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Pharmacognostic and Physiochemical Studies of *Artocarpus heterophyllus* Seeds

Vaishnavi Bhat, Ashmita Mutha, Myrene R. Dsouza*

Department of Chemistry (P. G. Biochemistry), Mount Carmel College, Palace Road, Bengaluru, India 560052

Abstract : Aqueous, hydroalcoholic and methanolic extracts of Jackfruit seed (*Artocarpus heterophyllus)* were assessed for antioxidant, anti-inflammatory and antibacterial activity by *in vitro* methods. Phytochemical analysis revealed the presence of carbohydrates, amino acid, alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides, xanthoproteins and phenols. The total phenolic and flavonoid content was found to be 0.4 GAE/g and 10.1 QE/g of fresh tissue respectively. The IC_{50} values for DPPH radical and H_2O_2 scavenging was found to be 398.8 µg/ml and 32.51 µg/ml respectively. *In vitro* anti-inflammatory activity was evaluated using lipooxygenase inhibition, albumin denaturation assay and membrane stabilization assay at different concentrations. Aspirin was used as a standard drug for the study of anti-inflammatory activity. Linear regression analysis was used to calculate IC_{50}value.The extract inhibited the lipoxygenase enzyme activity with an IC_{50} value of 242.85 µg/ml.Maximum inhibition of heat induced albumin denaturation of 97% was observed at 500 µg/ml, IC_{50} 36.63 µg/ml).Membrane stabilization assay attributed minor protection by the seed extract with an IC_{50} of 629.38 µg/ml. Antibacterial activity of the methanolic and seed oil extracts were studied using agar well diffusion method. Broad spectrum antibacterial activity was noted with maximum zone of inhibition reported for *E. coli* and *K. pneumonia.*
Key words : *Artocarpus,* anti-inflammatory, antibacterial, antioxidant, RBC stabilization, lipoxygenase, albumin denaturation, phytochemicals.

Introduction

Natural products are the basis of many standard drugs used in modern medicine. In addition to having adverse side effects on humans, pathogens and insects develop resistance on regular use of these synthetic drugs. This has led leading researchers to focus on finding good eco-friendly controlling agents with high biological activity, antimicrobial activity, insecticidal activity, solubility, stability, nontoxicity to humans and other animals etc. and sufficiently available as per the needs with less cost for betterment of human life[1]. According to The World Health Organization, more than 80% ofthe world population in developing countries dependsprimarilyon plant based medicines for basic healthcare needs[2].A number of Artocarpus species are used as food andfor traditional folk medicines in South-East Asia, Indonesia, Western part of Java and India[3].

The jackfruit (*Artocarpus heterophyllus*) belonging to family Moraceae have been used in traditional medicines[4]due to the presence of various flavonoids[5]. It possesses numerous medicinal properties such as antibacterial, antioxidant, antidiabetic, anti-inflammatory, anti-diuretic, immunomodulatory and have been useful in the treatment of fever, skin diseases, convulsions, constipation, ophthalmic disorders and snake bite[6]. The pulp and seeds ofjackfruit are used as a cooling tonic, roots in diarrheaand fever, leaves to increase lactation in women and animals, as asource to treat antisyphilic and vermifuge, leaf ash applied to

ulcerswounds[3]. The leaves and stem barks have been used to treat anemia, asthma, dermatitis, diarrhea, cough and as an expectorant[6].In addition, jack fruit seed powders are used in instant soups, snacks, bakery products, beverages, dairy products, candy, ice cream, baby foods, pastaetc[7]. Jack fruit seed extracts (JFE) has been reported to possess anti-inflammatory and antibacterial activity[5,8]. The presence of jacalin, a lectin in the seeds was found to inhibit herpes simplex virus type 2 and has proved to be useful for the evaluation of the immune status of patients infected with human immunodeficiency virus 1 (HIV1)[9]. The high antioxidant potential of the fruit is attributed to the presence of phenolic compounds[10]. Several researchers have cited the antidiabetic effects of JFE due to its high proanthocyanidin and flavonoid contents through inhibition of lipid peroxide formation, and via an alpha-amylase inhibitory effect[11,12,13,14,15]. Based on the above, the present study was carried out to evaluate the antioxidant, anti-inflammatory and antibacterial activities of the aqueous, hydroalcoholic and methanolic extract of *A. heterophyllus* seeds in different experimental model.

Materials and Methods

Collection and preparation of samples

The raw jackfruit(*Artocarpus heterophyllus* Lam) samples was collected from alocal market in Bengaluru, Karnataka in May 2016. The fruits were cleaned and separated into pulp and seeds. The seeds were rinsed in distilled water, sun dried and ground into a fine powder. Crushed jackfruit seeds were extracted using three different solvent systems: distilled water (aqueous extract), methanol (alcoholic extract) and 1:1water:methanol (hydroalcoholic extract). Extraction was carried out on an orbital shaker for 24 h at room temperature. Solvents were evaporated under vacuum and resulting extracts were stored at 4 ºC.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methodology to confirm the presence of phytoconstituents[16,17].

Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of alkaloids:

Extracts were dissolved individually in dilute HCl and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent. Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent. Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.

Detection of saponins

Foam Test: 500 mg of extract was shaken with 2 ml of water. If foam produced persists for 10 min it indicates the presence of saponins.

Detection of glycosides: Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of phytosterols:

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Detection of flavonoids:

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and amino acids:

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Millon's Test: A few drops of the reagent are added to the test solution, which is then heated gently. A reddish-brown coloration or precipitate indicates the presence of tyrosine residue which occur in nearly all proteins.

Detection of tannins:

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of terpenoids:

Salkowski test: 5 ml of fraction was combined with few drops chloroform, and then 3 ml of concentrated H_2SO_4. Change of reddish brown colour revealed terpenoids.

Total phenolic content: Total phenolic contents were estimated according to the spectrophotometric method using gallic acid as standard[18].The phenolic content in the extracts were expressed in terms of gallic acid equivalent (mg of GAE/g of tissue).

Total flavonoid content: Aluminum chloride colorimetric method was used for determination of total flavonoids[19]. The flavonoid content in extracts was expressed in terms of quercetin equivalent (mg of QE/g of tissue).

Evaluation of Antioxidant Activity

DPPH radical scavenging activity*:*A stock solution of ascorbic acid (1000 µg/ml) was diluted ranging from 100-500 µg/ml. 0.1 ml solution from different dilutions was pipetted out in respective tubes. The volume in each tube was made up to 3 ml with DPPH (20 µg/ml). The test tubes were incubated for 10 min at room temperature. The contents of each tube were mixed well and the absorbance was measured at 517 nm against a blank[20]. The percentage inhibition of DPPH by the samples was calculated as follows:

$$Scavenging\ effect\ (\%) = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control}\ x\ 100$$

Hydrogen peroxide scavenging activity: Plant extract at 30 µg/ml concentration in 3.4 ml phosphate buffer was added to 0.6 ml of 45 mM H_2O_2 solution absorbance of the reaction mixture was recorded at 230 nm[21]. A blank solution contained the sodium phosphate buffer without H_2O_2. Ascorbic acid was used as standard. The concentration of H_2O_2(mM) in the assay medium was determined using a standard curve (y = 0.1419x+45.462; R^2 = 0.448). H_2O_2scavenging ability was calculated as IC_{50}. The scavenging effect was calculated as follows:

$$Scavenging\ effect\ (\%) = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control}\ x\ 100$$

Evaluation of *In vitro* Anti – Inflammatory Activity

Lipoxygenase inhibition: Soybean lipoxygenase activity was assayed[22]. Briefly the reaction was carried out in a final volume of 3 ml containing 2.9 ml of 0.1M borate buffer pH 9.0 and 50 µl of 10 mM linoleic acid. The reaction was initiated by the addition of 50 µl of the soybean enzyme extract. The enzyme activity was measured by following the formation of the product, 12-HETE at 234 nm for up to 1 min. The enzyme inhibition was determined by pre-incubating the enzyme with the plant extract or standard phytochemicals prior to determining its 12-LOX activity. The results are expressed as percent inhibition of the 12-LOX activity. IC_{50} was calculated from y=0.1365x+4.0348; R^2=0.9859.

$$Inhibition\ (\%) = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control}\ x\ 100$$

Inhibition of heat induced protein denaturation*:* The anti-inflammatory activity was studied by using inhibition of albumin denaturation technique followed with minor modificationsfollowed with minor modifications[23,24]. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660 nm. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated from the following equation. IC_{50} was calculated from y=0.1421x + 43.929.

$$Inhibition\ (\%) = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control}\ x\ 100$$

hRBC membrane stabilization activity: Different concentration of extract (100-500 µg/ml), reference sample, and control were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of hRBC suspension. Diclofenac sodium (100 µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37^{o}C for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560 nm[25]. The percentage hemolysis was estimated by assuming the haemolysis produced in the control as 100%. The percentage inhibition of protein denaturation was calculated from the following equation. IC50 was calculated from y=0.0586x+11.176.

$$Inhibition\ (\%) = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control}\ x\ 100$$

Screening for Antibacterial Activity of methanolic extract and seed oil

Extraction of oil: 25 g of *A.heterophyllus* seed powder was filled in the thimble and extracted successively with n-hexane using Soxhlet extractor for 48 hat temperature not exceeding the boiling point of the solvent. The solvent was removed by distillation under reduced pressure in a rotary evaporator at 35 ºC and pure oil kept at 4 ºC in the dark, until analysis. The seed oil extract was subjected to antibacterial activity.

Agar well diffusion assay: The antibacterial activity was carried out by employing 24 h cultures of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The standard medium Mueller Hinton Agar, was poured to a depth of 4 mm in a 90 mm petridish. The bacterial inoculum was prepared from an 18 h broth culture of the microbe to be tested and was standardized with sterile physiologic saline to contain 106 cfu/ml. A well of 6 mm diameter was made using a sterile cork borer. The standard drug and extracts were placed in the well. Antibacterial assay plates were for overnight incubation.

Ciprofloxacin (5 µg/disc) was used as a positive control for antibacterial activity. After 24 h of incubation at 37^{o}C, zone of inhibition (ZOI) was observed and diameter measured.

Statistical analysis: Data of *in vitro* assays recorded were analyzed using Microsoft Excel to determine IC50. One-way analysis of variance (ANOVA) were conducted and $P<0.05$ was considered significant.

Results and Discussion

The search for new anti-inflammatory agents from medicinal plant sources is intensifying as they possess a beneficial effect not just in suppressing relevant aspects of the inflammatory cascade but also on diverse disease conditions. The present study was carried out to assess the validity of the folkloric uses of jackfruit seeds in the management and treatment of inflammatory disorders. In the present work, three solvent extracts of *A. heterophyllus* seeds were tested for evaluating the antibacterial and anti-inflammatory properties and the results obtained are discussed below.

Phytochemical screening: Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Results of the phytochemical screening of aqueous, hydro-alcoholic and methanolic extracts of *A.heterophyllus* seeds are depicted in Table 1. In general, our results indicate the presence of phytochemicals in the order of methanolic>hydroalcoholic>aqueous extract (Table 1). This shows a high level of its possible medicinal value. Our results are similar to those reported in *Tinospora cordifolia*[26] and *Leucas aspera*[27]. Saponins have been known for their medicinal uses, including antispasmodic activity, and toxicity to cancer cells. In addition, the anti-nutritional role of saponins reduce the uptake of certain nutrients including cholesterol and glucose[28]. Alkaloids derived from medicinal plants exhibit anti-inflammatory[29], antimalarial[30], antimicrobial[31], cytotoxic, antispasmodic and pharmacological effects[32]. Similarly, plant derived steroids possess cardiotonic, antibacterial and insecticidal properties[33].The presence of tannins supports its use in wound healing, varicose ulcers, hemorrhoids, frostbite and burn[34]. Cardiac glycosides have been used to treat congestive heart failure and cardiac arrhythmia[35]. Their mode of action starts by inhibiting Na^{+}/K^{+} pump which then increases the level of calcium ion, so more Ca^{+} would be available for the contraction of heart muscles which recover cardiac output and reduce the distension of heart[36].

Table 1: Phytochemical investigation of *Artocarpus heterophyllus* seed

Phytochemical analyzed	Tests performed	Results		
		Aqueous Extract	Hydro-alcoholic Extract	Methanolic Extract
Carbohydrates	Molisch	+	++	+++
	Benedict's	+++	+	++
	Fehling's	++	++	+
Alkaloids	Mayer's	-	-	-
	Wagner's	++	+	+
	Dragendroff's	+	-	++
Saponins	Foam Test	+	++	+++
Glycosides	Modified Borntrager's	+	+	+
Phytosterols	Salkowski's	-	-	-
Flavonoids	Flavonoid	+	+	+
Proteins and Amino Acids	Xanthoproteic	+++	++	+
	Ninhydrin	+	++	+++
	Millon's	-	-	-
Tannins	Ferric chloride	+	++	+++
Terpenoids	Salkowski's	+	++	+++

- denotes absence, +denotes presence, + +denotes average, +++ denotesabundance of phytochemicals

Total phenolic and flavonoid content: Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. The Artocarpus species are rich source of phenolic compounds and offers opportunities for development of value added products from edible fruits, neutraceuticals and food applications to enhance health benefits. The antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings[37]. Total phenolic content in the methanolic extracts of *A.heterophyllus* seeds was calculated using the standard curve of gallic acid (y = 1.4882x - 0.0067; R^2= 0.94964) was found to be 0.4 mg GAE/g dry weight (Table 2). Studies on medicinal plants/herbs with high phenolic contents have gained importance over the past few years due to the high antioxidant, anti-inflammatory and anti-carcinogenic activities and are of great value in decreasing the risk of many human diseases. Many studies have shown that polyphenols contribute significantly to the antioxidant activity and act as highly effective free radical scavengers which is mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[38].

Flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects. Hydroxyl assembles in flavonoids intercede their antioxidant impacts by scavenging free radicals as well as by chelating metal particles. The chelation of metals could prevent radical mediated biomolecule damage. As a dietary segment, flavonoids are thought to have wellbeing elevating properties because of their high antioxidant properties both *in vivo* and *in vitro* frame worksTotal flavonoid content was calculated using the standard curve of quercetin (y = 0.00148x; R^2 = 0.9988) and found to be 10mgQE/g dry weight(Table 2).The prenylated flavonoid, 5,7,40-trihydroxy-6,8-diprenylisoflavone, isolated from *A. heterophyllus* Lam. exhibited strong inhibitory effect on lipid peroxidation by interaction of hemoglobin and H_2O_2 than that of genistein, a non-prenylated isoflavone[39]. Artelastin, a prenylated flavone previously isolated from *A. elasticus*, proved to be an inhibitor of ROS production due to a strong O_2-scavenging activity as well as inhibitor of NO production without NO-scavenging activity[40].

Table 2: Quantitative analysis of total phenols and total flavonoids of *A. heterophyllus* seed extracts

Sample	Total Phenols (mg GAE/g Fwt)	Total Flavonoids (mg QE/g Fwt)
***A. heterophyllus* seed**	0.4± 0.01	10.1 ± 0.4

GAE – Gallic acid equivalents; QE – Quercetin equivalents

***In vitro* antioxidant activity of the extract**

The natural antioxidants in fruits and vegetables gained increasinginterest among food scientists, nutrition specialists andconsumers, as they reduce the risk of chronic diseases and promotehuman health.

DPPH scavenging activity: The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1,1- diphenyl 2-picrylhyorazyl (DPPH) free radical. Total antioxidant capacity of the extracts was calculated using % inhibition against concentration of ascorbic acid (y = 0.1065x + 7.717; R^2 = 0.794) (Fig 1A). At 500µg/ml concentration, methanolic extracts of *A. heterophyllus* seeds exhibited 31.06%inhibition(Table 3).The antioxidative activities observed can be attributed to the different mechanisms exhibited by polyphenolic compounds such as tocopherols, flavonoids and other organic acids and to the synergistic effects of different compounds. Studies on various parts of the jackfruit have shown the occurrence of higher antioxidant activity and phenolic content in seeds[3,41] with IC_{50} 410µg/ml).The seed extract of *A. odoratissimus*showed a much higherDPPH-scavenging activity (13.69mg AEAC/g) as compared to the flesh which showed 2.44mg AEAC/gDPPH scavenging[42].

H_2O_2scavenging Activity: H_2O_2 is an important reactive oxygen species because of its ability to penetrate biological membranes.H_2O_2 itself is not exceptionally reactive, but it can be lethal to the cell since it gives rise to hydroxyl radicals that are more damaging. Therefore, removal of H_2O_2 is imperative for the maintenance of redox balance within the cell. Scavenging of $H_2O_2$and its percentage inhibition in methanolic extracts of seeds of *A.heterophyllus*(Fig 1B) exhibited an IC_{50} value of 32.51 µg/ml (y = 0.1414x + 45.37; R^2 = 0.451), while that of the ascorbic acid standard (y = 0.1419x + 45.46; R^2 = 0.448) was found to be 31.99 µg/ml (Table 3).

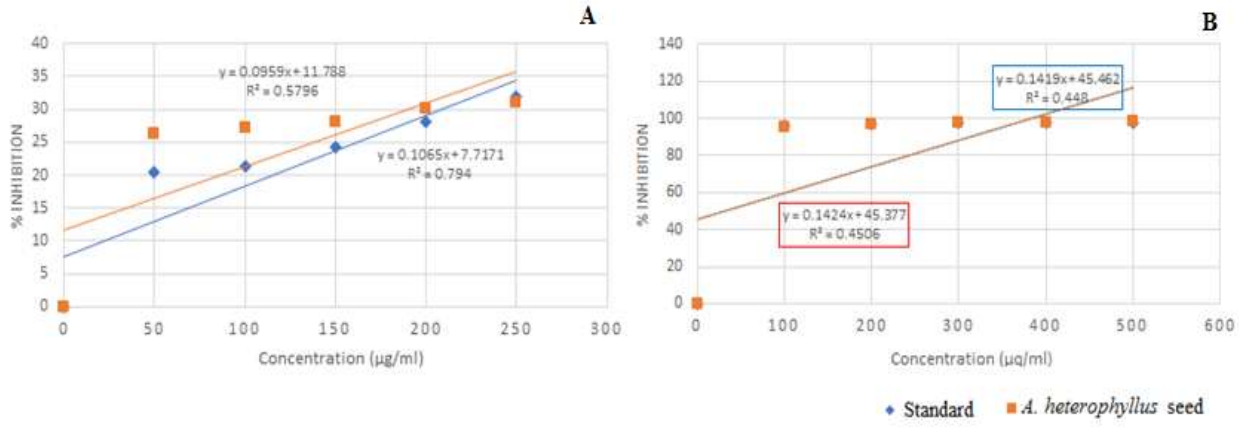

Fig1: DPPH radical scavengingactivity (A)andhydrogen peroxide savenging activityof*A. heterophyllus* seed extracts

Table3: DPPH radical scavengingactivityandhydrogen peroxide savenging activityof*A. heterophyllus* seed extracts. Ascorbic acid was used as standard. Linear regression analysis was performed to calculate IC50 value.

Sample	DPPH radical scavengingactivity			H_2O_2 scavenging activity		
	Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)	Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)
Ascorbic acid standard	50	20.38	397.1	100	95.8 ± 8.2	31.99
	100	21.35		200	96.5 ± 7.8	
	150	24.27		300	97.6 ± 2.3	
	200	28.15		400	97.8 ± 9.1	
	250	32.03		500	97.9 ± 9.9	
***A. heterophyllus* seed**	50	26.21	398.5	100	95.6 ± 5.5	32.51
	100	27.18		200	96.8 ± 6.2	
	150	28.15		300	97.4 ± 9.3	
	200	30.09		400	97.8 ± 8.8	
	250	31.06		500	98.2 ±2.9	

***In vitro* anti-inflammatory activity of the extract**

Inhibition of Lipooxygenase: The establishment of new *in vitro* test systems has resulted in the screening of plants aiming to find leads for the development of new drugs[43].LOXs are sensitive to antioxidants and the most of their action may consist in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy- radical formed in course of enzyme peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX. Methanolic extracts of *A. heterophyllu s*seeds were studied at 100, 200, 300, 400 and 500 µg/ml, and the inhibition obtained is as shown in Table 4 and Fig 2A. The standard exhibited 70.3 ± 3.3% inhibition at a concentration of 500 µg/ml. Percentage inhibition of lipoxygenase in methanolic extracts of seeds of *A. heterophyllus* showed IC_{50} value of 242.85µg/ml (y = 0.1382x + 16.46; R^2 = 0.831).Aspirin used as reference exhibited IC_{50} value of 336.74µg/ml (y = 0.1365x + 4.03; R^2 = 0.95). AC-5-1 isolated from *A. communis* was found to inhibit 5-lipoxygenase of cultured mastocytoma cells with a half-inhibition dose of 5±0.12×10−8 M. The AC-5-l at 10−5M inhibits 96% of leukotriene C4 synthesis of mouse peritoneal cells

Inhibition of heat induced protein denaturation: Denaturation of proteins is a well- documented cause of inflammation. Phenylbutazone, salicylic acid, flufenamic acid (anti-inflammatory drugs) etc. have shown dose dependent ability to thermally induced protein denaturation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. The methanolic

extract was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table 4 and Fig 2B. The IC_{50} value for the methanolic extracts of seed of *A.heterophyllus* was found to be 36.62 µg/ml (y=0.1409x + 44.84, R^2 = 0.452), while that of aspirin, a standard anti- inflammatory drug exhibited 42.78 µg/ml (y=0.1421x + 43.92, R^2 = 0.467) (Table 4).

RBC Membrane Stabilizing Activity: The erythrocyte plasma membrane closely resembles the lysosomal membrane and hence the stabilizing effect of drugs on erythrocyte membrane may correlate with its lysosomal membrane stabilizing effect. Stabilization of the later inhibits release of inflammatory mediators and consequent inhibition of the process of inflammation[44]. The highest inhibition of heat-induced hemolysis in the methanolic extracts of seed of *A. heterophyllus*(Fig 2C) was observed at 500 µg/ml with IC_{50} values of 629.38 µg/ml (y=0.0638x + 9.845, R^2 = 0.771). Aspirin showed the maximum inhibition at the concentration of 500 µg/ml with an IC_{50} value 662.62 µg/ml (y=0.0586x + 11.176, R^2 = 0.692) (Table 4).During inflammation, lysosomal enzymes are released into the cytosol, causing damage to the surrounding tissues, thereby triggering inflammation. Most of the anti-inflammatory drugs stabilize lysosomal membrane and inhibit the inflammatory process by restricting the release of lysosomal enzymes.

Table 4: Evaluation of *In vitro* anti-inflammatory activity of *A. heterophyllus* seed extracts. Aspirin was used as a standard drug. Linear regression analysis was performed to calculate IC_{50} value.

Sample	Conc. (µg/ml)	LOX inhibiting activity		Inhibition of albumin denaturation		hRBC stabilization activity	
		% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)
Aspirin standard	100	20.2 ± 1.3	336.74	92.9±11.4	42.78	26.7 ± 5.5	662.62
	200	32.6 ± 2.4		94.8 ± 7.8		28.2 ± 6.2	
	300	48.9 ± 5.6		95.5 ± 4.6		30.9 ± 8.9	
	400	56.9 ± 8.1		96.1 ± 9.9		31.7 ± 2.1	
	500	70.3 ± 3.3		97.4 ± 8.9		37.5 ± 3.4	
***A. heterophyllus* seed**	100	38.7 ± 2.7	242.85	94.8 ± 6.9	36.62	22.5 ± 4.8	629.38
	200	59.1 ± 6.6		95.5 ± 1.3		28.7 ± 4.1	
	300	63.3 ± 8.2		96.1 ± 8.9		32.7 ± 7.1	
	400	64.4 ± 3.1		96.7 ± 1.7		33.7 ± 5.7	
	500	80.4 ± 7.9		97.4 ± 3.1		37.1 ± 2.8	

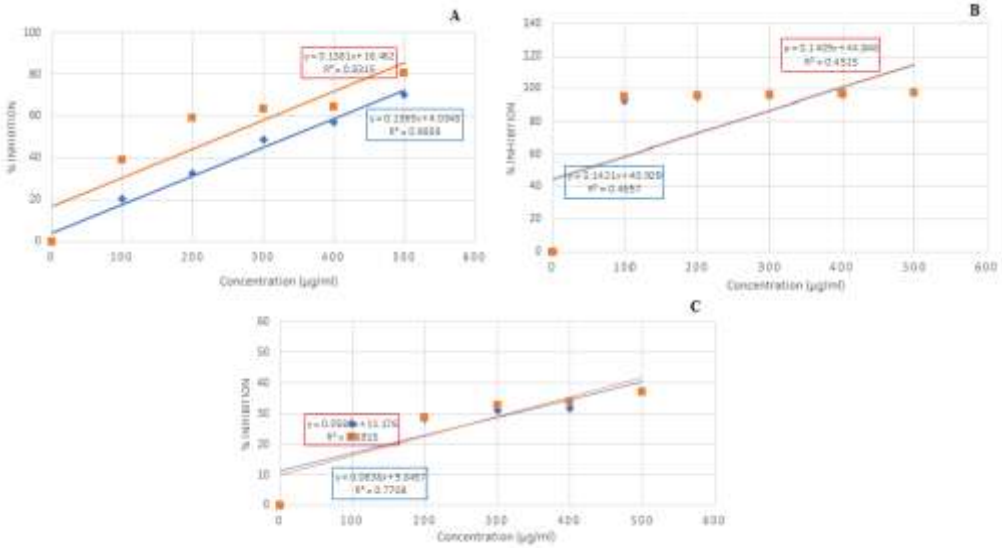

Fig 2: Lipooxygenase inhibitory action (A), inhibition of heat induced protein denaturation (B) and RBC stabilization activity of*A. heterophyllus* seed extracts (C).

Antimicrobial activity of extracts

Since plants are rich in various types of secondary metabolites including tannins, terpenoids, alkaloids and flavonoids, they have been found to exert *in vitro* antimicrobial property. Because of their potent pharmacological activity, low toxicity and economic feasibility, investigation of the medicinal properties of plant has been performed. In our study, *in vitro* antimicrobial activity of *A. heterophyllus* seed was determined by Agar well diffusion method (Table 5). Among gram negative bacteria, *K. pneumoniae* was found to be maximally inhibited by the methanolic extract of *A. heterophyllu s* with a zone of clearance of 19 mm (Table 5). On the other hand, aqueous extracts of *A. heterophyllus* seeds produced the largest zone of inhibition for the gram positive *S. aureus* at 16 mm. On the whole, methanolic extracts of all three samples were found to produce better antibacterial activity than control ciprofloxacin. This could be attributed to the presence of minute concentrations of bioactive compounds in the crude plant extract[45]. Two active isoprenyl flavones, artocarpin and artocarpesin that inhibited the growth of primary cariogenic bacteria at concentration of 3.13–12.5 µg/ml and alsoexhibited the growth inhibitory effects on plaque-forming Streptococci were isolated[46].This finding showed that phytochemicals from *A.heterophyllus* would be potent compounds for the prevention ofdental caries[46].Since the plant extracts were found to be effective against both Gram-positive and Gram-negative bacteria, it is a strong indication of the presence of broad spectrum antimicrobial compounds.

Table 5: Antibacterial activity of *A. heterophyllus* seed extracts. The zone of inhibition is expressed in mm.

Strain	Ciprofloxacin		*A. heterophyllus* seed		Oil extract of *A. heterophyllus* seed
	Positive	**Negative**	**MeOH**	**Aqua**	**MeOH**
S. aureus	10	0	7	16	9
E. coli	10	0	10	11	12
K. pneumoniae	17	0	19	8	11
P. aeruginosa	10	0	10	9	7

Essential oils are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use. The essential oil extracted from the seeds of *A.heterophyllus* was used in the present study to investigate their antibacterial potential. The results obtained and screening of antibacterial activity of essential oil of *Artocarpus heterophyllus* are summarized in (Table 5).The data indicated that *E. coli* was the most sensitive strain tested to the oil of *A. Heterophyllus* with the strongest inhibition zone (12 mm) followed by *K. pneumoniae*(11 mm). Modest activities were observed against *S.aureus*(9 mm) and *P. aeruginosa*(7 mm). These results are similar to those documented[47,48]. The antimicrobial activities are attributed to aromatic terpenes and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils.

Conclusion

The present study discusses the significance of *A. heterophyllus* as a valuable source for medicinally important compounds besides its edible fruit which is a store house of minerals, vitamins, antioxidants and other nutrients. It provides opportunities for the seed to be used as functional food. The antioxidant constituents present play important role in scavenging free radicals and ROS which are responsible for number of human disorders. Hence, a correlation between ethnomedical employment and the pharmacological activities is possible. However, there also arises a need to minimize the gap between the studies conducted so far and to exploit medicinal properties of *Artocarpus* maximally. This can be achieved by determining accurate and precise chemical composition of the seed followed by understanding metabolic engineering to enhance the synthesis and accumulation of these compounds.

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