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Antibacterial, Antifungal and Phytotoxic Pharmacological Profiling of Different Extracts of Lespedesea Gerardiana

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Abstract: *This study is based on phytochemical properties of L. gerardiana, as well as the pharmacological and biological activities of crude methanolic extract (Crd. MeOH Ext.) and other fractions. A range of pharmacological and biological activities, such as antibacterial, antifungal, phytotoxic, were examined in Crd. MeOH Extract and different plant fractions. The results of the pharmacological and biological examinations showed that the chloroform (CHCl3) fraction had good antibacterial activity against Salmonella typhi (48.0%), Bacillus cereus, Proteus merabilus (50.0%), and Klebsiella pneumoniae (57.1%). Also demonstrating strong (55.5%) antibacterial activity against S. typhi was the n-hexane fraction. Low phytotoxicity was demonstrated by the plant materials at 1000 and 100µg/ml concentrations.*

Key Words: Methanolic Extract, Salmonella Typhi, Bacillus Cereus, Proteus Merabilus

Introduction

Plants are an excellent source for making medications, and traditional medicine in particular is helpful in treating a wide range of illnesses. Ancient Greeks practised the use of herbal remedies, and the Iliad records that Achilles, the ultimate hero of the Trojan War, discovered that using yarrow stopped the bleeding from wounds sustained in combat. Current research has demonstrated that this plant has compounds with blood-clotting and anti-inflammatory qualities (Heinrichet al., 2012). Throughout the Middle Ages, yarrow tea was still used by Europeans to halt internal bleeding. The same plant has long

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been used by Indians to treat respiratory illnesses(Jason & Adams, 2023).

Globally, there is a growing trend towards the use of botanicals due to the high cost of developing pharmacological drugs from natural products. Examples of such products include Pneumocandin B, which is antifungal, Ascomycin, which is effective against atopic dermatitis, and the potent antibiotics Cephalosporin, Thienamycin, and Daptomycin. (Brahmachari, 2012). Ninety-five percent of therapeutic plants in South Asia are harvested from the wild. We have chosen L. gerardiana, a shrub leaf used as fodder that belongs to the Fabaceae family. Legumes' roots anchor bacteria that fix nitrogen from the air into the soil, which can then be absorbed by subsequent plants and lower the need for fertiliser inputs (Abrahamet al., 2022). Members of the fabaceae Desmodium styracifolium and Desmodium gyrans have historically been used to cure a variety of illnesses, including rheumatism, diarrhoea, pyrexia, wounds, malaria, cough, hepatitis, and hemoptysis (Opryshko et al., 2024; Bako et al., 2005).

Material and Method

General Experimental Condition

All chemical, pharmacological/biological, and instrumental analyses were carried out at the International Centre for Chemical and Biological Studies (ICCBS) at Karachi University (KU) and the Centre of Biotechnology and Microbiology (COBAM), University of Peshawar (UOP). Following double distillation, solvents that could be purchased commercially were utilised.

Physical Constants

Glass capillaries were utilised to determine the melting point(s) of the separated compounds utilising Gallenkamp 30/MF-370. The optical rotation was measured using a digital polarimeter (JASCO DIP-360) (Elenitoba *et al*., 2008)

Spectroscopy

For ultraviolet spectroscopy, a Shimadzu UV-240 fully automated spectrophotometer was utilised. For the IR spectra, a JASCO-A 320 Infrared (IR) spectrophotometer was utilised.

Low-resolution electron impact mass spectra were acquired using Finnigan MAT 311 on the MASPEC data system. Field desorption, field ionisation, and peak matching were measured using the Finnigan mass spectrometer (MAT 312). On the Jeol-JMS-HX-110 mass spectrometer, high-resolution mass measurements and fast atom bombardment (FAB) were carried out.

Proton Magnetic Resonance 1 H-NMR on Burkers was recorded at 500, 400, and 300 MHz as well as AMX-500, AM-400, and AM-300 at a digital resolution of 32 K 13 C-NMR on Aspect 3000 data systems. Experiments using 90 o and 135 o Distortionless Enhancement by Polarisation Transfer (DEPT) were used to identify the CH 3, CH, and CH 2 groups. The quaternary carbons were determined by subtracting the signals from the broadband (BB) 13 C-NMR spectrum (Rabbi & Nisar, 2023; Shi et al., 2024)

Isolation and Purification of Compounds (Techniques)

Following chromatographic techniques were used for separation and compound refinement.

Thin-Layer Chromatography (Tlc)

TLC was carried out on precoated cards using a silica gel card (20 20 cm, 0.25 mm thickness, Merck PF 254, Type 60).

Column Chromatography

Several organic solvents of analytical grade were utilised for the CC mobile phase, whereas Silica gel-GF 254, 60 (Merck) was utilised for the stationary phase (Unger & Janzen, 1986).

Spray Reagents (for Visualization of Spot)

Three unique colouring reagents were sprayed on TLC cards: vanillin-phosphoric, ceric sulphate-sulphuric acid, and iodine solution (Bolliger et al., 1965)

Vanillin-Phosphoric Acid

A mixture of 1g vanillin and 100ml of 50% phosphoric acid aqueous was prepared [32]. Terpenes were sprayed and heated to a light pink or blue colour, while steroids produced a deep colour. When heated, terpenoidal and steroidal glycosides also produced a pink hue (Stahl, 1969).

Ceric Sulphate-Sulphuric Acid

Ceric sulphate (saturated solution) was added to 65% of sulfuric acid (Stahl, 1969). The TLC plates were sprayed with this solution to make them visible. When terpenoids are present, they appear pink when heated, whereas alkaloids give off a pale yellow or blackish colour when not heated. With this spray, most oxidizable compounds may be seen.

Iodine Solution

Inside the heated TLC tank, which was meant to be in the water bath at 40–49 o C for a few minutes, was a tiny quantity of iodine crystals. A spot developed in the middle of the plates after they were in the tank for some time (Stahl, 1969).

Pharmacological Investigations

Antibacterial Activity

Since ancient times, people have used medicinal herbs for therapeutic purposes. Researchers are becoming more and more interested in finding effective chemicals that function as antimicrobials as well as screening, investigating, and establishing plant potential against microorganisms. Plants were screened for resistance to different infectious agents once their clinical importance was determined. For example, Bacillus cereus is the primary cause of stomach illnesses because it can produce enterotoxins and emetic toxins. Additionally, after consuming the meals, three distinct heat-labile enterotoxins induce a watery food-borne infection in the small intestine during a stage of B. cereus' vegetative growth. (Lindbäck & Granum, 2006; Grabley, & Thiericke, 1998).

After being produced, autoclaved, and incubated for 24 hours at 37 o C to verify sterility, nutrient agar medium and nutrient broth were tested. A sterile loop dipped in the broth-containing bacterial culture was transferred to a new broth for dilution after a 24-hour period. One millilitre of broth culture was added to each nutrient agar plate. Spread evenly by going around to create a bacterial lawn. Using a 6mm borer, wells were created inside the nutrient agar plates and labelled. 3 mg of test materials were diluted in 1 millilitre of DMSO to create stock solutions.

Using a micropipette, 100µl of the stock solution was added to each well, and the mixture was incubated for 24 hours at 37 o C. DMSO was used as a control group. A common medication used as a positive control is amoxicillin. The zone of inhibition was assessed after a 24-hour period. The following formula was used to determine the percent zone of inhibition in comparison to the positive control.

$$\% \text{ inhibition} = \frac{\text{Zone of inhibition by sample}}{\text{x 100}}$$

Antifungal Activity

One of the biggest concerns among doctors and microbiologists worldwide is the rise of microorganism resistance to currently available antimicrobial medicines. We must create better, safer, and more effective antifungal medications. It is evident that natural materials have relatively mild effects on human pathogenic fungus when compared to commercially available synthetic antifungal medications (Ahmadi, et al., 2010).

The antifungal activity was performed as per the reported procedure of Bashir et al. To make the stock solution, 24 mg/ml of the test substance was dissolved in sterile DMSO. The SDA medium was ready, put into Petri plates, and autoclaved. To verify sterility, the plates were incubated for twenty-four hours at 28+1 o C. The test organisms were reconstituted using the sterilised plates. The aforementioned strains were injected into sterile plates and allowed to incubate at 28 C for five to seven days. Four millilitres (ml) of SDA were added to the test tubes and autoclaved. Sixty-six microliter test samples from each stock solution were added to the corresponding test tube. The test tubes were placed one-sided and incubated at 28 C to ensure sterility before being cut into

slants. A day later, the test tubes were filled with a seven-day-old culture and cultured for seven days at 25 o C. Miconazole was employed as the positive control and DMSO as the negative control, respectively. The results were documented on day seven by comparing the test tubes' linear growth to the negative control using the following formula:

$$\text{Percent inhibition} = \frac{\text{Linear growth in test (mm) x 100}}{\text{Linear growth in standard (mm)}}$$

Phytotoxic Activity

The test samples' phytotoxicity against L. minor was evaluated using the protocol that Bashir et al. published. L. minor was gathered from UOP, KPK, and Pakistan's Department of Botany.

The test sample stock solutions were made with methanol at a concentration of 20 mg/ml. Using a micropipette, 10, 100, and 1000µl of the stock solutions were poured into the flask. The flasks were left overnight to allow the methanol to evaporate. For the purpose of growing L. minor, e-media was created. 20 ml of e-media was then poured into each flask once the methanol had evaporated. Sixteen healthy plants were selected and placed into each flask. Every flask was incubated for seven days at 28+1 o C inside a growth chamber. Day 7: The number of ruined plants was counted to record the results. (Wei,et al., 2020: Mouffouk et al., 2023)Using the following formula, the percent growth inhibition was calculated:

$$\text{Growth inhibition (\%)} = \frac{\text{100 - Number of fronds in test x100}}{\text{Number of fronds in control}}$$

Results

Antibacterial Activity

The chosen pathogens Proteus merablus, Bacillus cereus, Salmonella typhi, Klebsiella pneumoniae, and Pseudomonas aeruginosa were tested against the test samples. Gram-positive Cocci and Gram-negative bacilli are growing increasingly resistant to the antimicrobials we now have on hand through a variety of processes. Increasing research into novel and inventive antimicrobial medicines while reducing marketing tactics that encourage the excessive and inappropriate use of these important drugs is one way to prevent microbial resistance.

Table 3.1 and Figure 3.1-3.6 reveal the antibacterial activity results of the Crd. MeOH Ext. and different proportions of L. gerardiana. Based on the available data, it is clear that the Crd. MeOH Ext. exhibited no activity against P. merabilus and B. cereus, low activity against K. pneumoniae (33.3%) and P. aeruginosa (35.7%), and moderate activity against S. typhi (40.7%) and E. coli (40.7%). Against S. typhi (55.5%), E. coli (48.1%), P. merabilus (42.3%), and B. cereus (40%), the n-hexane fraction exhibited moderate activity. The n-hexane fraction had low activity against K. pneumoniae (38.0%) and P. aeruginosa (35.7%). Against P. merabilus (50.7%), B. cereus (48.1%), S. typhi (48.1%), P. aeruginosa (42.8%), and K. pneumoniae (57.1%), the chloroform fraction exhibited moderate activity. There was little (33.33%) action against E. coli. The EtOAc fraction exhibited moderate efficacy against E. coli (44.44%) and P. aeruginosa (46.4%). Similar low activity was seen against P. merabilus (15.3%), K. pneumonia (38.0%), and S. typhi (37%). B. cereus showed no activity at all. The aqueous fraction exhibited no activity against S. typhi but moderate activity against P. merabilus (50.0%), P. aeruginosa (42.8%), B. cereus (48.0%), and E. coli (48.1%). It also had low activity against K. pneumoniae (38.0%).

Table 1

Antibacterial activity of crude extract and various fractions of Lespedeza gerardiana

Name of Bacteria	Zone of inhibition						
	-ive control	+ive Control	Crd. MeOH Ext.	*n* –hexane	$CHCl_3$	EtOAc	Aqueous

	Mm	mm	mm	Percent	mm	Percent	mm	Percent	mm	Percent	mm	Percent
P. merabilus	0	26	0	0	11	42.3	13	50.0	4	15.3	13	50.0
B. cereus	0	25	0	0	10	40	12	48	0	0	12	48
S. typhi	0	27	11	40.7	15	55.5	13	48.1	10	37	0	0
P. aeruginosa	0	28	10	35.7	10	35.7	12	42.8	13	46.4	12	42.8
E. coli	0	27	11	40.74	13	48.14	9	33.33	12	44.4	13	48.14
K.pneumoniae	0	21	7	33.3	8	38.0	12	57.1	8	38.0	8	38.0

Figure 1

Antibacterial activity of Crd. MeOH Ext. against various test pathogens

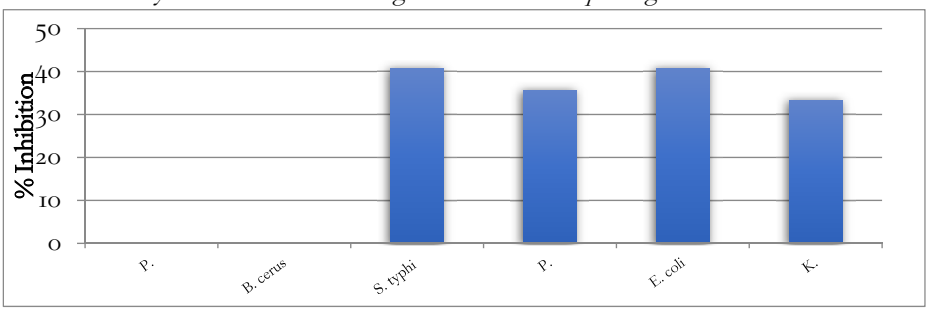


Figure 2

Antibacterial activity of n-hexane fraction against various test pathogens

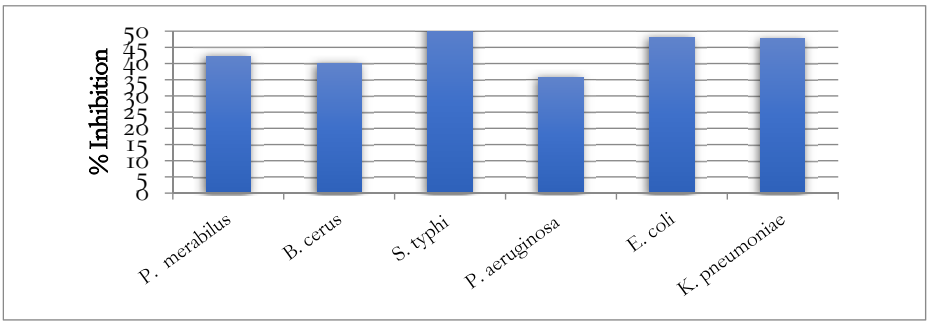


Figure 3

Antibacterial activity of $CHCL_3$ fraction against various test pathogens

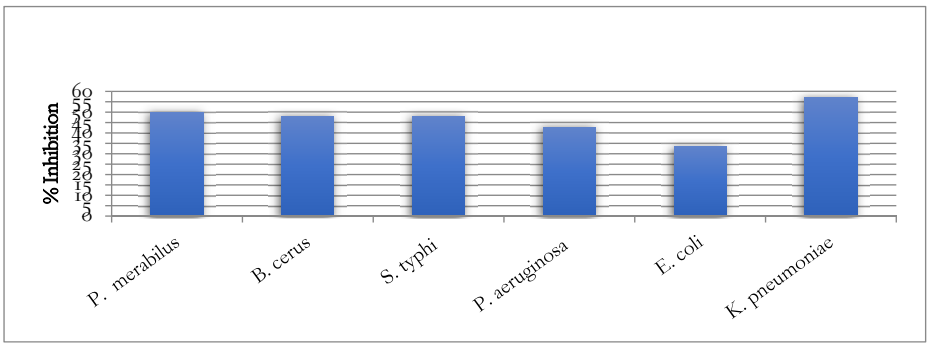


Figure 4

Antibacterial activity of EtOAc fraction against various test pathogens

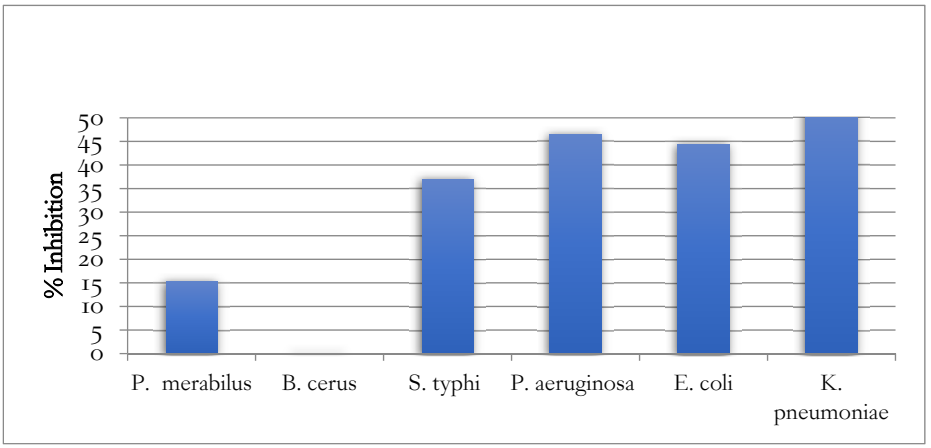


Figure 5

Antibacterial activity of aqueous fraction against various test pathogens

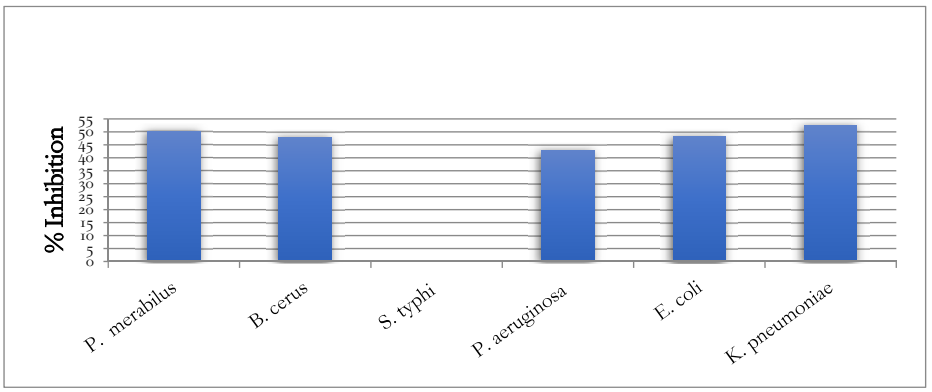
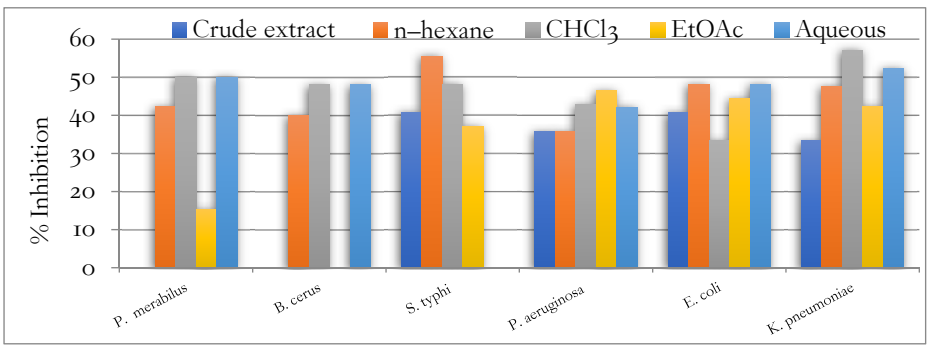


Figure 6

Antibacterial activity of Crd. MeOH Ext and various fractions of L. gerardiana against test pathogen



Antifungal Activity

A saprophyte found in soil, Aspergillus niger is the cause of black mould on garlic, onions, cotton boll rot, Sansevieria rot, shallot rot, and Dracaena root stalks and roots. dates, vanilla pods, figs, cashew kernels, and dried prunes spoilage. The most serious plant pest was caused by this organism, which also contributed to groundnut crown rot. The test samples were examined for the presence of A. fumigatus, A. flavus, and A. niger. The acquired results are shown in Figures 3.7-3.11 and are illustrated in Table 3.2. Miconazole was the standard medication. The percentage of inhibition against A. niger was as follows: Crd. MeOH Ext. (2.0%), n-hexane (0%), $CHCl_3$ (10.0%), and EtOAc (0%) aqueous (5.0%). The percentages of inhibition against A. fumigatus were as follows: aqueous (0%), n-hexane (0%), $CHCl_3$ (0%), EtOAc (0%) and Crd. MeOH Ext. (0%). The following compounds showed the highest percent linear inhibition against A. flavus: n-hexane (1.0%), $CHCl_3$ (2%), EtOAc (0%) and water (0%). The current findings demonstrated that neither the Crd. MeOH Ext. nor any of the L. gerardiana fractions exhibited any antifungal efficacy against the fungus species under investigation.

Table 2

Antifungal activity of Crd. MeOH Ext. and various fractions of L. gerardiana

Name of fungi	Percent Linear Growth Inhibition						
	-ive control	+ive control*	Crd. MeOH Ext.	*n.* hexane	$CHCl_3$	EtOAc	Aqueous
A. niger	0	100	2.0	0	10	0	5
A. fumigatus	0	100	0	0	0	0	0
A. flavis	0	100	0	1.0	2.0	0	0

Figure 7

Antifungal activity of Crd. MeOH Ext. against various test pathogens

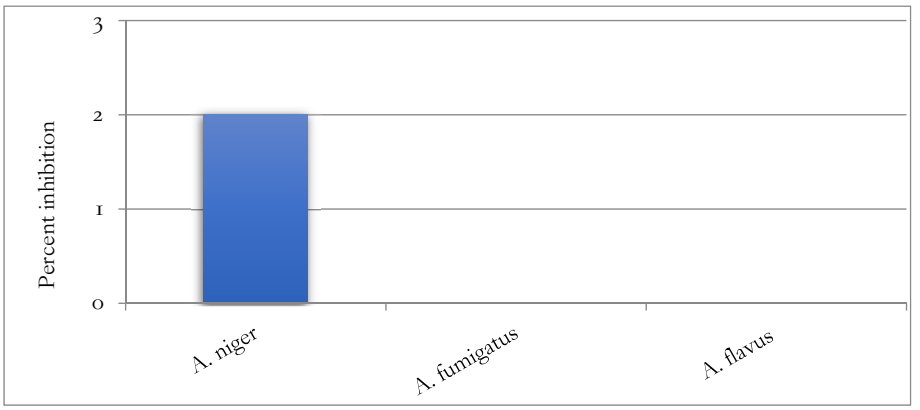


Figure 8

Antifungal activity of n-hexane fraction against various test pathogens

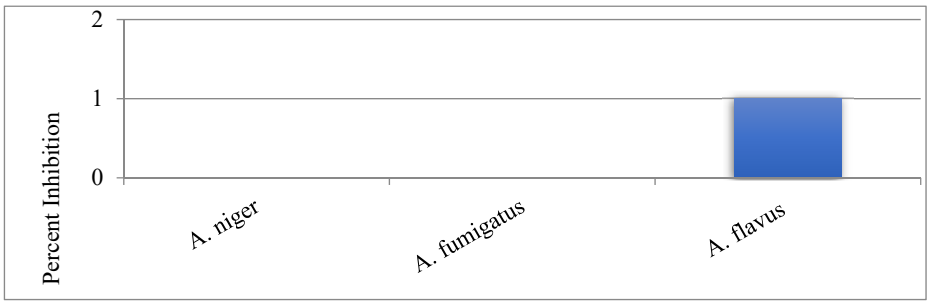


Figure 9

Antifungal activity of $CHCl_3$ fraction against various test pathogens

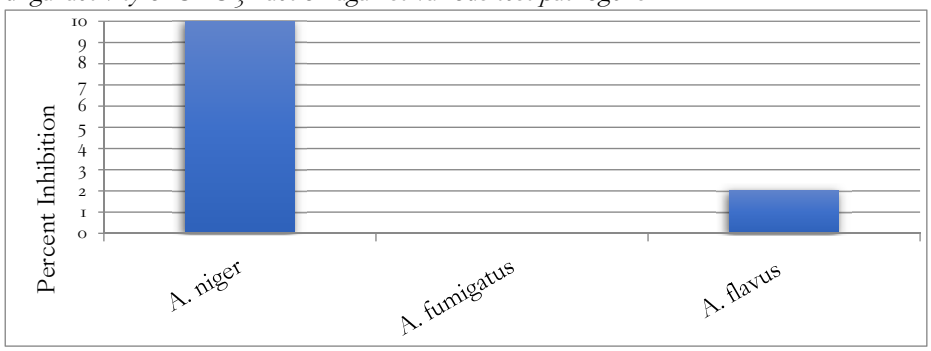


Figure 10

Antifungal activity of aqueous fraction against various test pathogens

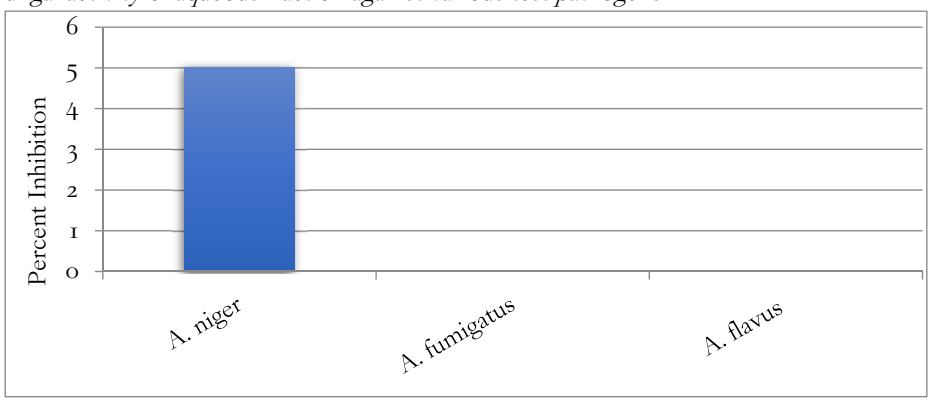
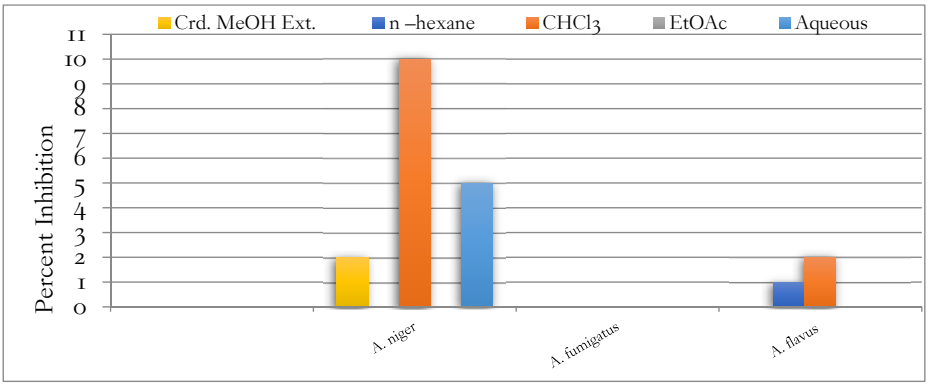


Figure 11

Antifungal activity of Crd. MeOH Ext. and various fractions of L. gerardiana.



Phytotoxic Activity

Due to its ability to preferentially gather specific chemicals, Lemna minor L, also referred to as duckweed, has been used to monitor aquatic contaminants and heavy metals as well as to investigate water quality. Because of their physiological characteristics, which include quick growth, tiny size, pH 5 and 9, vegetative propagation, and rapid growth, these plants provide an excellent test system.

The effects of the plant materials' phytotoxic activities are displayed in Figures 3.12 and in Tables 3.3 and 3.4. At 1000µg/ml, there was minimal phytotoxic activity for Crd. MeOH Ext. (31.2%), n-hexane (12.5%), CHCl3 (25%), EtOAc (6.25%), and aqueous fraction (6.25). At 100 and 10µg/ml, respectively, very little activity was seen for Crd. MeOH Ext. and other L.gerardiana fractions.

Table 3

Phytotoxic activity of Crd. MeOH Ext. and various fractions of *L. gerardiana*

Name of Plant	Concentration of test sample (µ/ml)	The number of fronds that survived					
		Crd. MeOH Ext.	*n.* hexane	$CHCl_3$	EtOAc	Aqueous	Control*
Lamina minor	1000	11	14	12	15	15	16
	100	13	15	14	15	13	16
	10	16	16	16	16	16	16

**Paraquat drug at a concentration of 0.015 (µg/ml) was applied as a positive control*

Table 4

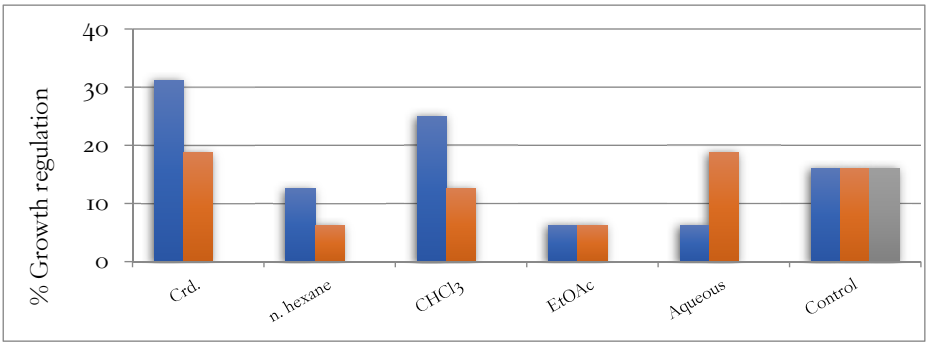
% reduction in growth regulation of the Lamina minor

Name of Plant	Test sample concentration (µ/ml)	% Growth Regulation					
		Crd. MeOH Ext.	*n.* hexane	$CHCl_3$	EtOAc	Aqueous	Control
	1000	31.2	12.5	25	6.25	6.25	100

Lamina minor	100 10	18.7 0	6.25 0	12.5 0	6.25 0	18.75 0	100 100

Figure 12

Phytotoxic Activity of the Crd. MeOH Ext. and various fractions of L. gerardiana



Discussion

Ultimately, clinical trials are conducted to assess the safety and efficacy of the plant extract or its constituents in humans. These trials are essential for determining whether the plant has therapeutic potential and for establishing guidelines for its use in clinical practice.

Based on the experimental work, it is concluded that the CHCl 3 fraction exhibited moderate antibacterial activity against *K. pneumoniae* (57.1%), *P. merabilus* (50.0%), *B.cereus* (48%) and *S. typhi* (48.0%). The n-hexane fraction also exhibited moderate antibacterial activity against *S. typhi* (55.5%). The plant materials showed low phytotoxicity at concentrations of 1000 and 100µg/ml). The phytotoxic activities of the plant materials are shown in (Figure 12) as well as in Tables 3 and 4. For Crd. MeOH Ext. (31.2%), n-hexane (12.5%), CHCl3 (25%), EtOAc (6.25%), and aqueous fraction (6.25), there was no phytotoxic activity at 1000µg/ml. Very little activity was observed for Crd. MeOH Ext. and other *L. gerardiana* fractions at 100 and 10µg/ml, respectively.

Given the limited research on Lespedesea gerardiana, it's challenging to provide a comprehensive evaluation of its pharmacological properties. More studies are needed to elucidate its potential therapeutic benefits, safety profile, and mechanisms of action. Researchers interested in this plant may consider exploring its traditional uses, conducting phytochemical analyses, and performing pharmacological studies to uncover its medicinal potential.

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