Journal of Applied and Natural Science
15(4), 1636 - 1645 (2023)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Evaluation of *Boerhavia diffusa* and *Eichhornia crassipes* plant extracts *in vitro* as potential antifungal agents against human pathogenic fungi *Candida albician* and *Candida tropicalis* : A comparative study

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Article Info
https://doi.org/10.31018/jans.v15i4.5010
Received: August 10, 2023
Revised: December 4, 2023
Accepted: December 9, 2023

How to Cite
Kumar, M. *et al.* (2023). Evaluation of *Boerhavia diffusa* and *Eichhornia crassipes* plant extracts *in vitro* as potential antifungal agents against human pathogenic fungi *Candida albician* and *Candida tropicalis :* A comparative study. *Journal of Applied and Natural Science*, 15(4), 1636 - 1645. https://doi.org/10.31018/jans.v15i4.5010

Abstract
Plant extracts are used to make herbal remedies with no side effects and little expense. During the COVID-19 pandemic, fungal species responsible for mucormycosis were found resistant to a variety of antifungals, including flucytosine, ketoconazole, fluconazole, voriconazole, itraconazole and echinocandins, due to their variable susceptibility. Amphotericin B is widely used as an antifungal agent due to its high inhibition capacity against various fungi. The present study aimed to compare the antifungal potential of Amphotericin B and herbal extract *in vitro*. The experiment was designed to measure zones of inhibition with the help of well-diffusion method. Four solvents, *viz.* methanol, chloroform, hexane and distilled water, were used to extract plant extract. The efficiency of plant extracts was found to be low compared to Amphotericin B (1.4mm). Chloroform extract of *Boerhavia diffusa* was found antifungal against *Candida albician* and *C. tropicalis* (0.45mm). Methanol and hexane extract of *Eichhornia crassipes* showed higher antifungal activity (1.35mm) and (1.75mm), respectively. The plant extracts also showed significant antifungal activity against *C. tropicalis*, revealing its potential to be used as a natural antifungal agent (1.1mm). Additionally, the findings showed that the chloroform and methanol extracts of *B. diffusa* and *E. crassipes* were also efficient against *C. albician* and *C. tropicalis*. The findings provide important insights about using plant extracts as a potential alternative to conventional antifungal agents.

Keywords: Amphotericin B, Antifungal, *Boerhavia diffusa*, *Eichhornia crassipes,* Weeds

INTRODUCTION

Humans have used plants as food and medication for a very long time. Due to their lack of side effects, herbal medicines are used by nearly 80% of people in developing nations (Agarwal *et al.,* 2019). Scientists are currently exploring medicinal plants because they are helpful as antimicrobial agents (Rafiq *et al.,* 2021). Herbal plants are also widely used to treat skin infections caused by pathogenic fungi. Common weeds such as *Achyranthes aspera, Chenopodium, album, Calotripis procera, Ocimum sanctum, Boerhavia diffusa*, *Parthenium hysterophorus*, *Tinspora cordifolia, Withania somnifera, Cannabis sativa* and *Colocynthis citrullus* are

widely used as medicinal plants.

Pathogenic fungi attack both plants and humans, which cause diseases. Fungal infections are becoming more common due to advancements in transplant surgery and the use of immunosuppressive medications, antibiotics and antimycotics in some cases. One pathogenic fungus frequently isolated from individuals with nosocomial infections is *C. albicans* and *C. tropicalis* (Sardi *et al.,* 2013). A forty per cent mortality rate is frequently associated with invasive candidiasis. The limited supply of antifungal medicines is a problem for various fungi-related diseases. Echinocandins, polyenes, and azoles are the three primary groups of antifungal drugs. Amphotericin B (AmB), a polyene developed in the 1950s that is one of the most effective antifungal drugs, is widely utilised in therapeutic settings (Oura *et al.,* 1955, Yan *et al.,* 2022). Amphotericin B has higher activity as compared to other antifungal creams and injections. However, in certain cases, the severe adverse effects of AmB, including fever, nausea, vomiting, rigours, and nephrotoxicity, lead to the early conclusion of the course of treatment (Laniado-Laborín *et al.,* 2009). Despite its propensity for toxicity, amphotericin B has long been regarded as the gold standard for treating invasive fungal infections. (Zareshahrabadi *et al,* 2022). Amphotericin B is as positive standard used to treatment of fungal infections. Amphotericin B was widely used in lockdown when coronavirus was declared pandemic. The rapid disease progression and angioinvasive nature of mucormycosis infection are additional factors that contribute to its severity (Prakash and Chakrabarti, 2021; Priya *et al.,* 2020; Alekseyev *et al.,* 2021). To prevent extensive angioinvasion, effective treatment should be started as soon as possible (Priya *et al.,* 2020; Garg *et al.,* 2021). They have variable susceptibility to itraconazole, making them resistant to a number of antifungals like flucytosine, ketoconazole, fluconazole, voriconazole, and echinocandins (Borman, *et al.,* 2017; Priya *et al.,* 2020; Garg *et al.,* 2021).

Fungal infections are a significant concern for public health due to their high morbidity and mortality rates, especially in immunocompromised individuals **(**Singh *et al.,* 2023). Traditional medicine has been used to treat fungal infections for many years, and natural products have gained attention as potential sources of antifungal agents **(**Singh *et al.,* 2020; Saleem *et al.,* 2020; Al Khoury *et al.,* 2021; Vrchotova *et al.,* 2021). However, the emergence of antifungal drug resistance has prompted the search for new antifungal agents from natural sources. Several studies have reported the antifungal activity of herbal plants against various pathogenic fungi (Dhale *et al.,* 2016; Chowdhary *et al.,* 2017; Alam *et al.,* 2019). The present study aimed to compare the antifungal activity of *B. diffusa* and *Eichhornia crassipes* plant extracts *in vitro* to that of Amphotericin B against human pathogenic fungi, *Candida albician* and *C. tropicalis*.

MATERIALS AND METHODS

Plants material

The plant samples were collected in September 2022 while still in their natural habitat from University campus. The collected plants were identified as *B. diffusa* and *E. crassipes* in the Department of Bio-Sciences and Technology, Maharishi Markandeshwar University (MMDU), Mullana, Ambala, Haryana (Singh *et al.,* 2020).

Extract preparation

The entire plants without root parts were collected, washed, shade-dried, and ground in an electric mixer grinder. The powder substance was stored in airtight glass bottles. Further extraction was performed using this bulk powder. In four separate conical flasks, 450 ml of methanol, chloroform, hexane and distilled water were added. Each conical flask added 50 gm of dried powder before being left at room temperature for 72 hours and shaken in an orbital mixer (Khan *et al.,* 2020). After incubation, the extracts were filtered using muslin cloth and Whatman filter paper. The extracts were permitted to evaporate. The dishes underwent evaporation before being weighed. 5% DMSO (Dimethyl Sulfoxide) was added and whole extract filtrate by syringe filter to purify liquid extract. The bio-efficacy of the extracts was evaluated *in vitro* using the well-agar diffusion technique (Onkar *et al.,* 1995). Fig.1 shows methodology. Amphotericin B powder was purchased from Hi-media Company.

Collection and maintenance of micro-organisms

The cultures *viz. C. albician (P.4071, F.8160) and C. tropicalis (P.4131, P.42680)* were procured from the Department of Microbiology, Postgraduate Institute of Medical Education and Research (*PGIMER*), Chandigarh. An experiment was conducted with human pathogenic fungi (*C. albician* and *C. tropicalis*) at the Department of Medical Microbilogy, MMDU Mullana, Ambala (Haryana). The minimum inhibitory concentration (MIC) was also determined. Fungal strains were maintained in PDA (Potato Dextrose Agar) and subculture on PDA slants.

Antifungal activity assay

To measure zones of inhibition, the punch well method was used (Zanna *et al.,* 2021). The 20 ml of nutritional agar was dispensed into sterilized Petri plates, and the plates were then left to set. Fungi culture spread on solid media. The four wells in the medium were made with a 4 mm cork borer evenly spaced apart. Each well received an injection of around 100 µl of the various extract strengths. For *C. albicans* and *C. tropicalis,* the

Petri dishes were incubated at 37 °C for 24 hours. Amphotericin B, a common antifungal, served as the positive control.

Experimental data on the antifungal activity of two plant extracts and Amphotericin B against two different species of *Candida* fungus were collected. The different solvents used for the *B. diffusa* and *E. crassipes* extracts were methanol, chloroform, hexane and distilled water. Each extract's minimum inhibitory concentration (MIC) was determined against the *C. albicans* and *C. tropicalis*.

Chacterization of plant extract

Plant extract was chacterized by using two techniques: FTIR and UV-visible. Fourier-transform infrared spectroscopy (FTIR) is a valuable analytical technique used in various fields, including chemistry and biology, to study plant extracts containing a variety of chemical components. Functional groups like hydroxyl (-OH), carbonyl (C=O), and numerous other bonds can all be detected by it. Dried powders of various plant material solvent extracts were used. To create a translucent sample disc, 100 mg of KBr pellet and 10 mg of the dried extract powder were combined. Each plant specimen's powdered sample was placed into an FTIR Spectroscope with a scan range of 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1} (Renuka 2023). UV-visible spectroscopy was used to identify the presence of the peak of the functional groups. A small amount of the material was placed in a cuvette and scanned at a wavelength between 200 and 800 na nometers using distilled water as the reference. Shimad zu UV-2600. A spectrophotometer was used to produce UV-visible spectrums.

Statistical analysis

In order to draw appropriate inferences from the above experimental data set, necessary statistical analysis was performed in two phases. In the first one, each variable parameter (Extract, Fungi and Solvent) has been analysed through one-way ANOVA (with the help of Minitab Statistical Software), as delineated in the following paragraphs. Required relations shipbuilding were performed by an appropriate Regression Analysis (during the second phase) by simultaneously taking all the variable factors as predictors for response MIC (mm).

RESULTS

The present study observed that both plant extracts of *B. diffusa* and *E. crassipes* had antifungal activity against both *C. albicans* and *C. tropicalis* species (Table 1 and 2). Numerous concentrations, including 100 mg/ml, 150 mg/ml, 200 mg/ml, and 250 mg/ml, have been used with this approach. 250mg/ml concentration inhibited the growth of both species of *Candida*. Stock prepared 1mg/ml concentration. Sterile water was applied as control and standard Amphotericin B.

The MIC values for the extracts ranged from 0 to 1.75 mm. *E. crassipes* extract shows the most potent antifungal activity against both *Candida* species compared to *B. diffusa* extract. The plant extract obtained using distilled water as a solvent showed relatively weak or no antifungal activity against both *Candida* species. *B. diffusa* distilled water extract showing alost no antifungal activity against *C. albician,* indicated that the observed antifungal activity was specific to the plant extracts. Fig. 8 and Fig.9 showed zone of inhibition of both plant extracts (*B. diffusa* and *E. crassipes*) against *C. albician* and *C. tropicalis*.

FT-IR Spectrum analysis

The FT-IR spectrum revealed that N-H, O-H, C=C, C-H, C-O, and CH3 functional groups were present (Fig. 1. A and B). The detection of biomolecule composition using FT-IR Spectroscopy has been shown to be accurate and sensitive.

E. crassipes having functional group such as N-H, 3298.77 cm^{-1}, O-H, 2922.58 cm^{-1}, C=C 1628.72 cm^{-1}, C -H, 1406.43 cm^{-1}, C-O 1318.08 cm^{-1}, CH_3 1054.47 cm^{-1} of and *B. diffusa* N-H 3256.02 cm^{-1}, O-H 2924.01 cm^{-1}, C=C 1734.17 cm^{-1}, C-H, 1399.31 cm^{-1}, C-O 1319.51 $cm^{-1,}$ CH_3 1040.22 cm^{-1} respectively (Pakkirisamy *et al*., 2017).

UV-Visible spectroscopy

The highest absorption was discovered with a broad peak between 209-300 nm, due to the surface Plasmon resonance effect in *E. crassipes* (Fig. 2A) and 220-300 nm of *B. diffusa* (Fig. 2B), respectively. The Plasmon resonance effects are an initial UV-visible absorption range at 200–500 nm (Vijay *et al.,* 2023). Similar UV visible absorbance for plant extracts was between 200 and 8 00 nm. Their peak was observed at 200 to 350 nm wavelength with 3.7 absorbance (Fig.2).

Fig. 3 illustrates each extract's means, standard deviations and 95% confidence intervals. In *Boerhavia diffusa* extract, the mean value for this extract is 0.2750 with a standard deviation of 0.1574. The 95% confidence interval is (0.1124, 0.4376), which indicates that it can be 95% confident that the true population mean lies within this interval.

In control, the mean value for this extract is 1.400 with a standard deviation of 0.000. The 95% confidence interval is 1.201, 1.599, which also indicates 95% confidence that the true population mean lies within this interval. The standard deviation of 0.000 suggests that there is no variability in the data for this extract, which could result from having a small sample size or a very homogeneous group of observations.

In E. crassipes, the mean value for this extract was 0.694, with a standard deviation of 0.629. The 95%

Table 1. Antifungal activity of *Boerhavia diffusa* against human pathogenic fungi in various solvents. MIC (Minimal inhibitory concentration) at 250mg/ml concentration

Plant Extract	Fungi	Methanol Extract MIC(mm)	Chloroform Extract MIC(mm)	Hexane Extract MIC(mm)	Distilled Water Extract MIC(mm)	Amphotericin B (+ve control) (mm)
Boerhavia diffusa	*Candida albician*	0.45±0.13	0.45±0.13	0.25±0.08	0	1.4±0.46
	Candida tropicalis	0.2 ±0.01	0.45±0.01	0.25±0.03	0.15±0.006	1.4±0.46

Table 2. Antifungal activity of *Eichhornia crassipes* against human pathogenic fungi in various solvents. MIC (Minimal inhibitory concentration) at 250mg/ml concentration

Plant Extract	Fungi	Methanol Extract MIC(mm)	Hexane Extract MIC(mm)	Distilled Water Extract MIC(mm)	Amphotericin B (+ve control)(mm)
Echhornia crassipes	*Candida albician*	1.35±0.45	1.75±0.003	0.6±0.21	1.4±0.46
	Candida tropicalis	0.1±0.04	0.65±0.03	1.1±0.36	1.4±0.46

confidence interval is (0.531, 0.856), which also indicated 95% confidence that the true population mean lies within this interval. The larger standard deviation for this extract compared to *B. diffusa* suggested more variability in the data for this extract.

Fig. 4 refers to more details. In summary, the results of the ANOVA indicated that there was no significant difference between the means of the two groups (fungi), and the means table provided further information on the mean and variability of each group.

In the present case, Dunnett multiple comparisons test for Fungi's is ignored, since Control level for Dunnett's is not one of the factor levels. The line connecting the means is almost horizontal, and the confidence interval range remains common in both fungi (around 0.59 to 0.83). This further emphasised the almost similar behaviour of both the Fungi and realized that there is not sufficient statistical proof to reject the null hypothesis.

It can be concluded that almost all the solvents behaved similarly during the entire experimentation. Fig. 5 delineated the mean, standard deviation, and 95% confidence interval for each solvent in the present set of data. In chloroform, **t**he mean value for this solvent was 0.519, with a standard deviation of 0.563. The 95% confidence interval was (0.225, 0.812). For distilled water, the mean value for this solvent was 0.697, with a standard deviation of 0.568. The 95% confidence interval was (0.403, 0.991). This solvent was used as a control.

In hexane Solvent, **t**he mean value for this solvent was 0.894, with a standard deviation of 0.627. The 95% confidence interval was 0.600, 1.187. The mean value for methanol solvent was 0.744, with a standard deviation of 0.590. The 95% confidence interval was 0.450, 1.037.

The line connecting the means of each solvent seems to have less slope due to the overlapping of MIC (Minimal inhibitory concentration) interval ranges (from 0.22 to 1.03). So statistically, it was found with 95% confidence that changing solvents did not significantly or substantially affect MIC (mm).

To further verify the simultaneous effect of all the factors, an appropriate Multi-Regression Analysis indicated that the heat map had been plotted for response MIC (mm) while simultaneously varying three predictors (Extract, Fungi's and Solvents). A heat map can be a useful visualization tool for understanding the relationship between the predictor and response variables (Fig. 6).

A colour scale (from blue to red) represents the relative strength of the relationship between the predictor variable and the response variables. The resulting heatmap shows the relationship between the predictor and response variables. Based on the experimental data (Table 1), the extract and solvent variables had a stronger relationship with the MIC value than the Fungi's variable. The methanol extract of *B. diffusa* showed sufficient antifungal activity against both strains of *Candida*, with MIC values of 0.45 mm for all three replicates, while the hexane extract of the same plant had lower but still significant activity against both strains. In contrast, the chloroform and distilled water extracts of the same plant had no activity against either strain of *Candida*. Similarly, the *E. crassipes* extracts showed different activity levels depending on the solvent and strain. The methanol extract of *E. crassipes* had moderate activity against both strains of *Candida*, while the

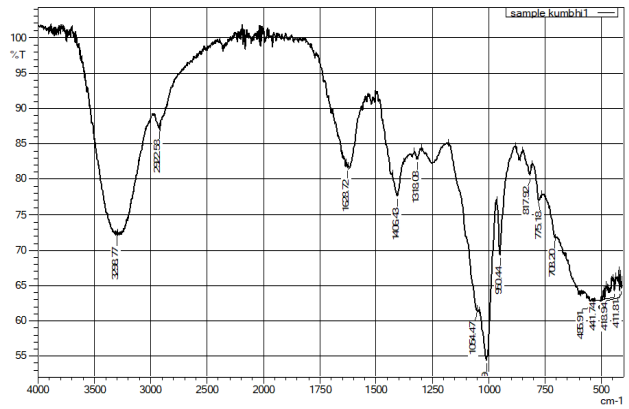

Fig. 1A. *FT-IR analysis of Eichhornia crassipes*

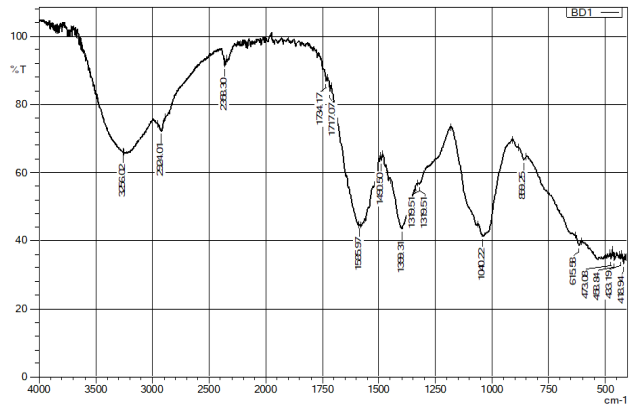

Fig.1B. *FTIR analysis of Boeheriva diffusa*

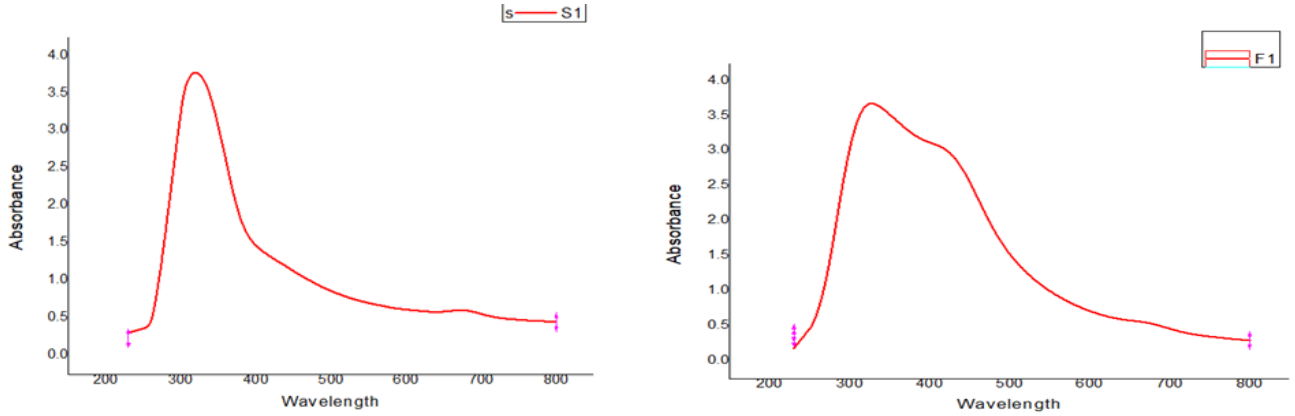

Fig. 2 (A). *UV-Visible of Eichhornia crassipes* (B). *UV-Visible of and Boerhavia diffusa.*

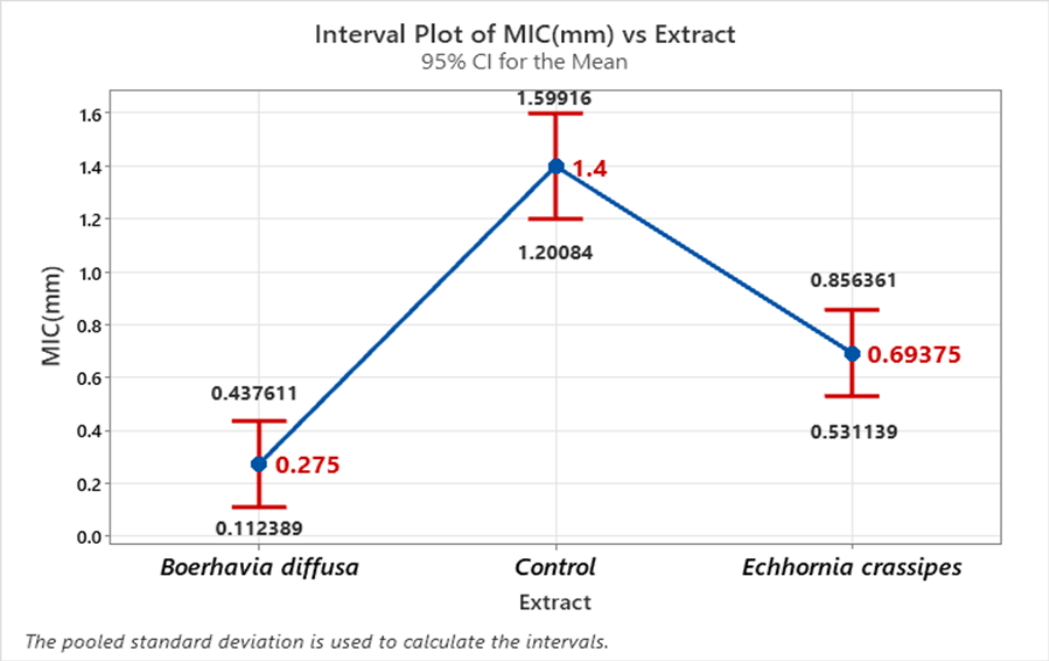

Fig. 3. *Significant effect of various plant extracts*

chloroform extract had no activity. However, the hexane extract of *E. crassipes* showed strong activity against both strains of *Candida*, with MIC values of 1.75 mm and 0.65 mm for *C. albicans* and *C. tropicalis*, respectively. The distilled water extract of *E. crassipes* had some activity against *C. albicans* (MIC=0.6 mm) but none against *C. tropicalis*. In brief, the heat map can be useful for visually exploring the relationships between the predictor variables and the response variable. It can help identify patterns and trends in the data that may not be immediately apparent when looking at the raw numbers. The maximum antifungal activity was

observed with plant extract *E. crassipes* with *C. albican* as a fungus taken along in Hexane solvent (see the dark red pattern in map Fig. 6).

At last, the regression equation has been formulated, which can be used to predict the value of the dependent variable (MIC) based on the values of the independent variables (Extract, Fungi, Solvent, and their combinations). The statistical model for MIC in mm is depicted below:

MIC = 0.3711 + 0.938Extract_Control - 0.231Extract_Eichhornia crassipes + 0.158Fungi_Candida tropicalis - 0.497Solvent_Distilled Water + 0.006Solvent_Hexane Solvent + 0.156Solvent_Methanol Solvent + 0.025ExtractFungi_Control Candida tropicalis - 0.438ExtractFungi_Eichhornia crassipes Candida tropicalis + 0.375Extract Solvent_Control Distilled Water + 0.200Extract Solvent_Control Hexane Solvent + 0.125Extract Solvent_Control Methanol Solvent + 1.225Extract Solvent_Eichhornia crassipes Distilled Water + 1.400Extract Solvent_Eichhornia crassipes Hexane Solvent + 0.850Extract Solvent_Eichhornia crassipes Methanol Solvent + 0.244Fungi Solvent_Candida tropicalis Distilled Water - 0.413Fungi Solvent_Candida tropicalis Hexane Solvent - 0.563Fungi X Solvent_Candida tropicalis Methanol Solvent

The equation has multiple terms, each with a corre-

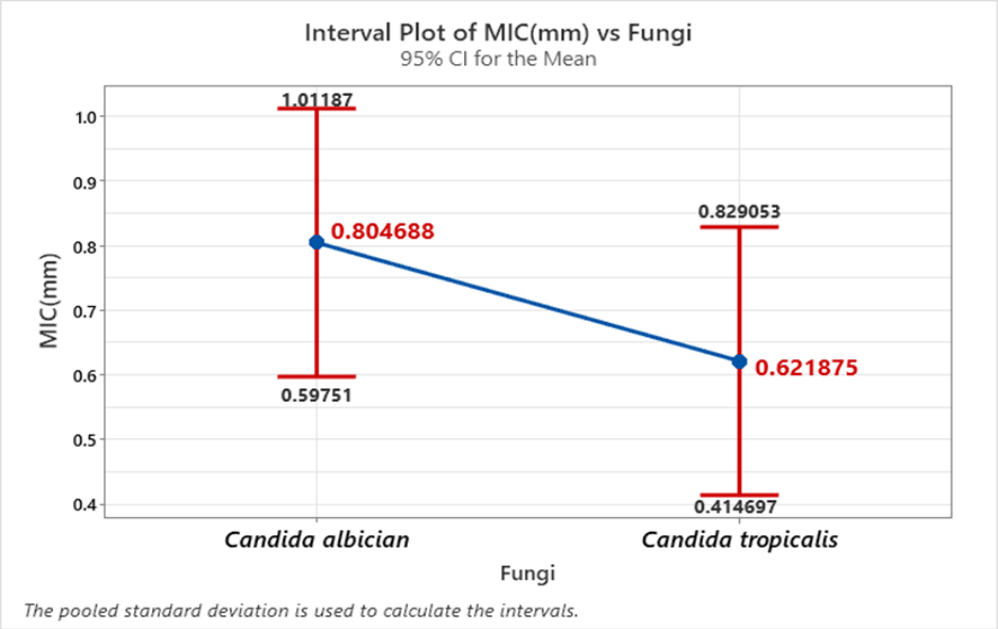

Fig. 4. *Non-significant effect of fungi*

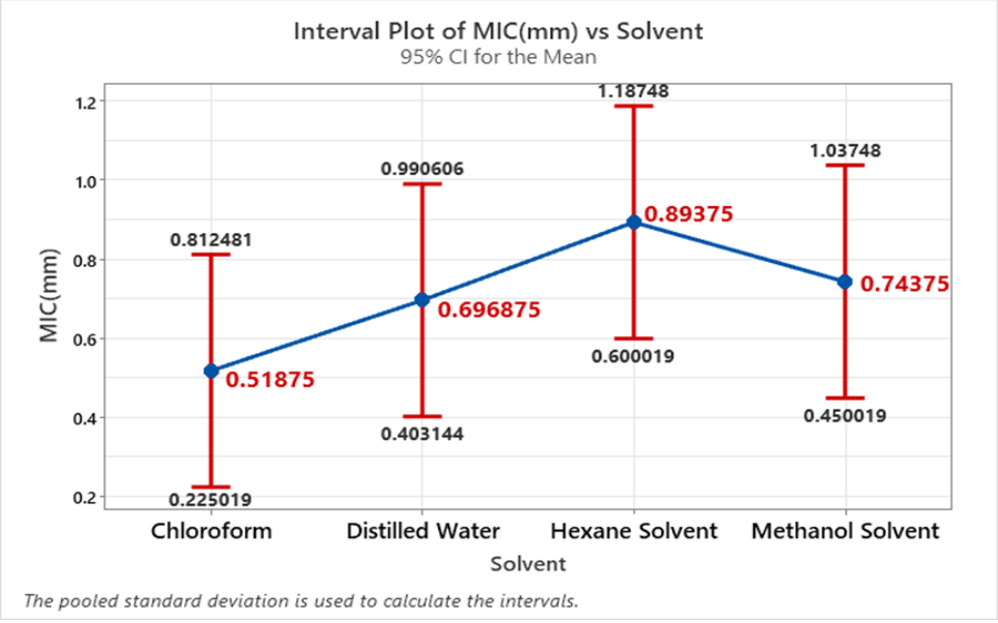

Fig. 5. *Non-significant effect of solvents*

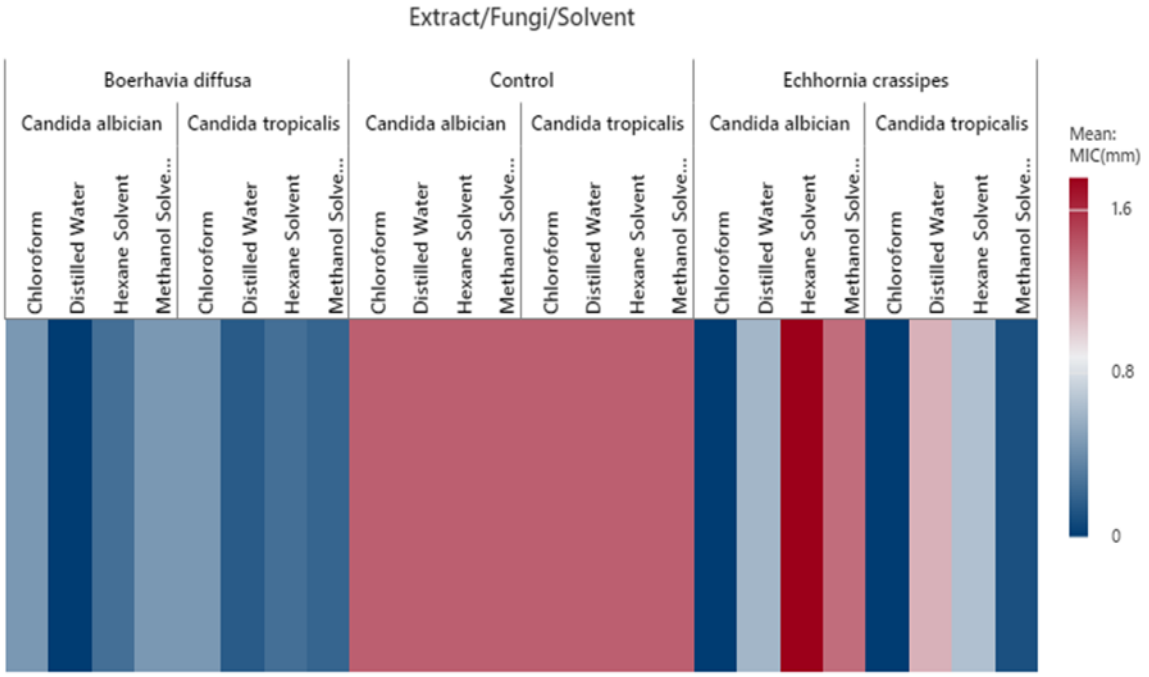

Fig. 6. *Heat Map for MIC (mm) ; color-coding to indicate the relative strength of a relationship between variables*

sponding coefficient representing the change in the dependent variable for a unit change in the independent variable, holding all other independent variables constant. The coefficient of 0.938 for extract (Control) indicates that a unit increase in the value of extract control was associated with an increase of 0.938 in the value of MIC, holding all other independent variables constant. Similarly, the coefficient of -0.231 for extract *E. crassipes* indicated that a unit increase in the value of extract *E. crassipes* was associated with a decrease of 0.231 in the value of MIC(Minimal inhibitory concentration), holding all other independent variables constant.

It is important to note that the regression equation assumes a linear relationship between the independent and dependent variables. It also assumes that the relationship between the independent variables and the dependent variable is additive, meaning that the effect of one independent variable on the dependent variable is independent of the values of the other independent variables. This modelled equation will be quite helpful to perform future research in this field. It can provide logical values of MIC (without lab experimentation) in various settings of Extract, Fungi's and Solvents. In Fig.7, different concentrations of 100mg/ml, 150mg/ml, 200mg/ml and 250mg/ml showed no output in starting three concentrations but 250mg/ml output to inhibit the growth of fungi.

Weeds extract helped control fungus growth. Methanol, chloroform, and hexane significantly inhibited the growth of pathogenic fungi such as *C. albician* and *C. tropicalis*. Two weeds (*B. diffusa* and *E. crassipes*) extracts (100 µL of 250mg/ml concentration) applied on human pathogenic fungi *in vitro* showed antifungal activity. Chloroform extract (0.5±0.4mm) of *B. diffusa* showed the best results against *C. albician* and *C. tropicalis* and the lowest results were with the extracts in distilled water (0.1±0.2mm). Another plant, *E. crassipes* methanol extract (1.2±1.5mm), activity was high and distilled water extract (0.7±0.5mm) showed low/nil activity. As comparable Amphotericin B activity as a positive control to check the difference between herbal extract and Amphotericin, Amphotericin B showed an inhibition zone of 1.2±1.6mm.

DISCUSSION

The fungal infections, either as a secondary or pre-existing state, aggravated the mortality during the COVID-19 pandemic. Besides increasing epidemiological awareness, pathogenesis and management of these fungal infections, fungal diseases using medicinal remedies, including Amphotericin B have been reported (Singh *et al.,* 2023). The study of medicinal plants as potential antifungal agents against human pathogenic fungi is a promising area of research. Ethnomedicinal plants offer a sustainable and natural alternative to synthetic drugs (Kumar *et al*. 2016; Singh *et al.,* 2020). Antifungal activity testing of weeds continues to be an area of interest (Singh *et al.,* 2016; Soni, *et al.,* 2018) . However, there are few studies on the use of weeds' antifungal properties including *E. crassipes* and *B. diffusa* , and there is little information on their use as antifungal agents (Garg *et al*., 2011; Joshi *et al.,* 2020). A

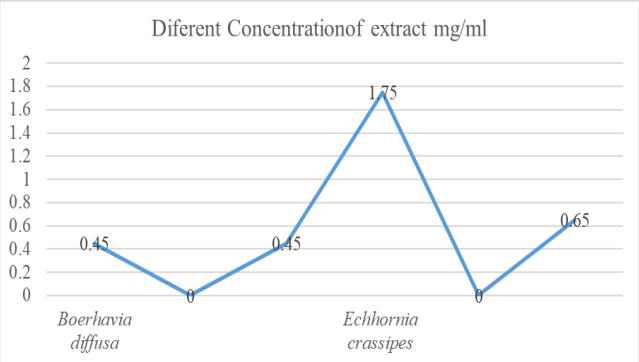

Fig. 7 *Representing different concentrations of plant extract against human pathogenic fungi*

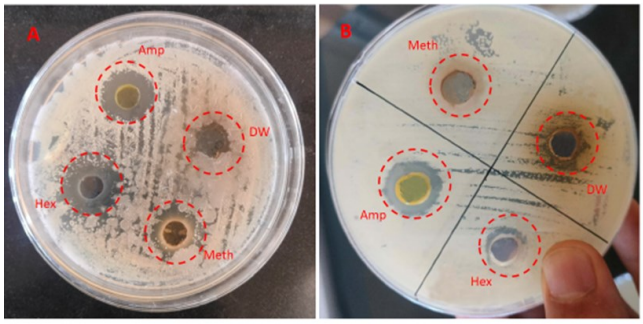

Fig. 8. *Antifungal activity of Eichhornia crassipes against- (A) Candida albician and (B) Candida tropicalis; Amp (Amphotericin), He (Hexane), DW (Distilled water), Meth (Methanol)*

wide spectrum of antifungal activity has been demonstrated by the solvent extract of *Calotropis gigantean, Withania somnifera, Ocimum sanctum and Tinospoa cordifoia* against human pathogenic fungus *Candida albician* and *Candida tropicalis* (Mawardi *et al.*, 2019; Veeraswamy *et al.*, 2022).

The plants contain various bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds, which may contribute to its antifungal properties (Baharuddin *et al.,* 2015; Dinesh *et al.,* 2021). The present study showed that the *B. diffusa* and *E. crassipe's* chloroform and methanol extract are very effective against *C. albician* and *C. tropicalis*.

The present study analysed both plant extracts with the help of FTIR and UV-Visible techniques. FTIR revealed the functional groups of the active components found in the extract of *Curcuma caesia* (Pakkirisamy *et al*., 2017). UV-visible spectrums provide the highest absorption with a broad peak between 209-300 nm, in *E. crassipes* and 220-300 nm in *B. diffusa*. The surface Plasmon resonance effect can be observed in an initial UV-visible absorption range of 200–500 nm (Vijay et al., 2023).

The present study found the UV-visible absorbance for plant extract was between 200 and 800 nm. The peak was observed at 200 to 350 nm wavelength and 3.7 absorbance. This knowledge is also useful for quality assurance and verification of plant extracts' chemical make-up. The findings provide further evidence of the potential antifungal activity of *B. diffusa* and *E. crassipes* against human pathogenic fungi. However, the lower activity observed compared to the conventional antifungal drug Amphotericin B suggests that further research is required to explore the potential of these plant extracts as an alternative or complementary therapy for fungal infections.

Conclusion

The fungal infections cause a serious threat to human beings and economic loss. Besides increasing epidemiological awareness, pathogenesis and management of these infections, the present study tried to treat human

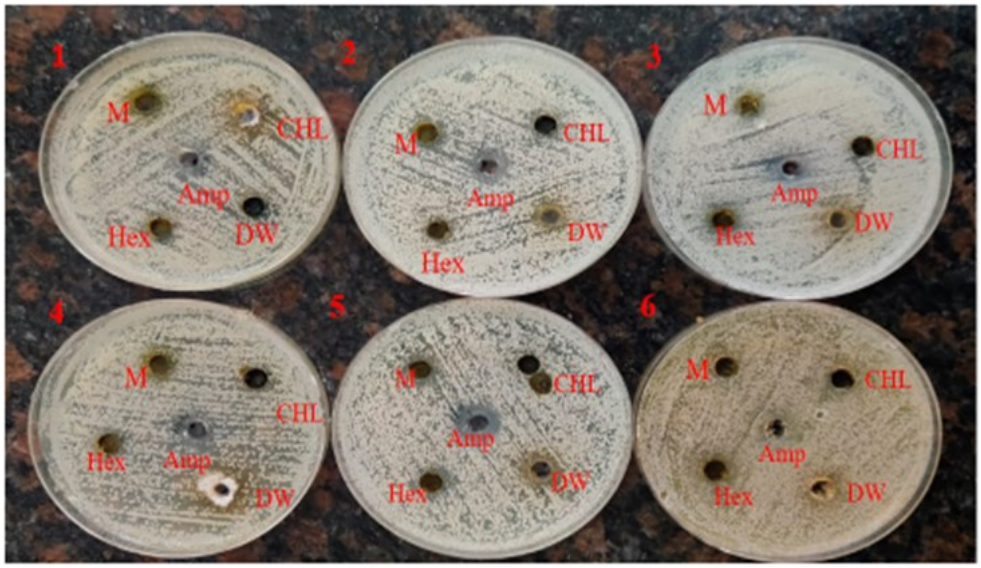

Fig. 9. *Representing (1-3) Candida albician and (4-6), Candida tropicalis 100mg/ml, 150mg/ml and 200mg/ml concentration; M (Methenol), Hex(Hexane), DW(Distilled water), CHL(Chloroform) and Amp(Amphotericin B)*

fungal *in vitro* using medicinal remedies, including Amphotericin B. Two weeds' extracts *B. diffusa* and *E. crassipes* applied on human pathogenic fungi, showed antifungal activity. The hexane extract was found more potent than methanol, chloroform, and distilled water to inhibit the growth of pathogenic fungi such as *C. albician* and *C. tropicalis*. This study emphasises the significance of looking into natural sources for creating new antifungal agents, particularly because of the growing fungal resistance to traditional medications. However, further research, including clinical trials and rigorous safety assessments, is needed to fully realize their potential for treating fungal infections in humans.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Shiva Parkash, Professor, Department of Medical Microbiology (PGIMER), Chandigarh, for providing both human pathogenic fungi and Dr. Narinder Kaur, Head of Department of Medical Microbiology, MMDU, Mullana, Ambala (Haryana) for laboratory facilities.

Conflict of interest

The authors declare that they have no conflict of interest.

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