.4).

The growth inhibition zone of 24 mm was observed for *Staphylococcus aureus* with treatment of methanolic leaf extracts of *Artocarpus altilis* at a concentration of 25µl (Table 4.). A low concentration of Ethyl acetate leaf extracts i.e. 10µl found effective against *Staphylococcus aureus* showing an inhibition zone of 12 mm (Table 4.).

The zone of inhibition for *Streptococcus mutans, Staphylococcus aureus, Pseudomonas aeruginosa* increases with increasing dose of methanolic leaf extract of *Artocarpus altilis*. MIC values of different leaf extract of *Artocarpus altilis* against different pathogenic microorganisms also varied significantly. The MIC values of leaf extract of *Artocarpus altilis* was found to be 0.6 mg/ml against *Streptococcus mutans* (Inhibition Zone: 9 mm) and *Pseudomonas aeruginosa* (Inhibition Zone: 10 mm) whereas these MIC values of different leaf extract of *Artocarpus altilis* ranges from 0.3mg/ml to 0.45 mg/ml for inhibition of *Enterococcus faecalis and Staphylococcus aureus* at different solvent media used[1]. Increased antimicrobial activity against *Pseudomonas aeruginosa* was shown by the action of steroid at a lower concentration i.e. up to 30µl (extracted from 0.9mg of leaf) whereas the application of 50µl of steroid was effective against *Staphylococcus aureus* (Fig. 1a). Purified flavonoid from leaf extracts at a higher concentration exhibit maximum zone of inhibition against *Streptococcus mutans* and effective against *Pseudomonas aeruginosa* at low dose (Fig 1b). Phytosterol has no effect on growth of *Pseudomonas aeruginosa* but most effective against *Streptococcus mutans* in comparison to other three phytochemicals (Fig1c). The growth of the organism *Enterococcus faecalis* was highly suppressed by phytosterols followed by steroids as revealed from their zone of inhibition (Fig. 1a, b and c).

DISCUSSION

The methanolic leaf extracts at a high concentration and ethyl acetate and petroleum ether leaf extracts of *A. altilis* at low concentration were found very much effective against all the four types of microbes studied and showed highest antimicrobial activity. This result showing the bioefficacy of different leaf extracts of *Artocarpus altilis* against various human pathogens might be due to the presence of different phyto-constituents which was further evidenced through their individual action on the growth of these pathogens, especially the presence of tannins[1]. An effective defense mechanism against these human pathogens was developed by the action of these secondary metabolites through inhibiting their growth[18,19]. The methanolic leaf extracts of *Artocarpus altilis* containing tannins have been found to form irreversible complexes with proline-rich proteins and these compounds are known to be biologically active resulting in the inhibition of the cell protein synthesis as a result of which microbial growth is inhibited [1]. Tannins also react with proteins and act as stable and potent antioxidants which fights against various toxins released from the microbes [7,20]. The activity of proteolytic enzymes used by plant pathogens were highly inhibited by tannins [19, 21]. Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens [21].

Screening of different phyto-constituents of medicinally important *Artocarpus altilis* for its multifarious antimicrobial activity has gained utmost importance in recent years. The present study on the effect of different phyto-constituents of *Artocarpus altilis* against human pathogenic bacteria is a first

kind of report which has its great medicinal application in the recent years. An insight for discovery of therapeutic agents and information on disclosure of new sources of tannins, oils, gums, flavonoids, saponins, essential oils, precursors for the synthesis of complex chemical substances in *A. altilis* have been provided by the knowledge of its various phytochemical constituents and the activity against tested pathogens [22]. There are a number of plants with immense ability to synthesize secondary metabolites and serve as plant defense mechanism against predation by microorganisms, insects and herbivores [19, 23] through providing unlimited prospects for the development of new drugs [24]. In this investigation, phytochemical constituent like flavonoids and steroids have immense importance for antimicrobial activity against more number of organisms. It was inferred from the present investigation that the leaves of *Artocarpus altilis* has significant antibacterial activity. Further purification of the secondary metabolites, structural studies and isolation of bioactive compounds from this plant on tested animal models against various other pathogens could lead to new inventions in pharmaceutical sciences.

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Table 1: TLC of purified samples of *Artocarpus altilis* leaf extracts.

Samples	Mobile Phase (Ratio of Solvents)	Spot colour (UV)	R_f value
Flavonoid	Petroleum ether: Ethyl acetate (2:1)	Blue	0.56
Phytosterol	n-hexane: Ethyl acetate (9:1)	Red	0.55
Steroid	Methanol: Water (95:5)	Red	0.88

Table 2: Phytochemical screening of leaf extracts of *Artocarpus altilis*.

Phytochemical constituents	Leaf Extracts		
	Petroleum ether	Ethyl acetate	Methanol
Alkaloid	-	-	-
Steroid	+	+	+
Phenol	-	+	+
Flavonoid	+	+	-
Saponin	-	-	-
Tannin	-	-	+
Phytosterol	+	+	+
Gums &Resins	+	+	+
Terpenoid	-	+	+

Source: Pradhan et al [1]

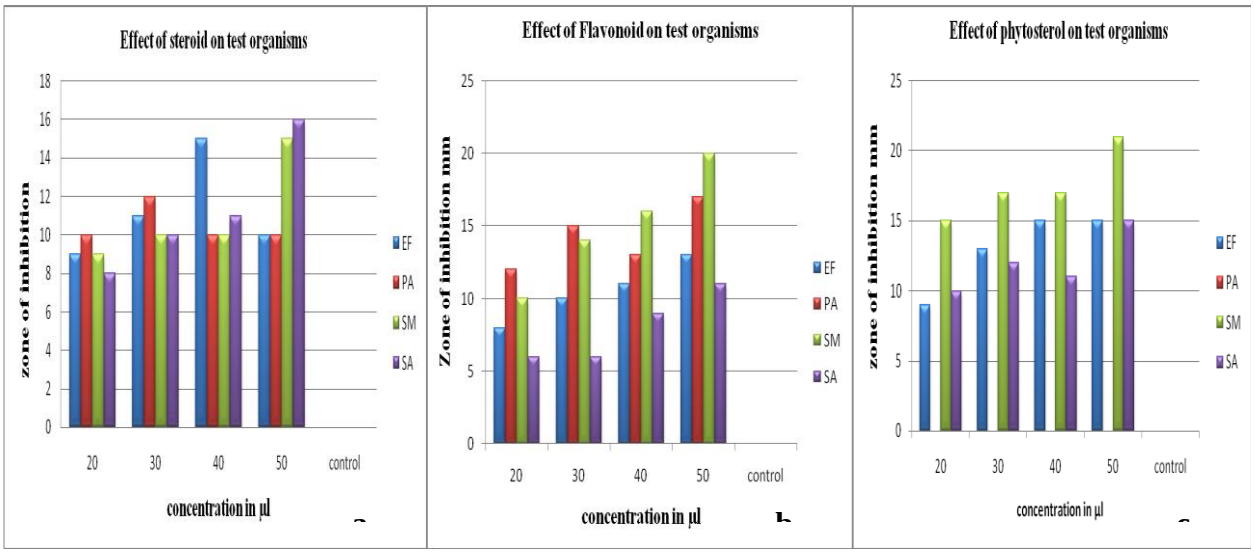
Table 3: Antimicrobial activity of leaf extracts of *Artocarpus altilis* against *Streptococcus mutans* and *Pseudomonas aeruginosa.*

Crude leaf extract (30 mg/ml)	Amount (µl)	Zone of inhibition (mm)	
		Streptococcus mutans	*Pseudomonas aeruginosa*
Petroleum ether	20	9	10
	30	11	12
	40	13	12
	50	13	15
Ethyl acetate	20	10	10
	30	13	12
	40	14	13
	50	15	13
Methanol	20	10	10
	30	11	13
	40	13	14
	50	16	18

Table 4 Antimicrobial activity of leaf extracts of *Artocarpus altilis* against *Enterococcus faecalis* and *Staphylococcus aureus*

Crude leaf extract (30 mg/ml)	Amount (µl)	Zone of inhibition (mm)	
		Enterococcus faecalis	*Staphylococcus aureus*
Petroleum ether	10	10	8
	15	15	10
	20	20	12
	25	22	14
Ethyl acetate	10	11	16
	15	11	20
	20	12	20
	25	15	21
Methanol	10	9	12
	15	10	17
	20	12	19
	25	15	24

Figure1: Effect of different phytochemicals (a-Steroid b-Flavonoid and c-Phytosterol) on test organisms showing zone of inhibition in mm.



NB: EF - *Enterococcus faecalis*, PA - *Pseudomonas aeruginosa*, SM - *Streptococcus mutans*, SA - *Staphylococcus aureus;* Control-Aqueous extract

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