Research Article

Evaluation of Antimicrobial and Antioxidant Property of Lychee's Seed for Therapeutic Purpose

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ABSTRACT

Lychee (*Litchi chinensis*) is a fruit which belongs to the genus Litchi and family Sapindaceae. It is a seasonal fruit which is an important source of various nutrient components. Due to its delicious taste and high nutrient density the consumption of fruit is very high this leads to the accumulation of lychee's seed as a waste. The aim of this study was to examine antimicrobial and antioxidant properties of the lychee's seed. The extract of lychee seed was prepared by using three different solvents ethanol, acetone and distilled water. These prepared extracts were used for evaluation of the antimicrobial property against six bacterial strains: *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The antimicrobial effect was evaluated by agar well diffusion assay and the results of these extracts were found to be positive for all the selected bacterial strains with different range of zone of inhibition. Subsequently, the antioxidant property of lychee's seed was also evaluated by ferric chloride reducing power assay and compared with 1% ascorbic acid solution. The results indicated that 1% of lychee's seed powder solution have more antioxidant property than 1% ascorbic acid solution. As a result from this investigation suggest that lychee's seed may hold several beneficial roles in the field of pharmacy and food technology so further investigations are required in this field.

Keywords: Lychee (*Litchi chinensis*), Antimicrobial activity, Well diffusion, Antioxidant property, ferric chloride reducing power assay.

INTRODUCTION

Lychee, (*Litchi chinesis*) is member of the family Sapindaceae[1]. Lychee is generally cultivated in tropical and subtropical country, this tree is native to Taiwan, china and southeast Asia but now a days it is cultivated in many part of the world like in Thailand, Japan, Vietnam, Bangladesh and northern India, In India mainly Muzaffarpur (Bihar) that accounts for 75% of total production. The fresh lychee fruit is covered by pink-red colour peel that is inedible but easily removed to expose delicate, whitish pulp called as aril [2-3]. Lychee is consumed as fresh or processed fruit. Processing and consumption of lychee's fruit lead to generation of by-products such as epicarps and seeds, which are discarded. This leads to their accumulation as waste and subsequently causes environmental pollution. However, with appropriate treatment and study, the seeds might possibly be used as a food ingredient, pharmaceutical drugs and even for other purposes. In the last few years various plant extract and fruit based waste extract like that of seeds, epicarps etc. have been used as alternative therapeutic agent for the treatment of various types of diseases and as antioxidants for preserving foods and neutralising free radicals of the body[4]. Free radicals production occurs during metabolism in animal as well as in plants cell, excess production of these free radicals leads to oxidative stress resulting in numerous disorders like cardiovascular, atherosclerosis, reperfusion injury, rheumatoid arthritis, inflammatory disorders and cancer etc.[5] Many synthetic antioxidants such as propylgallate, butylated hydroxyanisole etc. has been used to retard the oxidation process, however use of such synthetic antioxidant compound must be under strict regulation due to its potential possibility of causing health hazard [6-7]. Similar problems are faced while using therapeutic agents and synthetic antibiotics for treatment of various kinds of disease like acne, inflammatory disease, cough and cold or to cure infectious diseases. Among those, antibiotic like tetracycline, erythromycin, macrolide, clindamycin etc. are used, however long-term medication of these antibiotics may induce side effect such as appearance of resistant bacteria, organ damage immune-hypersensitivity etc.[8], due to these reasons many researchers have tried to find out a new therapeutic agent with minimal side effect, which has led to an increase in demand for screening of new bioactive compounds from natural resources. This research aimed to optimize the usage of lychee's seed, by implementing the design of its extraction, as a potential anti-bacterial and antioxidant that will be beneficial to mankind. Also, it will help to play a role in minimizing waste generation.

MATERIALS AND METHODS

Sample collection

The fresh fully ripen lychee fruits were harvested from an orchard in Muzaffarpur district (Bihar). The fruits were carefully selected on the basis of uniformity in size, shape and colour. The seeds were separated from the fruit by removing outer covering and aril. Dark brown colour seeds, thus obtained, were washed with distilled water and air dried at room temperature for four to five days.

Then, subsequently it was stored at room temperature for the further use.

Figure 1.1 Lychee's tree

Figure 1.2 Lychee's fruit with seed

Figure 1.3 Lychee's seed

Figure 1: Sequential steps for lychee's seed harvesting.

Extract preparation

Lychee's seed was extracted by the modified method of Abdullah *et al*. (2011). Initially the seeds under investigation were broken down into small pieces by using sterile pestle and mortar than it was ground to fine powder using high speed blender. Three different solvent such as ethanol, acetone and distilled water were used as a solvent for extract preparation. 7.5 grams of lychee's seed powder was dissolved in 25ml of ethanol, acetone and distilled water separately and then the mixture was kept for 24 hours in an incubator shaker at 100 RPM at 37ºC for continuous shaking. After 24 hours of continuous shaking the mixture were transferred to centrifuge tube and centrifuged at 6000 RPM for 5 minutes[8]. Supernatant was transferred to a fresh sterile falcon tube and stored at 4ºC for further use.

Antimicrobial test (well diffusion assay)

Antimicrobial susceptibility test was carried out by the well diffusion method. The Petri-dish containing nutrient agar was plated with 0.1 ml culture of different bacterial strain. A small round shape well was punched by well puncture of diameter 8 mm and filled with 100 µl of seed extract in different solvents as mentioned above. The plate inoculated with different microorganism were made in triplicate and incubated at 37ºC for 12 hours and the diameter of resultant zone of inhibition was measured with a ruler.

Evaluation of antioxidant property by reducing power assay

Reducing power assay was done by the modified method of Nikhat F. *et al* (2009), initially 1% ascorbic acid and 1% lychee's seed powder solution were prepared in distilled water and then further diluted to a working concentration ranged from 200 to 1000 µg/ml by diluting with phosphate buffer (pH-6.6) and then the solution was mixed with 2.5 ml of potassium ferricyanide (1% w/v) solution. The mixture was incubated at 50ºC for 20 minutes. Aliquots of trichloroacetic acid (2.5 ml of 10% w/v) were added into the mixture followed by centrifugation at 3000 RPM for 10 min. The supernatant of the solution was transferred into the fresh tube and equal volume of distilled water and 1 ml of freshly prepared ferric chloride (1% w/v) solution was added (if solution mixture is 5 ml). The absorbance of the reaction mixture was measured at 700 nm and absorbance of standard ascorbic acid solution was compared with lychee's seed powder solution. The higher the absorbance the higher is the reducing power [9].

Statistical Analysis

All the data obtained from antimicrobial activity test of different extract was analysed by using SPSS software version 12 for windows and presented as mean ± standard error of mean (S.E.M). The data were statistically analysed by paired sample T-test to check whether two different extracts have significant difference of inhibitory effect on the microbes. The level of statistical significance was set at $P \leq 0.05$[10-12].

RESULTS AND DISCUSSION

Antimicrobial assay

The study of antimicrobial effect of lychee's seed extract in different solvent is summarized in the Table 1. The inhibition zone formed in this study depends upon the bacterial species, quantity and type of extract filled in the well. Addition of higher concentration and large quantity of extract in the well could contribute to larger zone of inhibition. In this investigation four well were punched in the Petri dish, among the four, one well was filled with the distilled water as a control for comparison and interpretation of the inhibitory action of different extracts on bacterial species. Out of the six bacterial species chosen for this study, four species were gram negative and two species were gram positive bacteria. Gram negative bacteria were *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris while gram positive bacteria used were Staphylococcus aureus* and *Bacillus subtilis*. By comparing the inhibitory action of all the three kinds of extract, it was found that the acetone extract has most prominent effect on all bacterial species, followed by the ethanol extract. On the other hand moderate effect of water extract was observed on all gram negative as well as gram positive bacterial species. When the data obtained from this investigation was analysed by statistical analysis of paired T-test to

check whether different extract used in this study has similar kind of inhibitory effect against selected bacterial strain or different, it was revealed that ethanol extract and acetone extract has significant difference of inhibitory effect from water extract against all bacterium except *Proteus vulgaris.* On the other hand comparison of ethanol extract with acetone extract has shown significant difference in inhibitory effect only for *Klebsiella pneumoniae*. This investigation and statistical analysis result reveals that the different extract has different pattern of inhibitory action on the selected microorganism.

Table 1:

Name of organism	Control (water)	Ethanol extract	Acetone extract	Water extract
Escherichia coli	0.00	16.66±0.33	17.66±0.33	12.50±0.28
Staphylococcus aureus	0.00	17.50±0.28	18.00±1.15	12.00±0.00
Proteus vulgaris	0.00	19.00±0.57	19.66±0.33	17.66±0.33
Bacillus subtilis	0.00	19.33±0.33	18.00±0.00	13.83±0.16
Pseudomonas aeruginosa	0.00	18.33±0.33	19.33±0.88	13.00±0.00
Klebsiella pneumoniae	0.00	14.50±0.28	23.00±0.00	18.00±0.57

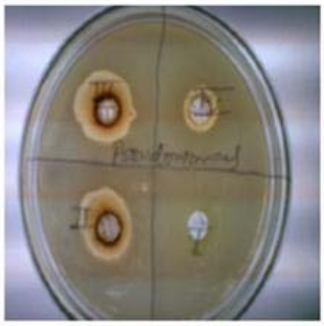

Figure 2.1 *Pseudomonas aeruginosa*

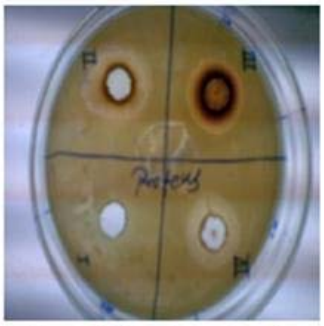

Figure 2.2 *Proteus vulgaris*

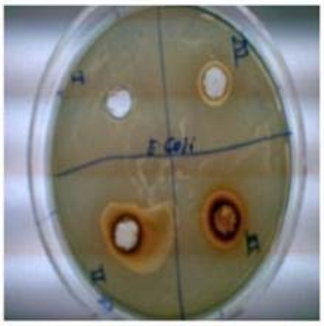

Figure 2.3 *Escherichia coli*

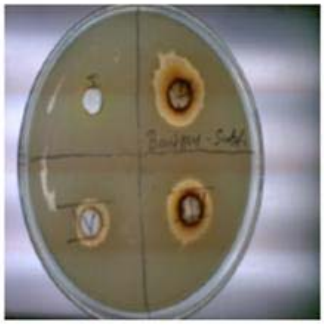

Figure 2.4 *Bacillus subtilis*

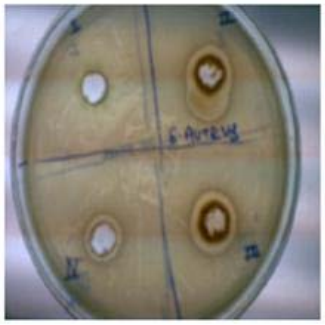

Figure 2.5 *Staphylococcus aureus*

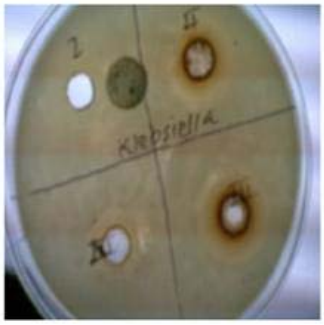

Figure 2.6 *Klebsiella pneumoniae*

Note: (well No. - [I] control (water), [II] Ethanol extract, [III] Acetone extract, [IV] water extract.)

Figure 2: The antimicrobial effect of different type of lychee's seed extract against different microorganism by well diffusion method.

Table 2: Free radical scavenging activity of Vitamin C and lychee's seed

Serial No.	Concentration of ascorbic acid (Vitamin C) (µg/ml).	Concentration of lychee's seed powder (µg/ml).	Absorbance at 700 nm	
			Vitamin C	Lychee
I	200	200	0.260	0.375
II	400	400	0.386	0.546
III	600	600	0.482	0.720
IV	800	800	0.598	0.895
V	1000	1000	0.812	0.910

The strongest effect of acetone extract was recorded against *Klebsiella pneumoniae with average diameter of zone of inhibition 23 mm, followed by Proteus vulgaris and Pseudomonas aeruginosa,* with average zone of inhibition 19.66 mm and 19.33 mm respectively, whereas least effect of acetone extract was estimated on *Escherichia coli* with average diameter of zone of inhibition 17.66 mm. While the investigation of ethanol extract against bacterial strains reveals that most sensitive bacterium was found to be *Bacillus subtilis* with average diameter of zone inhibition 19.33 mm, followed by *Proteus vulgaris* as second most sensitive bacterium with an average diameter of zone of inhibition 19.00 mm and then followed by *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* while *Klebsiella pneumoniae* showed the least effect against ethanol extract. *Moreover the most susceptible bacterium against water extract was found to be Klebsiella pneumoniae followed by second most sensitive bacteria Proteus vulgaris* whereas other bacterium has shown a moderate effect against water extract.

Reducing power assay

Estimation of reducing power of a particular compound or any type of extract indicates the capability of that compound to donate electron. For the measurement of reducing power of any extract or compound we measure the capability of extract to convert the ferric ion (Fe^{+3}) to ferrous ion (Fe^{+2}) by ferric chloride reducing power assay. In this assay yellow colour of the solution changes from yellow to green or blue as the ferric ion reduces from ferric to ferrous. Absorbance of the solution is measured at 700 nm. Increase in absorbance indicate the increased in antioxidant property of the compound.

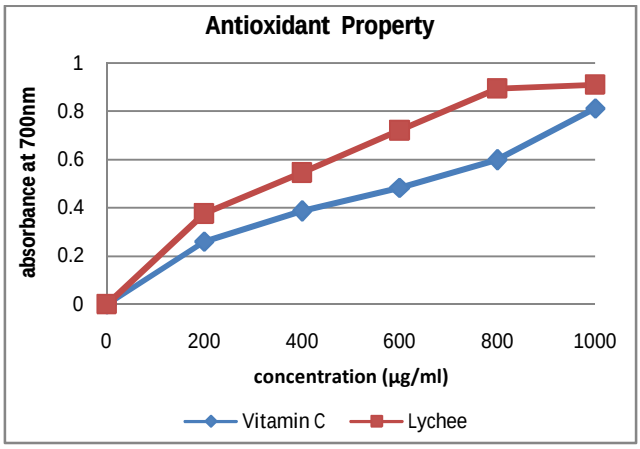

Figure 3: Comparison of radical scavenging activity of lychee's seed with Vitamin C.

Reducing power of compound serves as the functional indicator of an antioxidant capability of the compound. The antioxidant capability of the lychee seed and Vitamin C has been summarised in table 2 and graphically represented in figure 3. In the present study 1% lychee seed extract is compared with 1% ascorbic acid solution for its reducing power, during this investigation it was found that the antioxidant property of lychee seed extract was higher than the same concentration of ascorbic acid solution.

CONCLUSION

The result indicates that all types of lychee's seed extract used for the antimicrobial test against all the selected bacterial strain has shown a significant inhibitory effect, moreover 1% of lychee's seed powder solution has shown higher antioxidant property than 1% Ascorbic acid solution. So lychee's seed can be utilized as a therapeutic agent for the treatment of various types of diseases and as antioxidants for preserving foods and neutralising free radicals of the body**.** Since research in this area is very rudimentary so further studies must be done to find out the mechanism of action of the active compound in the sample extract which contribute to the antimicrobial and antioxidant property of lychee's seed. It is also crucial to find the effect of the compound on the animal models and human beings to establish the side effects and mechanism of action. Further it has to be ensured that the compounds are safe and has no adverse effect to the human health.

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A hands on Research oriented student, well knowledgeable with the tools and techniques of Biotechnology. His area of interest is Microbial technology, Biofuel, Bioenergy, genetic engineering, food biotechnology. The experience of Internships from various Industrial Organisations have given him the exposure to the practical Biotechnology and he has proved his work experience in the laboratory and has handled project in the field of microbial technology, Biofuel and food biotechnology. He is working under well guidance of Dr. Suneetha V, Associate Professor, VIT University Vellore, India.