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Short Communication

Phytochemical Screening and Anti-microbial Activity Studies on *Leea macrophylla* Seed Extracts

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Abstract

Seed extracts of medicinal plants *Leea macrophylla* were investigated for phytochemical as well as antibacterial screening. Phytochemical studies of the plant extracts revealed the presence of phenolic, saponin, glycosides, carbohydrate and protein types of compounds. The antibacterial effects of the plant extracts were also tested against several human multi-drug resistant pathogens, including one gram positive and three gram negative bacterial species and one fungal species using the disc-diffusion assay. All the plant extracts showed potent anti-microbial effects against gram positive micro-organism (inhibition zone 7-19 mm) whereas none of the extracts produced any potent anti-microbial effects against gram negative bacteria. Furthermore, it has been shown that the extracts exhibit antifungal activities against *candida albicans* except the extract n-hexane.

Keywords: Leea macrophylla; Phytochemical screening; Anti-microbial activity.

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1. Introduction

Natural products including plants have been the basis of treatment of human diseases. Medicinal plants occupied a major position in the practice of ethnomedicine, and herbal medicines remain the major source of health care for the world population. Phytochemicals obtained from traditional medicinal plants present an exhilarating prospect for the expansion of modern therapeutics to fight against complicated ailments. Natural products from traditional medicine have played a significant role throughout the world in treating and preventing individual diseases [1]. The medicinal plants *L. macrophylla*, locally known as Hastikarna Palash, belongs to the family Leeaceae. The plant is available in Dinajpur, Savar and Chittagong hill tracts of Bangladesh [2]. It is also distributed in Central and Eastern Nepal, Bhutan, Indo-China [3], Myanmar, Thailand, Cambodia and Laos [4]. Leaves simple, broadly ovate, nearly as broad as long, the lower leaves up to 60 cm long, the upper 15 -23 cm long, base cordate, apex acute or acuminate,

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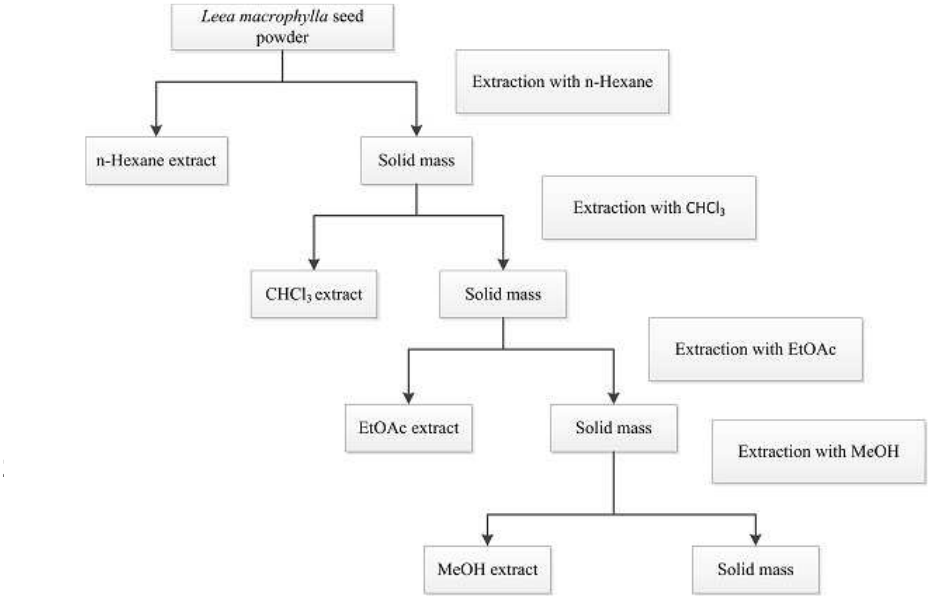
coarsely serrate or sublobed, dark green and glabrous above, pubescent beneath, main nerves opposite, 8-10 pairs, very prominent; petioles 5-12 cm long, deeply striate, glabrous. Inflorescence terminal, much branched, puberculous, corymbose cymes, up to 30 cm long, flower white. Berry globose, 6-8 cm in diameter, black, 3-6 celled, depressed-globose, usually 3-6 lobed. Leaf of *L. macrophylla* has been used for treatment of arthritis and boil [3]. Leaf juice is recognized as local anti-inflammatory agent and used in boils, arthritis, gout and rheumatism [6]. These are extensively used by the ayurvedic physicians in the preparation of seasonal tonic modaka preparation [5]. Dried powders of the roots with coconut oil are used on wounds and sores. Leaf juice is recognized as local anti-inflammatory agent and used in boils, arthritis, gout and rheumatism [7]. It has also ethno botanical uses in goiter, gastric tumor, lipoma and tetanus [8]. The present study, therefore, was designed to investigate the different fractions of *L. macrophylla* for screening their phytochemicals as well as anti-microbial activity as the plant has traditionally been used in the treatment of different diseases.

2. Materials and Methods

2.1. *Collection and extraction of plant materials*

The seeds of *L. macrophylla* were collected from the experimental medicinal plant garden of BCSIR Laboratories at Rajshahi. The collected seeds were then washed, dried in shade to protect them from direct sunlight. The shade-dried seeds were then crushed in a mecha nical grinder to fine powder of mesh 40. The powder was then successively extracted with



Scheme 1. Extraction flow chart.

different solvents on the basis of increasing polarity *e.g.*, n-hexane, chloroform, ethyl acetate and methanol fractions in an ultrasonic bath. Resulting extracts were filtered, concentrated under reduced pressure using rotary vacuum evaporator. A flow sheet of the extraction process of *L. macrophylla* seed powder is given in Scheme 1 above.

2.2. *Preliminary phytochemical screening*

The extracts were subjected to preliminary phytochemical screening to detect for the presence of different active components present in the seeds. Air-dried and powdered plant materials were screened for the presence of phenolic, alkaloids, glycosides, saponins, carbohydrates, starch and protein, as described in literatures [9-11].

Detection of alkaloids: A fraction of the extract was treated with Mayer's reagent (1.36g of mercuric chloride and 5g of potassium iodide in 100ml of distilled water) and observed for the formation of cream colored precipitate.

Detection of phenolics: A fraction of the extract was treated with 5% $FeCl_3$ reagent and observed for the formation of deep blue-black color.

Detection of saponins: In a test tube, about 5ml of extract and a drop of sodium bicarbonate were added. The mixture was shaken vigorously and kept for 3 minutes. The formation of a honey comb like froth showed the presence of saponins.

Detection of carbohydrate: In a test tube, about 0.5ml of extract and 5ml of Benedict's solution were added and boiled for 5 minutes. Formation of a colored precipitate indicated the presence of carbohydrate.

Detection of starch: A fraction of the extract was treated with iodine and potassium iodide solution. A blue color was produced.

Detection of glycosides: A fraction of the extract was treated with aqueous solution of NaOH. A pale yellow color indicated the presence of glycosides.

Detection of protein: A fraction of the extract was treated with 5-6 drops of Millon's reagent. A white precipitate was formed which turned red on heating.

2.3. *Determination of anti-microbial activity*

Anti-microbial tests were carried out with the disk diffusion method by applying the procedure described in [12]. Antibacterial activity was defined by measuring the diameter of the growth inhibition zone (mm) around a disk after 24 hours of incubation. The extracted fractions, namely n-hexane, chloroform, ethylacetate and methanol, were assayed for their antibacterial activity against the pathogenic bacteria one gram positive and three gram negative namely *staphylococcus aureus*, *escherichia coli, pseudomonas aeruginosa* and *salmonella typhi*. The antifungal activity of the fractions was assayed against the pathogenic fungal strains *candida albicans*.

2.4. *Preparation of the test plates*

The micro organism was transferred from the fresh subculture to the test tube containing 15ml autoclaved medium with the help of an inoculating loop in an aseptic condition.

Then the test tube was shaken by rotation to get a uniform suspension of the organism. The bacterial suspensions were immediately transferred to the sterile petridishes in an aseptic area. The petridishes were rotated several times; first clockwise and then anticlockwise to assure homogenous distribution of the test organisms. The media were poured into petridishes in such a way as to give a uniform depth of approximately 4mm. Finally, after the medium was cooled to room temperature in laminar air flow unit, it was stored in a refrigerator at 4°C.

2.5. *Preparation of test samples and discs*

The extracts (n-hexane, chloroform, ethyl acetate, and methanol 10mg each) were dissolved in 200µL DMSO in separate test tubes. Thus the concentrations were 10mg/ 200µL for each extract. Three types of discs were prepared for antibacterial screening: (a) sample discs (n-hexane, chloroform, ethyl acetate, and methanol extracts), (b) standard discs (kanamycin), and (c) control discs. *Sample discs:* sterilized metrical (BBL, Cocksrvile, U.S.A) filter paper discs (4mm diameter) were taken in a blank petridish. Sample solution of desired volume (10µL) was applied on the discs with the help of a micro-pipette in an aseptic condition. The discs were left for a few minutes in an aseptic condition for complete removal of solvent. To compare the antibacterial activity of test materials, kanamycin (10µL/disc) was used as standard discs [13], and control discs were made by applying solvent of 10µL on a sample disc in the present study.

2.6. *Placement of the discs and incubation*

By means of a pair of sterile forceps, the dried samples discs and standard discs were placed gently on the solidified agar plates seeded with the test organisms to ensure contact with the medium. The plates were then kept in a refrigerator at 4°C for 24 hours in order to provide sufficient time to diffuse the extracts into the medium. Finally, the plates were incubated at 37.5°C for 24 hours in an incubator.

3. Results and Discussions

Medicinal plants are a rich source of anti-microbialagents [14, 15]. Many secondary metabolites of plant are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated by tissue damage or pathogen attack [16]. Currently, majority of the pharmaceutically important secondary metabolites are isolated from wild or cultivated plants as their chemical synthesis is not economically feasible [17]. Major groups of anti-microbial compounds from plants include simple phenols and phenolic acids, quinones, flavones, flavonoids and flavonols, tannins, coumarins, alkaloids, terpenoids and essential oils, lectins and polypeptides [18]. Anti-microbial compounds identified have shown promising activity [19]. Different *in vitro* methods used for determining anti-microbial susceptibility include broth dilution assay, disc diffusion assay and well diffusion assay.

The qualitative phytochemical examination carried out are summarized in Table 1. Phytochemical features of various extracts of Leea macrophylla listed in Table 1 revealed the presence of phenolic, saponin, carbohydrate, glycoside and protein type of compounds.

Table 1. Phytochemical screening of *Leea macrophylla* seed extracts.

Class of compounds indicated	Extracts			
	n-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	-	-
Phenolics	-	-	+	+
Starch	-	-	-	-
Saponins	+	+	-	-
Proteins	-	-	+	+
Glycosides	-	-	+	+
Carbohydrates	-	-	+	+

Table 2 shows the inhibitory activity of n-hexane, chloroform, ethyl acetate and methanol extracts from *Leea macrophylla* seed against gram positive bacteria *staphyllococcus aureous* by the disc diffusion susceptibility assay. On the other hand, all the extracts (n-hexane, chloroform, ethylacetate and methanol) did not inhibit the growth of gram negative bacteria *escherichia coli*, *pseudomonas aeruginosa* and *salmonella tiphy*. The data of Table 2 showed that ethyl acetate extracts were more effective against *staphylococcus aureus* compared to two other three extracts (n-hexane, chloroform and methanol). The inhibition by the ethyl acetate extract was 19mm, whereas the zones of inhibition of methanol, chloroform and n-hexane were 13mm, 10mm and 7mm, respectively. The highest antibacterial activity was shown by the ethyl acetate extract while the lowest activity was seen from the n-hexane extract.

Table 2. Antibacterial activity of different extracts of *L. macrophylla* and Kanamycin standard.

Test organisms	Zone of inhibition (mm)				
	n-Hexane ext.(500µg/disc)	Chloroform ext. (500µg/disc)	Ethyl acetate ext. (500µg/disc)	Methanol ext. (500µg/disc)	Kanamycin (30 g/disc)
Staphylococcus aureus	7	10	19	13	28
Pseudomonas aeruginosa	-	-	-	-	15
Escherichia coli	-	-	-	-	18
Salmonella typhi	-	-	-	-	32

The data presented in Table 3 showed that three fractions, *i.e.* ethyl acetate, methanol and chloroform were active against *candida albicans*, whereas n-hexane extract did not show any activity against the tested fungi *candida albicans.* Among the four extracts ethyl acetate extract showed the highest activity than the other two extracts (chloroform and methanol) while n-hexane extract did not inhibit the growth of *candida albicans*. The zone of inhibition was found to vary between 10-15mm. Results show that plant rich in phenolic, glycosides, carbohydrate and protein compounds have been shown to possess anti-microbial activities against a number of microorganisms.

Table 3. Anti-fungal activity of different extracts of *L. macrophylla* and Kanamycin standard.

Test organisms	Zone of inhibition (mm)				
	n-hexane ext. (500µg/disc)	Chloroform ext. (500µg/disc)	Ethyl acetate ext. (500µg/disc)	Methanol ext. (500µg/disc)	Kanamycin (30g/disc)
Candida albicans	-	10	15	12	25

4. Conclusions

From the present study remarkable anti-microbial inhibition was shown against the tested organisms. The phytochemical analyses of seed extracts of the plants were carried out and found to have contained chemical constituents like phenolic, saponin, glycosides, carbohydrate and protein. The microbial activity of the *L. macrophylla fistula* was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may lead to the development of new pharmaceuticals research activities. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the anti-microbial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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