Original Article

Phytoconstituent Analysis and Comparative Bioefficacy Assessment of Breadfruit Leaf and Fruit Extracts for Antipathogenic Potentiality

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ABSTRACT

Present study reveals the comparative bioefficacy assessment of leaf and fruit extracts of breadfruit (*Artocarpus altilis*) using different solvents (petroleum ether, methanol and ethyl acetate) against various pathogenic organisms like *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans* and *Enterococcus faecalis* by disc diffusion assay through determination of MIC (minimal inhibitory concentrations). Steroids, phenols, phytosterols, gums & resins and terpenoids were found in 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*. Methanolic and ethyl acetate fruit extracts exhibits the presence of steroids, phenols and flavonoids. Zone of inhibition for *Streptococcus mutans*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were noted maximum with 50 µl of leaf extracts in ethyl acetate and methanol, 20 µl of petroleum ether leaf extract, 25 µl of petroleum ether leaf extract and 50µl of methanolic leaf extract respectively. The above observation for fruit extracts varies. 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. Maximum growth inhibition of *Streptococcus mutans* and *Enterococcus faecalis* was noticed with 25 µl of methanolic fruit extracts with a MIC value of 0.45 and 0.3 mg/ml respectively. *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed maximum zone of inhibition by 25 µl of ethyl acetate fruit extract with a MIC value of 0.45 mg/ml for both.

Keywords: *Artocarpus*; Antimicrobial activity; Leaf and fruit

extract; Phytochemicals; MIC.

Abbreviations

MIC: Minimal Inhibitory Concentrations; EF- *Enterococcus faecalis*,; PA- *Pseudomonas aeruginosa*; SM- *Streptococcus mutans*; SA- *Staphylococcus aureus*

INTRODUCTION

In recent years researches on the emergence of multiple drug resistance to various human pathogenic bacteria has gained utmost importance all over the world which necessitated a search for new antimicrobial substances from other sources including plants. In the present scenario, there has been a startling enhancement in the occurrence of new and re-emerging infectious diseases. 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 and lesser costs[1,2,3]. Plants are ample 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 and 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 antimicrobial 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 breadfruit includes food, medicine, clothing material, construction materials and animal feed. All parts are used medicinally in the Pacific and Caribbean, especially the latex, leaf tips, and inner bark. The latex is massaged into the skin to treat broken bones and sprains and is bandaged on the spine for relieving from sciatica.

It is commonly used to treat skin ailments and fungus diseases such as “thrush,” which is also treated with crushed leaves[9]. Diluted latex is taken internally to treat diarrhea, stomachaches, and dysentery. The sap from the crushed stems of leaves is used to treat ear infections or sore eyes.

The root is astringent and used as a purgative; macerated roots are used as a poultice for skin ailments. The bark is used to treat headaches in several islands. In the West Indies the yellow leaf is brewed into tea and taken to reduce high blood pressure and relieve asthma[9].The tea is also thought to control diabetes. A powder of roasted leaves is used as a remedy for enlarged spleen[11]. The other species of *Artocarpus* has been studied for its antimicrobial activity by several researchers[12,13]. Extracts from roots and stem barks showed some antimicrobial activity against Gram-positive bacteria and have potential use in treating tumors[14]. The root and stem bark extracts were being used against some bacteria[9,15]. The chromategraphic study of breadfruit revealed high content of amino acid, fatty acids, and carbohydrates[16]. Atrocarpin, isolated from Thai Breadfruit heartwood extract exhibits inhibitory effect on melanogenesis showing high antioxidant activity. These effects indicate the potential use of heartwood of Bread fruit in cosmetics[17,3]. 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 foremost regarding antibacterial potentiality of leaf and fruit extracts of breadfruit (*Artocarpus altilis*) against human pathogenic bacteria.

There was a very few reports on antibacterial properties of *Artocarpus* which is also largely restricted to the species of *A. heterophyllous*[12,13,18] and *A. communis*[19]. There is a huge dearth of information on the antimicrobial potentiality of various parts of breadfruit. 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* leaf and fruit extracts using different solvents. The investigations also comprise the bioefficacy of breadfruit leaf and fruit extracts against four strains of human bacterial pathogens viz *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Enterococcus faecalis* as evident from their MIC (minimal inhibitory concentrations) values. This study emphasizes the comparative growth inhibiting activity of leaf and fruit extracts against multi drug resistant organisms in different solvent media. The study is the newly developed original recent report on phytoconstituent analysis and bioefficacy assessment for antimicrobial activity using *A. altilis* fruit extracts in different solvent media.

MATERIALS AND METHODS

Plant Material Collection

The syncarpous fruits and leaves of Breadfruit [*Artocarpus altilis* (Parkinson) Fosberg)] (Fig. 1a) 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[3].

Bacterial Cultures

The cultures of different bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Enterococcus faecalis*) were procured from

IMTECH, Chandigarh, India and cultured in controlled condition for testing antimicrobial activity[3].

Preparation of Fruit and Leaf Extracts using Different Solvents

Fresh *Artocarpus* fruits and leaves 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]. 40 gram of ground powder was defatted with n hexane. The extraction was performed separately in a soxhlet apparatus by using 200 gram of finely macerated fruit powder and 100 gram of finely macerated leaf powder in 750 ml of different solvents (methanol, petroleum ether and ethyl acetate) individually for 3 days (at 30^0C to 40^0C) with inter-mittent shaking[22]. 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 and leaf extract was prepared in 10% dimethylsulphoxide (DMSO) to give a concentration of 60 mg/ml and 30 mg/ml respectively.

Minimum Inhibitory Concentration (MIC) and Antimicrobial Activity Testing

Maintenance of bacterial strains cultures, preparation of discs and study of inhibition zone showing growth retardation of different pathogenic bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis and Streptococcus mutans*) were undertaken following the methods of Pradhan *et al*., (2013a).

Phytoconstituent Screening

The screening of different phytochemicals viz. alkaloids, flavonoids, phenolics, glycosides, phytosterol, steroid, tannin, terpenoids, fats, oils, gums and resins were conducted in the laboratory as per the standard methods with little modification[21,23,24]. The crude extracts were stored in desiccators for maximum of 3 days and later preserved in deep freezer (-20^0C) for further use. The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in different solvent extracts of leaves and fruits[22].

Statistical analysis

The experiments were conducted in triplicates and the data were represented as the arithmetic mean values along with their Standard Error of Mean (SEM) values. A comparison is also made for the effectiveness of different solvent media against their antimicrobial activity.

RESULT AND DISCUSSION

Qualitative and Comparative Assessment of Phytoconstituents

The qualitative screening of phytoconstituents showed the presence of only 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).

Presence of all the necessary secondary metabolites such as steroids, phenols, tannins, phytosterols, gums & resins and terpenoids were reported in Methanolic leaf extracts of *Artocarpus altilis* except for saponins, flavonoids and alkaloids. Similar results were noted in ethyl acetate leaf extracts with a little deviation of absence of tannins and presence of flavonoids. But petroleum ether leaf extracts showed the presence of only four metabolites viz. steroids, flavonoids, phytosterols, gums & resins out of nine metabolites tested (Table.1).

Bioefficacy testing of fruit and leaf 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 1b.). The methanolic leaf extracts of *Artocarpus altilis* exhibited maximum growth inhibition activity against *Pseudomonas aeruginosa* (Zone of Inhibition: 18mm) followed by petroleum ether (Zone of Inhibition: 15mm) and ethyl acetate extracts (Zone of Inhibition: 13mm) (Fig 1c.) at a concentration of 50µl (≈1.5 mg dry leaf matter). The antimicrobial activity of methanolic leaf extract of *Artocarpus altilis* at a concentration of 50µl was found highest showing an inhibition zone of 16mm and most effective among all the different types of leaf extracts studied against *Streptococcus mutans* (Fig 1c). Growth of *Staphylococcus aureus* was highly inhibited by treatment with methanolic leaf extracts of *Artocarpus altilis* at a concentration of 25µl as evident from its inhibition zone of 24 mm (Fig 1c). The inhibition was highest among all the three types leaf extracts tested. Elevated growth inhibition of *Streptococcus mutans, Staphylococcus aureus, Pseudomonas aeruginosa* were observed with increased dose of methanolic leaf extract of *Artocarpus altilis* as evident from their diameter of the zone of inhibition.

Ethyl acetate leaf extracts at a concentration of 10µl also found effective against *Staphylococcus aureus* showing an inhibition zone of 12 mm (Fig 1c). 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.* 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.

Petroleum ether leaf extracts at a concentration of 25µl showed maximum zone of inhibition with a diameter of 22mm when tested against *Enterococcus faecalis* followed by methanol and ethyl acetate leaf extracts with an inhibition zone of 15mm. (Fig 1c).

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.

The comparative assessment of breadfruit fruit and leaf extracts for their phytoconstituent composition and antimicro-bial potential, reported that the methanolic fruit extracts at high concentration were more effective against *Staphylococcus aureus, Streptococcus mutans and Enterococcus faecalis* than ethyl acetate fruit extract. But it has negligible impact on growth of *Pseudomonas aeruginosa*. 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[25,26]. The inhibitory effect of Ethyl acetate fruit extract on *P. aeruginosa* may be due to the presence of a flavonoid, artonin E[19]. 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[27]. Methanolic leaf extracts were very much effective than others and showed highest antimicrobial activity. This result showing the efficacy of methanolic leaf extracts of *Artocarpus altilis* against various human pathogens is probably due to the presence of a wide range of phytochemical constituents, especially the presence of tannins. These secondary metabolites plays significant role in inhibiting growth of these human pathogens and act against them by developing an effective defense mechanism[25,26]. The tannins which were present in the methanolic leaf extracts of *Artocarpus altilis* have been found to form irreversible complexes with proline-rich proteins and these compounds are known to be biologically active that resulting in the inhibition of the cell protein synthesis as a result of which microbial growth is inhibited. Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect. Tannins act as stable and potent antioxidants which fights against various toxins released from the microbes[13,28]. Tannins are potent inhibitors of proteolytic enzymes used by plant pathogens[26,27]. Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens[27]. In recent years, screening of different phytochemical constituents of medicinally important plants like *Artocarpus altilis* for its multifarious antimicrobial activity has gained utmost importance. The present study is a first kind of report on the medicinally important plant *Artocarpus altilis* which has its great medicinal relevance in the recent years. The knowledge of its various phytochemical constituents and the activity against tested pathogens may not only provide an insight for discovery of therapeutic agents, but also such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances [29].

Natural products either extract or pure compounds contain diverse chemicals which provide unlimited prospects for the development of new drugs[30]. 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[26,31]. In this investigation, the methanol, ethyl acetate and petroleum ether leaf extracts with its different phytochemical constituent have immense importance for antimicrobial activity. It has been inferred

from the preset study that the leaves of *Artocarpus altilis* has significant antibacterial activity gainst 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.

Presence of diverse chemicals in the extract or pure compounds of natural products provide unlimited prospects for the development of new drugs[30]. 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[26,3,31]. In this investigation, the methanolic and ethyl acetate fruit extracts with its different phytoconstituents have immense importance for antimicrobial activity.

CONCLUSION

The present comparative study accentuates the potentiality of *Artocarpus altilis* leaf and fruit extracts in different solvent media for their antibacterial activity against various pathogenic bacteria. The bioefficacy potentiality of breadfruit extracts was attributed to the presence of flavonoids and phenolics in different plant parts. The plant leaf and fruit can be used as potential source for the development of a phytomedicine to act against infectious bacteria by purifying the economically important secondary metabolites. further investigations on the isolation of bioactive components synthesized from precursor secondary metabolites of medicinally important breadfruit plant and their administration with adequate dose as therapeutic agents for their action on microbial activity will be carried out. 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.

ACKNOWLEDGEMENT

The authors are grateful to the funding sources like DST-PURSE, Government of India and UGC-DRS (SAP) Programme for financial support and smooth running of the research work. The authors also acknowledge IMTECH, Chandigarh for providing the microbial strains.

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Table 1. Phytoconstituent analysis of fruit and leaf extracts of *Artocarpus altilis*

Phytoconstituents	Solvent Extracts					
	Methanol		Ethyl acetate		Petroleum ether	
	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf
Alkaloid	-	-	-	-	NA	-
Steroid	+	+	+	+	NA	+
Phenol	+	+	+	+	NA	-
Flavonoid	+	-	+	+	NA	+
Saponin	-	-	-	-	NA	-
Tannin	-	+	-	-	NA	-
Phytosterol	-	+	+	+	NA	+
Gums &Resins	-	+	-	+	NA	+
Terpenoid	-	+	-	+	NA	-

Figure 1a. Picture showing *Artocarpus altilis* plant with fruits

 ISSN 2321 – 2748

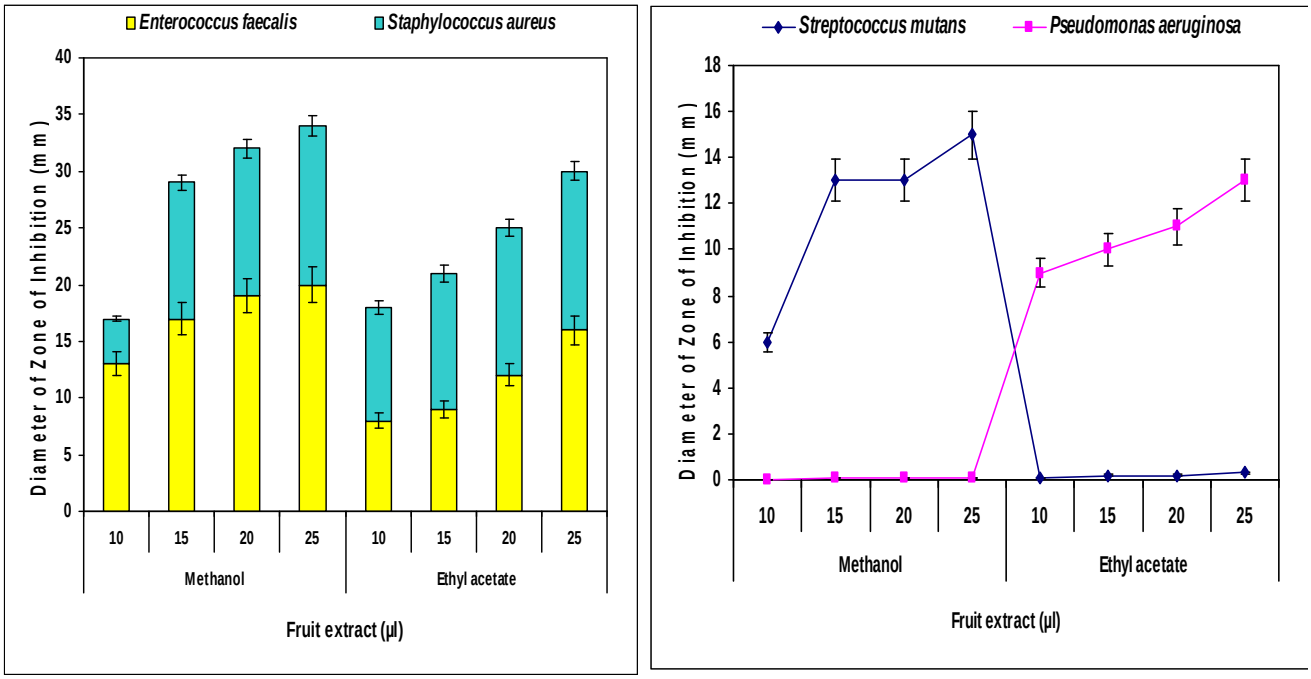

Figure 1b. Antibacterial activity of fruit extracts of *Artocarpus altilis* against *Enterococcus faecalis, Staphylococcus aureu, Streptococcus mutans* and *Pseudomonas aeruginosa s* (Values are Mean±SEM)

 ISSN 2321 – 2748

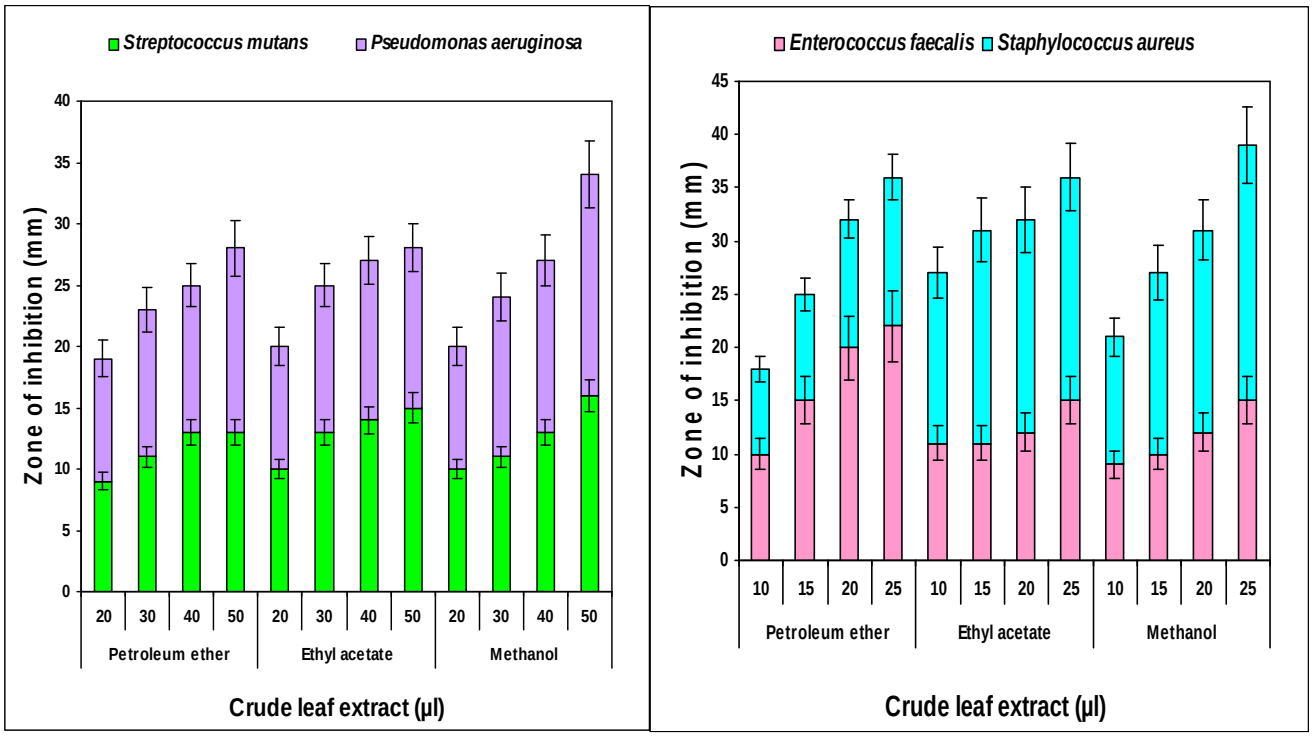

Figure 1c. Antibacterial bioefficacy of breadfruit leaf extracts showing growth inhibition against pathogens (*Streptococcus mutans* and *Pseudomonas aeruginosa, Enterococcus faecalis* and *Staphylococcus aureus*)
NB: (Values are Mean±SEM)