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In Vivo and In Vitro Analysis of Antimicrobial Potency of *Leea Macrophylla* Bioactives

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ABSTRACT

Bioactive extracts from different parts of Leea macrophylla. (Roxb.) member of Leeaceae family was investigated for antimicrobial properties. The perennial shrub has been utilized in floklore medicinal system. Phytochemical rich herb has shown significant therapeutic effect in treatment of ailments with explicit pharmacological response including anti-inflammatory, antilithotropic, hepatoprotective, neuroprotective and antidiarroheal as well. Phytochemical investigation revealed the high amount of primary and secondary metabolites enriching the nutritious and medicinal value of the herb in study. Presence of bioactives led researchers to conduct preclinical studies on the Leea macrophylla for its pharmacological response against pathogenic micro-organisms responsible for major setbacks in human health. The method implemented was well diffusion method for testing antimicrobial activity against gram-positive, gram-negative pathogenic bacterial strains isolated clinically and certain infective fungal strains. Medicinally important and bioactive rich medicinal plant Leea macrophyllatested for antimicrobial studies. The experimental study was carried out for antimicrobial efficacy in aerial parts, fruitning bodies, underground parts as well as invitro grown callus for comparative analysis. The observation and interpretation of the experiment conducted were discussed in the presented literature. The findings in the current analysis supports and clear recommend that Ethanolic extracts possess significant antimicrobial property against microbial clinical isolates in study.

***Keywords**: Anti-microbial, Agar Well diffusion, Clinical isolates, Bioactives, Flavonoids, Leea Macrophylla.*

Introduction

The rising awareness for herbal formulations and medications lead to the investigate novel antimicrobial mediators serving as an substitute course as compared to chemical-based drug and proven adverse side effects of same which is constantly a matter of concern. *Leea macrophylla*, recognized locally as Hastikarna Palash, member of the family Leeaceae (Singh and Singh, 1981). The herb is accessible in Savar, Dinajpur and Chittagong Mountain areas of Bangladesh *Leea macrophylla* has been researched for its *in vitro* antibacterial activity against infective strains of Staphylococcus, Micrococcus and Pasteurella bacteria in addition to its *in vivo* and *in vitro* anti-malarial, antioxidant, and antibacterial evaluations of *Leea* genus has been shown by Joshi *et al*., 2016 along with supplementary traditional practices in treatment of ailments by the genus. Leaves of *L. macrophylla* analysed for remedial treatment for arthritis, boils as well (Zaoui *et al.,* 2002). Suspension of dried powders of the roots immersed in coconut oil is used on lesions and abscesses. Foliage juice has been known for its local anti -inflammatory property (Dewanjee*et al*, 2013) and used in carbuncles, arthritis, swelling, gout and

rheumatoid atrophy of bones (Uddin *et al.,* 2006). Herb preparations are widely in use by the physicians of Ayurveda in the grounding regular tonic modaka (Singh and Singh,1981). It has also been recognized and researched for ethno botanical implementation in thyromegaly, lipoma, gastrointestinal tumor and tetanus (Garodia*et al.,*2007). The current work, consequently projected to examine the diverse bioactive fractions extracted from *L. macrophylla* as a part of phytochemical screening and for its bacteriocidal activity. The herb in study has been traditionally used as curatives of varying diseases. The initiation of orthodox medicinal system has not smeared the usage of medicinal flora (Sahoo *et al.,* 2010; Akinyemi *et al.,* 2005). According to W.H.O over more than 80% of the world's inhabitants counts on traditional medicine for their prime health care needs and wellbeing (Duraipandiyan*et al.,* 2006). Antidiarrhoeal and antimicrobial activities of *Emilia coccinea* (Teke *et al*., 2007) and antimicrobial effect of *P.amarus* and *P. nigrescens* has been reported by Oluwafemi and Debiri, 2008. This global attention in medicinally important plants mirrors acknowledgment of the rationality of many traditional claims concerning the worth of natural preparations and products (Borris *et al*., 1996) in human well-being and the development of resistance against microbes in contrast to the antibiotics, has directed the researchers to inspect the antimicrobial potential of medicinal plants (Veeramuthu *et al.,*2006). The purpose led to test essential oils and preparation of active principles, extracts of numerous plants screened for the antimicrobial property, as a consequence numerous reports reviewed in the previous research demonstrating the bioefficacy of phytoconstituents and secondary metabolites. Substantial consideration given to the plant extracts and pure extracts since for their fewer side effects in addition to high resistance near to innumerable microbes. Antimicrobials originated from plants characterize a huge untapped source for remedies and further investigation of plant antimicrobials is desirable to harness their massive therapeutic and healing potential (Rajesh *et al.,*2012) with gastro protective potential of *Leea macrophylla* (Swarnalatha *et al*., 2019) hepatoprotective activity (Blumrg*et al*., 1989; Akhter *et al*. 2015) to combat liver diseases by using herbal formulations (Dhiman and Chawla, 2005). Phytoconstituents employed as antimicrobial agents proved effective in the treatment of communicable ailments (Aswathanarayan and Vittal, 2013) though concurrently mitigating countless of the side effects that are frequently associated with use of synthetic antimicrobial drugs (Olowosulu*et al*., 2006). Roots of *Plectranthushereroensis* have been reported for its antimicrobial activity due to diterpenes was investigated with significant potency (Batista *et al*., 1994). The phytochemical study based on data provide ethnopharmacologically and evidences is common and effective method in sighting of novel anti-infective and anti-inflammatory agents from economic important herbs with high medicinal value.

Methodology

- **Test Microorganisms Used**

Antimicrobial activity of *Leea macrophylla* extracts was screened against a range of bacterial and fungalstrains. Bacterial strains employed were *Micromonospora purpurea*, *Acinetobacter calcoaceticus*, *Staphylococcus epidermidis*, and *Zymomonas mobilis*. Fungal strains employed were *Alternaria solani*, *Fusarium culmorum*, *Penicillium chrysogenum*, and *Phanerochaete chrysosporium*. Microorganisms were maintained on respective culture media under standard laboratory conditions before their use.

- **Plant Collection and Authentication**

Fresh plant parts of *Leea macrophylla* was obtained from the Department of Botany, University of Rajasthan, Jaipur, India. The *Leea s*pecies was recognized and validated by a taxonomist of the same department.

The plant samples were washed properly to eliminate any dust and debris, following which they were shade-dried at room temperature. Both *in vivo* (naturally occurring) plant tissues and *in vitro* (tissue culture-derived callus) material were dried and crushed into rough powder using a sterile mechanical grinder. The resulting powder material was transferred to airtight containers for further investigation**.**

Preparation of Extracts

Ethanolic extracts were separately prepared from aerial parts viz., leaves, stems, seeds, geopositive part root and in vitro grown callus tissues. Twenty grams of dry powder was packed in a cellulose thimble for each extraction and was extracted with a Soxhlet apparatus by using ethanol as a solvent. The extraction was repeated until the solvent became clear, indicating the cessation of extraction. The extracted samples were filtered and concentrated at room temperature by allowing the

solvent to evaporate. Dry weight and wet weight analysed for each sample extraction. The crude extracts were preserved in sterile glass tubes and employed for the subsequent antimicrobial assays.

Microbial Cell Suspension Standardization

To obtain standard inocula, five pure culture of separate test microorganism aseptically inoculated into sterile test tubes slants containing 10 mL of sterile Mueller-Hinton Broth for bacterial isolates or Potato Dextrose Broth for fungal isolates. The inoculated broths were then incubated at 37°C for 18 hours to ensure maximum microbial growth. At the completion of the incubation period, the turbidity of each culture was visually adjusted to the 0.5 McFarland standard, as described by the protocol of Cheesbrough(2004), to ensure a consistent microbial concentration per assay.

The microbial strains used in the current analysis were *Micromonospora purpurea*, *Acinetobacter calcoaceticus*, *Staphylococcus epidermidis*, *Zymomonas mobilis*, *Alternaria solani*, *Fusarium culmorum*, *Penicillium chrysogenum*, and *Phanerochaete chrysosporium*. Clinical isolates of the microbes were obtained from the Department of Microbiology, SMS Medical College, Jaipur, Rajasthan, India. The isolates were pure cultured and then stored on Nutrient Agar (for bacteria) or Potato Dextrose Agar (PDA) slants (for fungi) at 4°C until further need.

Antimicrobial Sensitivity Assay

- **Agar Well Diffusion Method**

Antimicrobial activity of *Leeamacrophylla* ethanolic extracts was examined by the agar well diffusion method. Isolated extracts were obtained from the leaves, stems, roots, seeds, and *in vitro* callus tissue of the plant. Standardized microbial cultures were seeded on sterile petri plates poured with either Mueller-Hinton Agar for bacterial strains and Potato Dextrose Agar (PDA) for fungal strains. Sterilized medium of approximately 15 to 20 mL was poured into each petri plate and left to solidify under aseptic conditions.

A sterile cotton swab was used to evenly spread each of the microbial suspensions on the surface of the solid medium. Wells having a diameter of around 6mm and 2cm center-to-center were drilled gently using a sterile cork borer followed by addition of 100 µL of corresponding plant extract into each well marked and labeled. The plates were incubated at 37°C for 24 hours under aerobic conditions.

Antimicrobial activity was quantified by measurement of inhibition zone diameter (inmm) around the wells in the form of clear zones. The assays were conducted in triplicate for reproducibility and accuracy. Negative controls were wells containing solvents (water, methanol, acetone)and standard antibiotics (ampicillin against bacteria and fluconazole against fungi) were used as positive controls.

Significant Outcomes

- ***Leea macrophylla* Extracts Antimicrobial Activity**

The ethanolic extracts from various parts of *Leea macrophylla* proved to possess a wide range of microbicidal activity against the tested bacterial and fungal strains. Plant material-based *in vivo and in vitro* studies showed effective inhibition, suggesting existence of bioactive phytocompounds.

- **Antibacterial Activity**

Antibacterial activity of the plant extracts is presented in Table 1. All extracts inhibited Gram-positive and Gram-negative bacterial strains. Remarkably, leaf extract inhibited *Micromonospora purpurea* with a maximum zone of inhibition (18 mm), which nearly matched that of the control antibiotic ampicillin (20mm). Similarly, both seed and leaf extracts inhibited *Acinetobacter calcoaceticus* strongly.

Rootextract inhibited Zymomonas mobilis with high efficacy (18mm), and seed extract inhibited Staphylococcus epidermidis strongly with high efficacy (17mm).Other plant parts also inhibited bacterial growth significantly, thereby confirming the extensive antimicrobial activity of the species.

- **Antifungal Activity**

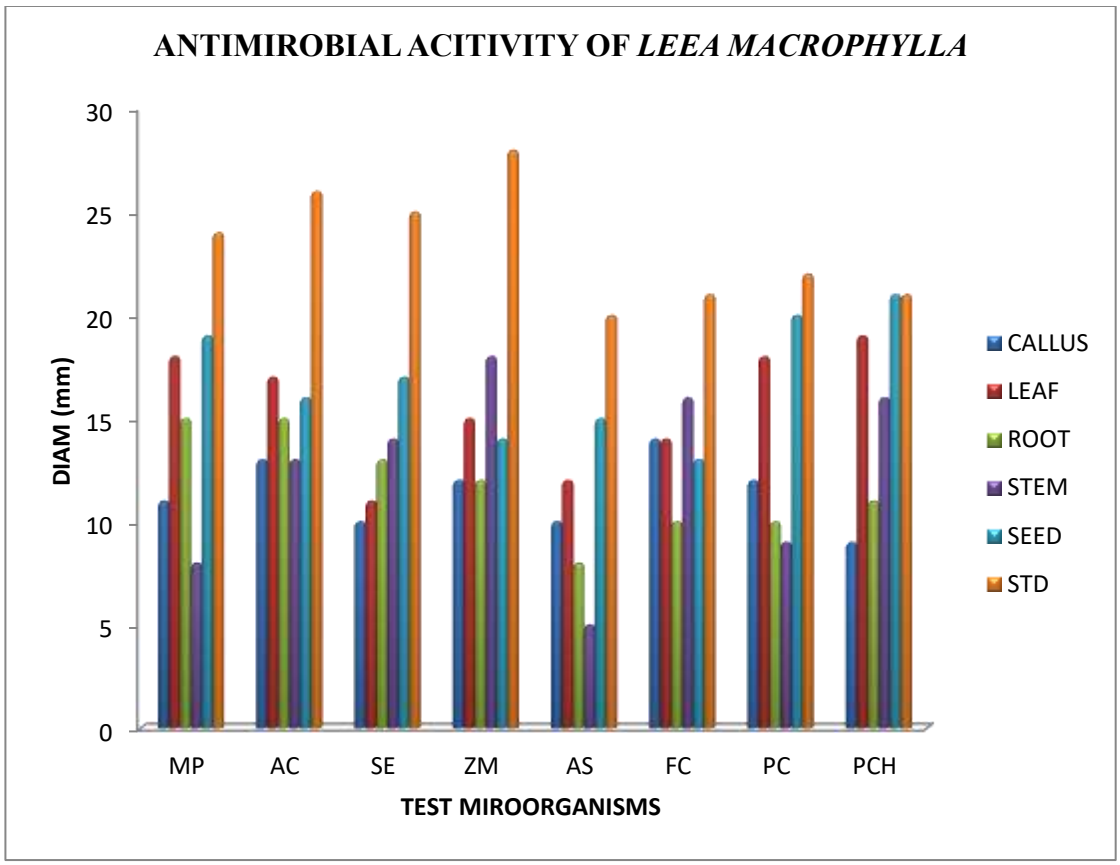
The extracts of the plant were also discovered to have high antifungal activity. From Table 1, it is clear that the leaf and seed extracts inhibited the tested fungal strains with inhibition zones of 20 mm and 18 mm, respectively, hence indicating antifungal activity comparable to that of fluconazole. This verifies the antifungal activity of Leea macrophylla, hence its potential use as a natural antifungal agent.

The overall results showed that all *Leea macrophylla* ethanolic extracts are strongly exhibited antimicrobial property. Mainly, the leaf and seed extracts have emerged to be most bioactive against a variety of pathogenic microorganisms.

The plant seems to be a promising candidate for additional pharmacognostic investigation, with prospects for development as alternative antimicrobial agents and there by surmounting the rising issue of drug resistance and thus augment reliance over herbal remedies to alleviate serious ailments caused by microbes under investigation.

Table 1: Antimicrobial Property of Ethanolic Extracts of *Leea Macrophylla*.

Test Organisms	Plant Parts Zones of Growth Inhibition (MM)					
	Standard	Callus	Leaf	Stem	Root	Seed
M. purpurea	20	11	18	15	8	19
A. calcoaceticus	16	13	17	15	13	16
S. epidermidis	17	10	11	13	14	17
Z. mobilis	28	12	15	12	18	14
A. solani	20	10	12	8	5	15
F. culmorum	21	14	14	10	16	13
P. chrysogenum	19	12	18	10	9	20
P. chrysosporium	21	9	19	11	16	21



MP: *M. purpurea,* AC: *A. calcoaceticus*, SE: *S. epidermidis,* ZM: *Z. mobilis*, AS: *A. solani,* FC: *F. culmorum* ,PC: *P. chrysogenum,* PCH: *P. chrysosporium*

Discussion

Numerous research had shown that plants possess antimicrobial constituents (El-Said *et al.*,1971, Lewis, 1980; Zaria *et al* 1975, Ibekwe *et al*., 2001, Akujobi*et al*., 2004, 2006). The outcomes of the experimental analysis is at par principally with researched work of the former researchers. The presence of flavonoids, tannins, saponins, cardiac glycosides and alkaloids shown to be associated with the plant extracts imparting antimicrobial potential (Boham and Kocipai, 1974) have been reported to possess antimicrobial properties (Bastista *et al.,* 1994; Boris, 1996; Olowusulu and Ibrahim, 2006; Akimnjobi *et al* 2006). Tsuchiya *et al*.,(1996) accredited the antimicrobial activities of flavonoids and the ability to compound with extracellular and soluble proteins of bacterial cell walls thereby disrupting microbial cells membranes. The findings of the experimental study suggests that *Leea macrophylla* possesses notable antimicrobial properties, supporting its traditional use as a natural remedy for treating microbial infections. The ethanol extracts, particularly from the leaves and seeds, demonstrated significant inhibitory effects against both bacterial and fungal pathogens. These results indicate that the plant contains bioactive compounds with broad-spectrum antimicrobial potential and also combat with the growing concern over antibiotic resistance, *Leea macrophylla* may serve as a valuable source for the development of substitute of antimicrobial agents.

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