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ASSESSEMENT OF PHYTOCHEMICAL PROPERTIES AND ANTIFUNGAL ACTIVITY OF *LAWSONIA INERMIS* (HENNA) AGAINST SOME SELECTED FUNGI

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ABSTRACT: *Lawsonia inermis*, also known as the henna tree, the mignonette tree, and the Egyptian privet, is used to treat many human illnesses, including ulcers, cough, bronchitis, leukoderma, scabies, boils, ophthalmic disorders, hair loss, and jaundice. It is a common herbal treatment. This study aimed to assess the phytochemical properties and antifungal activity of *L. inermis* extracts against some selected fungi. Following the extraction of the plant using methanol, ethanol, and aqueous solutions as solvents, the percentage yield was calculated by dividing the weight of the extract by the weight of the plant and then multiplying by 100. The phytochemical constituents in the plant extracts were also analyzed according to the standard procedure. The agar well diffusion technique was used to determine the antifungal activity of *L. inermis* extracts. The minimum inhibitory concentration (MIC) was determined using the tube dilution technique, and the tubes with positive MIC results were subcultured on freshly prepared potato dextrose agar (PDA) to determine the minimum fungicidal concentration (MFC). The results revealed that methanol extracts had the highest percentage yield at 23.18%. Phytochemical analysis showed the presence of saponins, terpenoids, flavonoids, alkaloids, steroids, triterpenes, and phenolic compounds. The antifungal activity of *L. inermis* showed that ethanol extracts had the highest activity, with a 22 mm zone at 200 mg/mL against *Aspergillus flavus* and *Candida albicans*, while the aqueous extracts showed the highest activity against *Aspergillus niger* with an 18 mm zone at 200 mg/mL. The MIC of the plant extracts ranged from 25 mg/mL to >100 mg/mL, while the MFC ranged from 50 mg/mL to >100 mg/mL. Overall, *L. inermis* extract contained a significant number of phytochemical constituents and exhibited antifungal activity against all tested fungi at different extract concentrations.

Keywords: Antifungal Activity, Henna, Minimum Fungicidal Activity, Phytochemical constituents.

INTRODUCTION

L. inermis (Henna) is a medicinal plant used in all parts of the world. It is a plant species that has been extensively used in traditional medicine for its medicinal and cosmetic properties (Chopra *et al*., 2014). The plant is native to tropical regions of Africa, Asia, and the Middle East, and its leaves, bark, and seeds have been used to treat various ailments, including skin infections, wounds, and inflammatory conditions (Kumar *et al*. 2017; Ahmed *et al*. 2018). The leaf powder of henna sap is used for staining hair, nails, and beard (Chengaiah *et al.*, 2010). Henna has been used cosmetically and medically for over 9,000 years.

Henna leaves, flowers, seeds, stem bark, and roots are used in traditional medicine to treat a wide variety of ailments, including rheumatoid arthritis, headaches, ulcers, diarrhea, and leprosy. The leaves are used in alleviating skin diseases (Gagandeep *et al*., 2011). The leaves of *L. inermis* are used to treat poliomyelitis and measles among the Yoruba, Igbo, and Hausa tribes of the Western, Southern, and Northern parts of Nigeria. Leaves are small, opposite in arrangement, and appear greenish brown to dull green in color. It measures about 1.5 to 5cm long and 0.5 to 2cm wide, sub-sessile, glabrous, entire margin is elliptic to broadly lanceolate, acute or obtuse apex with tapering base, Petioles very short, acuminate, having depressed veins on the dorsal surface. Studies have shown that *L. inermis* exhibits significant antimicrobial activity against a range of microorganisms, including bacteria, fungi, and viruses (Khan *et al*. 2018; Das *et al*. 2019). For example, a study by Habbal *et al*. (2011) demonstrated that the methanol extract of *L. inermis* leaves exhibited significant antimicrobial activity against several bacterial strains, including *Staphylococcus aureus* (MIC = 0.625 mg/mL) and *Escherichia coli* (MIC = 1.25 mg/mL).

The antimicrobial activity of *L. inermis* may be attributed to the presence of bioactive compounds that can inhibit the growth of microorganisms or disrupt their cellular processes (Ahmed *et al*. 2018). Lawsone, a naphthoquinone compound found in the leaves of *L. inermis*, has been shown to exhibit antimicrobial activity against several microbes, including bacteria and fungi (Gupta *et al*. 2015; Kumar *et al*. 2017). The phytochemical constituents of *L. inermis*, including lawsone, flavonoids, tannins, and phenolic compounds, are believed to contribute to its therapeutic properties (Habbal *et al*. 2011; Singh *et al*. 2013). The increasing prevalence of antimicrobial resistance has led to a growing interest in the search for novel antimicrobial agents from natural sources, including plants (WHO, 2020). *L. inermis*, with its demonstrated antimicrobial activity and rich phytochemical profile, may provide a valuable source of novel antimicrobial compounds. This study aims to investigate the phytochemical constituents and antimicrobial activity of *L. inermis* extracts.

MATERIALS AND METHODS

Study Area

This study was carried out in Modibbo Adama University, Yola, which is located in Girei Local Government Area, Adamawa State, Nigeria, It is within the central part of the state, between longitudes 12° 28′ 51″ E and latitude 9° 25′ 35″ N with an estimated population of 129,995 based on the 2006 census. The major occupation of the people in the area is farming and animal rearing, with the area providing much of the animal products available in the local government and the state (NPC, 2006). Girei has a tropical wet and dry season with distinct wet (April to November) and dry seasons characterized by low humidity with the average annual rainfall being 972mm. The area has average temperature of 33 °C (91.4°F), an average humidity of 24%, and average wind speed of 10 km/h.

Sample Collection and Processing

The leaves of *L. inermis* were collected from Girei town, put into a sterilized polythene bag, and transported to the microbiology laboratory of Modibbo Adama University, Yola. The sample was washed and dried in the shade for a week and blended into powder using a mortar and pestle. It was then sieved to get the equal-sized particles. The powder was kept in an air-tight container in a moisture-free environment for further analysis as described by Jeba *et al.* (2019).

Plant Extract Preparation

Fifty (50g) of the powdered and freshly milled plant material (leaves) was weighed into clean, sterile bottles separately. The weighed-out plant leaves were extracted using 250ml of water, ethanol, and methanol separately in a tightly covered bottle and left for 48 hours at room temperature. It was then filtered into sterile beakers, and filtrates were collected and re-filtered using Whatman No. 1 filter paper into sterile sample bottles, then concentrated using a freeze dryer. They were labeled appropriately and stored in plastic bags at -20°C for further analyses, as adopted by Umeh *et al*. (2005) and Hassan *et al*. (2022).

Percentage Yield of the Extract

After drying the extract, the percentage yield was calculated using the method described by Hanaa and Yusouf (2016).

The following formula was used:

$$\frac{\text{Weight of extract obtained}}{\text{Weight of plant sample}} \text{X1OO}$$

Phytochemical Analysis

Standard procedures, as adopted by Mamman and Isah (2013), Jaradat *et al.* (2015), and Danjuma *et al.* (2024), were used for the qualitative phytochemical screening of alkaloids, phenolic compounds, terpenoids, saponins, triterpenes, tannins, and flavonoids.

Test for Alkaloids

A quantity of 0.1g of the sample was boiled with 5ml of 2% hydrochloric acid on a steam bath. This was filtered, and a 1mL portion of the filtrate was reacted with 2 drops of Wagner's reagent (Iodine in potassium iodide solution), and a reddish-brown precipitate was observed.

Test for Phenolic Compounds

Half a gram (0.5g) of the sample was dissolved in water, and ferric chloride solution was added slowly and dropwise. A color change to violet or blue indicates the presence of phenolic compounds.

Test for Terpenoids

Half a gram (0.5g) of methanol extract was mixed in 2mL of chloroform. 1mL of concentrated H_2SO_4 was carefully added to form a lower layer. A reddish-brown coloration at the interface was taken as the presence of terpenoids.

Test for Saponins

Half a gram of extract was placed in a test tube, and then 0.5ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15 minutes indicated the presence of saponins.

Test for triterpenes

About 0.5 g of the dried powdered samples will be boiled in 20 ml of water in test tubes and then filtered. A few drops of 0.1% ferric chloride will be added and observed for brownish green or a blue-black coloration.

Test for Tannins

The extracts were diluted with distilled water, and 2-3 drops of 5% ferric chloride solution were added. A green-black coloration indicated the presence of tannins.

Test for Flavonoids

Two milliliters (2ml) of the filtrate obtained from the methanol extract of the plant samples was added to 2ml of dilute ammonia solution. The appearance of a yellow color was taken as the presence of flavonoids.

Preparation of Concentrations of Plant Extracts

The different concentration of plant extracts was prepared according to the method described by Ajose and Okozi (2017), Khadka *et al.* (2020), and Danjuma *et al.* (2024). 1 g of the powdered plant material was measured and dissolved in 5mL of dimethyl sulphoxide (DMSO) to obtain a 200mg/mL concentration of the extracts. Double serial dilution was performed to obtain dilutions of 10chee0mg/mL, 50mg/mL, and 25mg/mL.

Test Isolates

The test isolates used in this study were *A. niger*, *A. flavus*, and *C. albicans*, and they were obtained from the Microbiology Department, Modibbo Adama University, Yola. The isolates were verified by culturing on potato dextrose agar (PDA), and the standard protocol was used to further confirm the identity of the isolates as described by Cheesbrough (2006).

Inoculum Preparation

A loop full of the test isolates was inoculated in PDA broth and incubated for 72hours at room temperature. The suspension was adjusted and compared 0.5 McFarland turbidity standard (Rabi, 2020).

Determination of Antimicrobial activity of the extract

Agar well diffusion assay techniques, as adopted by Garba *et al.* (2018) and Danjuma *et al.* (2024), were used to carry out the bioassay. A standardized inoculum of each isolate was swap on PDA using a sterile cotton bud with a continuous rotation. The plates were allowed to air dry. Five wells were made on each plate using a sterile cork-borer. Four different concentrations of the extracts, 200mg/mL, 100mg/mL, 50mg/mL, and 25mg/mL, were added to each of the wells, and each concentration was poured into the wells labeled against it. The other well was filled with Ketoconazole (20ug/mL) as a positive control. All plates were incubated at room temperature for 48-72 hours, and the zone of inhibition around each concentration was measured in millimeters using a ruler (Rabi, 2020).

Minimum Inhibitory Concentration (MIC)

The MIC values of the methanol, ethanol, and aqueous extract were determined against the test isolates using the tube dilution assay. This was done by adding 2ml of PDA broth into seven test tubes. 2mL of extract was added to the first test tube and mixed. Then 2mL of the contents from tube one was removed and aseptically added to tube two, followed by mixing. The process was repeated up to tube numbered six, but no plant extract was added to tube numbered seven, as it was kept as a positive growth control. All seven test tubes

were then inoculated with a drop of the overnight culture of the test isolates. Another set of tubes was prepared in the same way, but no culture was added to them as a negative control. The MICs were regarded as the lowest concentration that inhibited visible growth of the test organisms after incubation at room temperature for 48 -72 hours (Rabi, 2020).

Minimum Fungicidal Concentration (MFC)

The MFC was determined by sub-culturing from each of the test tubes in the MIC that showed the absence of turbidity and streak on the PDA plate. The plates were incubated at 25 °C for 24hours. After incubation, the minimum concentration at which no visible growth was observed is considered the MFC (Bashir *et al.*, 2024).

RESULTS

Percentage Yield of *L. inermis* Plant Extracts

The percentage yield of the L. inermis extracts, as presented in Table 1, showed that methanol extracts have the highest yields with 23.18%. This is followed by ethanol extracts with a percentage yield of 14.37%. The lowest yields obtained among the three solvents used in the extraction were 7.13% against aqueous as the extraction solvent.

Table 1: Percentage Yield of *L. inermis* Plant Extracts

S/N	Solvent	Percentage yield
1	Ethanol	14.37
2	Aqueous	7.13
3	Methanol	23.18

Phytochemical Constituents of *L. inermis* Extracts

The results of the phytochemical analysis of *L. inermis* extracts revealed the presence of saponin, terpenoids, flavonoids, alkaloids, steroids, triterpenes, and phenolic Compounds. The results also showed that aqueous extracts contained only saponins, flavonoids, alkaloids, and steroids. However, the methanol extracts contained all the tested phytochemical compounds except glycosides, steroids, phenols, and anthraquinones as described in Table 2.

Table 2: Phytochemical Constituents of *L. inermis* Extracts

S/N	Phytochemical	Ethanol extracts	Aqueous extract	Methanol extracts
1.	Saponins	+	+	+
2.	Tannins	-	-	+
3.	Terpenoids	+	-	+
4.	Flavonoids	+	+	+
5.	Alkaloids	+	+	+
6.	Glycosides	-	-	-
7.	Steroids	+	+	-
8.	Phenols	-	-	-
9.	Triterpenes	+	-	+
10.	Anthraquinones	-	-	-
11.	Phenolic compounds	+	-	+

Antifungal Activity of the Methanol Leaf Extracts of *L. inermis*

The antifungal activity of the methanol leaf extracts of *L. inermis* revealed that the extracts have more antifungal activity at 200mg/mL of the extracts. The extracts showed the highest zone of inhibition of 22mm at 200mg/mL against *A. flavus*, followed by *C. albicans* with a 19mm zone of inhibition. *A. niger* was found to have no activity at a concentration of 50mg/mL and 25mg/mL, and hence, only the well diameter (6mm) was recorded. The extracts were also found to show antifungal activity against *C. albicans* with 19mm, 15mm, 14mm, and 8mm at 200mg/mL, 200mg/mL, 50mg/mL, and 25mg/mL, respectively, as revealed in Figure 1.

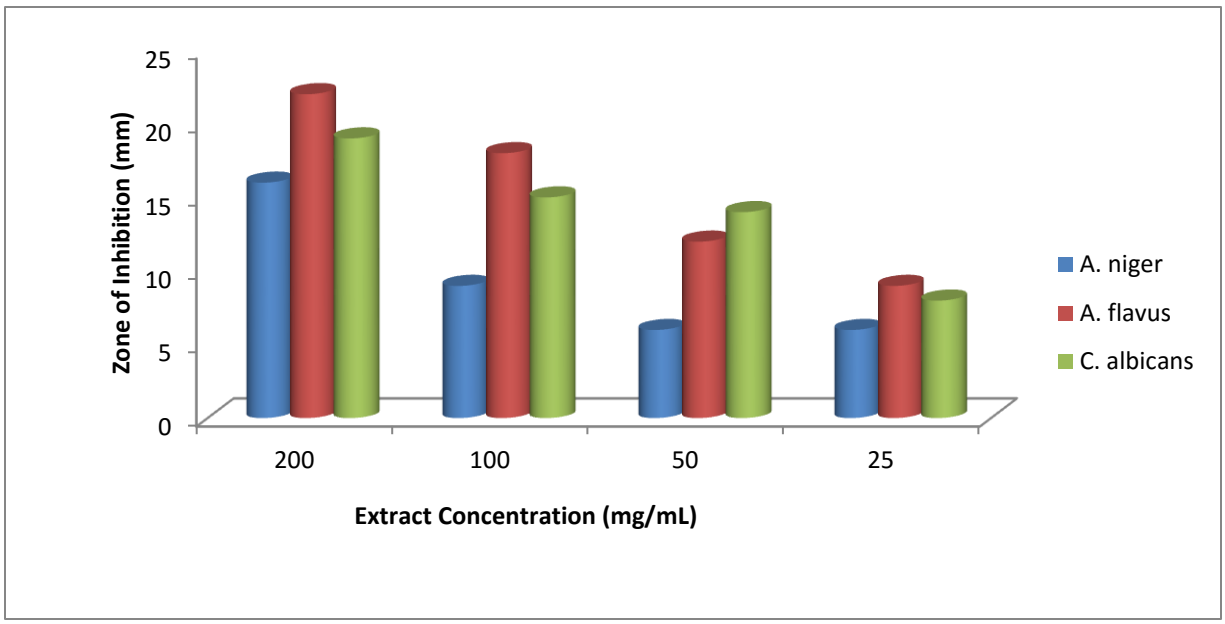

Figure 1: Antifungal Activity of the Methanol Leaf Extracts of *L. inermis* in Millimeters (mm)

Antifungal Activity of the Ethanol Leaf Extracts of *L. inermis*

The results of the antifungal activity of the ethanol extracts of *L. inermis* are reported in Figure 2, and the results revealed that ethanol extracts were more effective against *C. albicans*, with a highest zone of inhibition of 22mm and 18mm at 200mg/mL and 199mg/mL, respectively. 15mm, 13.2mm zones of inhibition were obtained at 200mg/mL and 100mg/mL against *A. niger*. However, *A. flavus* showed no activity at 50mg/mL and 25mg/mL, but it showed 14mm and 9mm zones of inhibition at 200mg/mL and 100mg/mL, respectively.

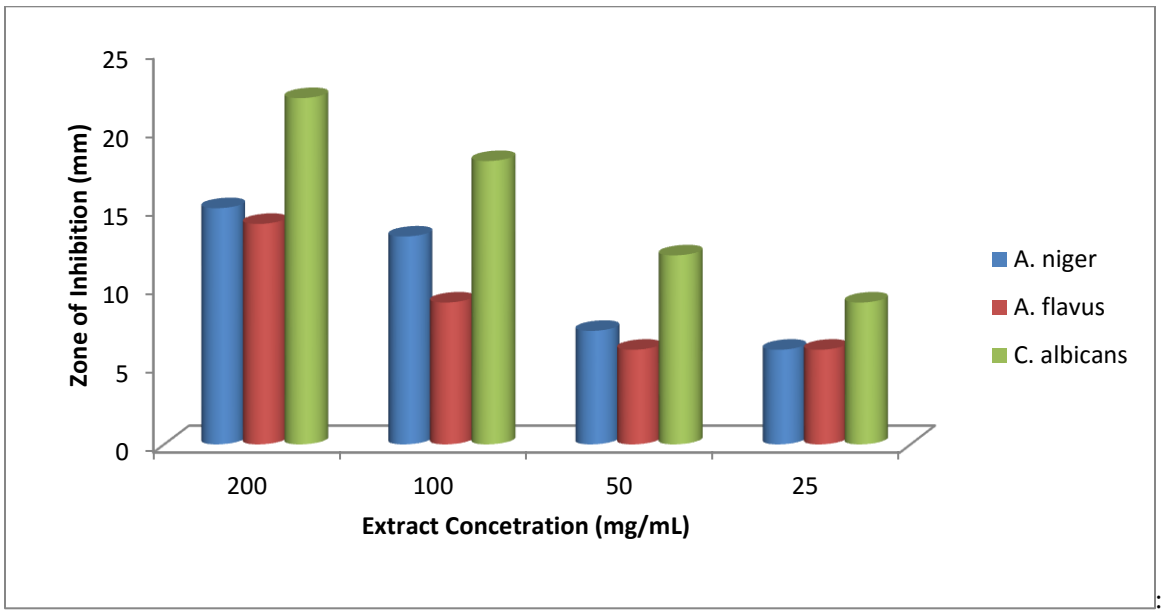

Figure 2: Antifungal activity of the Ethanol Leaf Extracts of *L. inermis* in Millimeters (mm)

Antifungal Activity of the Aqueous Leaf Extracts of *L. inermis*

Aqueous extracts of *L. inermis* also showed a wide range of activity against the test organisms. The result depicted that the highest zone of inhibition of 18mm against *A. niger*, 16mm against *A. flavus*, both at 200mg/mL of the extracts. None of the extracts showed any sign of activity against all the isolates at 25mg/mL. Furthermore, at 50mg/mL, the extracts showed activity against *C. albicans*, only with an 8mm zone of inhibition. At 100mg/mL, 12mm, 10mm, and 8mm were reported against *A. niger*, *C. albicans*, and *A. flavus*, respectively, as shown in Figure 3.

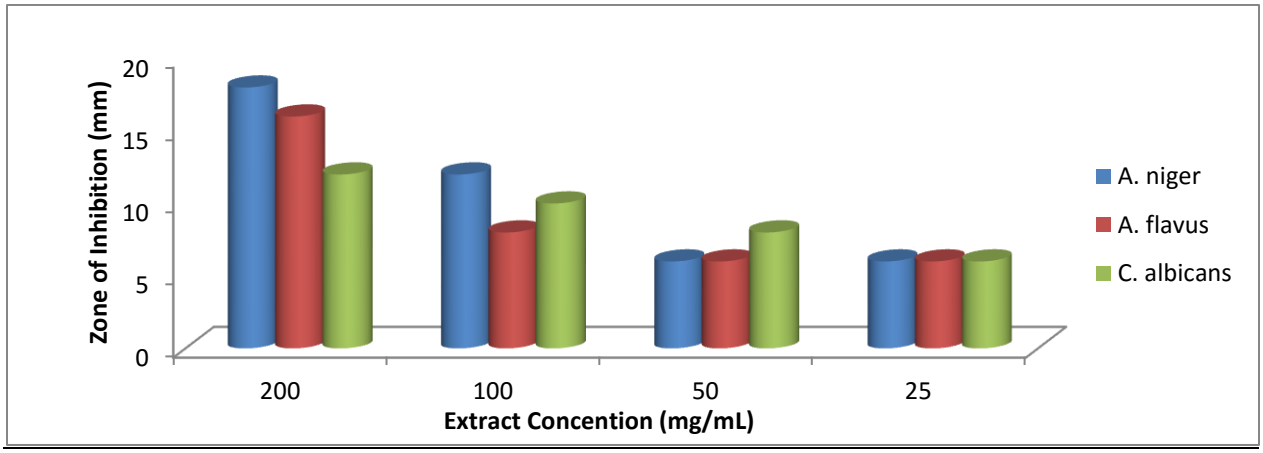

Figure 3: Antifungal Activity of the Aqueous Leaf Extracts of *L. inermis* in Millimeters (mm)

Minimum Inhibitory Concentration (MIC) of *L. inermis* Extracts

Table 3 describes the results of the MIC of the *L. inermis* extracts. The results revealed that *A. flavus* can be inhibited by methanol and ethanol extracts at a concentration of 100mg/mL. Still, aqueous extracts were shown to inhibit *A. flavus* even at 50mg/mL. *A. niger* was inhibited with aqueous extracts at 50mg/mL, but it requires a concentration of ethanol extracts that is >100mg/mL to be inhibited. *C. albicans* was inhibited at 25mg/mL, 50mg/mL, and 100mg/mL of aqueous, methanol, and ethanol extracts, respectively.

Table 3: Minimum Inhibitory Concentration of *L. inermis* Extracts

Test Organisms	Methanol Extracts (mg/mL)	Ethanol Extracts (mg/mL)	Aqueous Extract (mg/mL)
A. flavus	100	100	50
A. niger	50	>100	50
C. albicans	50	100	25

Minimum Fungicidal Concentration (MFC) of *L. inermis* Extracts

The results of the MFC of the *L. inermis* extracts indicated that methanol extracts have an MFC of 100mg/mL against *A. flavus* and *A. niger*, while 50mg/mL against *C. albicans*. Both *A. flavus* and *A. niger* require a concentration of ethanol extracts that >100mg/mL to be destroyed and eliminated. The aqueous extracts showed MFC of 100mg/mL against *A. flavus*, 50mg/mL against *A. niger*, and *C. albicans* as described in Table 4.

Table 4: Minimum Fungicidal Concentration (MFC) of *L. inermis* Extracts

Test Organisms	Methanol Extracts (mg/mL)	Ethanol Extracts (mg/mL)	Aqueous Extract (mg/mL)
A. flavus	100	>100	100
A. niger	100	>100	50
C. albicans	50	100	50

DISCUSSION

Although both ethanol, water, and methanol are polar solvents, the percentage of the *L. inermis* was obtained to be higher in methanol extracts. This finding is in contrast to the findings of El Bergadi *et al*. (2015), who reported that the percentage yield of *L. inermis* was highest in ethanolic extracts. The highest percentage yield observed in methanol extracts indicated that the chemical components are more soluble in methanol extracts than in water and ethanol extracts.

More phytochemical compounds were extracted in the methanol extracts. This explains why the percentage yield was higher in methanol extracts. However, saponins, flavonoids, and alkaloids were extracted in all the different extracts. Iliyasu *et al*. (2025) reported the presence of alkaloids, tannins, quinones, saponins, flavonoids, glycosides, and phytosterols in all three extracts, that is, ethanol, methanol, and aqueous. Variation in bioactive compounds in henna leaves can be related to factors like leaf maturity, climatic conditions, extraction method, solvents used in extraction, as well as season of harvest and storage conditions (Tiwari *et al*., 2011; Neeraj *et al*., 2019; Rabi, 2020).

The methanol extracts were more active against all the test isolates at a concentration of 200mg/mL, and *A. flavus* was inhibited more by the *L. inermis* extracts than *A. niger* and *C. albicans*. The antifungal activity of the ethanol extracts depends on the test isolates. It was observed that the extracts were more effective or active against *C. albicans* at all the tested concentrations. Hassan *et al*. (2022) reported that *L. inermis* leaf extracts have activity against *C. albicans.* The aqueous extracts were also found to be more effective at higher concentrations, and it was observed to be more active against *A. niger*. Lower minimum fungistatic concentration was observed against aqueous extracts of the *L. inermis* against all the isolates. The minimum fungicidal concentration of the *L. inermis* leaf extracts was found to range from 50mg/mL to >100mg/mL. Similarly, another study by Singh *et al*. (2013) found that the aqueous extract of *Lawsonia inermis* showed antifungal activity against several fungal strains, including *Aspergillus niger* and *Candida albicans*.

CONCLUSION

In this study, methanol was found to be the most effective solvent for extracting the leaves of *L. inermis.* Also, more phytochemical compounds, including tannins and terpenoids, were detected in methanol extracts than in ethanol and aqueous extracts. The extracts were observed to have antifungal activity against *A. niger, A. flavus,* and *C. albicans*. Therefore, the ability of the extracts to inhibit the growth of the tested fungal species is an indication of the broad-spectrum antimicrobial potential of *L. inermis*, which makes it a potent source of antifungal drugs.

RECOMMENDATION

1. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC-MS), or Nuclear Magnetic Resonance (NMR) should be employed to isolate, identify, and characterize the specific bioactive compounds responsible for antifungal activity. This could contribute to the development of novel antifungal agents.
2. Pharmaceutical formulations (e.g., creams, ointments, sprays) incorporating *Lawsonia inermis* extract could be developed and tested for clinical use.

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