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# Evaluation of Phytochemicals and Antimicrobial Activity of *Litchi Chinensis* Against the Various Disease Conditions

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## ABSTRACT

Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Preliminary Phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles. were carried out by employing standard methods for conducting Qualitative phytochemical analysis for studying the presence of active compounds like alkaloids, glycosides, phenols, saponins, tannins flavonoids, volatile oils, reducing sugars and steroids. Aqueous pericarp extract of *Litchi chinensis* showed presence of phenolic compounds, except flavonoids, volatile oils alkaloids, glycosides, saponins, tannins, reducing sugars and steroids. The antimicrobial activity of aqueous pericarp extract of *Litchi chinensis* was studied by agar well diffusion method *in vitro*. The effect of antimicrobial potential was examined *Salmonella typhi*, *Vibrio cholerae*, *Shigella dysenteriae*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Candida albicans*. The aqueous extract of the fruit pericarp has showed consistently significant inhibitory activity on different bacterial species tested and found the significance of antimicrobial activity of *Litchi chinensis*.

**Keywords:** *Litchi chinensis*, Phytochemicals, Antimicrobial activity

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## INTRODUCTION

In the recent years, antimicrobial resistance has become a major global problem[1]. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. Among the factors contributing to microbial resistance are indiscriminate use of antimicrobial agents by both healthcare professionals and patients, there is a need for production of substandard antimicrobials[2, 3]. Nowadays herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimum side effects and relatively low cost[4]. Phytochemicals are chemical compounds formed during the plants' normal metabolical processes [5]. These chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [6]. Many medicinal plants exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process[5]. There are reports on the curative potentials or abilities of medicinal plants and their products in the treatment of a wide range of infectious ailments such as urinary tract, gastrointestinal tract, respiratory tract and wound infections [7,8,9]. The potential value of such plant derived products prompted investigators to study new chemical constituents to improve the treatment of various diseases. Plants are the best source for the identification of new drug compounds. Litchi (Litchi chinensis Sonn.) is a subtropical fruit with a brightly red pericarp which contains a significant amount of phenolics such as epicatechin, procyanidins, cyanidin- 3-glucoside, and quercetin-3-rutinoside [10]. These phenolics exhibit good antioxidant ability [11], and anticancer and immunomodulatory activities [12].

## MARERIALS AND METHODS

### Plant Materials

The ripened litchees *(Litchi chinensis*) were obtained from local market. The peels were manually separated and shade dried. The pericarps were powdered in a grinder to get 40-mesh size powder. The moisture content of pericarp powder was found to be 13.5%. The powder was suspended in 2% gum acacia and used in the experimental studies.

**Phytochemical Analysis:** The extracts were analyzed by the following procedures [13]. To test for the presence of the flavonoids, volatile oils, unsaturated sterols and or/ triterpenes, alkaloids, saponins, tannins, terpenoids, glycosides and reducing sugars

**Flavonoids:** 4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

**Phenols:** The Solvent plant extract was treated with few drops of neutral ferric chloride solution 5%, intense colour developed indicates the presence of phenols.

**Volatile oils:** 2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

**Saponins:** Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

**Tannins:** To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins.

**Anthroquinones:** Borntreger's test was used for the detection of anthroquinones. 5 gm of plant extract was shaken with 10 ml of Benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammonical (lower) phase indicated the presence of free hydroxyl anthroquinones.

**Reducing Sugars:** To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

**Glycosides:** 25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

**Alkaloids:** 2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

**Steriods:** (Liebermann Burchard reaction: 200 mg plant extract in 10 ml chloroform, filtered), 2 ml filtrate + 2 ml acetic anhydride + conc. $H_2SO_4$. Blue green ring indicated the presence of steroids.

**Test Microorganisms**

*Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis, Escherichia coli* are gastrointestinal pathogens. *Klebsiella pneumonia*e is causative agent of pulmonary infections. *Staphylococcus aureus, Candida albicans* are causative agents of skin infections. *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis* are clinical isolates and remaining cultures are purchased from IMTEC, Chandigarh, India and NCL, Pune, India.

**Anti microbial assay by agar well diffusion method**

The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd., Mumbai, India) at 37 $^0$C while fungi were grown in Saboured Dextrose Agar media at 28 $^0$C and maintained on nutrient agar slants at 40 $^0$C and stored at -20 $^0$C. Inoculum of bacteria was prepared by growing pure isolate in nutrient broth at 37$^0$ C for overnight. The overnight broth bacterial cultures was sub-cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. 21 days old grown fungi culture was scraped with sterile scalpel and dissolved in sterile saline solution to make different dilutions. The diluted suspension which has the absorbance of 0.600 at 450nm determined spectroscopically (Electronics India) then it was used as inoculums for fungi. The agar plates were prepared by pour plate method using 20ml of agar medium. The sterile agar medium is cooled to 45$^0$ C and mixed thoroughly with 1ml of growth culture of concerned test organism (1 x 108 cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and test extracts were added. The agar plates were incubated at for 4days at 28$^0$C for fungi wile 24hours at 37$^0$C for bacteria. The diameter of inhibition zones was measured in mm using HiMedia zone reader. Ciprofloxacin (for Bacteria) and Griseofulvin (for Fungi) was used as positive control while Solvent (DMSO) used for negative control [14].

**Determination the Minimum Inhibitory Concentration by Broth Dilution Assay**

The minimum inhibitory concentration (MIC) of the plant extract was determined using broth dilution assay. The medium containing different concentrations of plant extracts viz., 1mg -1µg per ml prepared by serial dilution ($10^{-1}$ dilution). After inoculation of culture, the tubes were incubated for 72 hours at 28$^0$C for fungi while 24hours at 37 $^0$C for bacteria. The MIC of each sample was determined by measuring the optical density in the spectrophotometer (Electronics India) at 520 nm and compared the result with those of the non-inoculated broth used as blank. Control was prepared using media and inoculums without plant extract [14,15].

## RESULTS AND DISCUSSION

**Phytochemical Analysis:** Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [16]. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal science and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions [17]. Volatile oils are complex of compounds with strong odour. And known to have antiseptic, bactericidal, virucidal and fungicidal activities [18]. Phytochemical tests of the above extracts were performed through chemical reagents as described by Harbone 1998[19]. Nine chemical groups were screened for APLC. The detailed investigations of phytochemicals in APLC were shown in table 1. The APLC shown to have positive results for presence of phenolic compounds., The alkaloids, glycosides, flavonoids, volatile oils, saponins, tannins, reducing sugars and steroids were found to be absent in aqueous pericarp extract of *Litchi chinensis.*

**Antimicrobial activity on human pathogens**

Antimicrobial studies were carried out on human pathogenic bacteria and fungi. *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae Staphylococcus aureus* are bacterial sps. *Candida albicans* is a dermatophytic fungus. The APLC extract showed significant antibacterial activity and moderate antifungal activity. As shown in table 2, 15 mm was the highest zone of inhibition against *S. typhi* at 100 µg concentration of APLC extract. The MIC of APLC extract found to be between 1 - 100 µg/ml. 1 µg/ml was the lowest MIC value against *Salmonella typhi.* APLC extract showed comparable antibacterial activity with Ciprofloxacin (antibiotic) (Table. 3). *S. aureus, E. faecalis* are Gram positive bacteria which were showed sensitive to extract compared to gram negative bacteria. The APLC extract showed much effective against clinical isolates. The APLC extract was not active against *C. albicans*. 6 mm was the zone of inhibition at 100 µg/ml dose and 100 µg/ml was MIC against dermatophytic *C. albicans* (Table. 2 and 3). This indicates that APLC showed poor antifungal activity.

**Table 1: Phytochemical analysis of aqueous pericarp extract of *litchi chinensis*.**

| No | Phytochemicals | Aqueous pericarp extract of *litchi chinensis* |
|---|---|---|
| **1** | Flavonoids | --- |
| **2** | Volatile oils | --- |
| **3** | Phenols | +++ |
| **4** | Saponins | --- |
| **5** | Tannins | --- |

| | | |
|---|---|---|
| **6** | Reducing sugars | --- |
| **7** | Glycosides | --- |
| **8** | Alkaloids | --- |
| **9** | Steroids | --- |

**Table 2: Anti microbial activity of APLC extract on human pathogens.**

| S.No | Human pathogens | Causing disease | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | **25** µg | **50** µg | **75** µg | **100** µg | **Antibiotic*** (50 µg) |
| **1** | *Salmonella typhi* | Typhoid fever | 9 | 10 | 13 | 15 | 18 |
| **2** | *Vibrio cholerae* | Cholera | 8 | 10 | 11 | 13 | 17 |
| **3** | *Shigella dysenteriae* | Dysentry | **7** | 9 | 10 | 12 | 18 |
| **4** | *Enterococcus faecalis* | Gastro intestinal infections | 8 | 9 | 12 | 14 | 18 |
| **5** | *Escherichia coli* | Gastro intestinal infections | 10 | 11 | 13 | 14 | 17 |
| **6** | *Klebsiella pneumoniae* | Pulmonary infections | 8 | 10 | 11 | 13 | 16 |
| **7** | *Staphylococcus aureus* | Wound infections | 9 | 10 | 11 | 13 | 17 |
| **8** | *Candida albicans* | Dermatophycosis | 6 | 6 | 7 | 8 | 16 |

*Ciprofloxacin (for Bacteria) and Griseofulvin (for Fungi).

**Table 3: Anti microbial activity of APLC extract on human pathogens.**

| S.No | Human pathogens | Causing disease | MIC (µg/ml) | |
|---|---|---|---|---|
| | | | **APLC extract** | **Antibiotic*** |
| **1** | *Salmonella typhi* | Typhoid fever | 1 | 1 |
| **2** | *Vibrio cholerae* | Cholera | 10 | 1 |
| **3** | *Shigella dysenteriae* | Dysentry | 100 | 1 |
| **4** | *Enterococcus faecalis* | Gastro intestinal infections | 10 | 10 |
| **5** | *Escherichia coli* | Gastro intestinal infections | 10 | 1 |
| **6** | *Klebsiella pneumoniae* | Pulmonary infections | 10 | 10 |
| **7** | *Staphylococcus aureus* | Wound infections | 10 | 10 |
| **8** | *Candida albicans* | Dermatophycosis | 100 | 1 |

*Ciprofloxacin (for Bacteria) and Griseofulvin (for Fungi)

## CONCLUSION

In the present study was concluded that the presence of phytochemicals as phenolic compounds and anthocyanins were present in APLC. APLC showed strongest antibacterial activity, which was comparable to the commercially available antibiotic, Ciprofloxacin. APLC showed poor antifungal activity.

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