

PHYTOCONSTITUENT SCREENING AND COMPARATIVE ASSESSMENT OF ANTIMICROBIAL POTENTIALITY OF *ARTOCARPUS ALTILIS* FRUIT EXTRACTS

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ABSTRACT

The present study aims at bioefficacy assessment and phytoconstituent screening of *Artocarpus altilis* (breadfruit) fruit extracts in different solvents (methanol and ethyl acetate) for antimicrobial activity. The antimicrobial potentiality of plant fruit extracts obtained in different solvent media (methanol and ethyl acetate) were studied against various pathogenic organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Enterococcus faecalis*. Methanolic and ethyl acetate fruit extracts of *Artocarpus* exhibits the presence of only three phytochemicals viz. steroids, phenols and flavonoids. The MIC values of ethyl acetate and methanolic fruit extracts for microbes were determined by agar disc diffusion method which showed significant variation. *Streptococcus mutans* and *Enterococcus faecalis* showed maximum growth inhibition using 25 µl of methanolic fruit extracts with a MIC value of 0.90 and 0.6 mg/ml respectively. *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed maximum zone of inhibition using 25 µl of ethyl acetate fruit extract with a MIC value of 0.90 mg/ml for both. Methanolic fruit extract had no effect on growth of *Pseudomonas aeruginosa* and ethyl acetate fruit extract had negligible effect on growth of *Streptococcus mutans*. The fruit extracts of *Artocarpus altilis* has immense potentiality for antibacterial activities as evident from the phytochemical and MIC study. Further purification of the secondary metabolites and structural studies can aid in isolation of active compounds from this plant.

Keywords: *Artocarpus*; Antimicrobial activity; Fruit extract; Pathogens; Phytochemicals; MIC.

INTRODUCTION

In the present scenario, there has been a startling enhancement in the occurrence of new and re-emerging infectious diseases. In recent years researches on the emergence of multiple drug resistance to various human pathogenic bacteria has gained utmost attention all over the world which necessitated a search for new antimicrobial substances from other sources including plants. Early people confronted with illness and disease, discovered a wealth of useful therapeutic agents in the plant and animal kingdoms. The empirical knowledge of these medicinal substances and their toxic potential was passed on by oral tradition. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs. The plants with medicinal values and antimicrobial properties were in common use as a therapeutic agent to combat the detrimental side effects of conventional antibiotics as they have wide biological and medicinal activities, higher safety margins, easy reach to common people with affordable cost [1, 2, 3]. Plants are rich sources of various secondary metabolites such as tannins, alkaloids, terpenoids and flavonoids having been found *in vitro* with known therapeutic and antimicrobial properties which can be used as an effective as well as alternative treatment of diseases [4, 5]. Nowadays, multiple drug resistance associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions has developed due to indiscriminate use of commercial synthetic drugs [6]. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [7, 8].

Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg.) pantropical in its distribution is a multipurpose agroforestry tree crop which is primarily used for its nutritious, starchy fruit with rich source of carbohydrates, calcium and phosphorus, minerals and vitamins [9, 10]. It is a beautiful and prolific tree and an essential component of traditional Pacific island agriculture. It is originated from New Guinea and extensively grows in the Southern parts of India. The multifarious importance of different parts of breadfruit (latex, leaf tips, and inner bark) includes food, cosmetics,

medicine, clothing material, construction materials and animal feed [3, 9, 11]. Diluted latex is taken internally to treat diarrhoea. The sap from the crushed stems and leaves is used to treat ear infections.

The root is an astringent and used as a purgative. In West Indies the yellowing leaf is used in tea to reduce high blood pressure and relieve asthma [9]. Roasted leaf powder is used as a remedy for enlarged spleen [12]. The other species of *Artocarpus* has been studied for its antimicrobial activity by several researchers [13, 14]. Extracts from roots and stem barks showed some antimicrobial activity against Gram-positive bacteria and have potentiality in treating tumors [15, 16, 9]. The chromatographic study of breadfruit revealed high content of amino acid, fatty acids, and carbohydrates [17]. Atrocarpin, isolated from Thai Breadfruit heartwood extract exhibits inhibitory effect on melanogenesis showing high antioxidant activity. The medicinal uses of breadfruit are being actively researched; however still there is a huge dearth of information regarding the antimicrobial activity of different plant-parts of breadfruit. Comprehensive study aiming at evaluation of the potentiality of this plant against various bacterial pathogens is very much essential. This study might be reported to be the first regarding potentiality of fruit extracts of breadfruit in different solvents for their antimicrobial properties.

There was a very few reports on antibacterial properties of *Artocarpus* which is also largely restricted to the species of *A. heterophyllous* [13, 14, 18] and *A. communis* [19]. These unexplored therapeutic uses of bioactive compounds from breadfruit fruit extracts may reveal a great potential of the plant in pharmaceutical science. Therefore an attempt has been made to study the antibacterial activity of *Artocarpus altilis*. Present study revealed the comparative assessment of various phytoconstituents of *A. altilis* fruit extracts using different solvents. The investigations also comprise the bioefficacy assessment of breadfruit fruit extracts against four strains of bacterial pathogens viz *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Enterococcus faecalis* as evident from their MIC (minimal inhibitory concentrations) values. The study is the first report on phytoconstituent analysis and bioefficacy assessment for antimicrobial activity using *A. altilis* fruit extracts in different solvent media.

MATERIALS AND METHODS

Collection and Processing of Plant Material

The syncarpous fruits of Breadfruit [*Artocarpus altilis* (Parkinson) Fosberg]] were collected from OUAT, Bhubaneswar, India which were growing in natural condition. These are allowed to air dry followed by fine powdering in a Willey Miller. The plants were identified and authenticated at Herbarium Unit of Post Graduate Department of Botany, Utkal University, India. The fruiting season typically coincides with the wet, rainy summer months.

Bacterial Cultures

The cultures of different bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Enterococcus faecalis*) were procured from IMTECH, Chandigarh, India, and maintained at Laboratory of OUAT. Broth cultures of the four microbial strains showing an absorbance value ranging between 0.129-0.134 at a wavelength of 625 nm (i.e. equivalent to 0.5 McFarland of culture) were used for testing antimicrobial activity [3].

Preparation of Fruit Extracts using Different Solvents and Screening of Various Phytoconstituents

Fresh *Artocarpus* fruits were cleaned by thorough washing with deionized water, air dried at room temperature, powdered by a Willey Miller, sieved and the fine powder was stored in a clean glass container [20, 21]. 200 gram of ground powder was defatted with n hexane. The extraction was performed in a soxhlet apparatus by using 200 gram of finely macerated fruit powder in 750 ml of different solvents (methanol and ethyl acetate) individually with intermittent shaking (at 30°C to 40 °C). The distillation has been done to concentrate the extract with further concentration by evaporation using water bath at 100°C. The extracts were filtered using Whatman filter paper no. 42 (125 mm). The stock solutions of the fruit extract was prepared in 10% dimethylsulphoxide (DMSO) to give a concentration of 60 mg/ml. Different phytochemicals viz. alkaloids, flavonoids, phenolics, glycosides, phytosterol, steroid, tannin, terpenoids, fats, oils, gums and resins in methanolic and ethyl acetate fruit extracts of *A. altilis* were screened for their presence following standard methods with little modification [21, 22, 23].

Assessment of Antimicrobial Activity

Maintenance of bacterial strains cultures, preparation of discs, study of inhibition zone showing growth retardation of different pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Streptococcus mutans*) and calculations of MIC (minimum inhibitory concentration) values were undertaken following the methods of Pradhan et al., [3] after little modifications from Bauer et al., [24] and Prescott et al., [25].

Statistical Analysis

The results of the experiments were represented as the mean of the triplicates. Standard Error of Mean (SEM) values were calculated for

the data. A comparison is also made for the effectiveness of different solvent media against their antimicrobial activity.

RESULTS AND DISCUSSION

Qualitative and Comparative Assessment of Phytochemicals

The qualitative screening of phytochemicals showed the presence of steroid, phenols and falvonoids in both the Methanolic and ethyl acetate fruit extracts of *Artocarpus altilis*. Phytosterols are present in ethyl acetate fruit extracts only (Table.1). Previous reports on phytochemical analysis of leaf extract of breadfruit showed the presence of gums, resins, tannins and alkaloids in addition to the above [3].

Table 1: Phytochemical screening of fruit extracts of *Artocarpus altilis*.

Phytoconstituents	Fruit Extracts in different solvent media	
	Methanol	Ethyl acetate
Alkaloid	-	-
Steroid	+	+
Phenol	+	+
Flavonoid	+	+
Saponin	-	-
Tannin	-	-
Phytosterol	-	+
Gums & Resins	-	-
Terpenoid	-	-

Bioefficacy Assessment of Fruit Extracts against Bacterial Pathogens

Methanolic fruit extracts of *Artocarpus altilis* was more effective against *Enterococcus faecalis* in comparison to other pathogenic bacteria as shown by their high zone of inhibition. Maximum growth inhibition activity of *Enterococcus faecalis* (Zone of Inhibition: 20mm) was observed by 25µl methanolic fruit extracts of breadfruit (≈1.5 mg dry fruit matter) (Fig 1a.). This result showing the efficacy of methanolic fruit extracts of *Artocarpus altilis* against various human pathogens is probably due to the presence of phytoconstituents like flavonoids, sterols and phenols. These secondary metabolites play significant role in inhibiting growth of these human pathogens and act against them by developing an effective defense mechanism [26, 27]. Both the methanolic and ethyl acetate fruit extract have same zone of inhibition against *Staphylococcus aureus* at 25µl. Growth of *Staphylococcus mutans* was inhibited by treatment with methanolic fruit extracts of *Artocarpus altilis* at a concentration of 25µl (≈1.5 mg dry fruit matter) as evident from its inhibition zone of 15 mm (Fig 1b), whereas ethyl acetate leaf extract has no impact on its growth. Ethyl acetate fruit extract of *A. altilis* showed antibacterial activity against *Pseudomonas aeruginosa* whereas it has negligible impact on growth of *Streptococcus mutans*. The inhibitory effect of Ethyl acetate fruit extract on *P. aeruginosa* may be due to the presence of a flavonoid, artonin E [19].

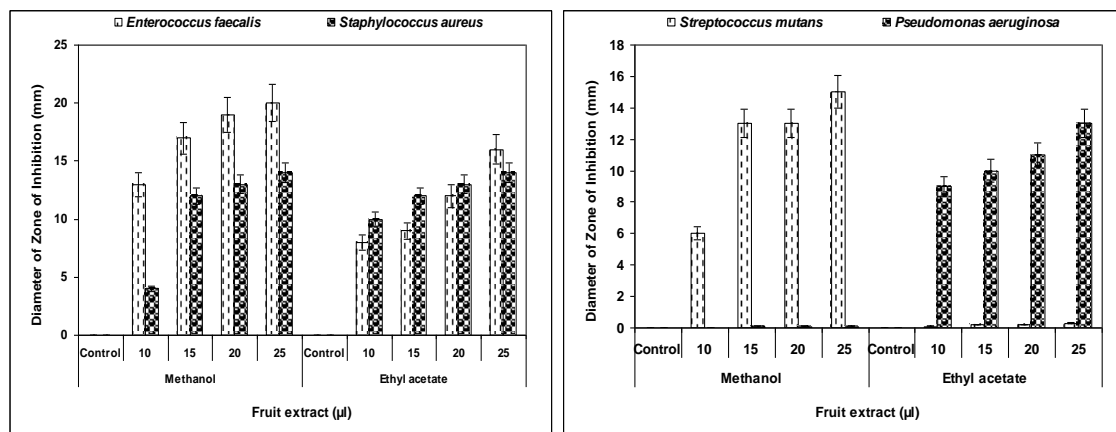


Fig. 1: Antimicrobial activity of fruit extracts of *Artocarpus altilis* against a) *Enterococcus faecalis* and *Staphylococcus aureus*, b) *Streptococcus mutans* and *Pseudomonas aeruginosa* (Values are Mean±SEM)

Methanolic fruit extract showed negligible zone of inhibition for *Pseudomonas aeruginosa* (Fig 1b.). Elevated growth inhibition of *Enterococcus faecalis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* were observed with increased dose of methanolic and ethyl acetate fruit extract of *Artocarpus altilis* as evident from their diameter of the zone of inhibition. MIC values of different fruit extract of *Artocarpus altilis* against different pathogenic microorganisms also varied significantly.

The MIC values of methanolic fruit extract of *Artocarpus altilis* was 0.9 mg for *Streptococcus mutans* (Inhibition Zone: 13 mm) and *Staphylococcus aureus* (Inhibition Zone: 12 mm) whereas only 0.6 mg of the extract showed growth inhibition for *Enterococcus faecalis*. Ethyl acetate fruit extract of *Artocarpus altilis* showed MIC values of 0.6 mg, 0.9 mg and 1.2 mg for inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* respectively. The antibacterial activity of fruit extract is attributed to the presence of phenolics which are released by hydrolysis of non-toxic glycosides. These phenolic constituents are mostly toxic to various microbial pathogens [28].

Presence of diverse chemicals in the extract or pure compounds of natural products provide unlimited prospects for the development of new drugs [29]. 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 [3,27,30]. In this investigation, the methanolic and ethyl acetate fruit extracts with its different phytoconstituents have immense importance for antimicrobial activity. The present study gives an insight about the antibacterial activity of *Artocarpus altilis* fruits against various pathogenic bacteria due to the presence of flavonoids and phenolics in different plant parts [3] which is a first kind of report. The plant fruit can be used as potential source for the development of a phytomedicine to act against infectious bacteria. Purification of economically important secondary metabolites as precursors for the synthesis of complex chemical substances, their action on microbial activity with proper administration of adequate dosage and subsequent structural studies will aid in isolation of bioactive compounds along with their nature and property, and discovery of therapeutic agents from the edible fruits of medicinally important breadfruit plant [31]. The components responsible for the anti-microbial activities of these promising plants will be further investigated. This study indicates the potentiality of breadfruit fruit which is widely used as functional foods for the treatment of various human pathogenic bacterial infections.

The present study provides scope for scientific studies to fully exploit the medicinal properties of breadfruit to support the traditional claims as well as, exploring some new and promising 'leads'. In view of the proven pharmaceutical credentials of *A. altilis* further identification, isolation, extraction and applications of bioactive compounds from breadfruit need to be investigated.

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