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Antifungal Activity of ethnomedicinal plants *Boerhavia diffusa, Chenopodium album* and *Ziziphus maurtiana* against *Alternaria solani* and *Mucor* Fungi

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ABSTRACT

Many ethnic groups use medicinal plants as a source of medicine to address a wide range of conditions in both domestic animals and people. The effect of solvent extracts was evaluated from whole/aerial plant parts of *Boerhavia diffusa, Chenopodium album* and *Ziziphus maurtiana* on growth of *Alternaria solani* and *Mucor* fungi. Pure culture of the fungus was established and their microscopic characteristics and colony morphology were observed. Furthermore, well diffusion method was used for identification of the extracted fraction showing bioactive potential. Among the four extracts Methanol and Hexane exhibited better activity than other solvents. Furthermore, this study revealed that extracts of *B. diffusa, C. album* and *Z. maurtiana* in hexane and methanol were particularly effective against *A. solani* and *Mucor*. Hexane solvent *B. diffusa* showed 1 cm zone of inhibition against both fungi. As a result, in a hexane extract experiment, *C album* demonstrated a 0.9 cm zone of inhibition against *A. solani* and a 0.4 cm zone of inhibition against *Mucor.*

Key words: Antifungal activity, bioactive compounds, *Boerhavia diffusa, Chenopodium album, Ziziphus maurtiana*

INTRODUCTION

Fungal phytopathogens cause diseases accounting for 20 to 40% of all known plant diseases. The most significant biotic stress that results in significant crop loss is caused by phytopathogens, such as fungus, which produce a variety of illnesses and poisons. The most popular method for preventing a fungal infection is to use fungicides in managing excessive yield or quality loss. Although chemical fungicides prevent and treat fungal infections, but simultaneously these are associated with serious issues that delimit their use in the future. Additionally, through food chain chemical fungicides are well known to cause fatal medical conditions including cancer (Olikh *et al.*, 2023). However, a variety of plants are frequently utilized in traditional medical systems to treat a range of plant ailments. Bioactive components of plants and herbs demonstrated to exhibit excellent antifungal activity against diverse phytopathogens.

Plant extracts are well known for their anti-quorum sensing and anti-biofilm forming properties, which inhibit the growth of microorganisms. Researchers focused on use of herbal extracts as comparable fungicides. Traditionally different parts of medicinal plants, such as roots, leaves, fruits, seeds, or entire plants, have been used to make herbal medicines from ethnomedicinal plants which are anti-inflammatory, anti-diarrheal, anti-oxidant, and anti-bacterial activities (Parveen *et al.*, 2021). Ethnomedicinal plants also have antifungal activity. *Boerhavia diffusa, Chenopodium album* and *Ziziphus maurtiana*

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containing secondary metabolites as flavonoids, tannins, saponins, phenolic groups, alkaloids, and other compounds having antifungal activity against fungus (Shah *et al.*, 2021). A soil-borne plant pathogen called *Alternaria solani* is well-known for causing a number of serious crop diseases and substantial losses in both agriculture and the economy. It has a large host range and a vast geographic distribution. *A. solani* is responsible for rice sheath blight, which can result up to 50% production losses in Asia. *Alternaria sp.* causes early blight diseases, which is responsible for severe loss in crop production. This has been linked in more than 400 different host species for spreading of leaf spots and other diseases (Roy *et al.*, 2019). There could be a huge loss of-fruits and vegetables if the *Alternaria. sp.* infects during postharvest period. Tomatoes, apples, grapes, blueberries, oranges, lemons, mandarins, and olives are naturally infected fruits that have been utilized to produce *Alternaria* mycotoxins. It is an opportunistic pathogen that causes rots, blights, and leaf spots on variety of plant parts (El-Shahir *et al.*, 2022, Sitara, *et al.*, 2023). Several ethnomedicinal plant ranges from small herbaceous *B. diffusa, C.* album (Amranthaceae) plants to tree *Z. maurtiana* (Rhamnaceae) species and their potential bioactive compound are well known in Ayurvedic literature. Thus, in the present study antifungal potential of three different weed species solvent extract against 2 fungal species *viz. A. solani* and *Mucor* fungi was evaluated. The results could be beneficial for the development of an herbal formulation for management of the phytopathogenic fungi.

MATERIALS AND METHODS

Three plant species (*Ziziphus maurtiana, Chenopodium album,* and *Boerhavia diffusa*) were collected in 2022 on the MMDU campus from their natural habitat. Plants were collected and brought to the laboratory and identified. Full grown plants of *Z. maurtiana, C. album,* and *B. diffusa* were collected, dried in the shade, and ground in an electric mixer grinder (Bandiola *et al.*, 2018). Glass bottles with airtight seals were used to keep the powdered samples.

Two fungi were isolated from potato leaf and bread. On the basis of their morphological characteristics and mycelium growth under microscope, Dr. Raj Singh, Professor of Botany; Department of Bio-Sciences and Technology MMDU, Mullana, Ambala, Haryana was identified the fungi *i.e Alternaria solani* and *Mucor* sp. and identified them as *Alternaria solani* and *Mucor* sp.

Whole plants of *B. diffusa* and *C. album* were uprooted and processed for solvent extraction. Both of these plants were collected from the nearby areas of MMDU, Mullana whereas fleshy fruits of *Z. maurtiana* were collected from the Mullana, Lat. 30.265438 and Long. 77.041874. Shade dried and a powdered form of each sample was used for solvent extraction as shown in the Fig. 1 A, B and C. Fig. 1 D. We took 3 solvents (Methanol, Chloroform, Hexane) for extraction. The bulk powder was used for further extraction. For solvent extraction, 350 ml each of methanol, chloroform, hexane, and distilled water were added to four different conical flasks filled with 50 gm of dried powder and agitated in an orbital mixer for 72 hours at room temperature (Khan *et al.*, 2020). After incubation, the extracts were filtered using muslin cloth and Whatman filter paper. The extracts were passed into a neat, crushable product that could be left to evaporate in the pre-weighted used crucibles. To purify the liquid extract, 5% DMSO was added, and the entire extract was filtered using a syringe filter. Air evaporated solvent extracts were equalized to a concentration of 100-500 mg/ml in DMSO to evaluate for the bioactive potential against *A. solani* and *Mucor* fungi. The agar well diffusion method was used to assess the extracts bio-efficacy *in-vitro* condition.

Fig. 1. Selected plants and their extracts (A) *Boerhavia diffusa,* (B) *Chenopodium album* and (C) *Ziziphus maurtiana* and (D) Dfferent solvents viz., methanol, chloroform, hexane and water solvents, respectively, from left to right.

The antifungal activity of crude plant extract was analysed using the agar well diffusion assay. Each sterile Petri dish was filled with PDA media before a single or small number of the pathogen's spores was added to the centre of the plates. In order to create a well with a diameter of 6 mm, the agar was punched six times at equal intervals with a sterile cork borer. Both of the fungi grew well on the standard potato dextrose Agar media. To see the radial growth, well diffusion assay was further used. The wells were filled with a total of 50 µl of crude plant solvent extracts, which were then incubated at 28 °C for 72 hours.

Growth inhibition (%) = 100 x (DC-DT)/DC

Where, DT stands for the diameter of the fungal colony after treatment, and DC for the diameter of the fungal colony under control. The low and higher concentration was chosen for the initial assessment of the bioactive potential of the metabolites.

5 g each of *B. diffusa, C. album,* and *Z. maurtiana* was added in 50 ml solvent for 72 hours, to create crude extracts of methanolic, ethyl acetate, and water. The mixture was vacuum-filtered via filter paper after extraction, and the resultant extract was then kept chilled until being analysed (Pillai *et al.*, 2020). The entire procedure was carried out under low light to prevent phenolic compounds. The two extracts (Methanolic and Distilled water) of *B. diffusa, C. album*, and *Z. maurtiana* were treated to various chemical assays for the detection of various phytocomponents using conventional protocols (Shah *et al.*, 2021).

Saponins test: To perform it, 500 ml of crude extract were combined with 2 ml of distilled water in a test tube, and violently shaken. Few olive oil drops were added. Development of stable foam was a sign that saponins were present.

Flavonoids test: 2 ml of dilute ammonia solution was added to 500 ml crude extract followed by addition of concentrated H_2SO_4. The presence of flavonoids was revealed by the yellow colouring of each extract. Standing faded this yellow colour.

Coumerin test: 500 ml of crude extract received diluted NaOH. The presence of quinines was indicated by blue, green, or red color. To 500 ml of extract, 10% NaOH and chloroform was added to see the yellow colour, indicating the presence of coumerin.

Glycosides test (Keller-Killani test): Two ml of glacial acetic acid containing one drop of ferric chloride solution were added to 500 ml of extract. Addition of 1 ml of concentrated H_2SO_4 denoted the cardinolide's deoxysugar property by interface's brown ring. Below the brown ring, a violet ring may emerge, and in the acetic acid layer, a thin layer of a greenish ring may progressively grow.

Terpenoid test: 500 ml of extract was added to one ml of 0.008 M potassium ferricyanide in a test tube. The presence of blue-black colouring was visible after the addition of 1 ml of 0.1 N HCl and 0.02 M ferric chloride. 1 ml of concentrated H_2SO_4 was carefully added after carefully combining 500 ml of extract with 2 ml of chloroform to create a layer. To demonstrate successful outcomes for terpenoids' presence, a reddish brown coloration of the interface was generated.

RESULTS AND DISCUSSION

Three plant extracts of *B. diffusa, C. album* and *Z. maurtiana* in Methanol, Chloroform, Hexane and distilled water solvents were used to assess antifungal activity against fungi *Alternaria solani* and *Mucor* (Table 1). *B. diffusa* showed the highest antifungal activity against these fungi. The extracts of *C. album* and *Z. maurtiana* also revealed antifungal activity against *A. solani* and *Mucor.* The DMSO was used as negative control and Amphotericin B (antifungal agent) as positive control. Concentration of plants extract 100 mg/ml was used against both fungi with well diffusion method, in which 50 µl extract applied in wells. *B. diffusa* dissolved in methanol had best results against *A. solani* showing (1.0 cm*)* zone of inhibition. While Methanol extract of *B. diffusa* inhibitory against *Mucor* shown 0.7 cm zone of inhibition. It was found that *C. album* chloroform extract showed greater zone of inhibition (1.5 cm) against *A. solani*. Whereas *C. album* methanol extract showed 0.8 cm against *Mucor*. *Z. maurtiana* in Methanol inhibited *A. solani* with a 0.7 cm zone of inhibition. *Z. Mautiana* extract of chloroform and hexane showed 0.7 cm zone of inhibition against *Mucor*. Amphotericin B exhibited 1.2 cm zone as standardization. It may be concluded that, methanol extract has a stronger antifungal activity as comparable to other three solvents.

The phytochemical properties demonstrated

Table 1. Antifungal activity of *Boerhavia diffusa, Chenopodium album* and *Ziziphus maurtiana* against *Alternaria solani* and *Mucor*

Plant	Fungus	Methanol (cm)	Hexane (cm)	Chloroform (cm)	Water (cm)	Amphotericin B (cm)	DMSO (cm)
Boerhavia diffusa	*Alternaria solani*	1.0	1.0	0.9	0.6	0.7	0.6
	Mucor	0.7	0.4	0.2	0.4	0.9	0.8
Chenopodium album	*Alternaria solani*	0.7	0.9	1.5	0.3	1.2	0.8
	Mucor	0.8	0.4	0.5	0.5	0.6	1.0
Ziziphus maurtiana	*Alternaria solani*	0.7	0.6	0.6	0.6	0.9	1.0
	Mucor	0.5	0.7	0.7	0.5	1.0	0.8

that all the three plants could be utilized as a medicine plants. (Table 2). All three plants contained saponin, flavonoids, glycosides, tannins, steroids, quinones, coumerin, and terponoids. The presence and absence of a phytochemical component was analyzed using two solvents (methanol and water). In the methanol solvent of *B. diffusa* (stem) and the water solvent of *Z. maurtiana* (leaf), coumerin and saponin were absent. Glycosides and quinones were absent in *C. album.*

The MIC values for the extracts ranged from 0 to 1.0 cm, with the lowest MIC values indicating the most potent antifungal activity (Figs. 2, 3 and 4). Among the three plant extracts, *B. diffusa* extract showed the most potent antifungal activity against both *A. solani* and *Mucor* species, as evidenced by the lowest MIC values observed for this extract in all tested solvents. The *B. diffusa* extract showed moderate antifungal activity, with lower MIC values observed for the methanol and chloroform solvents. The plant extract obtained using distilled water as a solvent showed relatively weak antifungal activity against both *A. solani* and *Mucor* species, as evidenced by the higher MIC values observed for this extract compared to the other two plant extracts. In order to draw appropriate inferences from above experimental data set, necessary statistical analysis was performed in two phases. In the first one, each variable parameter (extract, fungi and solvent) was analyzed through one-

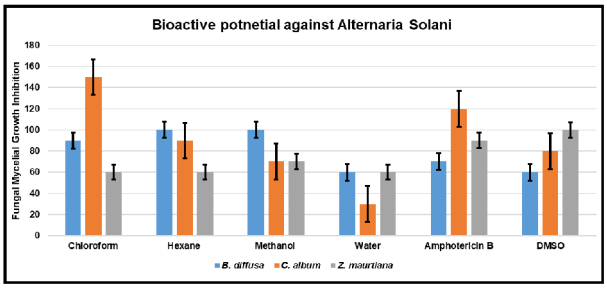

Fig. 2. Bioactive potential of plant extracts against *Alternaria solani.*

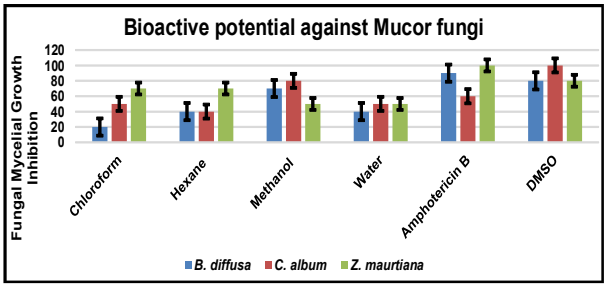

Fig. 3. Bioactive potential of plant extracts against *Mucor.*

way ANOVA (with the help of Minitab Statistical Software). Required relationsship building was performed by regression analysis (during second phase) by taking all the variable factors simultaneously as predictor for response MIC (mm).

The current study revealed the antifungal efficacy of the plant extracts *B. diffusa, C. album* and *Z. maurtiana* against several fungi causing fungal infection. The results made it evident that every plant extract examined had

Table 2. Phytochemical analysis of *Boerhavia diffusa, Chenopodium album* and *Ziziphus maurtiana*

Weeds	Solvent type	Saponin	Flavonoid	Steroids	Tannins	Glycosides	Terponoids	Quinones	Coumerin
B. diffusa (Leaf)	Methanol	+	+	_	+	_	+	_	+
	Water	+	+	+	_	_	+	+	+
B. diffusa (Stem)	Methanol	_	_	_	+	+	_	+	_
	Water	_	_	+	_	+	_	_	_
C. album (Leaf)	Methanol	+	+	+	+	_	+	_	+
	Water	+	+	+	+	_	+	_	+
Z. maurtiana (Leaf)	Methanol	+	+	+	+	+	+	+	_
	Water	_	+	+	+	+	+	+	_

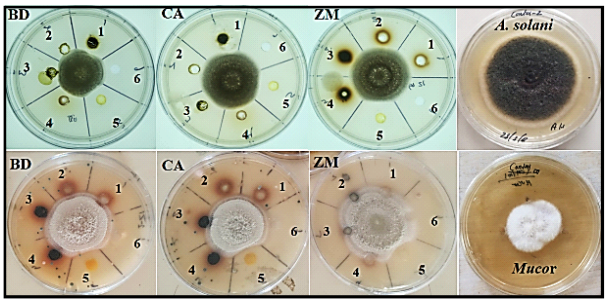

Fig. 4. Antifungal activity of *Boerhavia diffusa* (BD), *Chenopodium album* (CA) and *Ziziphus maurtiana* (ZM) against *Mucor* and *Alternaria solani* 1. Methanol, 2. Hexane, 3. Chloroform, 4. Distilled water, 5. Amphotericin B and 6. DMSO.

antifungal action and inhibited fungi's ability to proliferate mycelially *in vitro* condition. Plant extracts were subjected to phytochemical analysis, which identified components known to have physiological and therapeutic effects. Phytochemicals including phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids were discovered in the plant extracts after analysis. Phytochemical compounds may be the bioactive components, and these plants are proving to be a useful source of bioactive substances with significant medical value. Olikh *et al.* (2023) evaluated antifungal activity of selected medicinal plant extracts against *Alternaria alternata*. An investigation to find the biocontrol potentials of *Burkholderia gladioli* pv. *Agaricicola* plants against tomato-wilt disease caused by *Verticillium dahliae* was carried out; which revealed that bioactive secondary metabolites played a significant role as antifungal agents (El-shahir *et al.*, 2022). The acetone extracts *Psoralea corylifolia* and *Acorus calamus* completely inhibited *Phytophthora infestans* and *Terminalia bellerica, Asparagus racemosus* showed encouraging effect (Rani *et al.*, 2017). There are many similarities in different reports on antifungal activities of plant extracts. The present study also revealed the antifungal activities of *B. diffusa, C. album* and *Z. maurtiana* against *Mucor* as well as *A. solani*. It was revealed that extracts in hexane and methanol were more effective against *A. solani* and *Mucor*. Hexane extract of *B. diffusa* demonstrated 1.0 cm zone of inhibition against both fungus, whereas *C. album* showed 0.9 cm zone of inhibition against *A. solani* and a 0.4 cm zone of inhibition against *Mucor* in hexane extract. The results of this study provides significant insight on the antifungal potential of weed extracts, as a potential substitute for traditional antifungal agents.

CONCLUSION

The promising antifungal activity has been demonstrated by *Boerhavia diffusa, Chenopodium album*, and *Ziziphus mauritiana* against two fungi strains. These plants had bioactive chemicals that contributed to their antifungal effects. Moreover, there was little study was available on weeds extract as an antifungal against *Alternaria solani* and *Mucor* fungi. The methanol and chloroform extract were very effective against *A. solani* and *Mucor*. The precise method of action and the active ingredients responsible for their antifungal efficacy against certain fungal strains needs to be analysed. However, more phytochemical and pharmaceutical research is required to identify the bioactive compound (s). against a large variety of pathogenic fungi. Additionally, this research will be beneficial for novel finding of eco-friendly agents to control the plant pathogens and fungal contaminants.

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