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Evaluation of pharmacological activities of seed and pericarp of *Litchi chinensis*Sonn.

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Abstract

The phytochemical analysis of *Litchi chinensis*Sonn. (Litchi)seed and pericarp in water, methanol and hydromethanolic (1:1) extracts were investigated. The total phenolic, flavonoid, β-carotene and ascorbic acid levels were found to be higher in the pericarp than in seed extracts. Lycopene was not detected in the pericarp extract. *In vitro* antioxidant capacity was measured by assaying DPPH radical and H_2O_2 scavenging activities. Linear regression analysis was used to calculate the IC_{50} value. The IC_{50} values for DPPH scavenging were found to be 473.2 µg/ml (seed) and 437.1 µg/ml (pericarp). Similarly, the IC_{50} values for H_2O_2 scavenging activity were 34.94 µg/ml (seed) and 107.77µg/ml (pericarp). *In vitro* anti-inflammatory activities were evaluated using lipoxygenase inhibition, heat induced albumin denaturation and RBC membrane stabilization assays at different concentrations with aspirin as standard. The extracts inhibited lipoxygenase with IC_{50} values of 157.95 µg/ml (seed) and 98.29 µg/ml (pericarp). Maximum inhibition of heat induced protein denaturation with IC_{50} values of 45.85 µg/ml (seed) and 44.59 µg/ml (pericarp) was reported. RBC stabilization was seen maximally with IC_{50} values of 520.5 µg/ml (seed) and 711.7 µg/ml (pericarp). Agar well diffusion assay of extracts was performed on some common pathogens to determine the antibacterial activity. It was observed that the aqueous extracts of *L. chinensis*seeds produced the largest zone of inhibition for gram positive *S. aureus* while the methanolic extract of the pericarp produced the highest zone of clearance for *K. pneumoniae*.

Keywords:

Anti-inflammatory;
Antimicrobial;
Antioxidant;
Litchi;
Phytochemicals.

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1. Introduction

Plant-based natural solutions have been the foundation of treatment and cure for different physiological conditions and maladies in traditional systems of therapy like ayurveda and homeopathy. Parts of the plant utilized for this are leaves, flowers, fruit, seed, bark, root, and pericarp. The specific plant or plant parts to be used, its formulation and the method of application for a particular ailment were passed down through oral history [1], [2]. In more recent times, the use of plants as medicines has involved the isolation and characterization of pharmacologically active compounds.

Litchi (*Litchi chinesis*Sonn.), member of the family Sapindaceae is cultivated in tropical and subtropical countries. It is an evergreen tree native to Taiwan, China and southeast Asia but is now being cultivated in many parts of the world. The fruit is about 5 cm long and 4 cm wide, varying in shape from round, to ovoid and heart-shaped. The fruit peel is tough, inedible, covered with sharp protuberances and possesses a profound pink-red colour when ripe. It has a white, fleshy delicious aril surrounding a dark

brown inedible seed. Litchi is sold as fruit, mash, squash and wine. Processing and consumption of the fruit generates by-products such as epicarps and seeds, which are often discarded. However, with appropriate treatments, the seeds may be used as a food additive and in pharmaceutical drugs. With the search for alternative therapeutic agent intensifying, studies on plant extracts and fruit based wastes for treatment of various types of diseases, as antioxidants for preserving foods and neutralizing free radicals in the human body are being developed [3].

Recent work has shown that the litchi peel and seed possess high energy and nutritional potential, are rich in antioxidants such as ascorbic acid, phenolic compounds (especially gallic acid), flavonoids (procyanidin B4, procyanidin B2, epicatechin cyanidin-3-retinoside, cyanidin-3-glucoside, quercetin-3-retinoside and quercetin-3-glucoside) [4], [5], and anthocyanins [6]. Pharmacological studies indicate that the by-products of the litchi possess antioxidant, anti-inflammatory, anti-hyperlipidemic, anti-hyperglycemic, hepatic and cardioprotective activities [7], [8], [9]. The ethanomedicinal use of *L. chinensis*kernel oil on cardiovascular disease may be attributed to its ability of decreasing total cholesterol and LDL and increasing HDL and HDL/TC [10]. In addition, studies have shown that the aqueous seed extract increases insulin sensitivity and reduces the levels of blood glucose, triglyceride, leptin and tumor-necrosis factor in a Type-2 diabetes mellitus rat model [11]. Thus, the present investigation was undertaken to optimize the usage of the litchi seed and pericarp, by implementing the design of its extraction, and as a potential for antioxidant, anti-inflammatory and antimicrobial activities. It may also play a role in minimizing waste generation.

2. Research Method

2.1 Collection and preparation of samples

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

2.2 Fluorescence analysis

Fluorescence characteristics of the powdered seed and pericarp with different chemicals were observed in daylight and ultraviolet light [12].

2.3 Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methodology to confirm the presence of phytoconstituents [13], [14].

2.3.1 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 as follow:
Molisch's test: Formation of the violet ring at the junction with 2 drops of alcoholic α-naphthol solution indicates the presence of carbohydrates.
Benedict's test: Orange red precipitate with Benedict's reagent indicates the presence of reducing sugars.
Fehling's test: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Presence of reducing sugars was indicated by the formation of a red precipitate.

2.3.2 Detection of alkaloids:Extracts were dissolved individually in dilute HCl and filtered. Tests for the presence of alkaloids were as follows:
Mayer's test: Formation of a yellow coloured precipitate with Mayer's reagent indicates the presence of alkaloids.
Wagner's test: Formation of brown/reddish precipitate with Wagner's reagent indicates the presence of alkaloids.
Dragendroff's test: Formation of red precipitate with Dragendroff's reagent indicates the presence of alkaloids.

2.3.3 Detection of saponins:
Foam test: Persistence of foam after 10 mins with 0.5 ml extract and 2 ml water, shaken well indicates the presence of saponins.

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

Modified Borntrager's test: Extracts were treated with $FeCl_3$ 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.

***2.3.5 Detection of phytosterols*:**
Salkowski's test: The plant extracts were treated with $CHCl_3$ and filtered. The filtrate thus obtained was mixed with few drops of concentrated H_2SO_4, shaken and allowed to stand. The appearance of golden yellow colour was indicative of triterpenes.

***2.3.6 Detection of flavonoids**:*
Lead acetate test: Formation of yellow colour precipitate with the extract and a few drops of lead acetate solution indicates the presence of flavonoids.

2.3.7 Detection of proteins and amino acids:
Xanthoproteic test: Formation of yellow colour with extract and concentrated HNO3 indicates the presence of proteins.
Ninhydrin test: Formation of blue colour with extract and 0.25% w/v ninhydrin reagent boiled for a few minutes indicates the presence of amino acid.
Millon's test: A reddish-brown coloration with the extract and Millon's reagent gently heated indicates the presence of tyrosine residue.

2.3.8 Detection of tannins:
Ferric chloride test: Formation of bluish-black colour with the extract and 3-4 drops of $FeCl_3$ indicates the presence of phenols.

2.3.9 Detection of terpenoids:
Salkowski test: Appearance of reddish brown colour with 5 ml of extract, few drops of $CHCl_3$ and 3 ml concentrated H_2SO_4 revealed the presence of terpenoids.

2.4 Total phenolic, flavonoid, ascorbic acid, β-carotene and lycopene contents: Total phenolic contents were estimated according to the spectrophotometric method using gallic acid as standard [15] and expressed in terms of gallic acid equivalent (mg of GAE/g of tissue). Aluminum chloride colorimetric method was used for determination of total flavonoids [16] and expressed in terms of quercetin equivalent (mg of QE/g of tissue). Ascorbic acid estimation was carried out according to the procedure of Sadasivam and Manickam [17]. The levels of beta-carotene and lycopene were determined [18]. The powdered seed was extracted with acetone and hexane (4:6) (v/v) in an ultrasonic bath at 4 °C for 5 min. The absorbances of the filtered extracts were read in the 453 nm, 505 nm, 645 nm and 663 nm wavelengths.

2.5 Evaluation of Antioxidant Activity
2.5.1 DPPH radical scavenging activity: Standard ascorbic acid was pipetted out into different test tubes (100-500 µg/ml). 0.1 ml solution of each dilution was taken and 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 [19]. The percentage inhibition of DPPH by the samples was calculated as follows:

$$\%\ Inhibition = \frac{(OD\ of\ control - OD\ of\ sample)}{OD\ of\ control}\ x\ 100 \qquad \text{---------- (Eq. 1)}$$

2.5.2 Hydrogen peroxide scavenging activity: Plant extract in 3.4 ml phosphate buffer was added to 0.6 ml of 45 mM H_2O_2 solution and the absorbance of the reaction mixture was recorded at 230 nm against a blank containing only buffer [20]. 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 percentage inhibition was calculated as in Eq. 1.

2.6 Evaluation of In vitro Anti – Inflammatory Activity
2.6.1 Lipoxygenase inhibition: Soybean lipoxygenase activity was assayed [21]. The reaction contained 2.9 ml 0.1M borate buffer pH 9.0 and 50 µl 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 percentage inhibition was calculated as in Eq. 1. IC_{50} was calculated from y=0.1365x+4.0348; R^2=0.9859.

2.6.2Inhibition of heat induced protein denaturation: The anti-inflammatory activity of the extract was studied by using the inhibition of albumin denaturation technique [22], [23]. The reaction mixture comprised of the 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 ºC for 20 min. The turbidity was measured at 660 nm after cooling. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated using Eq. 1. IC_{50} was calculated from y=0.1421x + 43.92.

2.6.3 hRBC membrane stabilization activity: Various concentration of extract, 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 ^oC for 30 min and centrifuged at 3000 rpm. The supernatant was used to estimate the haemoglobin content using a spectrophotometer at 560 nm [24]. The percentage of hemolysis was estimated assuming that the control produced 100% haemolysis. The percentage inhibition of protein denaturation was calculated using Eq. 1. IC50 was calculated from y=0.0586x+11.176.

2.7 Screening for Antibacterial activity of methanolic extract

2.7.1 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 (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 ^oC, zone of inhibition (ZOI) was observed and diameter measured.

Statistical analysis: Data of *in vitro* assays recorded were analyzed using Microsoft Excel to determine IC_{50}. One-way analysis of variance (ANOVA) were conducted and $P<0.05$ was considered significant.

3. Results and Analysis

Natural products are a rich source of drugs since they provide a host of bioactive molecules, most of which probably evolved as chemical defenses against predation and infection. More intensive research involving the discovery of anti-inflammatory agents from medicinal plant sources is currently underway because of its known ability of suppressing relevant reactions of the inflammatory cascade. The present study was carried out to assess the validity of the folkloric uses of *L. chinensis*in the management and treatment of inflammatory disorders.

3.1 Fluorescence analysis: The mucilage of *L. chinensis*seeds produced different colours and fluorescence under UV light (254nm and 365nm) and day light when treated with various reagents (Table 1).

Table 1: Fluorescence analysis of seed powder of *Litchi chinensis.*

Treatment	UV light (254 nm)	UV light (365 nm)	Day light
Seed powder	Brownish	Black	Brownish-black
Seed powder + 1N HCl	Faint green	Black	Faint pink
Seed podwer + 1N $NaOH_{aq}$	Fluorescent green	Dull brown	Reddish
Seed powder + 1N $NaOH_{alc}$	Reddish	Dull red	Dark red
Seed powder + 1N HNO_3	Pink	Dull pink	Faint pink
Seed powder + acetic acid	Fluorescent green	Dull green	Pale pink
Seed powder + iodine	Black	Black	Bluish-black
Seed powder + ammonia	Fluorescent dark green	Dull green	Wine red
Seed powder + 50% KOH	Dull green	Black	Black
Seed powder + $FeCl_3$	Fluorescent green	Pitch black	Bluish-black

3.2 Phytochemical screening: The phytochemical screening of aqueous, hydro-alcoholic and methanolic extracts *L. chinensis*seeds and pericarp revealed the presence of some secondary metabolites such as alkaloids, steroids, flavonoids, tannins, terpenoids and cardiac glycosides (Table 2). These phytochemical

compounds are known to have medicinal importance. For example, alkaloids derived from medicinal plants show biological activities like, anti-inflammatory [25], antimalarial [26], antimicrobial [27], cytotoxicity, antispasmodic and pharmacological effects [28]. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties [29]. Tannins, according to research, are known to have antibacterial [30], antitumor and antiviral activities [31]. Other phytochemicals called cardiac glycosides have been used to treat congestive heart failure and cardiac arrhythmia [32]. Saponins were not detected in any of the extracts.

Table 2: Phytochemical investigation of *L.chinensis*seed and pericarp

Sl. No	Phytochemical analyzed	Tests performed	*L. chinensis*seed			*L. chinensis*pericarp		
			Aqueous	Hydro-alcoholic	Methanolic	Aqueous	Hydro-alcoholic	Methanolic
1	Carbohydrates	Molisch	+	-	++	+	++	+++
		Benedict's	+	++	+++	+	++	++
		Fehling's	+	++	+++	+++	++	+
2	Alkaloids	Mayer's	-	-	-	-	-	-
		Wagner's	+++	+	+	++	++	++
		Dragendroff's	-	-	++	+	-	++
3	Saponins	Foam Test	-	-	-	-	-	-
4	Glycosides	Modified Borntrager's	+	+	+	+	+	+
5	Phytosterol	Salkowski's	-	-	-	+	++	+++
6	Flavonoid	Flavonoid	+	+	+	+	+	+
7	Proteins and Amino acids	Xanthoproteic	++	+	+++	+	++	+++
		Ninhydrin	++	+	+++	+	++	+++
		Millon's	+	+	++	+	+	+
8	Tannins	Ferric Chloride	+	++	+++	+	++	++
9	Terpenoids	Salkowski's	-	++	+++	+	+	+

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

3.3 Total phenol, flavonoid, β-carotene and lycopene 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 antioxidant potential of such compounds is largely dependent on its structure, the number and position of hydroxy groups and the nature of aromatic ring substitutions.

The total phenolic contents of methanolic extracts of seed and pericarp of *L. chinensis*was found to 0.36 and 0.66 mg GAE/g of dry weight tissue respectively (Table 3).

Flavonoids are the most common group of polyphenolic plant metabolites known to provide health benefits through antioxidant activity and modulation of cell signalling pathways. The total flavonoid contents of methanolic extracts of seed and pericarp of *L. chinensis*was found to be 11.0 and 17 mg QE/g of dry weight tissue (Table 3). Extensive research has proposed defensive effects of flavonoids against numerous infectious and degenerative maladies, such as cardiovascular ailments, malignancies, and other age-related illnesses.

Ascorbic acid levels in seed and pericarp of *L. chinensis*was found to be 78.93 mg/100g and 231.87 mg/100g of dry weight tissue (Table 3). These values are consistent with those reported by Queiroz [33] for dried samples. The authors also reported a decrease in ascorbic acid levels upon drying when compared to tissues *in natura* and attributed this to degradation by temperature, pH, oxygen and degradative enzymes.

β-carotene, the main source of vitamin A was found to be higher in the litchi pericarp (195.09 mg/100 ml) when compared to the litchi seed (2.70 mg/100 ml) (Table 3). Drying as a method of processing could result in a significantly reduced level of β-carotene due to degradation of carotenoids [33] resulting from loss of tissue integrity, contact with oxygen, light and rise in temperature [34]. Nonetheless, drying did not result in complete loss of its nutritional potential and hence could be used for enrichment of food products if suitably treated. Such comparative reduction was not reported in dried seed when compared to seed *in natura*[33]. The lycopene content in the dried seed was found to be 4.33 mg/ 100 ml but was undetected in dried pericarp (Table 3).

Table 3: Quantitative analysis of total phenols, total flavonoids, β-carotene and lycopene of *L. chinensis*seed and pericarp extracts

Sample	Total Phenols	Total Flavonoids	β-carotene	Lycopene	Ascorbic acid

	(mg GAE/g DW)	(mg QE/g DW)	(mg/100 ml)	(mg/100 ml)	(mg/g DW)
Dried seed	0.36 ± 0.01	11.0 ± 0.13	2.63 ± 0.06	5.46 ± 0.4	78.93 ± 9.3
Dried pericarp	0.66 ± 0.02	17.0 ± 0.24	210.4 ± 11.31	0.00	231.87 ± 13.6

All values are represented as mean±SD (n=3). GAE – Gallic acid equivalents; QE – Quercetin equivalents.

3.4 *In vitro* antioxidant activity

DPPH scavenging activity: Total antioxidant capacity of the extracts was calculated using % inhibition against concentration of ascorbic acid (y = 0.1065x + 7.717; R^2 = 0.794). At 500 µg/ml concentration, methanolic extracts of seed and pericarp of *L. chinensis*exhibited maximum DPPH radical scavenging activity that was found to be 27.18% and 29.12% respectively as shown in Table 4. The percentage of antioxidant activity observed in both seed and pericarp was found to be considerably lower than reported [35], [33]. The differences seen could be attributed to climatic conditions, maturity, cultivation and agricultural practices. The extracts of seeds and pericarp of *L. chinensis* showed IC_{50} values of 473.2 µg/ml (y = 0.1451x + 44.94; R^2 = 0.465) and 437.1 µg/ml (y = 0.1556x + 33.2; R^2 = 0.646) (Fig 1).

H_2O_2 scavenging activity: Scavenging of hydrogen peroxide and its percentage inhibition in methanolic extracts of seeds and pericarp of *L. chinensis* showed IC_{50} values of 34.94 µg/ml (y = 0.1451x + 44.94; R^2 = 0.465) and 107.77µg/ml (y = 0.1556x + 33.2; R^2 = 0.646), respectively as given in the Table 4. Gallic acid taken as reference showed IC_{50} value of 31.99 µg/ml (y = 0.1419x + 45.46; R^2 = 0.448) (Fig 1).

The antioxidative activities observed can be attributed to either the mechanisms exhibited by different polyphenolic compounds such as, tocopherols, flavonoids and other organic acids. Studies have shown that many polyphenols contribute significantly to the antioxidant activity and act as highly effective free radical scavengers which is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [36].

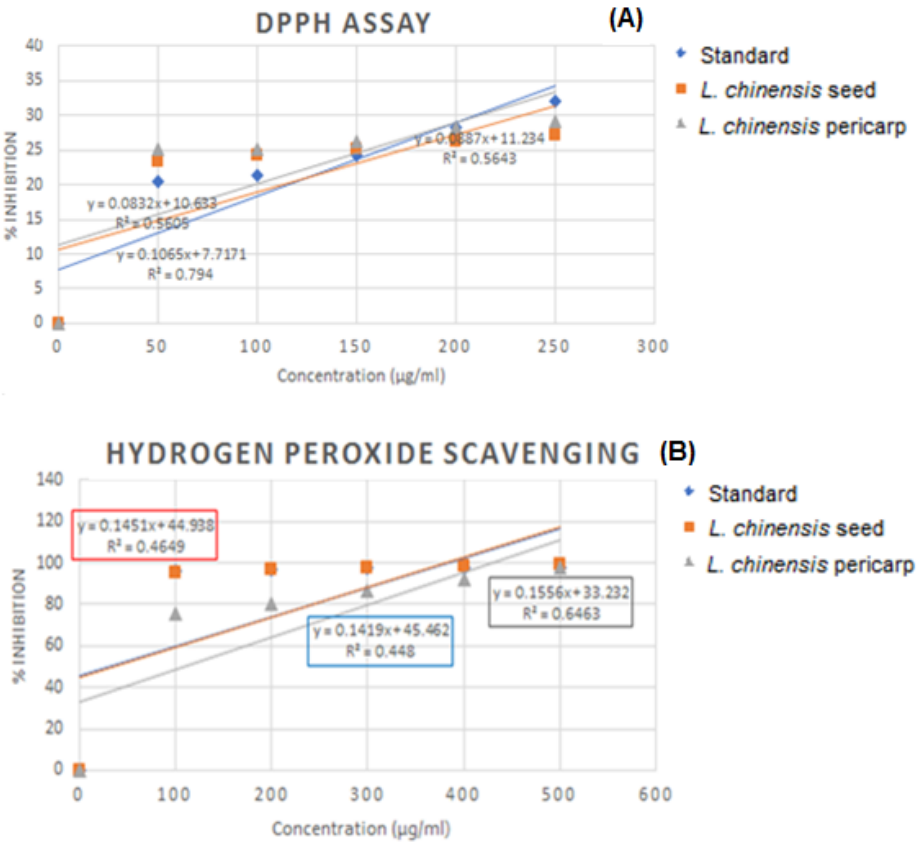

Fig 1: Antioxidant activities of seed and pericarp of *L. chinensis*; DPPH assay (A), H_2O_2 scavenging activity (B).

3.5 *In vitro* anti-inflammatory activity

Inhibition of Lipooxygenase activity: The plant lipoxygenase pathway is in many respects equivalent to the 'arachidonic acid cascades' in animals. For this reason, the *in vitro* inhibition of lipoxygenase constitutes a good model for the screening of plants with anti-inflammatory potential [37]. Methanolic extracts of seed and

pericarp of *L. chinensis* were studied at 100-500 µg/ml, and the inhibition obtained is as shown in Table 4. From these results, the strongest inhibition for all the samples was obtained at concentration of 500 µg/ml. The standard exhibited 70.3 ± 3.3% inhibition at a concentration of 500 µg/ml. Percentage inhibition of lipoxygenase in methanolic extracts of seed and pericarp of *L. chinensis* demonstrated IC_{50} values of 157.95 µg/ml (y = 0.153x + 25.77; R^2 = 0.723) and 98.29 µg/ml (y = 0.129x + 37.29; R^2 = 0.500), respectively (Table 4, Fig 2). Gallic acid taken as reference exhibited 336.74 µg/ml (y = 0.1365x + 4.03; R^2 = 0.95).

Table 4: *In vitro* antioxidant and anti-inflammatory activityof *L. chinensis*seed and pericarp extracts

	Assay	Control			*L. chinensis*seed		*L. chinensis*pericarp	
		Conc. (µg/ml)	% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)
***In vitro* antioxidant activity**	**DPPH scavenging activity**	50	20.3± 2.3	397.1	23.3 ± 1.1	473.2	25.2 ± 2.4	437.1
		100	21.3 ± 1.6		24.2 ± 0.9		25.2 ± 4.1	
		150	24.2± 0.7		25.2 ± 2.2		26.2 ± 0.8	
		200	28.1 ± 3.3		26.2 ± 3.3		28.1 ±1.6	
		250	32.0 ± 4.1		27.1 ± 1.7		29.1 ± 3.4	
	H_2O_2 scavenging activity	100	95.8 ± 8.2	31.99	95.0 ± 6.6	34.94	75.9 ± 3.2	107.77
		200	96.5 ± 7.8		96.8 ± 9.0		80.5 ± 6.1	
		300	97.6 ± 2.3		97.9 ± 3.2		86.7 ±0.6	
		400	97.8 ± 9.1		98.1 ± 4.2		92.0 ± 4.2	
		500	97.9±11.2		99.5 ± 7.1		97.9 ±7.3	
***In vitro* anti-inflammatory activity**	**LOX inhibiting activity**	100	20.2 ± 1.3	242.85	53.3 ± 3.1	157.95	79.6 ± 3.5	98.29
		200	32.6 ± 2.4		77.1 ± 3.3		82.0 ± 6.1	
		300	48.9 ± 5.6		82.8 ± 6.7		83.6 ± 4.4	
		400	56.9 ± 8.1		83.4 ± 8.2		86.1 ± 6.8	
		500	70.3 ± 3.3		88.2 ± 9.4		86.3 ± 8.2	
	Inhibition of heat induced protein denaturation	100	92.9±11.1	42.78	92.3 ± 9.8	45.85	92.9±12.5	44.59
		200	94.8 ± 7.8		93.5 ± 6.4		93.5 ± 7.9	
		300	95.5 ± 4.6		96.1 ± 7.2		94.2 ±9.0	
		400	96.1 ± 9.9		96.7 ± 8.3		95.5±10.6	
		500	97.4 ± 2.4		97.4 ± 6.5		96.1 ± 8.3	
	RBC membrane stabilization	100	26.7 ± 5.5	662.62	28.55±3.6	520.80	26.3 ±1.1	711.74
		200	28.2 ± 6.2		33.2 ±6.1		27.56±2.3	
		300	30.9 ± 8.9		38.2±4.4		29.8±1.8	
		400	31.7 ± 2.1		40.5 ±2.1		30.1 ±5.9	
		500	37.5 ± 3.4		42.3 ±0.8		35.4±6.1	

All values are represented as mean±SD (n=3).

Inhibition of heat induced protein denaturation: Denaturation of proteins is a well- documented cause of inflammation. As a part of the investigation of the anti-inflammatory activity of *L. chinensis*, ability of extract to inhibit protein denaturation was studied. Both seed and pericarp extracts were found to be effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table 4. Maximum inhibition was observed at 500 µg/ml for the methanolic extracts of seed and pericarp of *L. chinensis*(Fig 2) with an IC_{50} value of 45.85 µg/ml (y=0.1437x + 43.42, R^2 = 0.477) and 44.59 µg/ml (y=0.1397x + 43.77, R^2 = 0.459) respectively. Aspirin, a standard anti- inflammatory drug showed the maximum inhibition at the concentration of 500 µg/ml with an IC_{50} value 42.78 µg/ml (y=0.1421x + 43.92, R^2 = 0.467) (Table 4).

RBC membrane stabilizing activity: The plasma membrane of mammalian red blood cells has been particularly useful as a model for studies of membrane structure as they do not possess nuclei or internal membranes. The RBC plasma membrane resembles the lysosomal membrane and hence the stabilizing effect of drugs on RBC membrane may correlate with its lysosomal membrane stabilizing effect. Stabilization of lysosomal membrane leads to the inhibition of release of the inflammatory mediators and consequent inhibition of the process of inflammation. The highest inhibition of heat-induced hemolysis in the methanolic extracts of seed of seed and pericarp of *L. chinensis*(Fig 2) was observed at 500 µg/ml with IC_{50} values of 520.80 µg/ml (y=0.0721x + 12.45, R^2 = 0.733) and 711.74 µg/ml (y=0.0545x + 11.21, R^2 = 0.658). 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 9). Most anti-inflammatory drugs stabilize lysosomal membrane and inhibit the inflammatory process by restricting the release of lysosomal enzymes [38].

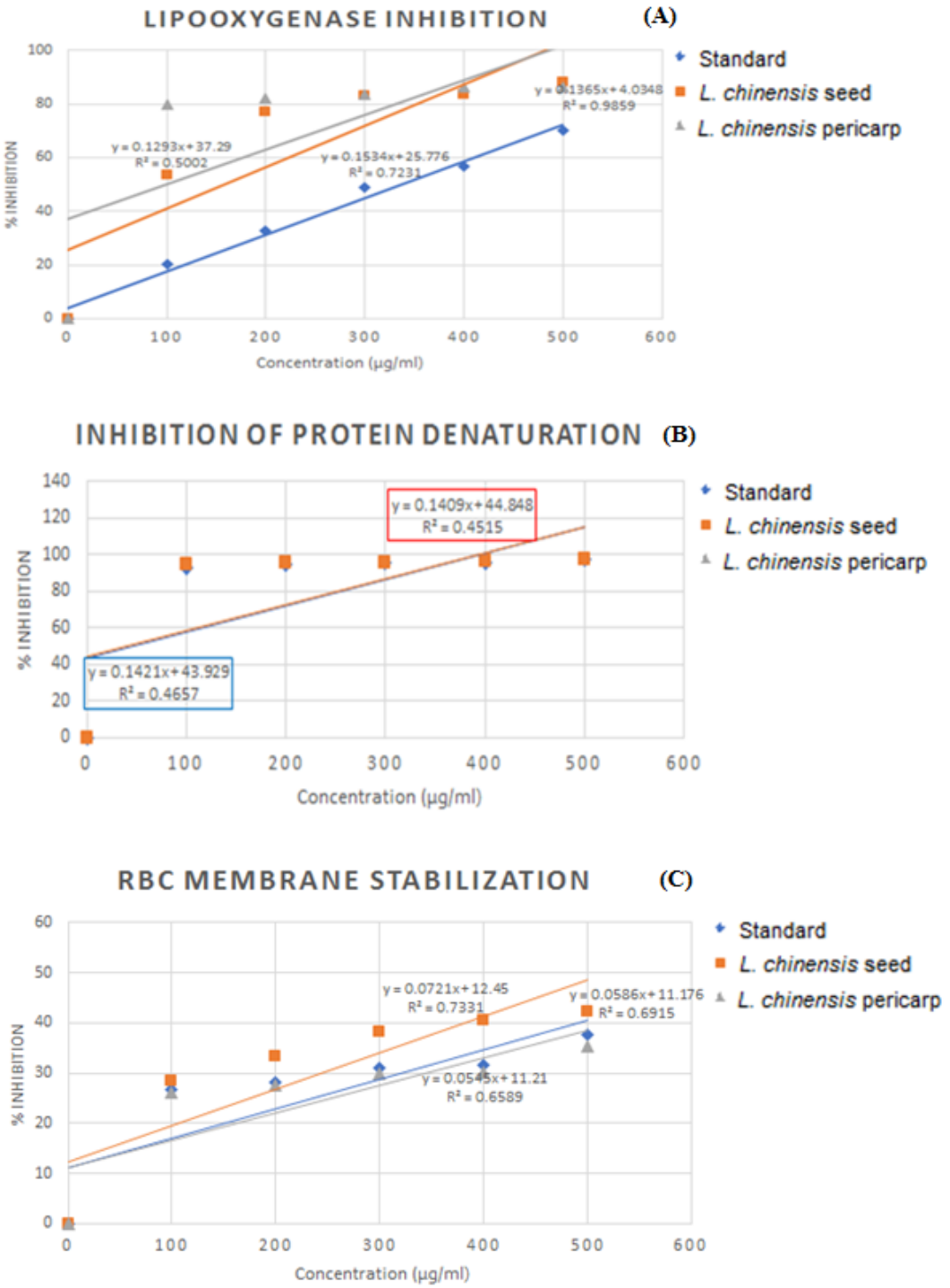

Fig 2: Anti-inflammatory activities of seed and pericarp of *L. chinensis*; lipoxygenase inhibition assay (A), inhibition of heat induced protein denaturation (B), RBC membrane stabilization assay (C).

3.6 Antibacterial activity of extracts: Plants products are the richest source of versatile chemical compounds for medicinal purpose and hence are greatly explored for bioactivity. 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 [39]. In our study, *in vitro* antimicrobial activity of *L. chinensis*seed and paricarp extractwas determined by agar well diffusion method (Table 5). The antimicrobial potential of the extracts was analyzed based on their zone of inhibition against the gram positive (*S. aureus*)

and gram negative bacteria (*P. aeruginosa, K. pneumonia*and *E. coli*). The inhibition zone formed in this study depends upon the bacterial species, quantity and type of extract filled in the well. By comparing the inhibitory action of both extracts, it was found that the methanolic extract has most prominent effect on all bacterial species, followed by the aqueous extract (Table 5).

Table 5: Antibacterial activity of extracts of seed and pericarpof *L. chinensis*. The zone of inhibition is expressed in mm.

Strain	***L. chinensis*seed**		***L. chinensis*pericarp**		**Ciprofloxacin**	
	MeOH	Aqua	MeOH	Aqua	Positive	Negative
S. aureus	9 ± 0.31	12± 0.22	11± 0.57	10± 0.17	10± 0.27	0
E. coli	7± 0.27	8 ± 0.13	15± 0.43	8± 0.12	10± 0.43	0
K. pneumoniae	15± 0.33	7± 0.11	17± 0.57	9± 0.16	17± 0.56	0
P. aeruginosa	12± 0.12	6± 0.32	18± 0.46	8± 0.11	10± 0.21	0

All values are represented as mean±SD (n=3).

The strongest effect of aqueous seed extract was recorded against gram positive *S. aureus* with a zone of inhibition (ZOI) 12 mm, followed by *E. coli* (ZOI, 8 mm)and *K. pneumoniae* (ZOI, 7 mm), whereas the least effect of aqueous extract was observed on *P. aeruginosa* (ZOI, 6 mm) (Table 5). While the investigation of methanolic seed extract against bacterial strains reveals that most sensitive bacterium was found to be and *K. pneumoniae* (ZOI, 15 mm), followed on *P. aeruginosa* (ZOI, 12 mm) and *S. aureus* (ZOI, 9 mm). Moreover, the most susceptible bacterium against aqueous pericarp extract was found to be *S. aureus* (ZOI, 10 mm), followed by *K. pneumoniae* (ZOI, 9 mm). Methanolic extract of *L. chinensis* pericarp demonstrated the largest clearance against *P. aeruginosa* (ZOI, 18mm), followed by *K. pneumoniae* (ZOI, 17 mm) and *E. coli* (ZOI, 15 mm). On the whole, methanolic extracts of both samples were found comparable in antibacterial activity to the control ciprofloxacin. This could be attributed to the presence of minute concentrations of bioactive compounds in the crude plant extract. 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 components [40].

4. Conclusion

The present study concludes that the hydro-alcoholic extract of seed and pericarp of *Litchi chinensis*Sonn. possess potential antioxidant, anti-inflammatory and anti-microbial activities. There is therefore further need to explore the bioactive constituents responsible for this activity as well as to elucidate the exact mechanism of action and to extrapolate the results on animal models to establish possible side effects. Additionally, the radical scavenging activities are related to the high levels of total phenolics and flavonoids especially in the litchi pericarp. This along with the absence of anti-nutritional factors in the pericarp indicates that there is immense potential for its use as functional ingredients for food and pharmaceutical applications. Thus, a preliminary correlation between ethnomedical employment and the pharmacological activities has been established. Accurate and precise chemical composition of the seed and pericarp has to be established along with an understanding of metabolic engineering to enhance the synthesis and accumulation of these compounds.

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