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Original Article

Comparative Antifungal Study of Methanolic Extracts of *Senna auriculata* and *Lawsonia inermis*

Shanmugapriya Ekambaram[1,*], Saravanan Devarajan[1], Seetharaman Rathinam[1], Vanitha Rani Nagasubramanian[2], Padmavathy Jagannathan[3]

[1]Department of Pharmaceutical Chemistry, Tagore College of Pharmacy, Chennai, Tamil Nadu, INDIA.
[2]Department of Pharmacy Practice, Tagore College of Pharmacy, Chennai, Tamil Nadu, INDIA.
[3]Department of Pharmaceutics, Tagore College of Pharmacy, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Background: Fungal infections pose a serious threat to human health. Infections commonly known as mycoses, particularly those caused by Candida pose a remarkable challenge, especially in immunosuppressed patients. These fungal infections are often treated by using topical or systemic antifungal agents. They mainly act by interrupting integrity of fungal cell membrane and inhibiting ergosterol synthesis. Nowadays many phytoconstituents are found to provide potential antifungal agents. The phytochemicals present in the plants *Senna auriculata* and *Lawsonia inermis* have promising antifungal properties. **Materials and Methods:** This study assessed the antifungal activity of methanolic extracts of *Senna auriculata* and *Lawsonia inermis* against *Candida albicans* using Infrared (IR) spectroscopy, preliminary phytochemical screening and antifungal testing by disc diffusion method using fluconazole discs (25 µg/disc) as positive control. **Results:** Both extracts demonstrated concentration-dependent antifungal activity against *Candida albicans* by producing a 9 mm inhibition zone at 2000 µg/mL. Also, at 4000 µg/mL, *Senna auriculata* and *Lawsonia inermis* demonstrated inhibition zones of 12 mm and 13 mm respectively. **Conclusion:** These findings indicate that both plant extracts possess significant antifungal properties, with *Lawsonia inermis* showing slightly greater antifungal activity at higher concentrations.

Keywords: Antifungal, Candida species, Disc diffusion method, Henna, Senna, Zone of inhibition.

Correspondence:
Dr. Shanmugapriya Ekambaram
Department of Pharmaceutical Chemistry, Tagore College of Pharmacy, Rathinamangalam, Chennai-6000127, Tamil Nadu, INDIA.
Email: priyasenthil01@gmail.com

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INTRODUCTION

Fungi are parasitic eukaryotic microorganisms which possess a cell wall and lack the ability to photosynthesize. Medically, fungi represent an important group of microbial pathogens, responsible for several potentially fatal diseases in humans. They commonly infect the dermis and deeper layers of the skin and most fungal infections can cause itching, blisters, swelling, and severe scaling on the dermis, epidermis or stratum corneum. It has been reported that *Candida* species colonize the skin and mucous membranes without causing any damage under normal conditions. However, factors like increased moisture, heat and weakened local or systemic immune defenses can create an ideal environment for their proliferation and pathogenicity (Nurdin *et al*., 2021). These infections may remain superficial, by affecting the epidermis or mucous membranes, or become systemic by entering the bloodstream and spreading to internal organs.

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Collection and authentication of plants material

Senna auriculata (Common name: Senna; Family: Fabaceae) and *Lawsonia inermis* (Common name: Henna; Family: Lythraceae) are commonly used in the folk medicine in Tamil Nadu (Jangid and Begum, 2022). The plant materials were collected from local areas of Chennai during the optimal time of rainy and early winter season and evaluated for antimycotic activity (Al-Snafi, 2019). The collected plant materials were authenticated at the Department of Botany, Madras Christian College, Chennai.

Cleaning and drying of plant material

The aerial parts of the plants were cleaned and washed with tap water to remove dirt and contaminants. The leaves were then separated and dried under shade until they were completely moisture-free (Ben *et al*., 2011). The dried leaves were finely powdered using a blender and stored in a clean airtight container for further analysis.

Despite the individual antifungal efficacy of *Lawsonia inermis* and *Senna auriculata* being studied, there has been no direct comparative study of their methanolic leaf extracts employing the same extraction techniques, phytochemical analysis, FTIR characterization, and antifungal testing against *Candida albicans*.

Thus, the current research aimed to undertake a comparative assessment of the phytochemical constituents and antifungal properties of these two traditionally employed medicinal plants under standardized experimental conditions to identify the comparatively more promising extract for future antifungal drug formulation.

MATERIALS AND METHODS

Extraction of Plant Material

The extraction process was done by Maceration with agitation method (Abubakar AR & Haque M (2020); World Health Organization, 2011). 30 g of leaf powder sample of Henna and Senna were taken in two different conical flasks, and 150 mL of methanol (analytical grade) was added in both the conical flasks and kept for 72 hr at 25°C temperature. The entire mixture of both the samples was filtered and concentrated to dryness by evaporating on a vacuum evaporator. The extracted crude sample was kept in airtight glass container and stored at 4°C until use. Percentage yield of extracts was calculated for the extracts (Table 1) using the following formula:

$$\text{Percentage yield} = \frac{\text{weight of concentrated crude extract (g)}}{\text{weight of dried crushed plant sample used(g)}} \times 100$$

Preliminary Phytochemical analysis

The preliminary phytochemical screening of the methanolic extracts of *Senna auriculata* and *Lawsonia inermis* was carried out using standard qualitative methods to identify the presence of major phytoconstituents, including flavonoids, tannins, saponins, alkaloids, glycosides, carbohydrates, and proteins (Evans, 2009; Harborne, 1998).

Infrared (IR) Spectroscopy Analysis of PLANT Extract

The dried crude extracts were mixed with KBr (Potassium Bromide) and pressed into pellets for Fourier-Transform Infrared (FTIR) spectroscopy analysis. The FTIR spectra were recorded in the 4000-400 cm^{-1} range, and the absorption peaks corresponding to various functional groups were analyzed and interpreted (Arockia *et al.,* 2015; Bhalodia, 2012).

Evaluation of Antifungal Activity

Preparation of control plate

The control plates were prepared for each extract. *Candida albicans* (ATCC strain) was used as the test organism. The inoculum was standardized to 0.5 McFarland turbidity. The targeted microorganisms were cultured in Potato dextrose broth and incubated for 24 hr. The petri dishes containing Potato Dextrose Agar (PDA) medium were cultured with diluted fungal strain.

Preparation of test concentrations of the extracts

A stock solution of 10 mg/mL was prepared by taking 100 mg) of each extract in 10 mL of sterile distilled water. From this solution, appropriate dilutions were made to obtain concentrations of 1000 µg/mL, 2000 µg/mL and 4000 µg/mL.

Evaluation of anti-fungal activity by Disc diffusion method

The antifungal activity was evaluated by disc diffusion method (Kavita, 2024). Sterile Whatman No.1 filter paper discs (6 mm) were impregnated with 20 µL of each extract solution to obtain final extract loadings of 20 µg/disc, 40 µg/disc and 80 µg/disc (corresponding to stock solutions of 1000 µg/mL, 2000 µg/mL and 4000 µg/mL). The impregnated discs were air-dried under aseptic conditions before being placed on inoculated Potato Dextrose Agar plates. Commercial fluconazole discs (25 µg/disc) served as the positive control, and sterile distilled water was the negative control. The inoculated plates were incubated at 28°C for 24 hr and the zone of inhibition was measured and expressed in millimetres (Akwongo *et al.,* 2024).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC of methanolic extracts of *Senna auriculata* and *Lawsonia inermis* against *Candida albicans* was determined by the broth microdilution method as per the guidelines of Clinical and Laboratory Standards Institute (CLSI) (M27-A3) with slight modifications. Each extract was serially diluted in sterile Potato Dextrose Broth to give a range of concentrations. Each well was inoculated with one standardized suspension of fungus equivalent to 0.5 McFarland standard and incubated at 35°C for 24-48 hr. The MIC was defined as the lowest concentration of the extract at which no visible growth of the fungus was observed. Samples from wells that did not show visible growth were plated onto Potato Dextrose Agar plates for determination of the Minimum Fungicidal Concentration (MFC). Plates were incubated for 24-48 hr. The minimum concentration that showed no visible growth of fungal colonies was recorded as MFC indicating complete fungicidal activity.

Statistical Analysis

Statistical analysis was performed using IBM SPSS statistics Version 23.0. Results are expressed as mean±standard deviation of three independent experiments. Comparisons among groups were performed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test. A p-value<0.05 was considered statistically significant.

RESULTS

As presented in Table 2, the percentage yield of methanolic extracts of *Lawsonia inermis* produced a higher extraction yield (17.36%) than *Senna auriculata* (10.14%), indicating a greater recovery of methanol-soluble constituents under identical extraction conditions. The preliminary phytochemical screening depicted in Table 3 revealed that the phytochemical profiles of the two plants were largely comparable. The methanolic extracts of both plants contained alkaloids, carbohydrates, cardiac glycosides, flavonoids, proteins, resins, saponins and tannins, whereas starch, steroids and triterpenoids were absent. The FTIR analysis (Tables 4 and 5; Figures 1 and 2) confirmed the presence of several functional groups including hydroxyl (O-H), carbonyl (C=O), aromatic (C=C), amine (C-N), carboxylic acid (C-O), alkane (C-H), and ester functional groups in both extracts. The variation in their chemical composition was indicated by the minor differences in peak positions between the two medicinal plants.

The antifungal susceptibility test showed that *C. albicans* was susceptible to *Senna auriculata* and *Lawsonia inermis* by having a clear zone of inhibition. Figures 3 and 4 show the growth inhibitory effect of standard drug and *Senna auriculata* and *Lawsonia inermis* at 1000 µg/mL, 2000 µg/mL and 4000 µg/mL. Table 6 explains the antifungal activity of the methanolic extracts against *Candida albicans*. No inhibition was observed with either extract at 1000 µg/mL. At 2000 µg/mL, both *Senna auriculata* and *Lawsonia inermis* produced comparable inhibition zones of approximately 9 mm. At 4000 µg/mL, the inhibition zone increased to 12.2±0.5 mm for *Senna auriculata* and 13.0±0.6 mm for *Lawsonia inermis*, demonstrating concentration-dependent antifungal activity. Fluconazole (25 µg/disc) produced the largest inhibition zone (20.4±0.2 mm). Statistical analysis showed significant inhibition at 2000 µg/mL ($p<0.05$) and highly significant inhibition at 4000 µg/mL ($p<0.01$) compared with the negative control.

The MIC and MFC values of the extracts are presented in Table 7. *Lawsonia inermis* exhibited lower MIC and MFC values than *Senna auriculata*, indicating comparatively greater antifungal potency.

DISCUSSION

The present study evaluated the antifungal activity of two commonly used medicinal plants, *Senna auriculata* and *Lawsonia inermis* against *Candida albicans* through preliminary phytochemical analysis, Fourier-Transform Infrared (FTIR) spectral studies and antifungal evaluation. The phytochemical analysis revealed that both methanolic extracts contained various phytoconstituents like alkaloids, carbohydrates, glycosides, flavonoids, proteins, resins, saponins, and tannins attributing to different biological activities.

Methanolic extracts of *Senna auriculata* and *Lawsonia inermis* showed concentration-dependent antifungal activity against *Candida albicans* by disc diffusion assay. The observed antifungal activity may be due to the presence of these phytoconstituents such as flavonoids, tannins and phenolic compounds which are known to inhibit fungal enzymes, disrupt cell membrane integrity

Table 1: Percentage yield of methanolic extracts.

Plant material	Solvent system	Weight of Plant powder (g)	Weight of Dried extract (g)	Percentage yield (%)
Lawsonia inermis	Methanol	30	5.209	17.36
Senna auriculata	Methanol	30	3.043	10.14

Table 2: Preliminary Phytochemical Screening.

Sl. No.	Phytoconstituents	Methanolic extract	
		Senna auriculata	*Lawsonia inermis*
1.	Alkaloids	(+)	(+)
2.	Carbohydrate	(+)	(+)
3.	Cardiac Glycoside	(+)	(+)
4.	Flavonoids	(+)	(+)
5.	Proteins	(+)	(+)
6.	Resins	(+)	(+)
7.	Saponins	(+)	(+)
8.	Starch	(-)	(-)
9.	Steroids	(-)	(-)
10.	Tannins	(+)	(+)
11.	Triterpenoids	(-)	(-)

Table 3: FTIR spectral analysis of methanolic extract of *Senna auriculata*.

Observed peak (cm^{-1})	Functional group	Interpretation
3307.13	O-H stretching	Phenols/alcohols
2924.12	C-H stretching	Alkanes
2853.05	C-H stretching	Alkyl groups
1721.81	C=O stretching	Carbonyl compounds
1613.22	C=O stretching	Ketones
1505.28, 1453.36	C=C stretching	Aromatic rings
1355.12	C-H bending	Methyl groups
1233.79	C-N stretching	Amines
1203.80	C-O stretching	Carboxylic acids
1155.21, 1109.33	C-O stretching	Ethers/esters
1009.29	C-O-R stretching	Esters
892.64	P-O-C stretching	Phosphate groups
834.35	C-H bending	Aromatic compounds

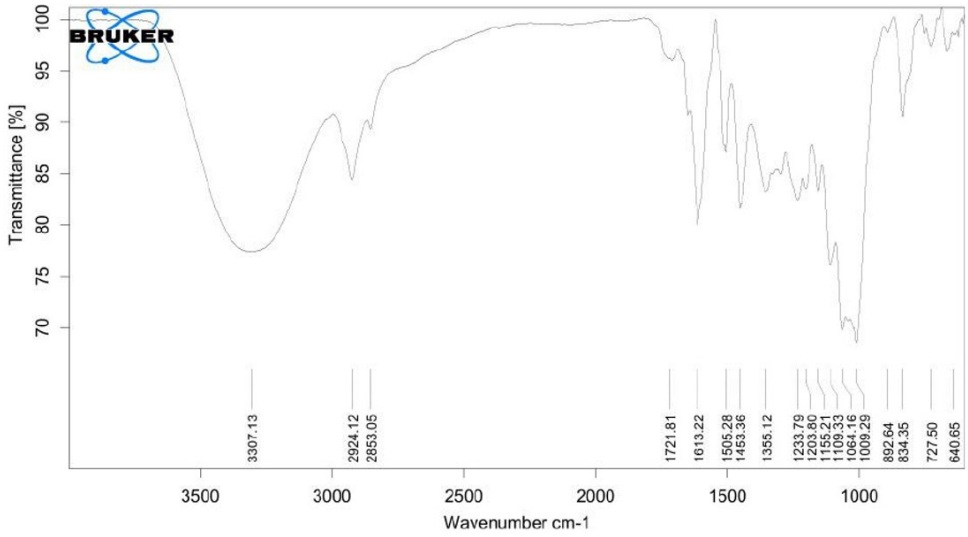


Figure 1: IR spectra of methanolic extract of *Senna auriculata*.

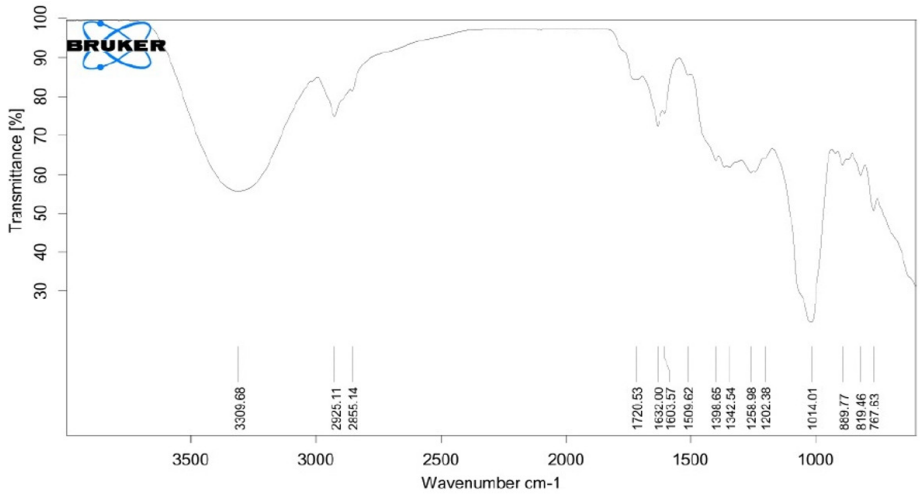


Figure 2: IR spectra of methanolic extract of *Lawsonia inermis*.

and interfere with fungal growth and metabolism (Clinical and Laboratory Standards Institute., 2008).

Lawsonia inermis showed a marginally greater zone of inhibition at the highest concentration tested, but the difference between the two extracts was relatively small and suggestive of similar antifungal efficacy under the experimental conditions employed. The FTIR analysis also confirmed the presence of functional groups associated with phenolic compounds, aromatic rings, alcohols, carbonyl compounds and amines which have been reported to contribute to antimicrobial activity (Doss, 2009).

Table 4: FTIR spectral analysis of *Lawsonia inermis*.

Observed peak (cm^{-1})	Functional group	Interpretation
3309.68	O-H stretching	Phenols/alcohols
2925.11	C-H stretching	Alkanes
2855.14	C-H stretching	Alkyl groups
1720.53	C=O stretching	Carbonyl compounds
1632.00, 1603.57	C=C/C=O stretching	Aromatic/ketones
1509.62	C=C stretching	Aromatic rings
1398.65	O-H bending	Phenolic compounds
1342.54	O-H bending	Hydroxyl groups
1258.98	C-N stretching	Amines
1202.38	C-O stretching	Carboxylic acids
1014.01	C-O-R stretching	Esters
889.77	P-O-C stretching	Phosphates
819.46	C-H bending	Aromatic compounds

In contrast to earlier research that examined these medicinal plants individually, the current study offers a direct comparative evaluation of the methanolic extracts of *Senna auriculata* and *Lawsonia inermis* sourced from the same geographical area and subjected to analogous extraction and laboratory conditions (Manoj *et al.,* 2024). The phytochemical analysis, FTIR characterization, and biological assessment under controlled experimental conditions provide valuable initial insights into their relative antifungal efficacy and help identify the more promising extract for future phytopharmaceutical development. These findings also establish a scientific foundation for the isolation and characterization of bioactive antifungal agents.

Consistent with the previous reports, the present study demonstrated the antifungal potential of *Lawsonia inermis*, particularly against *Candida* species and dermatophytes, which has led to the development of several herbal formulations, including nail lacquers for the management of onychomycosis caused by *Trichophyton rubrum* and *Trichophyton interdigitale*. Likewise, studies have also reported that *Senna auriculata* possesses antimicrobial activity attributable to its rich phytochemical composition (Bello, 2020 and Balasankar *et al.,* 2013). The comparable antifungal activity observed in the present study further supports its potential as a medicinal plant with antifungal properties. Future studies should evaluate these extracts against multiple clinically relevant fungal species, including other *Candida* species and filamentous fungi, to determine their broader antifungal spectrum.

Table 5: Antifungal activity of methanolic extracts against *Candida albicans*.

Treatment	Concentration (µg/mL)	Zone of inhibition (mm, Mean±SD, *n*=3)	Statistical significance
Negative control	-	0.0±0.0	-
Senna auriculata	1000	0.0±0.0	ns ($p>0.05$)
	2000	9.4±0.6	$p<0.05$
	4000	12.2±0.5	$p<0.01$
Lawsonia inermis	1000	0.0±0.0	ns ($p>0.05$)
	2000	9.0±0.4	$p<0.05$
	4000	13.0±0.6	$p<0.01$
Positive control (Fluconazole)	25 µg/disc	20.4±0.2	-

Data are expressed as Mean±SD ($n=3$). One-way ANOVA followed by Tukey's post-hoc test. Differences were considered statistically significant at $^{*}p<0.05$; $^{**}p<0.01$; ns=not significant.

Table 6: MIC and MFC determination.

Treatment	MIC (mg/mL)	MFC (mg/mL)	MFC/MIC ratio	Interpretation
/Senna auriculata	2.0	4.0	2	Fungicidal
Lawsonia inermis	1.0	2.0	2	Fungicidal
Fluconazole	0.016	0.032	2	Fungicidal

MIC = Minimum inhibitory concentration; MFC=Minimum fungicidal concentration. MFC/MIC ≤ 4 indicates fungicidal activity.

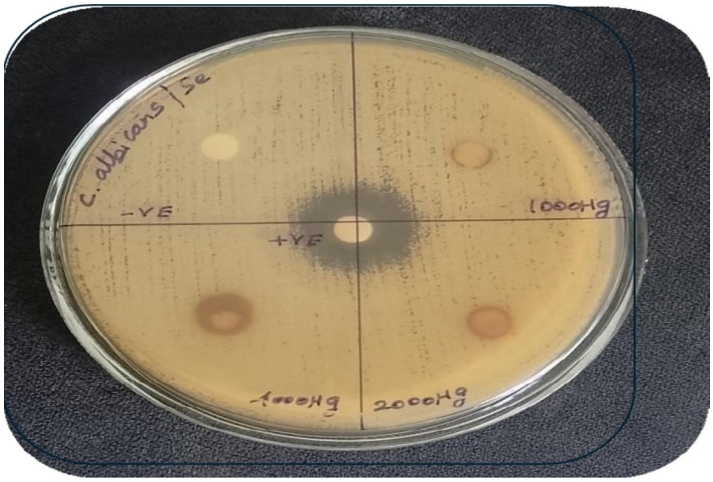


Figure 3: Disc diffusion assay showing Anti-fungal activity of *Senna auriculata*.

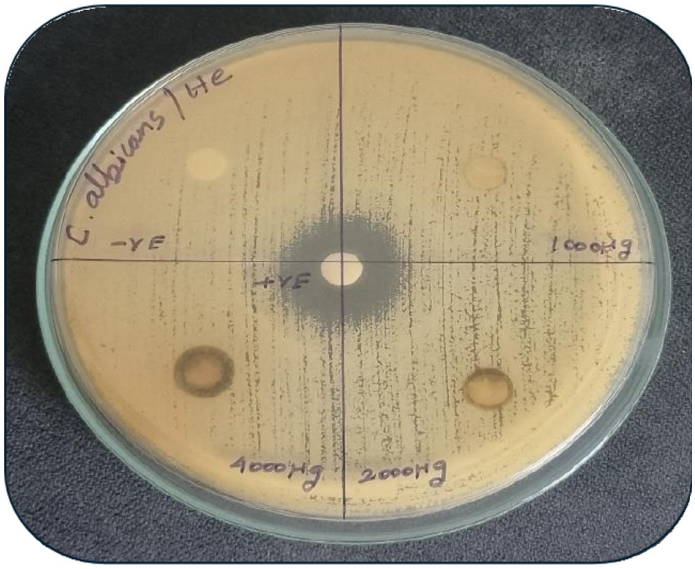


Figure 4: Disc diffusion assay showing Anti-fungal activity of *Lawsonia inermis*.

Table 7: Zone of inhibition of *Senna auriculata* and *Lawsonia inermis*.

Extract	Concentration (µg/mL)	Zone of inhibition (mm)
Senna auriculata	1000	-
	2000	9±0.57
	4000	12±0.86
Lawsonia inermis	1000	-
	2000	9±0.68
	4000	13±0.94

The MIC and MFC values further confirmed the disc diffusion results. *Lawsonia inermis* showed lower MIC and MFC values than *Senna auriculata,* indicating relatively higher antifungal activity. The MFC/MIC ratio of 2 for both extracts indicates that the extracts are fungicidal under the experimental conditions.

In the present study, *Senna auriculata* demonstrated antifungal activity comparable to that of *Lawsonia inermis*, suggesting that both plants may serve as promising, cost-effective sources of natural antifungal agents. However, this study has some limitations such as evaluation against a single fungal species and lack of *in vivo* validation. MIC and MFC values were determined but further studies such as isolation of active phytoconstituents, elucidation of antifungal mechanisms, toxicity evaluation and *in vivo* efficacy studies are required before clinical application.

CONCLUSION

The study concludes that *Lawsonia inermis* and *Senna auriculata* both have strong antifungal activity against *Candida albicans*. Both plants showed similar antifungal potential, however *Lawsonia inermis* exhibited a higher activity at higher concentrations.

Together, the phytochemical, FTIR, disc diffusion and MIC/MFC results support their potential as sources of bioactive compounds for the development of new antifungal agents. Further studies involving isolation of active compounds, elucidation of antifungal mechanisms and evaluation of toxicity and *in vivo* evaluation are recommended before clinical application.

ABBREVIATIONS

IR: Infrared; **FTIR:** Fourier-transform infrared spectroscopy; **PDA:** Potato Dextrose Agar; **CLSI:** Clinical and Laboratory Standards Institute; **MIC:** Minimum inhibitory concentration; **MFC:** Minimum fungicidal concentration; **ANOVA:** One-way analysis of variance; **SD:** Standard deviation; **ns:** Not significant; **KBr:** Potassium bromide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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