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Phytochemical investigation and antimicrobial screening of extracts of *Litchi chinensis* leaves from Dehradun region, India

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Abstract

The aim of the present study is to establish a correlation between litchi leaves extracts and their activity against human pathogenic bacteria. For this fresh litchi leaves were collected and dried in shade, powdered and extracted successively with solvents of different polarity. Phytochemical investigation was performed using conventional natural products identification tests and antimicrobial screening was done for two Gram negative and three Gram positive bacterial strains. Antimicrobial test were performed by disc diffusion method. All the tests were performed in a triplicate. Presence of majority of phytoconstituents in acetone extract may be responsible for its prominent activity against all the pathogens.

Keywords: *Litchi chinensis, successive extraction, phytochemical investigation, and antimicrobial screening*

Introduction

The wealth of India is stored in the enormous natural flora which has been gifted to her, endowed with a wide diversity of agro-climatic conditions. Thus, providing favorable condition for the growth for variety of medicinal and aromatic plants. Due to climatic diversity the chemical constituents in a same plant vary from region to region. In north India Uttarakhand is known for its climatic diversity, Dehradun is popular for its litchi production. Litchi belongs to the family sapindaceae. It is the plant native to South China but now exotic to other parts of the world. In India it is wildly cultivated for its high nutritive value. It is a rich source of vitamin C **(**Ahmad, 1956**)**. The chemical composition of litchi reveals that it had the edible portion 74.5%, moisture 78.5%, citric acid 1.2%, ash 0.69% and sugar 13.57 % **(**Cabin, 1954**)**. Medicinally the fruit of litchi is tonic to heart, brain, and liver **(**Kritikar & Basu, 1998**)**. The potential of cultivated plants as a source for new anti-microbial drug and botanical pesticides is still largely unexplored. This is also true in India and only a small percentage of plants of this region have been evaluated for antibacterial activity (Varma, 1999**).** The present study is designed to explore the preliminary phytochemical and anti-microbial screening of leaves extract of *Litchi chinensis* of Dehradun region and to establish a correlation between the phytoconstituents and their pharmacological activity.

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Material and Methods

Collection of plant material**: -** Fresh leaves of *Litchi chinensis* were collected from "Bombay bagh" Dehradun in the month of April, and authenticated at Botanical Survey of India, (BSI), Dehradun with Acc. No. – 113638. The collected leaves were dried in shade, grinded in to powder and stored in polythene bags before use.

Extraction of plant material: - 50 gm shade dried, grinded leaves of *Litchi chinensis* were extracted in a soxhlet sequentially in 700 ml of Petroleum ether (40-60° C) and acetone. The obtained extracts were concentrated at reduced pressure in a rotary vacuum evaporator.

Preliminary phytochemical investigation: - Phytochemical analysis for major phytoconstituents of the obtained extracts was undertaken using standard qualitative methods **(**Harborne, 1984**).**

Microorganism used**:** - Two gram negative and three gram positive human pathogenic bacterial

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strains were used in the present study. The bacterial species used are *Escherichia coli* (ATCC 433), *Bacillus cereus* (ATCC 11778), *Bacillus licheniformis* (ATCC1483), *Salmonella typhi* (ATCC 733), *and Staphylococcus aureus*. All the stock cultures in pure form were collected from S.G.R.R.I.T.S department of Life Sciences, Dehradun.

Culture media and inoculums: **-** Muller Hinton Agar and Agar Nutrient broth (Hi-Media Pvt. Ltd., Bombay, India) are used as culture for the test microorganism. Microbial cultures, freshly grown at 37°C were diluted in sterile normal saline solution and the turbidity of the suspension is adjusted equivalent to a 0.5 McFarland standard by adding more organisms, so as to obtain the cell suspension between 10^5 to10^8 CFU/ml.

Preparation of Test extract: **-** Solvent free extracts obtained were dissolved in sterilized and filtered DMSO (filtered with whatman filter of pore size 0.45 micron) to prepare a test solution of extract.

Antibacterial screening: **-** The anti-bacterial activity was performed by standard protocol of Kirby- Bauer disc diffusion susceptibility methods **(**Bauer *et al*.,1966**).** The disc devoid of extract and presence of DMSO served as control. Tetracycline (30 µg/ disc) was used as standard. All the test processes were performed in a triplicate in laminar chamber.

Results and Discussion

Extractive yield: **-** *Litchi chinensis* leaves when subjected to sequential soxhlet extraction with petroleum ether and acetone and further their concentration yields the extracts with different yield and consistency. Table No. 1 represents the % yield w/w of the obtained extract.

Preliminary Phytochemical Investigation**: -** The phytochemical screening test shows the presence of various bioactive secondary metabolites. Table No. 2 represents the presence of various phytoconstituents in different extracts.

Antimicrobial Screening: The antimicrobial activity showed significant reduction in bacterial growth in terms of zone of inhibition. The zone of inhibition was recorded and tabulated in Table No. 3. Acetone extract exhibit the maximum inhibitory effect against all bacterial strain. Petroleum ether shows zone of inhibition aginst *E. coli*, *B. licheniformis*, and *S. typhi* only.

Table 1: Percentage extractive values and physical characteristics of different extract of *Litchi chinensis* leaves.

Extracts	Weight of sample (gm)	Weight of extract (gm)	w/w % yield	Colour	Consistency
Petroleum ether	50	1.526	3.052	Greenish	Waxy
Acetone	50	2.726	5.452	Dark brown	Sticky

Table No. 2:- Phytoconstituents present in various concentrations in different extract of *Litchi chinensis* leaves.

Phytoconstituents	Extracts	
	Petroleum ether	Acetone
Carbohydrates	-	-
Alkaloids	-	+++
Amino Acids	-	-
Steroids	+++	+
Terpenoids	-	++
Protein	-	-
Tannin & Phenols	++	++
Flavonoids	-	+++

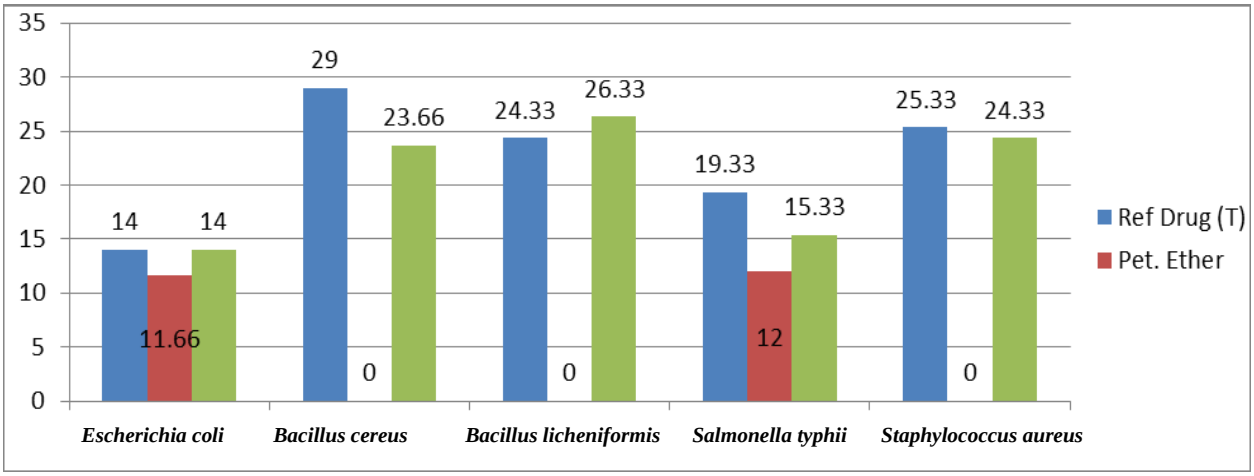
(+++) = high concentration, (++) = moderate concentration, (+) = low concentration, (-) = absent.

Table No. 3:- Antimicrobial screening of different extracts of *Litchi chinensis* leaf. All the values are mean zone of inhibition ± SD

Bacterial strain	Zone of inhibition in mm. (Mean ± SD)		
	Ref drug (T)	Pet. ether	Acetone
Escherichia coli	14 ± 1.732	11.66 ± 2.08	14.0 ± 2.0
Bacillus cereus	29.0 ± 1.00	-	23.66 ± 1.52
Bacillus licheniformis	24.33 ± 2.08	+	26.33 ± 2.08
Salmonella typhi	19.33 ± 7.09	12.0 ± 2.0	15.33 ± 3.05
Staphylococcus aureus	25.33 ± 1.52	-	24.33 ± 3.78

(+) = light zone of inhibition, **(-)** = no zone of inhibition

Plate 1:- Graphical representation of zone of inhibition of different extract against the selected pathogens.



Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. In the present study extracts from leaves of *Litchi chinensis* were tested against drug-resistant gram negative and gram positive bacteria. Table No. 1 shows that the acetone fraction contains a greater proportion by mass of the component compound. Phytochemical screening shows the presence of various bioactive secondary metabolites which are well known to have curative activity against several human pathogenic microorganisms and therefore could suggest the use traditionally for the treatment of various diseases **(**Usman and Osuji 2007**).** The literature revealed that the presence of polyphenolic compounds including condensed tannin and flavonoids in *Litchi chinensis* are responsible for its potential anti-cancer, anti-oxidant, **(**Wang *et al*. 2006 & Jiang and Li 2007**)** and cardio-protective activity **(**Deng *et al*. 2010**).** Acetone extract is evidenced for the presence of Terpenoids which shows cytotoxic, **(**Xinya *et al*. 2010**)** anti-tumor, anti-inflammatory, anti-viral and anti- bacterial activity **(**Sashi and Sucharitra 1997**)**. On correlating the results an inference can be drawn that presence of majority of phytoconstituents in acetone extract may be responsible for its prominent activity against all the pathogens. The active components usually interfere with growth and metabolism of microorganisms in a negative manner **(**Aboba and Etuwape 2001**).** The reason for the difference in sensitivity between the gram-positive and gram-negative bacteria could be ascribed to the morphological differences between them. Gram- negative pathogens have an outer phospholipidic membrane carrying the structural lipopolysccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to hydrophilic solutes. The gram positive bacteria should be more

susceptible having only an outer peptidoglycone layer which is not an effective permeability barrier **(**Nikaido and Vaara 1985**).** Phenolic content of plant extract possess antimicrobial activity **(**Acar *et al*. 2010**)** and highly oxidized phenols are more inhibitory because of phenolic toxicity to microorganisms **(**Ciocan and Bara 2010**).** The above mechanism also confirms that due to the presence of appreciable amount of Phenolic compounds in acetone extract, it shows potent antimicrobial activity but it is also evidenced that more hydrophilic phytoconstituents are present in acetone extract; due to which it shows more prominent antimicrobial activity against gram-negative bacteria. Lastly further explorations on plant derived antimicrobials are needed, to determine the identity of that particular compound in this plant and also to determine their full spectrum.

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