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Research Article

Exploration of Antimicrobial Activity and Antioxidant Potential of *Loranthus cordifolius* Plants From Mirpur Azad Kashmir, Pakistan

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Abstract

Loranthus cordifolius is a parasitic plant, and its extracts are traditionally used in some cultures as antimicrobial and immunological medicines. Literature suggests that bioactive compounds produced by a parasitic plant could potentially be influenced by the host plant's own chemical composition, playing a role in the adaptogenic properties of the parasitic plant. In the present research, *L. cordifolius* shrubs were collected from host species *Phyllanthus emblica* and *Juglans regia*, and extracts were obtained using chloroform, petroleum ether, methanol, and water. The phytochemical profile, encompassing alkaloids, flavonoids, cardiac glycosides, tannins, terpenoids, and saponins, was qualitatively and quantitatively assessed, revealing varying concentrations based on solvents and hosts. Extracts from *L. cordifolius*, particularly those from the *P. emblica* host, showed superior antimicrobial and antioxidant activities. Notably, leaf extracts from *L. cordifolius* (Host *P. emblica*) exhibited effective results, including minimum inhibitory effect, Minimum Bactericidal Concentration, and Minimum Fungicidal Concentration. Activity indices were highest for extracts derived from the P. emblica host. Stem extracts from *L. cordifolius* plants (Host *P. emblica*) demonstrated higher DPPH scavenging effect, total antioxidant activity, and total phenolic contents. The study concludes that *Loranthus cordifolius* exhibits notable antimicrobial and antioxidant properties, suggesting its promising potential as a natural source for developing green medicines and alternative therapeutic agents.

Keywords: Antibacterial activity, Antifungal activity, phytochemical analysis, Antioxidant Potential, *Loranthus cordifolius*

1. Introduction

Phyto-medicines, derived from plants, are globally recognized, constituting approximately 80% of current medicinal compounds (Maqbool *et al.* 2019). The intricate relationship between humans and plants has strengthened over time, leading to the emergence of ethnobotany as a pivotal discipline within plant sciences. Technological advancements have facilitated the identification, screening, purification, and application of phytochemicals (Behera and Gosh, 2018). Antibiotic resistance has created a need for new antimicrobial drugs. Plant-based bioactive compounds, or botanicals, present a promising avenue (Mishra *et al.* 2020). Researchers globally are exploring the antimicrobial properties of plant-based chemicals against bacteria such as Bacillus cereus, Staphylococcus aureus, Pseudomonas

aeruginosa, Escherichia coli, and Salmonella enterica (Alsheikh *et al.* 2020).

Oxidative stress caused by free radicals contributes to ailments like tumors, aging, and inflammatory diseases such as arthritis and vasculitis. Free radicals, derived from metabolism or environmental exposure, require antioxidants to prevent cellular damage. Medicinal plants and edibles serve as vital antioxidant sources, aiding disease prevention (Lobo et al. 2010). Similarly, fungal infections like otomycosis and diseases caused by *Aspergillus niger*, *A. oryzae*, and *Fusarium oxysporum* remain global challenges. Limited and costly antifungal drugs, coupled with rising fungal resistance, highlight the need for novel plant-based therapies (Roaemer and Krysan, 2014).

The Loranthaceae family, constituting around 1% of flowering plants, encompasses approximately 4500 angiosperm parasitic plant species. *Loranthus cordifolius* Wall., identified as a parasitic shrub in Mirpur, AJK, Pakistan, holds cultural significance locally, known as 'Parwikh.' The plant is found on a variety of hosts, such as *Juglans regia*, *Quercus dilatata*, and various members of the genus Platanus (Adesina *et al.* 2013). Notably, these host plants contribute to the unique chemical composition of *Loranthus cordifolius*, endowing it with distinctive therapeutic properties. Moreover, 'Parwikh' has been traditionally employed in local folk medicine for its perceived medicinal benefits. The plant is believed to possess various healing properties, and extracts from *Loranthus cordifolius* have been used in traditional remedies to address ailments and promote well-being (Piwowarczyk *et al.* 2020).

Ongoing research continues to explore the full extent of its medicinal potential, shedding light on the diverse bioactive compounds present in the plant that may contribute to its therapeutic efficacy. In addition to its cultural and medicinal significance, *Loranthus cordifolius* serves as a valuable subject for ecological studies (Marvier, 1996; Smith *et al.* 2016). This study focuses on the phytochemical profile of *L. cordifolius* from two host species, *P. emblica,* and *J. regia*, exploring antibacterial and antimycotic potential using various solvents, stem and bark extracts, and highlighting its role as an alternative to antibiotics, i.e., green medicine. *Phyllanthus emblica* (Indian gooseberry) and *Juglans regia* (walnut) were chosen as host plants due to their established medicinal significance and their potential to influence the bioactivity of parasitic plants like *Loranthus cordifolius*. Both hosts are rich in bioactive compounds such as antioxidants and antimicrobial agents, which may synergistically enhance the therapeutic properties of *Loranthus cordifolius*. Exploring these associations can provide deeper insights into the ecological and pharmacological interactions between parasitic plants and their hosts. The antioxidant potential of the plant from both hosts will be assessed, providing valuable insights into its antimicrobial efficacy as a potential green medicine (Marvier, 1996; Smith *et al.* 2016).

2. Materials and Methods

2.1. Collection of *L. cordifolius* and Its Phytochemical Screening

Loranthus cordifolius plants were gathered from *Juglans regia* and *Phyllanthus emblica* tree species located in the local peasant association. The collected plant samples were examined and confirmed using the Flora of Pakistan (Nasir, 1981). Subsequently, the plant samples were dried, placed on herbarium sheets, and stored in the herbarium of the Department of Botany MUST, AJK with voucher number MUH-899 (Figure S1). Figure S1 shows an original image of the plant along with its mounted herbarium specimen.

The plant parts, namely the leaf and stem, were chosen for extract production. Mature leaves and fresh stem parts were collected from the field area and packed in polythene bags. After washing, they were shadow-dried and subjected to the maceration procedure for further extraction. The extracted material was analyzed phytochemically

Table 1: Qualitative phytochemicals of *L. cordifolius* using methanolic leaf and bark extracts.

S. No.	Phytochemicals	Significant findings
1	Alkaloids	Higher alkaloid concentrations were observed in methanolic extracts of *L. cordifolius* from *P. emblica* leaves.
2	Flavonoids	Flavonoids were most abundant in *L. cordifolius* extracts from *P. emblica* bark.
3	Proteins	*L. cordifolius* extracts from *P. emblica* showed the highest protein levels, followed by moderate levels in bark and extracts from J. regia.
4	Saponins	Saponins were present in moderate concentrations in both leaves and bark of *L. cordifolius* from *P. emblica.*
5	Tannins	*L. cordifolius* extracts from *P. emblica* had the highest tannin levels compared to other extracts.
6	Anthraquinones	Anthraquinones were most prominent in *L. cordifolius* extracts from *P. emblica* leaves.
7	Terpenoids	Terpenoids were detected at higher levels in *L. cordifolius* extracts from *P. emblica.*
8	Cardiac glycosides	Cardiac glycosides were present in moderate concentrations in *L. cordifolius* extracts from *P. emblica.*

for antimicrobial activity. Four solvents, namely petroleum ether (PE), chloroform (Chl), methanol (MeOH), and water (Aq), were used for extraction in subsequent order of polarity. To begin the extraction process, 250g of the plant powder was soaked in 250 ml of PE in tightly sealed beakers. After seven days at room temperature (RT), the soaked material was filtered using What man filter paper No. 42. The filtrate was then concentrated using a rotary evaporator, and the remaining residue was re-soaked in Chl solvent, followed by MeOH and Aq solvents. The same extraction process as for the first one was repeated (Kebede *et al.* 2021).

The 80% methanol extracts of each plant were subjected to standard preliminary phytochemical qualitative analysis to identify various plant constituents and screen for the presence or absence of biologically active compounds or secondary metabolites. Modified standard procedures were used, and the major phytochemical constituents identified were alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenolic compounds, saponins, tannins, and terpenoids (Mamta and Jyoti, 2012).

- Approximately 200 mg of plant material was boiled in 10 mL of methanol, filtered, and mixed with 1% HCl and Dragendorff reagent. The formation of a brownish-red precipitate indicated the presence of alkaloids.
- For anthraquinones, 1 mg of the extract was mixed with 2 mL of benzene, filtered, and shaken with 2.5 mL of 10% ammonia. A pink, red, or violet color in the lower phase confirmed their presence.
- To test for cardiac glycosides, 1.25 mg of the extract was mixed with 0.5 mL of chloroform, and 0.5 mL of concentrated sulfuric acid was added. A reddish-brown color at the interface indicated the presence of glycosides.
- Flavonoids were identified by dissolving 7.5 mg of the extract in ethanol, concentrated HCl, and magnesium turnings. The formation of a yellowish color indicated flavonoids.
- Phenolic compounds were detected by treating the crude extract with ferric chloride solution or dissolving 5 mg in 1%

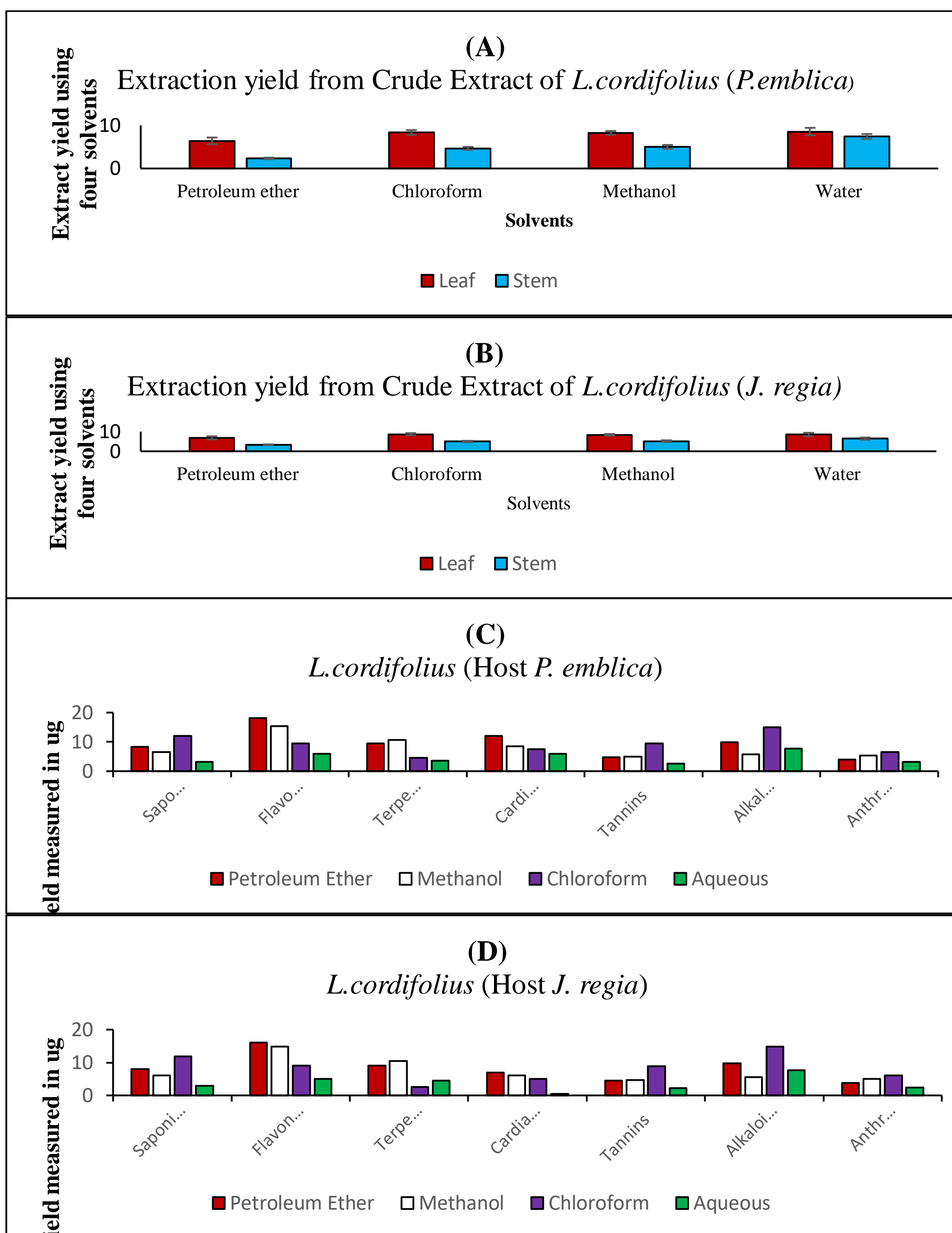

Figure 1: Extraction and yield of *L. cordifolius* (A) extract yield from *P.emblica* (B) extract yield from *J. regia* (C) phytochemical yield *P.emblca*, (D) phytochemical yield *J. regia*

- ferric chloride solution. A bluish-black color confirmed their presence.
- Saponins were tested by mixing 2.5 mg of the extract with 5 mL of water and shaking the mixture. Persistent foam formation upon warming indicated the presence of saponins.
- Tannins were detected by boiling 2.5 mg of the extract in 5 mL of water, filtering, and adding 0.1% ferric chloride. A brownish-

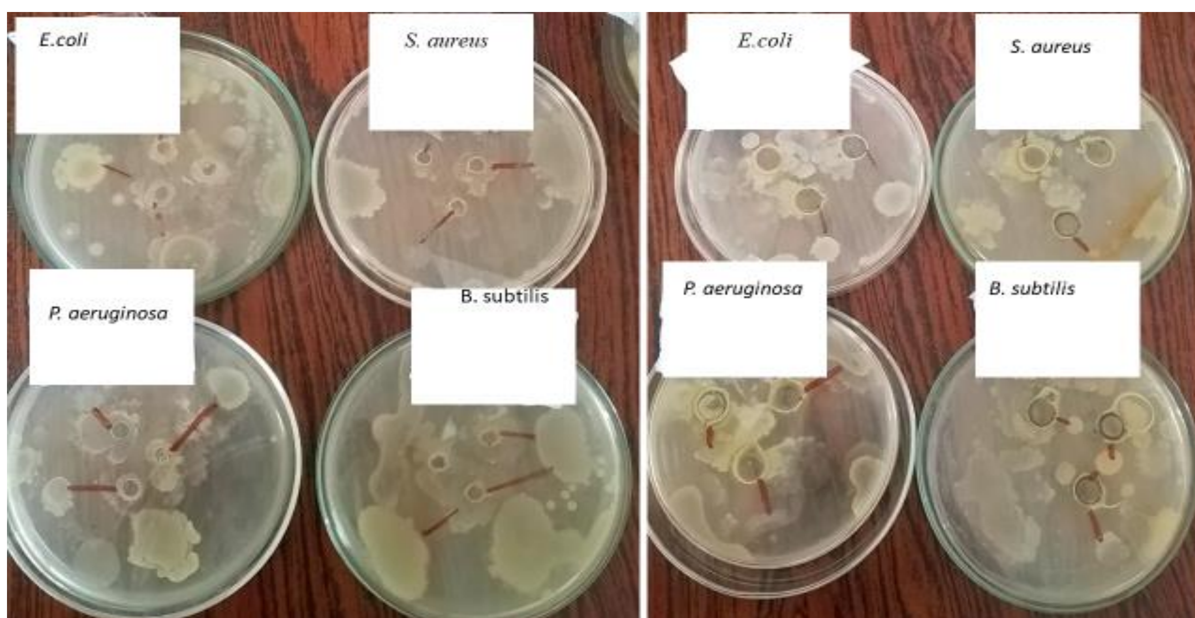

Figure 2. Zone of inhibition images from the microbiology laboratory against tested bacterial strains, demonstrating the efficacy of leaf extracts from the semi-parasitic plant *L. cordifolius.* (A) Extracts from the host *J. regia* and (B) extracts from the host *P. emblica.*

green or blue-black precipitate confirmed tannins.

- For terpenoids, the chloroform extract was evaporated to dryness and heated with concentrated sulfuric acid. The appearance of a grey color confirmed terpenoids.

2.2. Antibacterial Activities

2.2.1. Test Organisms for Antibacterial Activities

In this study, we employed four distinct bacterial strains to investigate the antibacterial properties of a specific plant extract. The selected test organisms included two Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 10145) and *Escherichia coli* (ATCC 10799), as well as two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 29213) and *Bacillus subtilis* (ATCC 11774). These bacterial strains were sourced from a local Pharmacology Lab.

2.2.2. Preparation of Nutrient Agar Medium for Bacteria

Nutrient agar medium was prepared by dissolving nutrient agar and broth powders in 500 ml of distilled water, following the manufacturer's instructions. The mixture was boiled for sterilization, poured into sterile Petri dishes and tubes, and allowed to solidify. Unused plates were stored at 4°C. Aseptic techniques were employed throughout to prevent contamination (Maqbool et al. 2017).

2.2.3. Minimum Inhibitory Concentration (MIC)

Bacterial and fungal growth was prepared through serial dilutions based on WHO guidelines (2006). MIC was determined by inoculating microorganisms on plates, incubating for 72 hours at 28°C, and identifying the lowest concentration that inhibits growth (Maqbool et al. 2017).

2.2.4. Minimum Bactericidal Concentration (MBC)

MBC was determined by observing plates under microscopes and identifying the concentration that eradicates 99.5% of bacterial growth. Subcultures from MIC plates were streaked onto fresh agar plates and incubated for 72 hours at 28°C. The effective concentration was recorded as MBC (Ajaib et al. 2013).

2.2.5. Zone of Inhibition

Agar plates were prepared, microbial cultures streaked, and antimicrobial disks placed on the

Table 2: Zone of inhibition for bacterial strains using leaf and stem extracts of *L. cordifolius* taken from hosts *P. emblica* and *J. regia*

Plant Part	Solvent	*E.coli*		*S. aureus*		*P. aeruginosa*		*B. subtilis*	
		P. emblica	*J. regia*	*P. emblica*	*J. regia*	*P. emblica*	*J. regia*	*P. emblica*	*J. regia*
Stem	Pet. Ether	36±0.9	34±0.4	24±0.8	23±0.8	25±0.2	22±0.2	22±0.9	21±0.3
	Chloroform	27±0.5	25±0.5	29±0.5	27±0.4	31±0.5	29±0.2	19±0.7	17±0.2
	Methanol	37±0.7	36±0.6	33±0.5	31±0.5	26±0.1	24±0.4	18±0.5	15±0.4
	Aqueous	14±0.9	12±0.9	22±0.8	19±0.4	20±0.5	18±0.5	14±0.8	11±0.1
Leaf	Pet. Ether	44±0.2	43±0.2	40±0.2	39±0.3	36±0.9	33±0.6	21±0.7	20±0.7
	Chloroform	42±0.9	41±0.2	39±0.7	37±0.7	31±0.3	29±0.3	23±0.7	19±0.8
	Methanol	42±0.1	42±20.1	38±0.4	39±0.4	34±0.8	31±0.8	24±0.4	23±0.4
	Aqueous	23±0.7	20±0.4	24±0.2	21±0.2	26±0.1	25±0.2	17±0.4	16±0.3
Standard		45±0.4		41±0.6		35±0.5		28±0.5	

Table 3: MIC and MBC of leaf and stem extracts of *L. cordifolius* taken from two host species *P. emblica* and *J. regia* against four bacterial strains using four different solvents.

Plant Part	*L. cordifolius* Host	Solvent Type	Minimum Inhibitory Concentration (MIC) Minimum Bactericidal Concentration (MBC)							
			E. Coli		*S. aureus*		*P. aeruginosa*		*B. subtilis*	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Stem	*P. emblica*	Pet. Ether	60.1±0.4	92.5±0.4	73.6±0.8	113±0.4	72.3±0.2	112±0.4	81.6±0.2	124±0.4
		Chloroform	69.4±0.5	109±0.3	67.4±0.4	121±0.5	76.2±0.2	117±0.5	80.2±0.5	115±0.5
		Methanol	63.6±0.6	101±0.2	65.4±0.5	102±0.6	67.7±0.4	103±0.6	74.1±0.4	116±0.6
		Aqueous	81.2±0.9	121±0.9	87.2±0.4	141±0.9	84.2±0.5	142±0.9	99.2±0.1	139±0.9
Leaves		Pet. Ether	51.3±0.3	83.2±0.2	64.5±0.3	103±0.2	63±0.5	103±0.2	72.4±0.7	113±0.2
		Chloroform	57.4±0.2	94.5±0.2	62.5±0.7	100±0.2	70.1±0.3	111±0.2	69±0.8	111±0.2
		Methanol	53.6±0.8	89.2±0.1	59.7±0.4	98.3±0.1	61.4±0.6	99±0.1	64.2±0.4	104±0.1
		Aqueous	69.2±0.4	113±0.4	72.2±0.2	97.3±0.4	78.6±0.2	121±0.4	86.2±0.3	136±0.4
Stem	*J. regia*	Pet. Ether	64.7±0.6	94±0.4	75.8±0.8	119±0.4	81±0.1	121±0.2	92.1±0.1	134±0.3
		Chloroform	75.2±0.3	115±0.5	71.3±0.9	125±0.5	85.3±0.2	125±0.5	89.9±0.3	125±0.6
		Methanol	68.9±0.6	103±0.6	67.6±0.4	116±0.6	74.9±0.8	116±0.7	82.4±0.4	116±0.6
		Aqueous	86.5±0.8	127±0.9	99.6±0.4	161±0.9	98.6±0.6	148±0.5	99.9±0.1	161±0.7
Leaves		Pet. Ether	53±0.2	90±0.1	66.5±0.5	113±0.6	68.7±0.6	108±0.2	83.7±0.6	123±0.1
		Chloroform	61±0.2	98.5±0.5	66.1±0.7	108±0.2	71.7±0.3	115±0.2	78.2±0.6	120±0.8
		Methanol	55±0.1	99.3±0.1	63.7±0.4	104±0.5	63.2±0.8	103±0.1	73.2±0.2	113±0.1
		Aqueous	75±0.4	120±0.4	91.2±0.2	132±0.4	98.9±0.2	135±0.4	96±0.3	136±0.3

agar. Plates were incubated at appropriate temperatures, and zones of inhibition were measured after 24 hours to evaluate antimicrobial efficacy (Ajaib et al. 2013).

2.2.6. Activity Index

The activity index was calculated by dividing the zone of inhibition diameter by the antimicrobial disk diameter. A higher activity index indicated greater potency (Ajaib et al. 2013).

2.3. Antifungal Activities

2.3.1. Test Organisms for Antifungal Activities

In this antifungal study, four distinct fungal strains were utilized as test organisms. These strains include:

1. *Aspergillus oryzae* (ATCC 42149)
2. *Aspergillus niger* (FGSC A1513)
3. *Fusarium oxysporum* (KP297995)
4. *F. solani* (TIMM1303)

These fungal strains were employed to evaluate the effectiveness of antifungal agents or natural

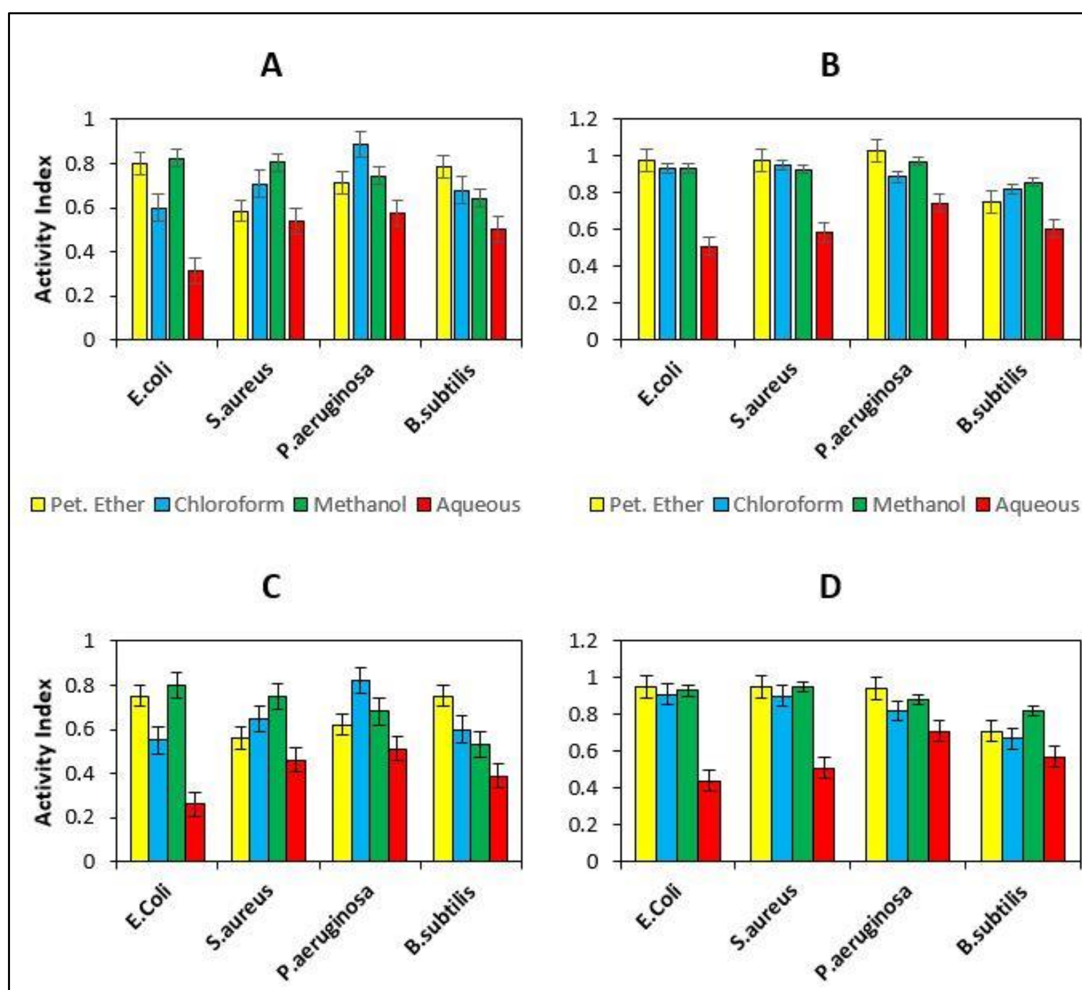

Figure 3: Activity indices of (A) Stem extracts (B) Leaf extracts of *L. cordifolius* taken from host *P. emblica* and (C) Stem extracts (D) Leaf extracts of *L. cordifolius* taken from host *J. regia* using test organisms and solvents followed in the current experiment.

products against them. The use of different fungal strains allows researchers to assess the potential broad-spectrum or specific antifungal activity of the tested compounds, contributing to a comprehensive understanding of antifungal properties and potential applications in medicine, agriculture, and related fields.

2.3.2. Potato dextrose Agar (PDA) Medium for Fungal Analysis

The PDA medium was prepared by boiling 200 g of peeled, chopped potatoes in 1 L of distilled water for 30 minutes. The extract was strained, and 20 g dextrose and 15 g agar were added, stirred, and boiled briefly for sterilization. The hot medium was poured into sterile Petri dishes, solidified, labeled, and stored at 4°C. Aseptic techniques were followed to prevent contamination (Mitscher et al. 1972; Ajaib et al. 2013).

2.4. Measurement of Antioxidant Activity

2.4.1. DPPH Protocol for Measuring Antioxidant Activity

2.4.1.1. Preparation of Stock Solution (SS)

A 0.1 mM DPPH stock solution was prepared by dissolving the required amount of DPPH powder in methanol with gentle stirring until fully dissolved. The solution was transferred to an amber vial to protect it from light and stored in a refrigerator to maintain stability. Fresh working solutions were prepared by diluting the stock

Table 4: Zone of inhibition for the fungal strains using leaf and stem extracts of *L. cordifolius* taken from hosts *P. emblica* and *J. regia.*

Plant Part	Solvent	A. niger		A. oryzae		F. oxysporum		F. solani	
		P. emblica	J. regia	P. emblica	J. regia	P. emblica	J. regia	P. emblica	J. regia
Stem	Pet. Ether	39±0.5	38±0.7	25±0.6	22±0.9	23±0.2	20±0.2	28±0.6	26±0.8
	Chloroform	31±0.6	29±0.8	33±0.8	32±0.9	29±0.6	28±0.1	26±0.4	25±0.8
	Methanol	33±0.5	33±0.6	35±0.7	34±0.4	37±0.3	33±0.3	34±0.6	32±0.6
	Aqueous	28±0.4	26±0.4	25±0.9	23±0.4	29±0.4	24±0.5	26±0.3	24±0.5
Leaf	Pet. Ether	48±0.3	46±0.2	38±0.5	36±0.2	32±0.5	29±0.5	35±0.6	33±0.6
	Chloroform	50±0.3	49±0.3	53±0.6	51±0.7	43±0.3	42±0.3	33±0.3	31±0.2
	Methanol	45±0.5	43±0.4	45±0.7	41±0.7	40±0.1	39±0.3	37±0.1	34±0.1
	Aqueous	37±0.4	34±0.4	27±0.2	26±0.3	25±0.2	24±0.2	26±0.2	25±0.1
Standard		51±0.4		53±0.6		43±0.5		38±0.5	

solution with methanol before the assay (Gautam et al. 2007).

2.4.1.2 Preparation of Dilution Series (DS)

A 0.1 mM stock solution of DPPH in methanol was prepared, and subsequent dilutions were made for antioxidant activity assessment. The reduction of DPPH was measured using spectrophotometry or colorimetric methods, with absorbance reduction indicating antioxidant capacity (Gautam et al. 2007; Prieto et al. 1999).

2.4.1.3 Preparation of 0.1 mM DPPH Solution

0.1 mmol (26.9 mg) of DPPH powder was dissolved in methanol to create a 0.1 mM solution. The mixture was stirred until fully dissolved and transferred to an amber vial to protect it from light. The stock solution was stored in a cool, dark place (Gautam et al. 2007; Prieto et al. 1999).

2.4.2. Total Antioxidant Activity Analysis

The total antioxidant activity of the sample was measured using the Phosphomolybdenum method. A reagent was prepared by mixing ammonium molybdate with sulfuric acid in a 1:1 ratio. The sample extract was diluted to fall within the spectrophotometer's linear range, and a blank solution was prepared. A standard curve was constructed using known concentrations of ascorbic acid. After incubating the sample, blank, and standard solutions at a specified temperature, they were cooled to room temperature. Absorbance was measured at a specific wavelength using a UV-Vis spectrophotometer. The antioxidant activity was calculated using the standard curve and expressed as ascorbic acid equivalents per gram or milliliter of sample. The analysis was performed in triplicate, and potential interferences were considered (Gautam et al. 2007; Prieto et al. 1999).

2.4.3. Determination of Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteu method. Sample extracts were prepared by extracting phenolic compounds with methanol. The Folin-Ciocalteu reagent was mixed with water in a 1:10 or 1:20 ratio, depending on the expected phenolic content. The extracts were diluted to fall within the spectrophotometer's linear range, and blank solutions were prepared without the sample. A standard curve was created using known concentrations of gallic acid. The Folin-Ciocalteu reagent and diluted extracts were mixed and incubated at room temperature for 30 minutes to 2 hours. Absorbance was measured at 750 nm using a UV-Vis spectrophotometer. Total phenolic content was calculated using the standard curve and expressed as gallic acid equivalents per gram or milliliter of sample. The analysis was done in triplicate, with potential

Table 5: MIC and MFC of leaf and stem extracts of *L. cordifolius* taken from two host species *P. emblica* and *J. regia* against four fungal strains using four different solvents.

Plant Part	Name of the *L. cordifolius* Host	Solvent Type	Minimum Inhibitory Concentration (MIC) Minimum Fungicidal Concentration (MFC)							
			A. niger		*A. oryzae*		*F. oxysporum*		*F. solani*	
			MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Stem	*P. emblica*	Pet. Ether	64.2±0.4	124±0.4	79.5±0.8	152±0.4	72.3±0.2	134±0.4	61.4±0.3	114±0.9
		Chloroform	69.2±0.5	134±0.2	77.3±0.4	148±0.3	69.5±0.7	128±0.9	57.5±0.2	105±0.5
		Methanol	66.3±0.6	132±0.6	71.4±0.5	142±0.6	64.6±0.9	116±0.6	52.4±0.2	99±0.6
		Aqueous	78.5±0.9	156±0.4	91.3±0.4	172±0.2	98.5±0.5	171±0.9	76.5±0.5	165±0.9
Leaves		Pet. Ether	53.7±0.2	114±0.4	69.5±0.3	137±0.2	63.3±0.5	123±0.2	51.6±0.6	103±0.2
		Chloroform	58.7±0.2	123±0.2	67.6±0.7	134±0.2	59.3±0.5	117±0.2	49.4±0.1	99.3±0.2
		Methanol	55.4±0.1	120±0.9	69.6±0.4	131±0.1	55.4±0.8	111±0.1	47±0.3	92.5±0.8
		Aqueous	66.3±0.4	143±0.6	81.6±0.8	156±0.4	87.5±0.2	171±0.4	65.4±0.2	113±0.8
Stem	*J. regia*	Pet. Ether	74.5±0.3	134±0.5	84.3±0.8	154±0.4	75.3±0.2	137±0.4	65.4±0.3	117±0.4
		Chloroform	78.2±0.4	143±0.4	82.4±0.4	150±0.5	72.3±0.2	131±0.4	61.6±0.2	108±0.5
		Methanol	74.3±0.9	141±0.6	77.5±0.5	143±0.6	67.4±0.4	119±0.6	58.4±0.4	102±0.6
		Aqueous	86.7±0.8	161±0.9	92.5±0.4	174±0.9	99.5±0.5	176±0.8	79.6±0.1	165±0.9
Leaves		Pet. Ether	69.6±0.1	129±0.7	74.6±0.1	143±0.2	66.4±0.7	128±0.2	55.3±0.6	108±0.5
		Chloroform	73.2±0.6	137±0.2	72.4±0.4	141±0.9	62.2±0.4	122±0.2	52.4±0.4	102±0.4
		Methanol	69.8±0.4	136±0.1	72.6±0.4	142±0.1	58.6±0.6	116±0.1	53.2±0.2	95.7±0.3
		Aqueous	81.4±0.3	152±0.8	87.8±0.6	170±0.5	95±0.5	172±0.4	69.6±0.1	131±0.4

interferences considered (Ajaib et al. 2013; Maqbool et al. 2017).

3. Results and Discussion

3.1. Phytochemicals Extraction

The anti-microbial and antioxidant potential of the plants taken from both host species *P. emblica* and *J. regia* was studied after qualitative analysis of the phyto-constituents. The yield of fractions of phytochemicals obtained has been shown in figure 1. Presence of various phytochemicals suggested that plant can serve as potential green medicine (Table 1). Detailed numerical values are given in table S1. Highest contents of the phytochemicals were detected using petroleum ether and methanol. Maximum proportion of alkaloids was obtained through extraction with methanol and petroleum ether whereas chloroform and water provided lower concentration as shown in figure 2. Highest extraction of alkaloids with methanol has been reported previously by Truong *et al.* (2019). The alkaloids not only provide the protection to the plants but they also regulate their growth. Medicinally, the alkaloid act as major cardio-protective agents and anti-inflammatory species (Heinrich *et al.* 2021). More alkaloids contents were detected in *L. cordifolius* leaf extracts taken from host *P. emblica* whereas lower ratio was found from *L. cordifolius* taken from *J. regia.*

Looking at saponins and their extraction quality via various solvents, it is evident that again the better qualitative yield of the saponins has been obtained through extraction with methanol and petroleum ether and the *L. cordifolius* from the host specie *P. emblica* provided better saponins output. The bark extract from the host *J. regia* showed no detectable saponins, which are key protective chemicals against fungal pathogens (Hussain et al. 2019) and exhibit antibacterial properties (Arabski et al. 2012). The highest concentration of cardiac glycosides was obtained using chloroform from plants associated with *P. emblica*, while lower yields were recorded with methanol, petroleum ether, and water from *J. regia*. Cardiac glycosides function as anti-rhino viral agents and exhibit cytotoxic activity (Tholl, 2015; Morsy, 2017). Higher terpenoid contents were extracted with water and petroleum ether, but none were detected with methanol from *J. regia*, consistent with Li et al. (2015). Flavonoids were most

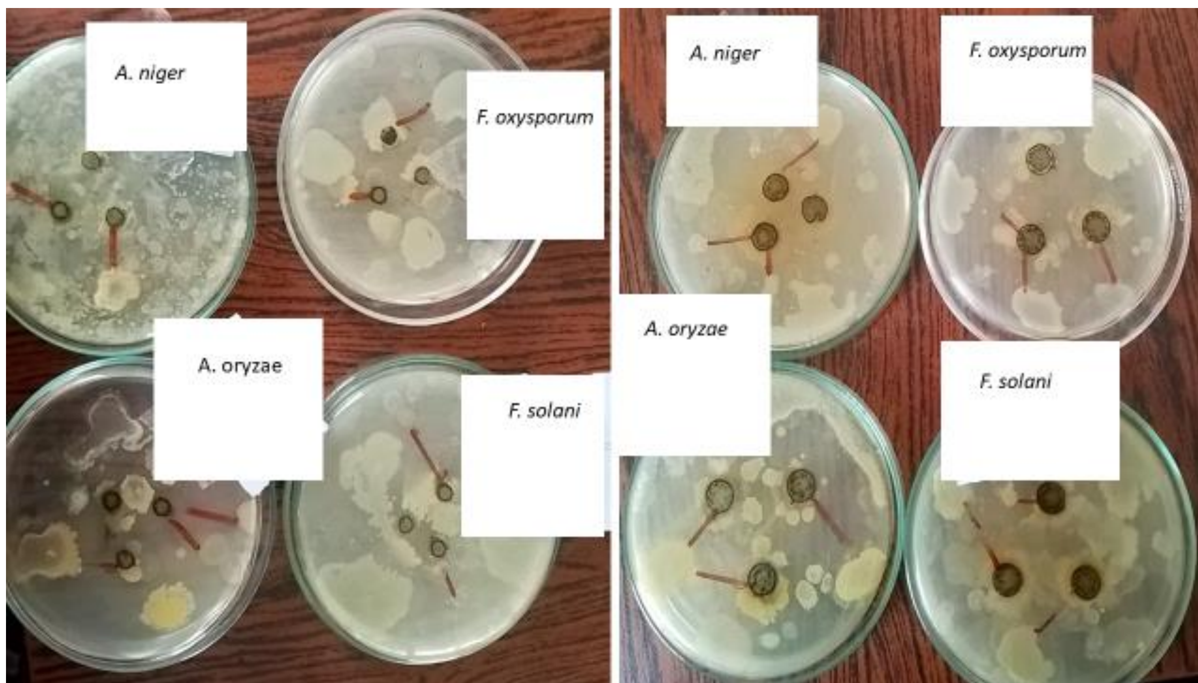

Figure 4. Zone of inhibition images from the microbiology laboratory against tested fungal strains, demonstrating the efficacy of leaf extracts from the hemi-parasitic plant *L. cordifolius.* (A) Extracts from the host *J. regia* and (B) extracts from the host *P. emblica.*

abundant in petroleum ether extracts from *P. emblica* and least in water extracts from *J. regia*. Flavonoids serve as vital bioactive compounds, acting as allelopathic agents, detoxifiers, and antimicrobials (Samanta et al. 2011). The highest tannin content was extracted using methanol from *P. emblica*, whereas water extracts from *J. regia* contained minimal tannins. Tannins are important secondary metabolites with antifungal and antibacterial properties (Furlan et al. 2011). Anthraquinones were highest in the leaf extracts of *P. emblica* using methanol and chloroform, while lower yields were obtained from *J. regia*. Anthraquinones are noted for their cytotoxic, anti-inflammatory, and antihyperlipidemic activities (Wang et al. 2021).

3.2. *Antibacterial Activity*

Extractions in all four solvents and both the stem and leaf parts of *L. cordifolius* proved beneficial against the bacterial strains. However, in terms of activity indices, the leaf extractions were more effective compared to the stem. Among the solvents, the best activity indices were achieved with petroleum ether, methanol, and chloroform, while aqueous extractions were the least effective (Table 2; Figure 3). Host-wise comparison suggests that *L. cordifolius* taken from the host *P. emblica* is more effective, as evident from the zone of inhibition values mentioned in Table 2. Table 3 presents antibacterial results in the form of minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC). The data from Tables 2 and 3 suggest that *L. cordifolius* exhibits strong antibacterial properties, especially when taken from the host *P. emblica.*

In Figure 4, the values of activity indices for antibacterial action are shown. Higher trends in activity indices were observed for leaf and stem extracts of *L. cordifolius* (Host *P. emblica*) compared to those of *L. cordifolius* (Host *J. regia*). The leaf extracts of *L. cordifolius* (Host P. emblica) produced the best activity indices of 0.97, 0.97, 1.02, and 0.85 against the test organisms *E. coli, S. aureus, P. aeruginosa*, and *B. subtilis*, respectively, as compared to leaf extracts of *L. cordifolius* (Host *J. regia*) with values of 0.95, 0.95, 0.94, and 0.82

Table 6. Summary table for antibacterial and antifungal activity indices for *Loranthus cordifolius* leaf and stem extracts from *P. emblica* and *J. regia*.

Test Organism	Leaf Extracts of *L. cordifolius* (Host *P. emblica*)	Leaf Extracts of *L. cordifolius* (Host *J. regia*)	Stem Extracts of *L. cordifolius* (Host *P. emblica*)	Stem Extracts of *L. cordifolius* (Host *J. regia*)
E. coli	0.97	0.95	0.82	0.8
S. aureus	0.97	0.95	0.8	0.75
P. aeruginosa	1.02	0.94	0.88	0.82
B. subtilis	0.85	0.82	0.78	0.75
A. niger	0.98	0.96	0.76	0.74
A. oryzae	1	0.96	0.66	0.64
F. oxysporum	1	0.97	0.86	0.76
F. solani	0.97	0.89	0.89	0.84

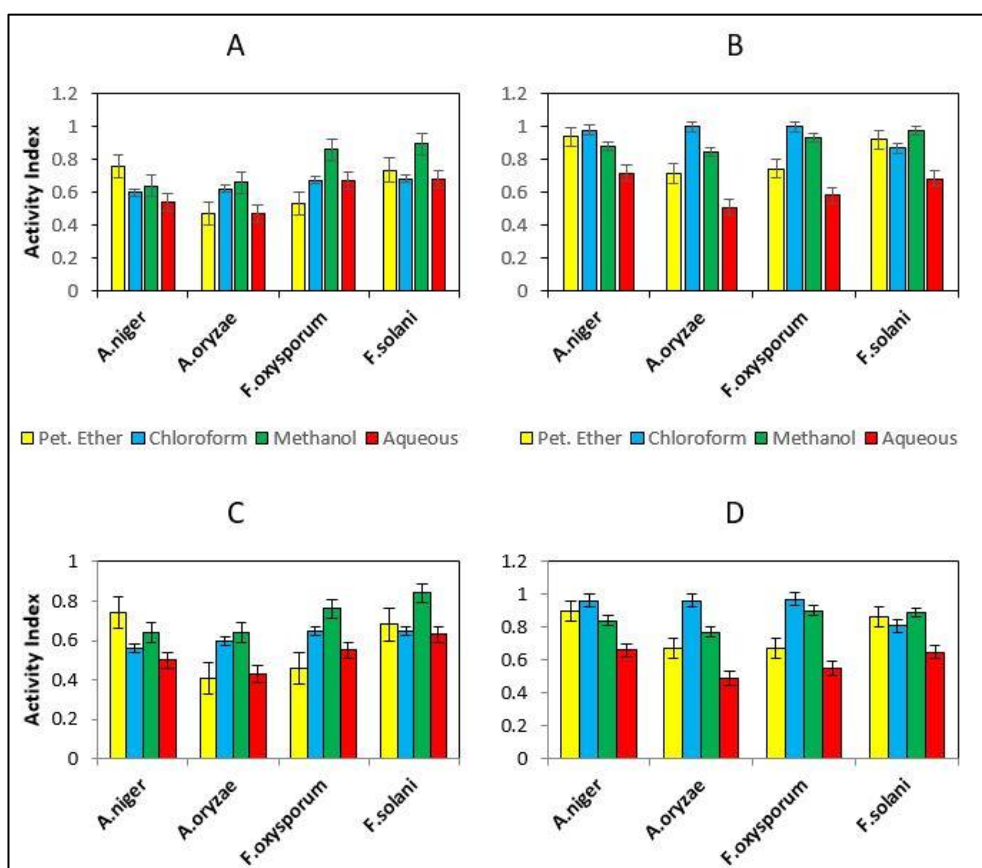

Figure 5: Activity indices of (A) Stem extracts (B) Leaf extracts of *L. cordifolius* taken from host *P. emblica* and (C) Stem extracts (D) Leaf extracts of *L. cordifolius* taken from host *J. regia* using test organisms and solvents followed in the current experiment.

(Figure 4). The stem extracts of *L. cordifolius* (Host *P. emblica*) also produced the best activity indices of 0.82, 0.80, 0.88, and 0.78 against the test organisms *E. coli, S. aureus, P. aeruginosa*, and B. subtilis, respectively, compared to stem extracts of *L. cordifolius* (Host *J. regia*) with values of 0.80, 0.75, 0.82, and 0.75 (Figure 4).

Table 7: Antioxidant activity of different concentration of solvents followed in the present study using leaf and stem extracts of *L. cordifolius* taken from hosts *P. emblica* and *J. regia*.

Plant Part	Nature of Solvent	Volume (µL)	%age Scavenging effect of DPPH Host *P. emblica*	%age Scavenging effect of DPPH Host *J. regia*
Stem	Pet. Ether	500	93±0.4	90±0.3
		250	90±0.9	85±0.4
		125	82±0.5	71±0.9
		50	64±0.4	65±0.7
	Chloroform	500	88±0.4	87±0.5
		250	83±0.3	80±0.5
		125	82 ±0.4	70±0.4
		50	65 ±0.7	62±0.8
	Methanol	500	85±0.6	78±0.7
		250	76±0.2	69±0.6
		125	65±0.4	58±0.3
		50	57 ±0.4	44±0.2
	Aqueous	500	67 ±0.7	61±0.2
		250	59 ±0.2	43±0.3
		125	51 ±0.4	41±0.4
		50	47±0.3	36±0.6
Leaf	Pet. Ether	500	87 ± 0.4	85±0.9
		250	58 ±0.3	54±0.4
		125	44 ±0.4	45±0.4
		50	35 ±0.4	33 ±0.6
	Chloroform	500	86±0.5	81±0.1
		250	77±0.9	74±0.1
		125	56±0.6	51 ±0.3
		50	43 ±0.6	42±0.5
	Methanol	500	71±0.2	64±0.6
		250	63 ±0.3	59±0.5
		125	51±0.1	48±0.3
		50	41±0.4	33±0.3
	Aqueous	500	62±0.5	59±0.5
		250	49±0.5	50±0.5
		125	48±0.8	43±0.6
		50	42±0.3	40±0.5

These results clearly demonstrate the potential of the plant for use as an antibacterial medicine. These findings align with previous research on the mistletoe family Loranthaceae by Adesina et al. (2013). The observed antibacterial effects may be attributed to the presence of alkaloids in *L. cordifolius* extracts, as alkaloids often serve as a foundation for various antibacterial drugs (Cushnie *et al.* 2014). Additionally, Xie *et al.* (2015) proposed that the flavonoids present in plants exhibit various antibacterial mechanisms, including inhibiting plasma membrane functioning, disrupting energy metabolism, and weakening bacterial cells by blocking porins in the plasma membrane. Given the identified presence of flavonoids in the phytochemical profile of *L. cordifolius*, it is plausible that these compounds acted against bacterial strains. Moreover, the tannins found in the plant extracts possess antibacterial properties (Kaczmarek, 2020). These findings align with our results. Notably, extracts from plants obtained from the host *P. emblica* were more effective compared to those from *J. regia*. Gaire and Subedi (2014) extracted antibacterial

Table 8: Total antioxidant activity (TAA) of leaf and stem extracts of *L. cordifolius* taken from hosts *P. emblica* and *J. regia*.

Plant Part Used	Nature of Solvent Used for Extraction	Absorbance at 695 nm Host *P. emblica*	Absorbance at 695 nm Host *J. regia*
Stem	Pet. Ether	3.55±0.40	3.29± 0.77
	Chloroform	2.98±0.52	3.02±0.54
	Methanol	2.60±0.43	2.77±0.34
	Aqueous	2.45±0.35	2.31±0.35
Leaf	Pet. Ether	2.49±0.34	2.20± 0.41
	Chloroform	1.65±0.32	1.41±0.52
	Methanol	2.31±0.61	2.12±0.95
	Aqueous	1.80±0.21	1.75±0.25
Standard (BHT)		4.60±0.55	4.60±0.55

compounds from *P. emblica* using bioassay-guided fractionation, while Khan *et al.* (2013) investigated the antibacterial nature of phytochemicals produced by *J. regia*. These studies support a strong host-parasite interaction, corroborating our current findings. The higher phytochemical contents extracted from plants obtained from the *P. emblica* host provide additional robust evidence of their antibacterial action.

This study represents the first comparative analysis of *Loranthus cordifolius* collected from different host plants, focusing on its antimicrobial activity and antioxidant potential. By evaluating phytochemical profiles and bioactivities, our findings reveal significant variations based on the host plant. Notably, *L. cordifolius* parasitizing *P. emblica* exhibited superior antimicrobial activity and higher concentrations of key bioactive compounds, such as cardiac glycosides, flavonoids, and anthraquinones, compared to samples from *J. regia*. These results highlight the influence of host species on the medicinal properties of *L. cordifolius*, offering novel insights into its potential for developing antimicrobial and antioxidant agents.

3.3. Antimycotic Activity

Data presented in Figure 5 reflects the values of the activity indices for the stem and leaf extracts of *L. cordifolius* from both hosts. Generally, leaf extracts showed better values of activity indices as compared to bark extracts (Figure 5) whereas chloroform and methanol proved as better solvents for extracting plant tissues for antimycotic activity.

The data values for the zone of inhibition were recorded against each fungal strain and were compared with standard anti-fungal drugs available in the market showing a zone of inhibition value of 51±0.4 for *A. niger* 53±0.6 for *A. oryzae* for *F. oxysporum* 43±0.5 and for *F. solani* 38±0.5 as control. In table 5 minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) for *L. cordifolius* plants from both hosts are shown. It is clear that leaf extracts proved more suitable for antimycotic action as compared to stem extracts (Table 5). The *P. emblica* imparts better antifungal traits to the *L. cordifolius* phytochemicals as compared to *J. regia*.

The extraction using water was found least effective in all cases and the activity indices for the plant extracts dropped for each fungal strain using water as an extraction medium for the phytochemicals (Figure 6). High values of activity indices depict that the plant has great anti-fungal potential and the leaf extract of the plant can serve as potential antimycotic drugs. The higher trends in activity indices for anti-fungal action were shown by leaf and stem extracts of *L. cordifolius* (Host *P. emblica*) as compared to the same of *L. cordifolius* (Host *J. regia*). The stem extracts of *L. cordifolius* (Host *P. emblica*) produced the best activity

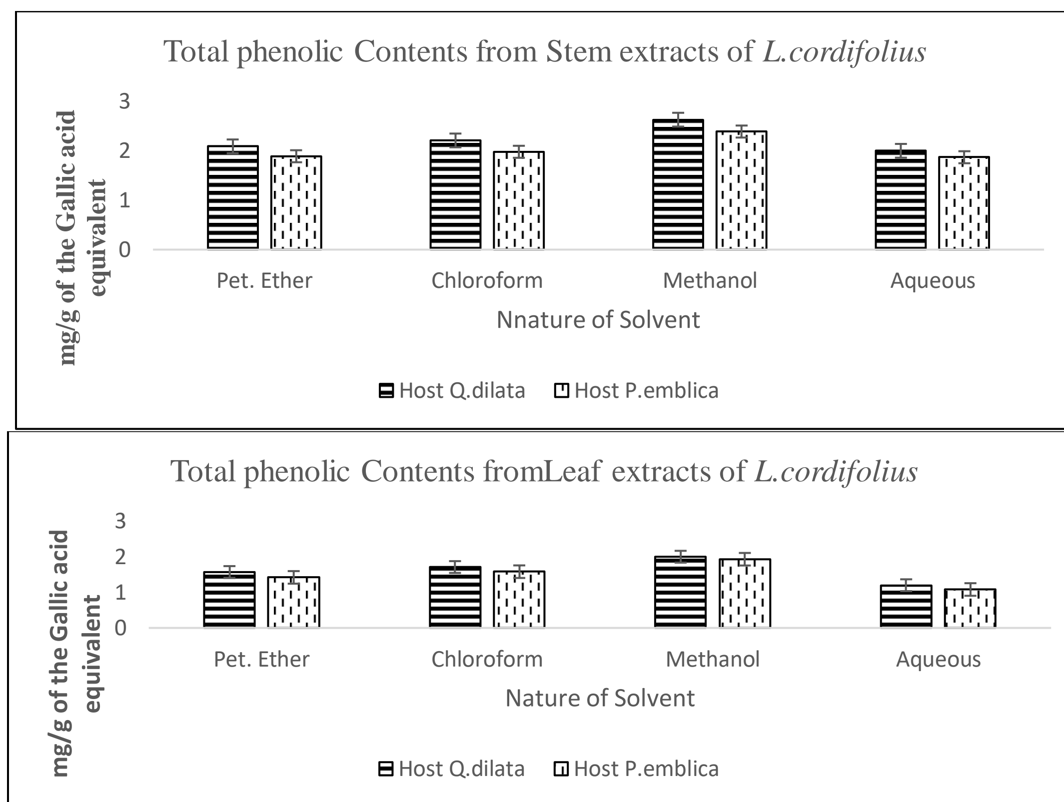

Figure 6. Total phenolic contents measured in mg/g of the Gallic acid.

indices of 0.76, 0.66, 0.86, and 0.89 against test organism *A. niger, A. oryzae, F. oxysporum,* and *F. solani* respectively as compared to stem extracts of *L. cordifolius* (Host *J. regia*) showing values of 0.74, 0.64, 0.76 and 0.84. The leaf extracts of *L. cordifolius* (Host *P. emblica*) produced best activity indices of 0.98, 1, 1, and 0.97 against test organism *A. niger, A. oryzae, F. oxysporum,* and *F. solani* respectively as compared to leaf extracts of *L. cordifolius* (Host *J. regia*) showing values of 0.96, 0.96, 0.97 and 0.89 (Figure 6). Detailed description of activity indices against both fungal and bacterial test organisms have been mentioned in table 6.

The enhanced antifungal activity observed in *L. cordifolius* plants taken from the host *P. emblica* could be attributed to the presence of tannins in *P. emblica.* Studies have indicated that *P. emblica* produces secondary metabolites such as gallic acid, ellagic acid, castalagin, and tannic acid, all of which exhibit potent antifungal activity. These compounds are known to damage fungal cell walls and cell membranes, leading to the leakage of sugars and other vital osmolytes (Ahmad *et al.* 2021; Zhu *et al.* 2019). Furthermore, mistletoes, including *L. cordifolius*, have been reported to contain antifungal compounds such as viscotoxins, caprylamide, linleliadic acid, and methyl esters, which act as natural toxins against fungi (Akalazu *et al.* 2016). These findings align with prior research highlighting the antifungal and antimicrobial properties of plant extracts (Mahizan *et al.* 2019). The antifungal action observed in the experiment may be attributed to flavonoids causing mitochondrial dysfunction and preventing fungal cell wall formation, a mechanism associated with flavonoids and terpenoids. Additionally, the well-known antimycotic activity of saponins involves their potent inhibition of efflux pumps and fungal protein synthesis (Porsche *et al.* 2018).

3.4. Anti-oxidant Potential

In the present study, the antioxidant potential of *L. cordifolius* plants taken from the hosts *J. regia* and *P. emblica* was assessed by monitoring the scavenging effect of DPPH and conducting total antioxidant analysis. Additionally, total phenolic contents were studied to evaluate polyphenols, prime botanical antioxidants. The data in Table 6 suggests that *L. cordifolius* plants taken from the host *P. emblica* exhibit superior antioxidant potential in terms of DPPH scavenging activity compared to plants from host *J. regia*. The scavenging activity varies significantly for each solvent and the host's nature. The highest scavenging activity percentages were observed as 93±0.4 and 90±0.3 for the host *P. emblica* and *J. regia*, respectively, using stem extraction of *L. cordifolius* in petroleum ether (Equivolumetric concentration). The lowest activity was observed with aqueous extraction of leaves, i.e., 42±0.3 and 40±0.3 for *P. emblica* and *J. regia*, respectively. Bark extracts provided better results than leaves, possibly due to the high flavonoid content of the bark. The petroleum ether stem extracts of plants from both hosts proved better in terms of % age DPPH scavenging effect, i.e., 93±0.4 and 90±0.3 from the host *P. emblica* and *J. regia*, respectively.

Similarly, total antioxidant activity as depicted in Table 7 suggests stem extracts are better than leaves. The highest total antioxidant activity (TAA), i.e., 3.55±0.40, was observed with the bark extracts of the plant taken from host *P. emblica* compared to 3.29±0.77 shown by the plants of host *J. regia*. Petroleum ether proved to be the best among all solvents assayed for antioxidant analysis, while water proved to be the least effective. Data presented in Figure 7 shows the total phenolic contents (TPC) of bark and leaves of *L. cordifolius* plants taken from both hosts. Higher TPC was observed with bark extracts of *L. cordifolius* (*P. emblica*). These results underscore the antioxidant potentials of *L. cordifolius* taken from different hosts and extracted in different solvents. The findings indicate that *L. cordifolius* plants taken from the hosts *P. emblica* exhibit better antioxidant activity compared to *J. regia*. Similarly, petroleum ether and methanolic extracts have shown better promise for antioxidant activity.

The antioxidant potential of *L. cordifolius* can be attributed to the presence of polyphenols in the plant (Siraichi *et al.* 2013). Specifically, the plant contains flavonoids, which are polyphenolic compounds known for donating free electrons to reactive oxygen species (ROS) and free radicals, thus playing a significant role as cardio-protective and anti-inflammatory agents. These findings align with the work reported by Maqbool *et al.* (2017). The superior results obtained from plants taken from the host *P. emblica* suggest a robust host-parasite chemistry between *L. cordifolius* and *P. emblica*. Studies have identified important polyphenols in *P. emblica*, including gallic acid, quinic acid, gentisic acid, ferulic acid, homogenistic acid, and vanilic acid (Wu *et al.* 2022). These polyphenols likely act as antioxidants, reflected in the phytochemical profile of the parasite, i.e., *L. cordifolius.* Researchers have isolated quercetin as a major antioxidant compound from the bark of *P. emblica* (Wu *et al.* 2022). It is well-established that the bioactive compounds and chemical composition of the parasite are affiliated with the host's phytochemical profile (Okubamichael *et al.* 2016). As mistletoes are obligate hemi parasites, they may extract certain antioxidants from the host, serving both defensive and offensive purposes. While *L. cordifolius* (P. emblica) has demonstrated the best antioxidant activities, the study also validates the antioxidant ability of *L. cordifolius* from the other host, i.e., *J. regia*, using stem and leaf extracts in all four solvents analysed in the current study. The antioxidant potential of *J. regia* has been discussed by various researchers from various localities and cultures worldwide (Liu *et al.* 2008; Li *et al.* 2015). However, the variability in the antioxidant ability is host-specific, treatment-specific, solvent-specific, and plant-part-specific. The above findings suggest that *L. cordifolius* might

serve as a potent antioxidant agent and could be explored for use in hepatic protection and cardio-protective medicines.

Further studies are recommended to purify the bioactive compounds from *L. cordifolius* extracts to identify the specific constituents responsible for its antimicrobial and antioxidant activities. Advanced techniques such as chromatography and spectroscopy should be employed to isolate and characterize these compounds. Additionally, exploring *L. cordifolius* from a broader range of host plants beyond *P. emblica* and *J. regia* could provide a more comprehensive understanding of host influence on its phytochemical and therapeutic profiles. Such studies would not only enhance the pharmacological potential of this parasitic plant but also contribute to its sustainable utilization in traditional and modern medicine.

4. Conclusion

The present study concludes that *L. cordifolius*, particularly from *P. emblica* hosts, is a promising source of antimicrobial and antioxidant agents. Leaf extracts in various solvents demonstrated significant antibacterial and antifungal efficacy, with chloroform extracts achieving the highest activity against fungal strains. Antioxidant potential was strongest in stem extracts, especially in petroleum ether, with plants from *P. emblica* exhibiting superior activity. To fully realize the therapeutic potential of *L. cordifolius*, advanced fractionation techniques should be employed to isolate and identify specific bioactive compounds for further pharmacological testing. Future studies should also explore *L. cordifolius* from a wider range of host species to expand its medicinal applications.

Competing Interests

The authors declare no competing interests.

Ethics Approval

This study was conducted in accordance with ethical guidelines for the collection and use of plant materials. All plant samples used in this research were obtained from sustainable sources and with the necessary permissions. The study did not involve human or animal subjects, and no harmful or unethical practices were involved in the collection, handling, or analysis of the plant materials. Ethical approval for this study was granted by the Departmental Ethics Committee (DEC), Mirpur University of Science and Technology, Mirpur, Pakistan.

Consent Forms

Not applicable

Data Availability

All the data related to this study is available with the authors.

Author Contributions

MI, MM, MWM, WM, and TH contributed to the study's conception and design. MWM, FIJ, MM, and AS performed the experiments, FIJ, WM, and MM performed data collection and analysis, and the first draft was written by MWM. Review and editing were performed by all authors Supervision was done by MI. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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