

Studies on phytochemical analysis and antimicrobial activity of *Artocarpus heterophyllus* fruit latex against selected pathogenic microorganisms

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Abstract:

The latex is widely used in cosmetics, pharmaceuticals and food industry as in paper, textile and petroleum industries also. The present study was carried out to assess the potential antimicrobial activity of methanolic, ethanolic and chloroform extracts of fruit latex of *Artocarpus heterophyllus* against different pathogenic bacteria and fungus. All three extracts were found to show good to moderate activity against bacteria namely Gram +ve (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*) and Gram -ve (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungal strains namely *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. In methanolic extract major activity was perceived on bacteria *Bacillus subtilis* and among fungi *Aspergillus flavus*. In ethanolic extract maximum activity perceived on bacteria *Pseudomonas aeruginosa* and amongst fungi *Aspergillus flavus*. In chloroform extract determined activity was observed on bacteria *Staphylococcus aureus* and between fungus *Aspergillus flavus*. Maximum activity was observed on bacterial strains compared with fungal strains. In all three extracts Minimum inhibitory concentrations were in the range of 12.5-25 mg/ml. In addition, quantitative phytochemical and Biochemical analysis estimations of assured active compounds like Total phenolics, flavonoids, tannins, proteins, carbohydrates, glycosides, and alkaloids were found to be in considerable quantities.

KEYWORDS: *Artocarpus heterophyllus* fruit latex, Phytochemical analysis, Biochemical analysis, antimicrobial activity.

INTRODUCTION

Artocarpus heterophyllus, (Moraceae family) is one of the most important trees in tropical home gardens and perhaps the most widespread and useful tree in the significant genus *Artocarpus*.¹ (Raja Sekhar K.K. 2010). The tree is reportedly native to the rainforests of Malaysia, the Western Ghats of India and also found in central and Eastern Africa, south-eastern Asia, the Caribbean, Florida, Brazil, Australia, Puerto Rico and many Pacific Islands.

All parts of the tree exude sticky, white milky latex when injured.² (Rahman A.M. 1999). The whole tree has a valued place in research due to its medicinal and nutritive properties. All parts of the tree are said to have medicinal properties. The young fruits are acrid, astringent, and carminative. The ripe fruits are sweet, cooling, laxative, aphrodisiac and also used as a brain tonic. The seeds are diuretic, and constipating. The wood is nervine, antidiabetic, sedative and is useful in convulsions.³ (Hemborn pp. 1996). Plant latex has been used in folk medicine since it has clot inducing and dissolving properties in human hemostasis, wound healing and antimicrobial activity.⁴ (Osoniyi O, 2003,⁵Bohlooli S 2007). including the treatment of various forms of cancer.⁶ (Hartwell J.L, 1967). the latexes are source of various biologically active compounds, including glycosides,

tannins and many proteins.⁷ (wititsuwannakul D2002. 8Dubey VK 2003).

The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent.⁹ (Sato M 1996). Mixed with vinegar, the latex promotes healing of abscesses, snakebite and glandular swelling.¹⁰ (Vaidyagote V.M, 2000). Hot water extract of mature jack leaves is recommended by Ayurvedic and traditional medical practitioners as a treatment for diabetes mellitus.¹¹ (Jayaweera D.M.A, 1982). Phytochemical screening has revealed that the hot water extract contains flavonoids, leucoanthocyanins, anthocyanins and tannins as components. It is an important source of compounds like morin, dihydromorin, artocarpetin, norartocarpetin, jacalin, ellagic acid, cycloheterophyllin, sapogenins, carotenoids, cycloartinone, betulinic acid, artocarpone and heterophyllol.¹² (prakash o, 2009). Some scientific evidences of the bioactivity of *A.heterophyllus* were reported on the extract or isolated compound. However few reports are related to the antimicrobial activity of this taxon. The present study was carried out to evaluate the potential antimicrobial activity of methanolic, ethanolic, and chloroform extracts of fruit latex of *Artocarpus heterophyllus*.

MATERIALS AND METHODS

Collection of plant latex

A.heterophyllus, fruit latex was collected between April 2007 to June 2007 from the surrounding area of Simhachalam, Paderu and Aruku of Visakhapatnam district, Andhra Pradesh, India. Plant was identified as by a plant Taxonomist Dr. M. Venkaiah, Associate Professor, Department of Botany, Andhra University, Visakhapatnam.

Extraction of latex

A.heterophyllus, fruit latex were aseptically collected and subjected to drying at room temperature for 12 hrs. The dried matter (10 g) of latex was extracted by using 100ml of organic solvents (chloroform, ethanol and methanol). The suspended solutions were left to stand for 5 days, and the resulting extract was filtered and evaporated to dryness under reduced vacuum 60°-80°C and a brown gummy residue was obtained. The obtained residue was used to determine the antimicrobial activity.¹³

Phytochemical and Biochemical analysis

Analysis of total phenolics

The total phenolics were determined by Javanmardiet, al.(2003)¹⁴method. 50µl of methanolic, Ethanolic and chloroform extracts of the fruit latex, 2.5ml diluted Folin-ciocalteu reagent and 2.0ml of 7.5% (w/v) sodium carbonate were added and incubated at 45°C for 15min. The absorbance of all samples was measured in a spectrophotometer (*Hitachi, Germany*) at 765nm and the results were expressed as mg of Gallic acid equivalents.

Estimation of total tannins

The total tannins were determined using the Folin-Ciocalteu method (1927)¹⁵. To 0.1ml of fruit latex in methanol, ethanol, and chloroform extracts added 6.5ml of water and 0.5ml of Folin-ciocalteu reagent and 1.5ml of 20% sodium carbonate at overnight standard solution and incubated for 1hour and the absorbance of all samples were measured in a spectrophotometer at 725nm, and the results were expressed as mg of Tannic acid equivalents.

Estimation of total flavonoids

Total flavonoids content was carried by the method of Chang *et al.* (2002)¹⁶To 0.5ml of fruit latex in methanol, ethanol, and chloroform extracts added 1.5ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium

acetate and 2.8ml of distilled water, it remained at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415nm on U/V visible spectrophotometer. The results were expressed as mg of Quercetin equivalents.

Estimation of Alkaloids

Total alkaloid content was estimated by the method of Sreevidya and Mehrotra(2003)¹⁷. A standard solution was prepared by dissolving 5mg of boldine and fruit latex extract separately in 5ml of warm distilled water each. Five ml of boldine solution/sample extract was adjusted to pH2-2.5 (with 0.01 M HCl), and 2ml of DR was added to form an orange precipitate that was centrifuged at 5000 rpm for 15min.Afterward ,DR was added to the supernatant to check for complete precipitation. A 2ml amount of 1% sodium sulfide was added to the residue to form a brownish black precipitate which was centrifuged at 5000rpm for 15min. Complete precipitation was checked by further adding1% sodium sulfide. The resulting residue was dissolved in 2ml of nitric acid with warming and sonication and then made up to 10ml with distilled water. A 5ml amount of 3% thiourea was added to 1ml of the resulting solution to form a yellow bismuth complex, of which the absorbance was measured at 435 nm. All the assays were performed in triplicate. The amount of bismuth present in the boldine solution /extract was achieved from the calibration curve of bismuth nitrate. The results were expressed as boldine, considering that is a monobasic alkaloid, and therefore the complex formed with bismuth follows a 1:1 stoichiometry. Plant materials containing alkaloids were determined. The method was compared with other methods. It can be used for routine analysis of commercial samples by industries dealing with herbal drugs for standardization of plant materials containing alkaloids and for alkaloid-containing pharmaceutical products.

Estimation of total Hexosamine

Total hexosamine content was carried by the method of Wagner *et al.*(1979)¹⁸0.5ml of the fruit latex extract was made up to 1.0ml with distilled water. Standard galactoseamine (in the range of 10-40 µg) was also made up to 1.0ml. Blank comprised of 1.0ml distilled water, 0.6ml of acetyl acetone reagent was added to all the tubes and heated in a boiling water bath for 30 min. After cooling, 2.0ml of Ehrlich's reagent was added and the contents were shaken well. The pink colour developed was read at 540nm against a blank using Photochem colorimeter. The results were expressed as mg of galactosamine equivalents.

Estimation of Sialic acid

Total Sialic acid was determined by the method of Warren, (1959)¹⁹. To 0.5 ml of fruit latex in methanol, ethanol, and chloroform extracts 0.25 ml of periodate was added and incubated at 37°C for 30 min. After incubation, the reaction was arrested by the addition of 0.25 ml of arsenite. The tubes were shaken well and 2.0 ml of TBA was added and the tubes were heated in a boiling water bath for 6 min. After cooling, 5.0 ml of acidified butanol was added and the butanol phase was separated after shaking well. The absorbance was read at 540 nm against a blank treated similarly using a Photochem colorimeter. Standard solutions containing 10-50 µg of n-acetyl neuraminic acid were also treated similarly.

Estimation of proteins and carbohydrates

Total protein content was estimated by Lowry (1951)²⁰ method and total carbohydrate content was estimated by Anthrone method as per Yemm and Willis (1954)²¹. Total fructose was estimated by Ashwell G. (1957)²² method. Other carbohydrates like starch Hedge et al. (1962)²³ and cellulose Updegroff, D. M. (1969)²⁴ were estimated by Anthrone method.

Test Organism

The selected test organisms were purchased from Microbial Type Culture Collection (MTCC) bank, Chandigarh as a freeze dried pure culture. The bacterial cultures were revived by using MTCC specified selective growth medium. The test organisms are *Staphylococcus aureus* (MTCC 3160), *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 420), *Aspergillus niger* (MTCC 961), *Aspergillus flavus* (MTCC 3396), *Candida albicans* (MTCC 227), *Saccharomyces cerevisiae* (MTCC 170). They were separately sub-cultured and the pure culture resub-cultured on Nutrient Agar and Sabouraud Dextrose Agar media, respectively and stored at 4°C for further studies.

Inoculum preparation

Fresh microbial cultures were prepared by streaking loop full of bacterial suspension into organism specific selective media and incubated at optimal temperature in order to maintain approximately uniform growth rate of each organism. The bacterial cultures from fresh media were compared with 0.5 McFarland turbidity standards, which is equivalent to approximately 1×10^8 bacterial cell count per ml, were maintained throughout the experimentation²⁵.

Antimicrobial assay

The antimicrobial activities were determined by the agar well diffusion method²⁶. The plant latex extract along with standards Ampicillin and Fluconazole was screened *in vitro* for antimicrobial activity against gram positive bacteria *S. aureus*, *B. subtilis* and *B. cereus*, gram negative bacteria *P. aeruginosa* and *E. coli* and antifungal activities against *C. albicans*, *S. cerevisiae*, *A. niger* and *A. flavus*. The different concentrations of plant latex extract (100 mg/ml, 50 mg/ml and 25 mg/ml and 12.5 mg/ml) were used for testing antimicrobial activities. The sterile nutrient agar medium and Sabouraud dextrose agar media plates, was inoculated with test organism. The inoculation has to be completed under aseptic conditions and when the medium was in molten state. The inoculated medium was transferred to sterile Petri dishes, evenly distributed and allowed to solidify. The wells (6 mm diameter) were made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. Into each of these wells, 0.05 mL (50 µg) of the fruit latex extract /reference standard/control was added by using a micropipette. The plates were incubated at 37°C for 24 h and 28°C for 48 hrs, respectively for the bacterial and fungal cultures. The observed inhibitory zones were measured excluding 6 mm diameter wells.

RESULTS

The Results of the phytochemical and biochemical screening and quantitative estimations showed that *A. heterophyllum* fruit latex of three solvent extracts like methanolic, ethanolic and chloroform extracts contain phenolics, flavonoids, tannins, proteins, carbohydrates, glycosides, and alkaloids. The total phenol content was expressed as µg of Gallic acid equivalents. The total phenol content of the *A. heterophyllum* fruit latex was ranged from 7.63 ± 0.25 to 7.11 ± 0.11 µg /gm of extract, and decreased in the following order ACC > ACM > ACE. The total flavonoid content was expressed as mg of Quercetin equivalents. The total flavonoid content of the extracts *A. heterophyllum* fruit latex ranged from 7.76 ± 0.15 to 7.63 ± 0.45 µg/gm of extract and decreased in the following order ACM > ACE > ACC. The total tannin content was expressed as tannic acid equivalent per mg. The total tannin content of the extracts of *A. heterophyllum* fruit latex ranged from 2.13 ± 0.15 to 1.76 ± 0.05 µg /gm of extract and decreased in the following order ACM > ACC > ACE. The total Alkaloids content was expressed boldine as equivalent per mg. The monobasic alkaloids content of the extracts of *A. heterophyllum* fruit latex ranged from 8.56 ± 0.30 to 7.26 ± 0.25 mg /gm. And decreased in the following order ACM > ACC > ACE. The total protein content is expressed as mg of bovine serum albumin equivalents. The total protein content of the *A. heterophyllum*

fruit latex was ranged from 3.57 ± 0.36 to 2.94 ± 0.04 mg /gm, and decreased in the following order ACC > ACM > ACE. The total carbohydrate content was expressed as mg of Standard Glucose equivalents. The total carbohydrate content of the extracts *A.heterophyllum* fruit latex ranged from 4.72 ± 0.20 to 4.46 ± 0.24 µg/gm of extract and decreased in the following order ACC > ACM > ACE. The total fructose content is expressed as mg of Standard fructose equivalents. The total fructose content of the *A.heterophyllum* fruit latex was ranged from 6.46 ± 0.29 to 4.28 ± 0.33 µg /gm of extract and decreased in the following order ACM > ACC > ACE. The total starch content was expressed as mg of Standard Glucose equivalents. The total starch content of the extracts of *A.heterophyllum* fruit latex ranged from 6.5 ± 0.11 to 5.91 ± 0.22 mg /gm, and decreased in the following order ACM > ACE > ACC. The total cellulose content was expressed as Standard cellulose equivalent per mg. The total cellulose content of the extracts of *A heterophyllum* fruit latex ranged from 6.03 ± 0.57 to 4.9 ± 0.61 µg/gm of extract in the following order ACM > ACC > ACE. The total hexose amine content was expressed as mg of galactosamine equivalents. The total hexose amine content of the extracts of *A. heterophyllum* fruit latex ranged from 6.91 ± 0.09 to 6.69 ± 0.80 mg /gm, and decreased in the following order ACC > ACM > ACE. The total Sialic acid content was expressed as mg of n-acetyl neuraminic acid equivalents. The total Sialic acid content of the extracts of *A. heterophyllum* fruit latex ranged from 8.82 ± 0.04 to 8.53 ± 0.33 µg /gm of extract and decreased in the following order ACM > ACE > ACC. The antibacterial potency of *A. heterophyllum* fruit latex extract was evaluated by the presence or absence of inhibition zones and zone diameters (mm). From the results, it is evident that the Methanolic, Ethanolic, and Chloroform extracts of *A.heterophyllum* fruit latex showed a maximum inhibitory zone in a dose dependant manner (Table.2). The bacteria used were clinical stains of Gram +ve (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*) and Gram -ve (*Escherichia coli* and *Pseudomonas aeruginosa*) However, there was no significant difference between the levels of zone of inhibition at the concentration of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml. The bacterial potency of *A.heterophyllum* fruit latex in methanolic extract on Gram-positive bacteria, *B.subtillis* showed that result 24.6 ± 4.28 (100 mg/ml) larger diameter of clearance than that of the other ethanolic and chloroform extracts of Gram positive bacteria used in this study. Similarly, *A.heterophyllum* fruit latex methanolic extract showed a maximum zone of clearance in the Gram negative bacteria. *P.aeruginosa* 19.3 ± 2.16 (100 mg/ml) than that of other ethanolic and chloroform extracts of Gram negative bacteria. Moreover, the zone of clearance achieved by *A.heterophyllum* fruit latex

extracts is comparable to that of standard antibiotic Ampicillin. The fungal stains namely *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. The antifungal activity of *A.heterophyllum* fruit latex methanolic, ethanolic and chloroform extracts were evaluated using agar well diffusion method, these extracts were tested against *A.niger*, *A.flavus*, *C.albicans*, *S.cerveisia* at concentrations of 12.5mg/ml, 25mg/ml, 50mg/ml, and 100mg/ml. Among the various solvent extracts of *A.heterophyllum* fruit latex methanolic extract showed significant zone of inhibition against fungi where as ethanolic and chloroform extractions showed significant inhibition against *A.niger* and *C.albicans* (Table.3). The fungal potency of *A.heterophyllum* fruit latex methanolic extract on *A.flavus* 18 ± 2 (100 mg/ml) showed larger diameter of clearance than that of other ethanolic and chloroform extracts.

DISCUSSION

Polyphenolics are known to function as antioxidants through a number of mechanisms including radical scavenging by H-donation, prevention of chain initiation by donating electrons or by binding of transition metal ion catalysts. 27 (Deepikagupta, 2011). The total estimations of phenols, flavonoids, tannins, proteins, carbohydrates, glycosides and alkaloid content of the *A.heterophyllum* fruit latex shown in Table-1. The chloroform extracts of the *A.heterophyllum* fruit latex had higher phenol content than the methanolic and ethanolic extracts. The methanol extracts of the *A.heterophyllum* fruit latex had higher tannin content than the ethanol and chloroform extracts. These phenolics and tannins have similar results reported in seed extracts of *Artocarpusheterophyllum* plant²⁸. (K.Shanmugapriya 2011) Polyphenolic compounds tend to be potent free radical scavengers and their abilities to act as antioxidants mainly depends on their chemical structure, capability to donate/accept electrons, thus delocalizing the unpaired electron within the aromatic structure and the polyphenols are broadly classified into two categories, flavonoids and phenolic acids²⁹ (Augustin. S, 2005). The Flavonoids prevent platelet stickiness and hence platelet aggregation. 27 (Deepikagupta, 2011) The basis of the presence of quercitrin flavonoid that could attenuate the diabetic state by decreasing oxidative stress and preserving pancreatic β-cell integrity. 30 (Coskun, 2005) The methanol extract had higher flavonoid content than other extracts of ethanol and chloroform. Flavonoids, a family of phytochemical compounds, are widely distributed in foods of plant origin such as vegetables, fruits and in medicinal plants, display a wide range of pharmacological properties including anti-oxidative, anti-inflammatory, anti-proliferative, anti-mutagenic, anti-carcinogenic and anti-

cancer effects^{31,32,33,34,35} (Kuo, 1997; Birt et al., 2001; Ren et al., 2003; Yamamoto and Gaynor, 2001; Li et al., 2007). These flavonoids are similar *A.heterophyllus* fruit latex results were reported earlier in leaf extract of *Artocarpus heterophyllus* plant³⁶ (Chandrika U). Some alkaloids function as spasmolytic, anti-cholinergic and anesthetic agents. 27 (Deepikagupta,2011). The methanol extracts of the *A.heterophyllus* fruit latex had higher alkaloid content than the chloroform and ethanol. Similar results were reported in *Ficus thoningii* plant³⁷ (Ndukwe, I.G,2007) Plant proteins usually are produced to resist potential pathogens and diseases.38.(Glandorfdcm. 1997) The total protein content of similar *A.heterophyllus* fruit latex results were reported earlier in tree latex extract of *Artocarpus heterophyllus* plant³⁹ (Jaruwansiritapetawee 2011) The chloroform extracts of the *A.heterophyllus* fruit latex had higher protein content than the methanolic and ethanolic extracts. Carbohydrates are the important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharides which cannot be split by hydrolysis into more simpler sugars. The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides.23 (Hedge,JE 1962). In our *A.heterophyllus* fruit latex the chloroform extract had higher carbohydrate content than other extracts of methanol and ethanol. Similar results were reported earlier in latex extract of *Euphorbia splendensvarhislopii latex* plant⁴⁰ (Clélia Christina Mello-Silva.2010). Fructose is a keto-hexose is usually accompanied by sucrose in fruits. 22 (Ashwell,g 1957). The methanol extracts of the *A.heterophyllus* fruit latex had higher fructose content than the chloroform and ethanol Similar *A.heterophyllus* fruit latex results were reported earlier in *Phoenixdactylifera* plant⁴¹. (Moustafa A.2008). Starch is a mixture of polysaccharides amlopectin and amylose. Starch is used as an universal binder. It is fine white powder, odorless, and useful in food industry. 42 (The wealth of India-dictionary. 2004). In our methanol extract of the *A. heterophyllus* fruit latex had higher starch content than the ethanol and chloroform extracts. Similar results were reported in seed extracts of *Artocarpusheterophyllus* plant.⁴³ (NarkhedeSachin B.,2011) The methanol extract of the *A. heterophyllus*fruit latex had higher cellulose content than the ethanol and chloroform extracts. Similar results were reported in seed extracts of *Artocarpus heterophyllus* plant.²⁷.(K.Shanmugapriya 2011)The biochemical compounds of Hexosamine has an important role in the synthesis of sialic acid, a family of acetylated derivatives of N-acetyl neuraminic acid, occurs as a terminal component of carbohydrate chain of glycolproteins and gangliosides. It forms structural

component of glycolipids present on the surface of tumour cells 44 ,45 (Voet and Voet, 1995; Erbil et al., 1985.). The chloroform extract of the *A.heterophyllus*fruit latex had higher hexose amine content than the ethanol and methanol extracts. The methanol extract of the *A. heterophyllus* fruit latex had higher Sialic acid content than the ethanol and chloroform extracts. Both of these hexose amine and Sialic acid contents are similar results were reported in latex extracts of *Jatropha curcas* plant⁴⁶ (R. Balaji 2009) Various studies have reported the significance of sialic acid as a tumour marker in various cancers including melanoma. Aberrant glycosylation processes in tumour cells contribute to the biosynthesis of certain oligosaccharides, hence, malignant or transformed cells contain increased sialic acid residues on their surfaces 47,48. (Yogeeswaran, 1981; Hulbert et al., 1979). These metabolites present in various parts are known to have varied pharmacological actions in humans and animals³⁷(Ndukwe, I.G,2007).

Antimicrobial and phytochemical analysis of *Artocarpus heterophyllus* plant seed extract²⁸. (K. Shanmugapriya 2011) Hence these fruit latex extracts might be exhibiting similar effect, furthermore these results is in concert with the reports on the activities of the fruit latex against micro-organisms. Similar results were reported in latex extracts of *Artocarpus heterophyllus* plant⁴⁹ (Ramappa, Raghavendra.2011). *Escherichia coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination 58,59 *Pseudomonas aeruginosa* is a common bacterium that can cause disease in animals, including humans. An opportunistic, nosocomial pathogen of immun-ocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections.(60). *Bacillus cereus* is an endemic, soil-dwelling, Gram-positive, rod-shaped, beta hemolytic bacterium. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals.*B. cereus* is responsible for a minority of foodborne illnesses (2–5%), causing severe nausea, vomiting and diarrhea (61)

Staphylococcus aureus is a bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning.(62). *Aspergillus niger* or *A. niger* is a fungus and one of the most common species of the genus *Aspergillus*. It

causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. *A. niger* is less likely to cause human disease than some other *Aspergillus* species, but, if large amounts of spores are inhaled, *A. niger* can be deadly. This is due to a serious lung disease, aspergillosis, that can occur. Aspergillosis is, in particular, frequent among horticultural workers that inhale peat dust, which can be rich in *Aspergillus* spores. It has been found in the mummies of ancient Egyptian tombs and can be inhaled when they are disturbed (63). *Aspergillus flavus* is a saprotrophic and pathogenic fungus with a cosmopolitan distribution. It is best known for its colonisation of cereal grains and legumes. In addition to causing pre-harvest and post-harvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins, which when consumed are toxic to mammals (64).

From the results of the antibacterial studies as shown table 2 the methanolic extracts had more activity on the organisms than the ethanolic and chloroform extracts. Methanolic *A.heterophyllum* fruit latex extract had activity against *P.aeruginosa* and *E.coli* while the ethanolic and chloroform extracts did not show any activity on 12.5 mg/ml of these organisms. In our methanolic *A.heterophyllum* fruit latex extract and Antibiotic had equal activity shows *B.subtillis* organism. The moderate activity shows *P.aeruginosa* organism the concentration of 100mg/ml in methanolic *A.heterophyllum* fruit latex extract. *P.aeruginosa* is widespread in nature. It can be found in soils, surface water, on plants, in human and animal intestines.⁵⁰(Kayser fh. 2005). The main infections are pneumonias in patients on respiratory equipment, infections of burn wounds, postoperative wound infections, chronic pyelonephritis endocarditis in drug addicts, sepsis, and malignant otitis externa.⁵⁰ (Kayser fh. 2005). Moreover, it frequently causes nosocomial infections.⁵⁰ (Kayser fh. 2005). Alteration of bacterial morphology, shape and size after treatment of any agent can be used to define the antibiotic mechanism. ⁵¹ (yourassowsky E. 1982). The result also revealed that the Ethanolic extract of the plants were active at very high concentrations (50-100mg/ml) unlike the Methanolic extracts (12.5-25mg/ml) which means that Methanol extract active components of the plant responsible for their antimicrobial activities. The study also revealed that *S.aureus* and *P.aeruginosa* have become resistant to Ampicillin drugs ⁵²(Ajaiyeoba, 2000). From the results of antifungal studies as shown table 3 the methanolic extracts had more activity on the organisms than the other ethanolic and chloroform extracts. Methanolic, Ethanolic and chloroform extracts *A.heterophyllum* fruit latex extract had highest activity against on *A.flavus*,

lowest activity on *S.cervisiae*. Methanolic, Ethanolic and chloroform *A.heterophyllum* fruit latex extracts did not show any activity on *A.niger*, *A.flavus*, *C.albicans* and *S.cervisiae* (12.5) mg/ml of these organisms. *C.albicans* also the important pathogen since at least 70% of all human candida infections are caused by this microorganism, this *C.albicans* is opportunistic mycosis, can cause endogenous infection, primary infection of mucosa and skin with secondary dissemination for instance, in HIV patients⁵⁰. (Kayser fh. 2005). In our methanolic *A.heterophyllum* fruit latex extracts show potent activity on *C.albicans* organism of the concentration (100mg/ml). The different concentrations of *A.heterophyllum* fruit latex respectively exhibited significant activity against all the tested fungi compared with the standard drug fluconazole. Compared to our study fruit latex extracts are more active than seed extracts on human pathogenic bacteria and fungi, However further research is needed to identify the active agents responsible for the antibacterial and antifungal activities in methanolic, ethanolic and chloroform extracts of *A.heterophyllum* fruit latex. There are various reports on the literature regarding characterization of medicinal plant extracts that may inhibit the above mentioned bacteria. For example the antibacterial potential of *A.heterophyllum rtocarpusrigida* blume bark extract has been reported ⁵³(Parekh J and ChandaS) Based on our results, it is concluded that fruit latex extracts have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. *A.heterophyllum* showed stronger activity than the other plants against all the tested bacterial strains as reported earlier in *Artocarpusrigidablume*⁵⁴ (TatiSuhartati 2008). *A.heterophyllum* can be selected for further analysis. It can be used in further research on bioactive natural products that may result in the development of new pharmaceuticals that address therapeutic needs and such screening of various natural organic compounds and identification of active agents is the need of the novel molecule and therapeutic properties at the onset of drug discovery, which shall pay off later in drug development. The significant phytochemical constituents and antimicrobial effects of *A.heterophyllum* fruit latex extracts suggest that the latex may be a useful source for the development of novel antibiotics against pathogenic bacteria and fungi. It looks that still there is a scope for scientific studies to fully pursue its medicinal properties to support the traditional claims as well as exploring some new and promising 'leads'. The Phenolic compounds such as flavonoids, phenolic acid and tannins possess diverse biological properties such as anti-inflammatory, anticarcinogenic and anti-atherosclerotic activities. These biological properties

might be due to their antioxidant activities⁵⁵ (Li HB, Wong CC, 2007) Searching of compounds with antimicrobial properties has generally targeted to the plants with a history of ethno botanical uses 56,57(sokmenA,jones B. 1999). (shrinivasan D 2001). Present study targeted to

search the antibacterial activity of randomly selected fruit latex. They may contain strong antimicrobial activity and plant may provide the good source of antimicrobial compounds.

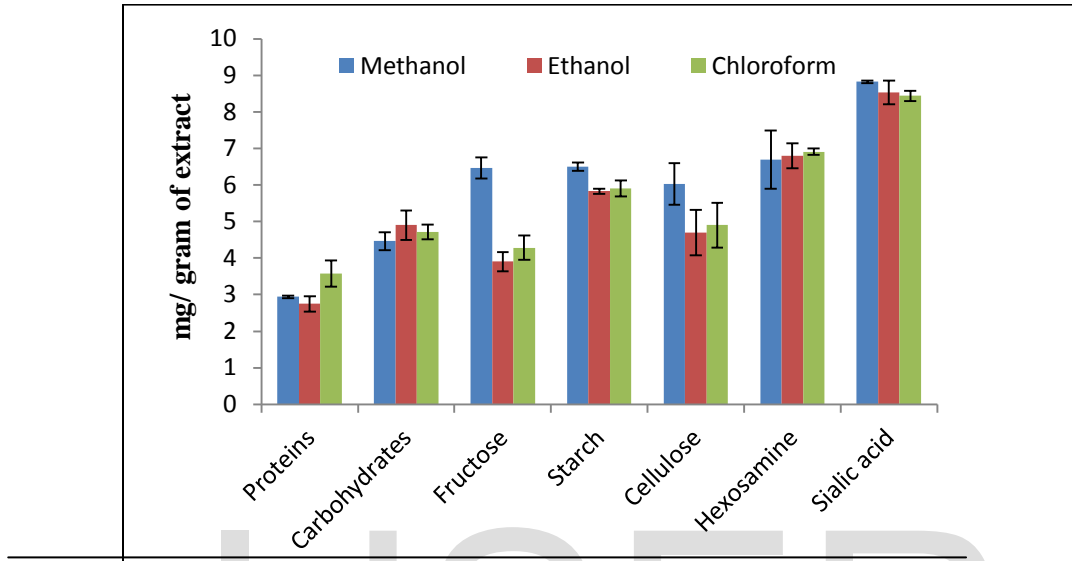


Figure: 1 Biochemical analysis of Methanolic, ethanolic and chloroform extracts of *Artocarpus heterophyllum* fruit latex in mg/gm values.

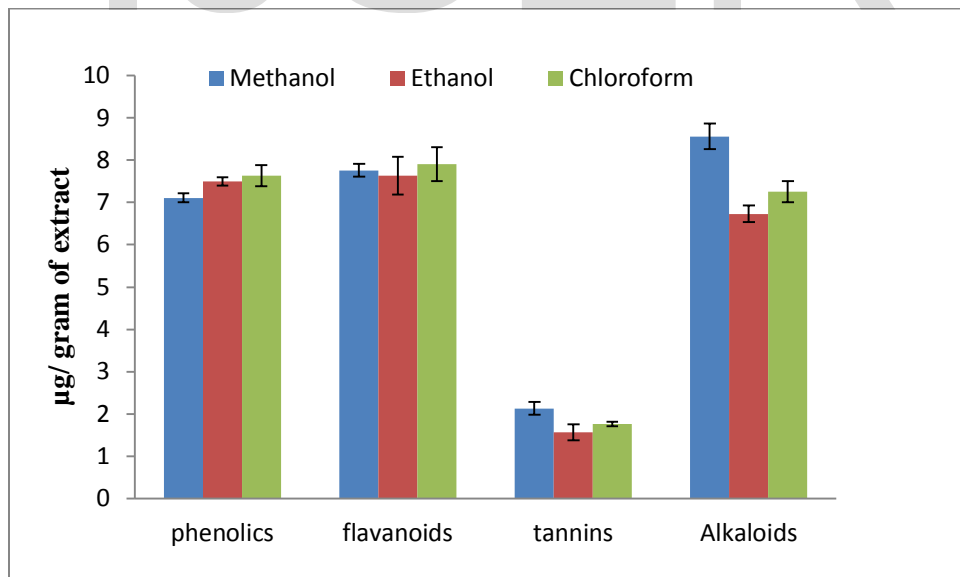


Figure: 2 Phytochemical analysis of Methanolic, ethanolic and chloroform extracts of *Artocarpus heterophyllum* fruit latex µg/gm values.

S.No	Name of Bacterial Sps	Zone of inhibition with Methanolic extract.				
		12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Antibiotic 1mg/ml
1	E.coli	ND	ND	14±2.82	16.3±3.55	18.3±3.51
2	S.aureaus	10.3±1.52	12±2.82	15±1.41	17.3±2.16	23.6±2.51
3	P.aeruginosa	ND	13.6±2.56	16±2.82	19.3±2.16	21.3±2.08
4	<i>Bacillus cereus</i>	11±2	12.6±2.51	13.6±2.51	15.3±3.55	18.3±2.51
5	B.subtillis	11.6±2.51	12.6±2.55	14.3±1.52	24.6±4.28	24.3±1.52

2B

S.No	Name of Bacterial Sps	Zone of inhibition with Ethanolic extract.				
		12.5mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Antibiotic1 mg/ml
1	E.coli	ND	ND	10.3±1.53	14±2	37±1.41
2	S.aureaus	10±1	11.3±2.16	14±2.82	15±1.41	33±2.82
3	P.aeruginosa	ND	12.6±1.52	13±1	17.6±3.34	22±1.41
4	<i>Bacillus cereus</i>	10.6±2.16	11±1.41	13±1.41	14±1.41	26±2.82
5	B.subtillis	11±2	12±2.82	13.6±1.52	15±2	18±1.41

2C

S.No	Name of Bacterial Sps	Zone of inhibition with Chloroform extract.				
		12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Antibiotic1 mg/ml
1	E.coli	ND	10± 1	13.6±1.52	15.3±1.52	27±2.94
2	S.aureaus	11.3±2.16	12.6±2.16	14±1.41	16±1.41	30±1.41
3	P.aeruginosa	ND	11±1	12.6±2.51	14.3±1.58	18±1.41
4	<i>Bacillus cereus</i>	10±1	12±2.82	13.3±3.51	15.3±3.51	23±2.82
5	B.subtillis	10.3±1.52	11.3±2.16	15±1	18.3±3.51	19.3±2.16

Table 2: Antibacterial activity of Methanolic (2A), Ethanolic (2B) and Chloroform (2C) extracts of *Artocarpusheterophyllus* fruit latex. Zone of inhibition expressed in mm. ND indicates Not detecte

3A

S.No	Name of Fungi	Zone of inhibition with Methanolic extract				
		12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Antibiotic1mg/ml
1	A.niger	ND	ND	12±1.41	15.3±1.52	17.6±1.56
2	A.flavus	ND	11.6±1.52	15±1.41	18±2	23±1
3	C.albicans	ND	ND	11.3±2.16	15±1	22±3
4	S.cervisiae	ND	ND	ND	12±1	20.6±2.08

3B

S.No	Name of Fungi	Zone of inhibition with Ethanolic extract				
		12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Antibiotic1mg/ml
1	A.niger	ND	ND	11.6±2.52	14±2.82	18.0±1.0
2	A.flavus	ND	11.3 ±2.16	13.6±1.52	16.3±3.55	16.3±1.52
3	C.albicans	ND	ND	10.6±2.16	13.6±1.52	17.3±1.52
4	S.cervisiae	ND	ND	ND	11.63±2.51	15.3±2.16

3C

S.No	Name of Fungi	Zone of inhibition with Chloroform extract				
		12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	Antibiotic1mg/ml
1	A.niger	ND	ND	10.3±1.52	13.3±3.51	13±1.0
2	A.flavus	ND	10.6±2.16	12.6±2.51	14.3±1.58	15.3±1.52
3	C.albicans	ND	ND	10±1	12.6±1.52	17±2
4	S.cervisiae	ND	ND	ND	10.3±1.52	15±2.82

Table 3: Antifungal activity of different extracts Ethanolic (3A), methanolic (3B) and chloroform (3C) of fruit latex of *Artocarpusheterophyllus*. Zone of inhibition expressed in mm. ND indicates Not detected.

Conclusion

Our work concludes that the Ethanolic, Methanolic and Chloroform extracts of *A.heterophyllus* fruit latex are rich in flavonoids and alkaloids respectively and when checked for anti- bacterial and anti-fungal properties these extracts have shown fairly well and significant anti-bacterial and anti-fungal properties in comparison with standard anti-bacterial and anti-fungal drugs. This work paves a way to develop a therapeutic strategy from natural resources, against various bacterial and fungal infections.

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