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ANTIFUNGAL ACTIVITY OF DIFFERENT ORGANIC SOLVENT EXTRACT PARTS OF *ALTERNANTHERA PHILOXEROIDES* AGAINST SOME PATHOGENIC FUNGI

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Abstract

Fungal phytopathogens greatly affect the agriculture industry. At present these pathogens are being controlled by synthetic fungicides but all of these fungicides have serious environmental effects and human health hazards. In search of ecofriendly natural fungicidal extracts, antifungal activity of leaf, stem and root of alligator weed [*Alternanthera philoxeroides* (Mart). Griseb] was evaluated in the present investigation. The extracts were prepared by soaking the dry powder of plant parts (Leaves, stem and root) into four organic solvents viz. methanol, *n*-hexane, chloroform and ethyl acetate. Antifungal activity was examined by using food poisoning technique against four plant pathogenic fungal species; *Alternaria alternata* (Fr.) Keissl., *Aspergillus flavus* Link, *Aspergillus niger* Tiegh. and *Macrophomina phaseolina* (Tassi) Goid. Antifungal activity was checked at 5 concentrations ranging from 5-25 mg/ml. Synthetic fungicide, defeater plus was used as positive control while dimethylsulfoxide (DMSO) was used as negative control. It was found that *n*-hexane fraction of *A. philoxeroides* leaves exhibited the highest (40%) antifungal activity against *A. alternata* and *A. flavus* while there was 32% decline in case of *M. phaseolina* and 25% decrease in fungal biomass in *A. niger* at concentration of 25 mg/ml. The present study concluded that *A. philoxeroides* has antifungal constituents that can be isolated and identified to be used as natural ecofriendly fungicides in future.

Key words: Alligator weed; *Aspergillus;* Fungicide.

Introduction

Plants have been a source of nutrition and medicine for human since long. Almost 80% people from developing countries directly or indirectly depend upon the herbal medicines due to less or no side effects (Agarwal *et al*., 2019). Dramatic increase in the use of medicinal plants by the native people indicates the trust on plants making it feasible for researchers to carry out pharmacological studies on these plants (Baptista *et al*., 2018). Medicinal plants have great importance because they are useful as antimicrobial agents due to the occurrence of different phytochemicals which are being explored by the researchers (Rafiq *et al*., 2021).

Weeds are undesirable plants that interfere with main crop (Akbar *et al*., 2017). These weeds belong to different plant families. Amaranthaceae family consists of mostly weeds and only few shrubs and there are 64 genera and 800 species in this family (Pamila & Karpagam, 2017). Genus *Alternanthera* consists of 80 plant species. One of the important species of Genus *Alternanthera* is alligator weed (*Alternanthera philoxeroides*). *A. philoxeroides* is common weed found in different zones in the world ranging from tropical to subtropical regions. Different species of *Alternanthera* such as *Alternanthera sessilis* and *A. philoxeroides* are being used to treat different ailments by many tribes in the world (Kumari & Krishnan, 2016).

In spite of the fact that advancement in science and technology is at its peak but on the other hand, hazardous plant diseases are also spreading rapidly (Jakhar & Dahiya, 2017). Now a days, synthetic fungicides are being used to control these fungal invasions but these synthetic fungicides impose number of ill effects to human and environment (Jayaraj *et al*., 2016). Damages to non-target species and induction of cross resistance in fungal pathogens are other problems associated with these synthetic fungicides e.g., *Candida albicans* has become resistant to the synthetic antifungal drugs like imidazoles (Fuentefria *et al*., 2018). Overdose of these fungicides cause multi drug resistance in microbial pathogens, which triggers the demand for alternative novel substances of herbal origin (Abuga & Gaobotse, 2019).

Fungal pathogens have irreversible effects on plants as *Aspergillus* sp. infects rice plants and causes rice blast disease. *Azadirachta indica* inhibited the growth of *A. flavus* by 22% through its ethanolic fraction, while, it inhibited the growth of *A. niger* upto 38% (Keta *et al*., 2019). Charcoal root rot disease is caused by *M. phaseolina* which possess a wide host range of more than 500 weeds and crops (Waghmare *et al*., 2019). Similarly, *A. philoxeroides* showed inhibitory action against *A. niger* (17.3mm), *A. flavus* (20.8mm) and *Trichoderma viride* (16.2mm) at 1000 microgram/ml concentration, in agar well diffusion method (Pulipati *et al*., 2016). Other species of *Alternanthera* such as *A. pungens* also exhibited antimicrobial activity against several microbial pathogens such as acetone extract of *A. pungens* showed antifungal activity with inhibition zone of (9.66±0.57 mm) against pathogenic fungus, *Aspergillus fumigatus* (Jakhar & Dahiya, 2017). Aqueous extract of *A. sessilis* exhibited antifungal activity against *A. niger* with inhibition zone of 5.5 mm keeping miconazole as control, however *T. viride* was resistant to its extract (Patil & Rothe, 2016). Similarly, aqueous extract of *Alternanthera bettzickiana* exhibited antifungal activity of 9 mm zone of inhibition at concentration of 50 µg/ml against

Epidermophyton floccosum. Ethanolic extract of *A. bettzickiana* showed antifungal activity against *A. niger* to 10, 11 and 13 mm zone of inhibition by using the concentrations of 30, 40 and 50 mg/ml, respectively, while ketoconazole, used as control, exhibited 23 mm zone of inhibition (Pamila & Karpagam, 2017b).

In recent years, bioactive constituents from plants have been characterized for their antifungal activities and now are being used as effective fungicides but there is great need for more botanical products to be used as fungicides. e.g., ellipticines have been reported as natural antifungal agents found potent against *Phytophthora infestans* (McKee *et al*., 2020).

In past, only few investigations have been made that described antifungal potential of genus *Alternanthera*. There are no reports on antifungal activity of different organic fractions (methanolic, *n*-hexane, chloroform and ethyl acetate) of *A. philoxeroides* against *A. alternata, A. flavus, A. niger* and *M. phaseolina,* so in the present study, *in vitro* antifungal activity of these extracts has been investigated.

Materials and Methods

Preparation of organic solvent extracts of alligator weed: The *A. philoxeroides* was collected from marshy places in Kapurowali (32.5338° N, 74.4511 °E), District Sialkot, Punjab, Pakistan to check its antifungal activity against selected pathogenic fungal species. Fresh sample of leaves, stem and root of *A. philoxeroides* was picked up at flowering stage and washed thoroughly first with tap water and then with distilled water. Then samples were placed under sunlight for 7 days at day time and at night the samples were placed in room under fan for the sake of full aeration. Then, the desired parts of the weed were crushed separately with pestle and mortar to make it fine powder. 200 g dried sample of leaves, stem and root was added separately in separate glass jars containing 500 ml methanol and incubated for 1 week at 45°C. The methanolic extract was first filtered by using muslin cloth and then filtered by Whatman filter paper No.1. The filtered extract was then evaporated in rotary evaporator at 45°C to evaporate extra solvent (methanol). The methanolic extract was dissolved in dH_2O and fractioned by three organic solvents viz. *n*-hexane, chloroform and ethyl acetate. Extra solvents from fractions were evaporated *in vacuo* as described for methanolic extract. These organic extracts thus obtained were kept in a refrigerator for subsequent use (Akbar *et al*., 2020a; 2021).

Culturing of selected plant pathogenic fungal strains: Fungal cultures viz. *Alternaria alternata, Aspergillus flavus, Aspergillus niger* and *Macrophomina phaseolina* were obtained from the microbial repository, University of the Punjab, Lahore, Pakistan. These fungi were then sub cultured on fungal growth medium for sufficient mycelial growth. These grown cultures were kept at 4°C for subsequent use.

Preparation of control and stock solution: For the determination of antifungal activity, procedure described by Akbar *et al.,* (2020b) was adopted with slight modifications. For the preparation of negative control, 166 µl DMSO was mixed with 333 µl autoclaved distilled water to make the final volume of 500 µl. For the preparation of positive control, 150 mg synthetic fungicide (defeater plus) was dissolved in 166 µl DMSO and 333 µl autoclaved distilled water to make the final volume of 500 µl. Defeater plus is trade name of fungicide having 50% WG (5% + 45%) & Tech. (Flumorph 95% + Fosetyl Aluminium 95%). For the production of stock solutions, 150 mg organic extracts were separately taken out and then dissolved into 166 µl DMSO and 333 µl autoclaved dH_2O to make 500 µl solution.

Preparation of culture medium: Potato dextrose broth (PDB) was used for the growth of all test fungi. For the preparation of medium, 20 g potato dextrose (PD) powder was mixed in 1000 ml distilled water in a conical flask. Culture medium was then autoclaved at 121°C for 20 minutes. Commercial antibacterial compound, penicillin was added in to the medium to eradicate the bacterial contamination before pouring the material into culture tubes.

Antifungal activity: Six concentrations viz. 0, 5, 10, 15, 20 and 25 mg/ml were made by the addition of 0, 20, 40, 60, 80 and 100 µl stock solutions in 100, 80, 60, 40, 20 and 0 µl control solution into the 1.1 ml PDB solution to make the final volume of 1.2 ml, in each 10 ml culture tube. There were 3 replications in each treatment. After it, 2 µl suspension containing spores of each test pathogenic fungus was added separately into growth medium and then incubated at 27± 2°C for 72 hours for attaining the optimal growth of fungi. Afterwards, filtration of the fungal mass was performed by pre-weighted filter papers and placed it in hot air oven at 65°C till dryness. After dryness, the filtered material was calculated by electric balance to find out the dry fungal biomass. Likewise, this process was used to record the biomass of other selected fungal pathogens by applying following formula:

$W2 - W1 = W$ (W1=Weight of filter paper before filtration, W2= weight of filter paper after filtration and drying, W= weight of fungal biomass).

Formula of the measurement of antifungal activity was following as described by Akbar *et al*., (2020c).

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Statistical analysis

ANOVA ($p \leq 0.05$), followed by Fisher's LSD test was performed for all statistical analyses using statistical software Minitab 19.

Results

In general, extracts of *A. philoxeroides* at concentrations ranging from 15-25 mg/ml significantly declined fungal growth as compared to control, while, extract concentrations at 5-10 mg/ml failed to induce any observable fungicidal effect. Defeater plus used as positive control completely arrested the fungal growth at

all concentrations investigated, while DMSO kept as negative control, did not cause any inhibitory effect on all fungal species.

Antifungal activity of *A. philoxeroides* leaves extract: Figure 1A shows the antifungal activity of *A. philoxeroides* leaves extract against *A. alternata*. In this experiment, different concentrations of *A. philoxeroides* leaves extract viz., 0, 5, 10, 15, 20 and 25 mg/ml were used. DMSO was used as negative control and defeater plus was used as positive control. Different concentrations of methanolic extract of *A. philoxeroides* leaves ranging from 5-25 mg/ml declined fungal growth to 7, 9, 13, 19 and 29%. *n*-hexane extract of *A. philoxeroides* leaves reduced the fungal biomass of *A. alternata* to 8, 12, 20, 28 and 40%, while DMSO did not exhibit any reduction in fungal growth. Chloroform extract reduced the fungal biomass to 5, 8, 14, 20 and 27%. Effect of ethyl acetate fraction of *A. philoxeroides* leaves as having antifungal activity against *A. alternata* is shown in Fig. 1A. Ethyl acetate extract showed less inhibition to fungal biomass compared to other extracts. It showed inhibition of fungal biomass by 3, 5, 8, 11 and 17% by using the extract concentrations of 5-25 mg/ml.

Figure 1B shows the antifungal activity of *A. philoxeroides* leaves against *A. flavus.* Different concentrations of the extract ranging from 5-25 mg/ml were used to evaluate the tendency of the antifungal activity of *A. philoxeroides.* There was a reduction in fungal biomass to 6, 11, 15, 26 and 35% by using the methanolic extract at concentrations of 5-25 mg/ml. Likewise, *n*-hexane fraction ranging from 5-25 mg/ml reduced the fungal biomass to 7, 13, 22, 29 and 40%. Similarly, chloroform extract of *A. philoxeroides* leaves inhibited the growth of *A. flavus* to 6, 9, 15, 21 and 26%. Ethyl acetate extract of *A. philoxeroides* reduced the fungal biomass of *A. flavus* to 4, 6, 12, 16 and 19%.

Inhibitory activity of *A. philoxeroides* leaves extract against *A. niger* is presented in figure 1C. There was decrease in fungal growth of *A. niger* to 3, 6, 11, 14 and 17% against the conc. of 5-25 mg/ml of methanolic extract. There was reduction in fungal growth of *A. niger* to 5, 8, 12, 17 and 25% against concentrations viz. 5-25 mg/ml of *n*-hexane extract. The different concentrations of chloroform extract ranging from 5 to 25 mg/ml reduced the fungal biomass of *A. niger* to 4, 8, 13, 18 and 23% respectively. Ethyl acetate extract inhibited the fungal growth of *A. niger* to 5, 9, 14, 18 and 21%.

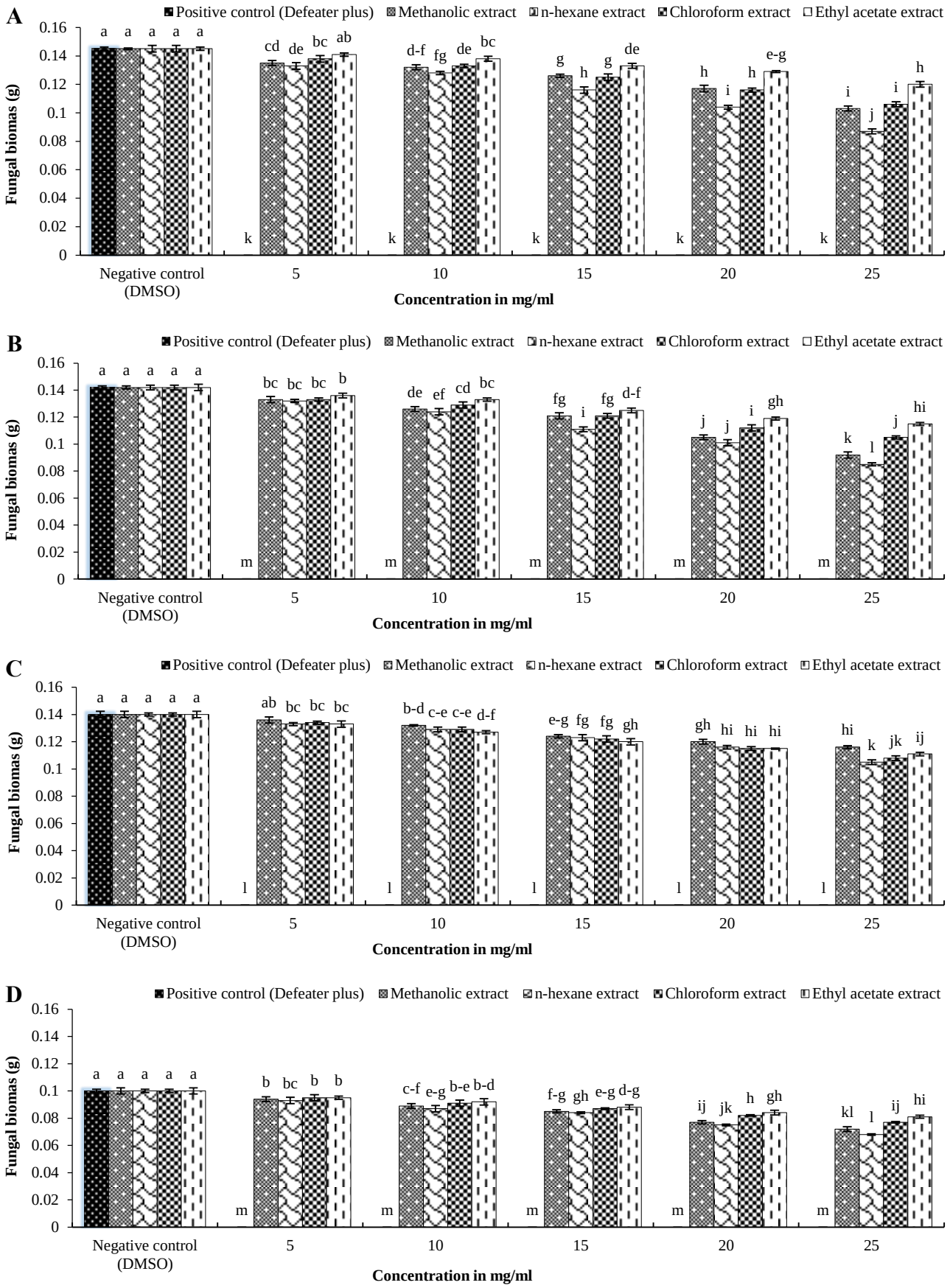
Data regarding antifungal activity of *A. philoxeroides* leaves extract at conc. ranging from 5-25 mg/ml are shown in figure 1D. Methanolic extract of *A. philoxeroides* leaves reduced the fungal biomass of *M. phaseolina* to 6, 11, 15, 23 and 28%, respectively. Different concentrations of *n*-hexane extract ranging from 5 to 25 mg/ml reduced the fungal biomass to 7, 13, 16, 25 and 32%. There was 32% reduction of fungal biomass by *n*-hexane extract of *A. philoxeroides* leaves but it was lowest as compared to defeater plus as it reduced the fungal biomass of *M. phaseolina* to 100% at all concentrations investigated. Similarly, chloroform extract of *A. philoxeroides* leaves showed reduction of fungal biomass of *M. phaseolina* to 5, 9, 13, 18 and 23%. In a similar way, ethyl acetate extract inhibited the fungal biomass of *M. phaseolina* to 5, 8, 12, 16 and 19% at different concentrations of 5-25 mg/ml. The maximum reduction was 19% by ethyl acetate extract of *A. philoxeroides* leaves which was the lowest than *n*-hexane and chloroform extracts.

Antifungal activity of *A. philoxeroides* stem extract: Data about antifungal activity of *A. philoxeroides* stem extract against *A. alternata* are shown in Figure 2A. Methanolic extract inhibited the biomass of *A. alternata* to 3, 6, 8, 10 and 15%. Likewise, *n*-hexane extract reduced the fungal biomass of *A. alternata* to 4, 7, 12, 17 and 22%. Defeater plus reduced 100% fungal biomass of *A. alternata* while DMSO did not inhibit any growth. Effect of chloroform extract of *A. philoxeroides* stem against growth of *A. alternata* is shown in figure 2A. Reduction in fungal biomass of *A. alternata* at different conc. ranging from 5-25 mg/ml was 6, 8, 11, 15 and 18%, respectively. Ethyl Acetate extract reduced the fungal biomass of *A. alternata* to 3, 7, 9, 10 and 13%. Significant results were obtained at 10-25 mg/ml while non-significant results were obtained at 5 mg/ml.

Data of antifungal activity of *A. philoxeroides* stem extract against *A. flavus* are presented in figure 2B. Different concentrations of organic extract of *A. philoxeroides* stem were used to evaluate their fungistatic effect on the growth of *A. flavus.* Different conc. ranging from 5-25 mg/ml of methanolic fraction reduced the growth of *A. flavus* by 1, 4, 7, 9 and 11%, respectively. On the other hand, *n*-hexane extract inhibited the fungal biomass of *A. flavus* to 4, 6, 9, 11 and 15%. Chloroform extract of *A. philoxeroides* stem reduced in fungal biomass of *A. flavus* to 5, 9, 13, 14 and 16% by using different conc. of 5-25 mg/ml. There was decline in fungal biomass to 5, 8, 11, 13 and 15% by the application of ethyl acetate extract which showed the lowest antifungal activity as compared to methanolic and chloroform extracts.

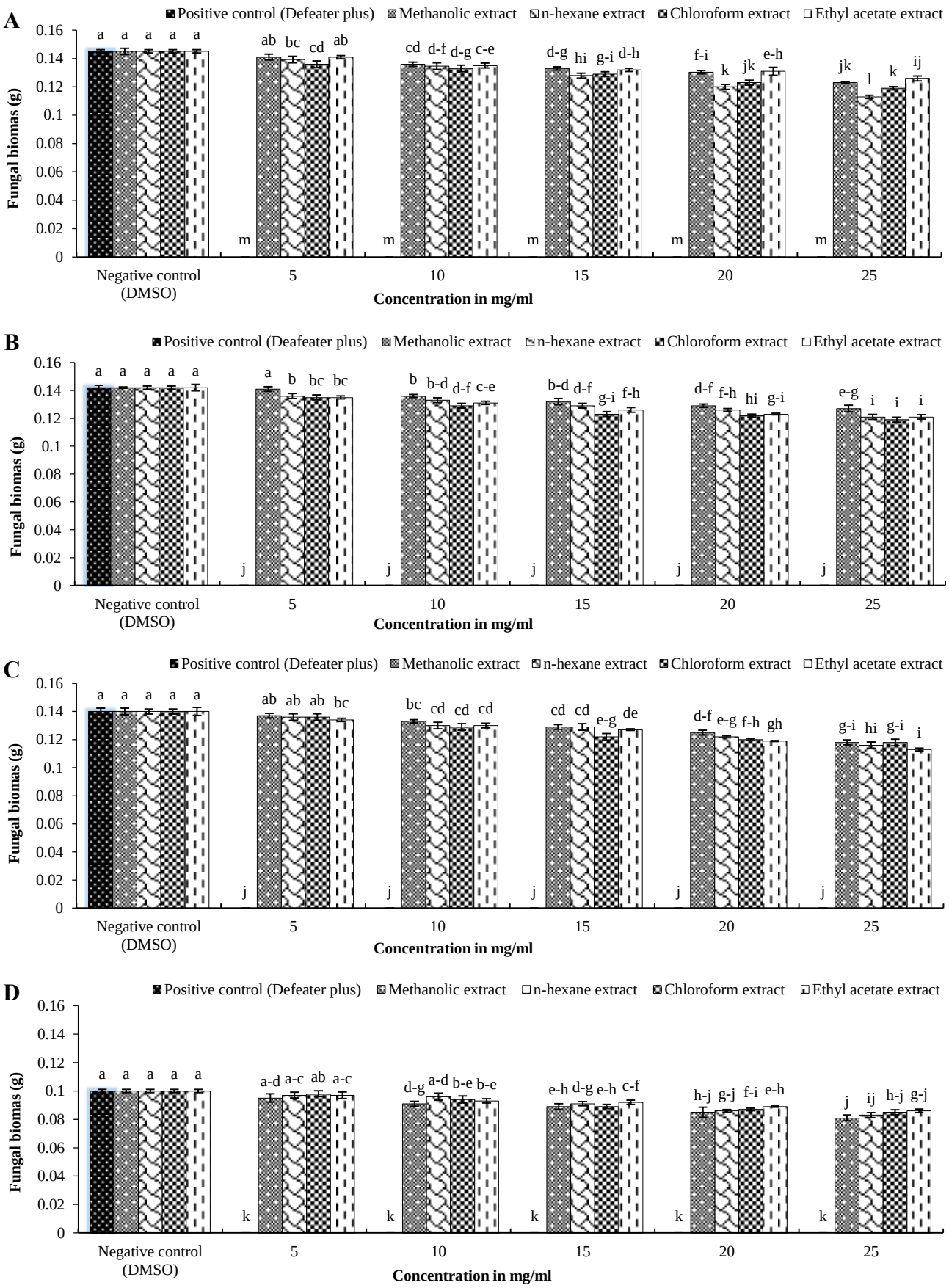
Antifungal activity of *A. philoxeroides* stem extract against *A. niger* is presented in figure 2C. Different concentrations of methanolic extract ranging from 5-25 mg/ml showed reduction in fungal biomass of *A. niger* to 2, 5, 8, 11 and 16%. *n*-hexane extract reduced the fungal biomass of *A. niger* to 3, 7, 8, 13, 17%. Chloroform extract of *A. philoxeroides* stem reduced the fungal biomass of *A. niger* to 3, 8, 13, 14 and 16%. Ethyl acetate fraction inhibited the growth of *A. niger* to 4, 7, 9, 15 and 19%.

Effect of different conc. (5-25 mg/ml) of *A. philoxeroides* stem extract against *M. phaseolina* is presented in figure 2D. Antifungal activity of methanolic extract of *A. philoxeroides* stem against *M. phaseolina* have shown reduction in fungal biomass of *M. phaseolina* to 5, 9, 11, 15 and 19%. Significant results were obtained at 10-25 mg/ml. Thereafter, *n*-hexane extract reduced the fungal biomass of *M. phaseolina* to 3, 4, 9, 14 and 17%, respectively. Similarly, chloroform extract of *A. philoxeroides* stem reduced the growth of *M. phaseolina* to 2, 6, 11, 13 and 15%. Ethyl acetate fraction of *A. philoxeroides* stem reduced the fungal biomass to 3, 7, 8, 11 and 14%.



Vertical bars show the standard error of means of three replicates. Different letters with values showed significant differences ($p \leq 0.05$) as determined by ANOVA followed by fisher's LSD test using Minitab 19 statistical software

Fig. 1(A-D). Effect of different organic solvent extracts of *Alternanthera philoxeroides* leaves on the growth of A) *Alternaria alternata*, B) *Aspergillus flavus*, C) *Aspergillus niger* and D) *Macrophomina phaseolina.*



Vertical bars show the standard error of means of three replicates. Different letters with values showed significant differences ($p \leq 0.05$) as determined by ANOVA followed by Fisher's LSD test using Minitab 19 statistical software

Fig. 2 (A-D): Effect of different organic solvent extracts of *Alternanthera philoxeroides* stem on the growth of A) *Alternaria alternata*, B) *Aspergillus flavus*, C) *Aspergillus niger* and D) *Macrophomina phaseolina.*

Antifungal activity of *A. philoxeroides* root extract: Methanolic extract of *A. philoxeroides* root reduced the fungal biomass of *A. alternata* to 2, 6, 9, 12 and 15%. Significant results were obtained at 10-25 mg/ml. Likewise, *n*-hexane extract reduced the fungal biomass of *A. alternata* to 4, 7, 11, 16, 19%. Chloroform extract of *A. philoxeroides* root inhibited the fungal biomass of *A. alternata* to 1, 3, 6, 8 and 11%. Different conc. of ethyl acetate extract of *A. philoxeroides* root ranging from 5 to 25 mg/ml inhibited the growth of *A. alternata* to 2, 6, 7, 11 and 12%. Significant results were obtained at 10-25 mg/ml (Fig. 3A).

Different conc. of *A. philoxeroides* root extract ranging from 5, 10, 15, 20 and 25 mg/ml were used to record reduction in the growth of *A. flavus.* Different concentrations of methanolic extract of *A. philoxeroides* root ranging 5-25 mg/ml inhibited the fungal biomass of *A. flavus* to 4, 7, 9, 13 and 14%. Similarly, different concentrations of *n*-hexane extract of *A. philoxeroides* root viz. 5-25 mg/ml reduced the fungal biomass of *A. flavus* to 3, 5, 8, 12 and 14%. Chloroform extract of *A. philoxeroides* root have shown antifungal activity against the growth of *A. flavus* and reduced its biomass to 4, 9, 13, 16 and 18%. Different concentrations of ethyl acetate extract ranging from 5-25 mg/ml, reduced the fungal biomass of *A. flavus* to 2, 5, 9, 10 and 12% (Fig. 3B).

Different concentrations of methanolic extract ranging from 5-25 mg/ml reduced the fungal biomass of *A. niger* to 0, 4, 9, 11 and 16%. *n*-hexane extract of *A. philoxeroides* root reduced the biomass of *A. niger* to 4, 7, 9, 14, 19%. In a similar way, chloroform extract reduced the fungal biomass of *A. niger* to 2, 5, 7, 10 and 12%. Ethyl acetate extract reduced the fungal biomass of *A. niger* to 2, 7, 11, 17 and 22% (Fig. 3C).

Fungicidal effect of *A. philoxeroides* root extract against *M. phaseolina* is shown in figure 3D. There were different concentrations of methanolic extract of *A. philoxeroides* root viz. 5, 10, 15, 20 and 25 mg/ml which reduced the fungal biomass of *M. phaseolina* to 5, 7, 11, 14 and 19%. Different concentrations of *n*-hexane extract used in this study, reduced the growth of *M. phaseolina* to 3, 6, 10, 16 and 18%. Different concentrations of chloroform extract of *A. philoxeroides* root reduced the growth of *M. phaseolina* to 3, 5, 7, 10 and 12%, while, ethyl acetate root extract reduced the fungal biomass to 4, 6, 9, 11 and 12%.

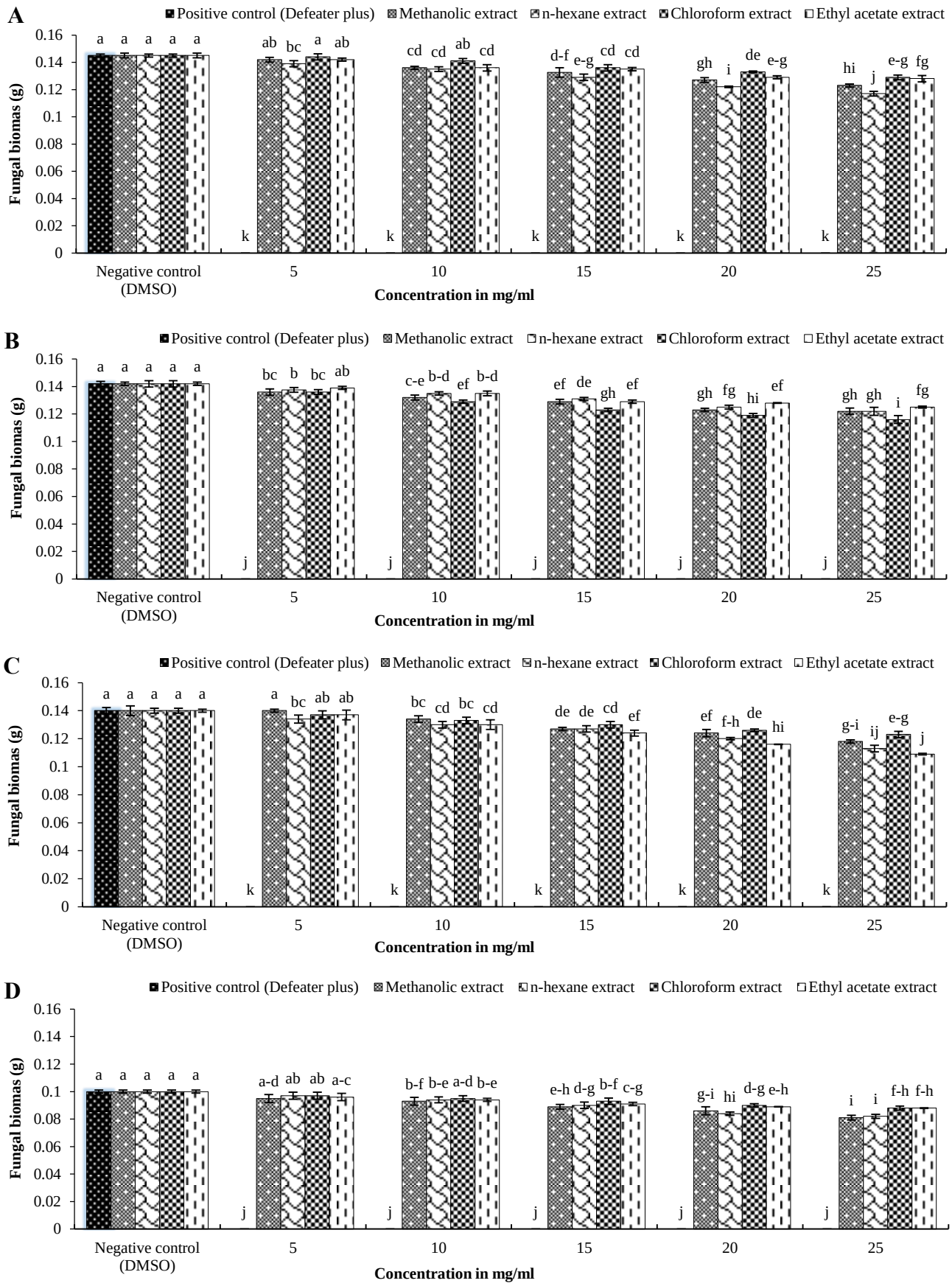
Discussion

Plants has always been focused due to their bioactive metabolites which helps to defend against several harmful pathogens. Several researchers have reported these beneficial effects in the form of antifungal activity. Likewise, in a previous study, chloroform extract of *Malvastrum coromandelianum* and *Lantana camara* inhibited the growth of *A. alternata* to 56% and 48%, respectively (Mushatq *et al*., 2012). In another studies, *Ageratina adenophora* inhibited the growth of *A. alternata* to 48% as compared to nystatin, the reference compound (Zheng *et al*., 2018), and *Helichrysum buddleioides* inhibited the biomass *A. alternata* up to 50% (Raghavendra *et al*., 2017). But in our present study, 40% was the highest inhibition recorded against *n*-hexane extract of *A. philoxeroides* meaning that less fungal growth inhibition was recorded in our study and this can be due to number of reasons. The quantity of bioactive metabolites comprising the extract, methodology employed to assess bioactivity and use of different isolates of microorganisms greatly influence the outcome of bio efficacy.

In present experiment, the highest antifungal activity of 40% was recorded at concentration of 25 mg/ml while in previous research; aqueous extract of *Azadirachta indica* leaves inhibited the *A. flavus* isolates to 17% (Keta *et al*., 2019). In earlier work, it was shown that methanolic extract of *Coleus forskohlii* reduced the fungal biomass of *A. flavus* to 45% (Saklani *et al*., 2011). Difference of results may be due to the different plant species, different plant parts, methodology (disk diffusion method), or the extract concentrations.

Likewise, in the case of *A. niger*, our results have shown the antifungal activity of 25% at the concentration of 25 mg/ml. These resembled with results of Arasu *et al*., (2019) which revealed that *Sesamum indicum* L. inhibited the growth of *A. niger* to 53%. Similarly, Qadir *et al*., (2017) revealed that ethanol extract of garlic inhibited *A. niger* to 37%. However, on the other hand, our results were contrary to findings of Kumari & Krishnan (2016) who reported that aqueous extract of *A. sessilis* and *A. philoxeroides* were found inactive against test fungi *A. niger* and *C. albicans*. It may be either due to the differences in methodology, like utilization of whole plants and their aqueous extracts as against separate plants parts and use of different organic fractions (methanolic, *n*-hexane, chloroform and ethyl acetate) in our study.

In previous studies, various concentrations of extract of *Cirsium arvense* leaves were shown to inhibit the growth of *M. phaseolina* to 10-74% (Banaras *et al*., 2017). Similarly, essential oil of *Ocimum gratissimum* inhibited the growth of *M. phaseolina* up to 25% as compared to control (Mohr *et al*., 2017). This increased inhibition in their study can be attributed to higher concentration of antifungal compounds in essential oil as essential oil is concentrated extract but, in our study, less inhibition % age was observed as this study was not related to use of essential oils. But in our results, the highest inhibition in fungal biomass of *M. phaseolina* was 40%. In another investigation, ethyl acetate leaf fraction of *Amaranthus viridis* reduced the fungal growth up to 45% in *M. phaseolina* at 25 mg/ml extract concentration (Akbar *et al*., 2020c). This antifungal activity of ethyl acetate leaf fraction of *A. viridis* was higher as compared to antifungal activity reported in the present study. These differences in antifungal activity can be attributed to antifungal activity of different plants under investigation which may possess different sets of biochemical constituents, leading to different levels of bioactivities.



Vertical bars show the standard error of means of three replicates. Different letters with values showed significant differences ($p \leq 0.05$) as determined by ANOVA followed by fisher's LSD test using Minitab 19 statistical software

Fig. 3 (A-D). Effect of different organic solvent extracts of *Alternanthera philoxeroides* root on the growth of A) *Alternaria alternata*, B) *Aspergillus flavus*, C) *Aspergillus niger* and D) *Macrophomina phaseolina*.

Conclusions

Leaves extract of *A. philoxeroides* showed more antifungal activity as compared to stem and root fractions. *n*-hexane fraction of *A. philoxeroides* leaves exhibited the highest (40%) antifungal activity against *A. alternata* and *A. flavus* while there was 32% decline in fungal biomass in case of *M. phaseolina* and 25% decrease in fungal biomass in *A. niger*, at concentration of 25 mg/ml of *n*-hexane extract of *A. philoxeroides* leaves.

References

Abuga, H. and G. Gaobotse. 2019. Antibacterial potential of extracts of the roots of *Zingiber officinale* against bacterial strains commonly associated with nosocomial infections. *J. Med. Plant. Res.*, 13(2): 41-46.

Agarwal, S., P.H. Ramamurthy, B. Fernandes, A. Rath and P. Sidhu. 2019. Assessment of antimicrobial activity of different concentrations of *Tinospora cordifolia* against *Streptococcus mutans*: An *In vitro* study. *J. Dent. Res.*, 16(1): 24-28.

Akbar, M., A. Amin, T. Khalil, M.S. Iqbal, A. Nazir and A. Taswar. 2021. Antibacterial activity of *Alternanthera philoxeroides* (Mart.) Griseb. against bacterial phytopathogens: *Erwinia carotovora*, *Ralstonia solanacearum* and *Xanthomonas axonopodis. Allelopathy J.*, 53(1): 83-92. doi.org/10.26651/allelo.j/2021-53-1-1329

Akbar, M., I.N. Sherazi, M.S. Iqbal, T. Khalil and H.M. Waqas. 2020b. Antibacterial and antioxidant activities of slender amaranth weed. *Planta. Daninha*, 38: 1-8.

Akbar, M., I.N. Sherazi, T. Khalil, M.S. Iqbal, S. Akhtar and S.N. Khan. 2020c. Identification of antifungal compounds from slender amaranth. *Planta Daninha*, 38:e020207096. https://doi.org/10.1590/S0100-83582020380100063

Akbar, M., M.S. Iqbal and T. Khalil. 2017. Isolation and characterization of natural herbicidal compound from *Drechslera rostrata*. *Planta. Daninha.*, 35: 1-6.

Akbar, M., U. Ali, T. Khalil, M.S. Iqbal, A. Amin, R. Naeem, A. Nazir, H.M. Waqas, Z. Aslam, F.I. Afri, N. Aslam and S.A. Chohan. 2020a. *Cornus macrophylla*, the antibacterial activity of organic leaf extracts and the characterization of the more lipophilic components by GC/MS. *Molecules*, 25: 2395; doi:10.3390/molecules25102395.

Arasu, M.V., P. Viayaraghavan, S. Ilavenil, N.A. Al-Dhabi and K.C. Choi. 2019. Essential oil of four medicinal plants and protective properties in plum fruits against the spoilage bacteria and fungi. *Ind. Crops. Prod.*, 133(2019): 54-62.

Banaras, S., A. Javaid, A. Shoaib and E. Ahmed. 2017. Antifungal activity of *Cirsium arvense* extracts against phytopathogenic fungus *Macrophomina phaseolina*. *Planta. Daninha.*, 35: 1-10.

Baptista, A., R.V. Gonçalves and J. Bressan MDCG Peluzio. 2018. Antioxidant and antimicrobial activities of crude extracts and fractions of cashew (*Anacardium occidentale* L.), cajui (*Anacardium microcarpum*), and pequi (*Caryocar brasiliense* C.): a systematic review. *Oxid. Med. Cell. Longev.*, 2018: 1-13 https://doi.org/10.1155/2018/3753562.

Fuentefria, A.M., B. Pippi, D.F. Dalla Lana, K.K. Donato and S.F. de Andrade. 2018. Antifungals discovery: an insight into new strategies to combat antifungal resistance. *Lett. Appl. Microbiol.*, 66(1): 2-13.

Jakhar, S. and P. Dahiya. 2017. Antimicrobial, antioxidant and phytochemical potential of *Alternanthera pungens* HB&K. *Int. J. Pharm. Sci. Res.*, 9(8): 1305-1311.

Jayaraj, R., P. Megha and P. Sreedev. 2016. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdiscip. Toxicol.*, 9(3-4): 90-100.

Keta, J.N., H.A. Suberu, K. Shehu, U. Yahayya, N.K. Mohammad and G.B. Gudu. 2019. Effect of neem (*Azadirachta indica* a. Juss) leaf extract on the growth of *Aspergillus* species isolated from foliar diseases of rice (*Oryzea sativa*). *Sci. World. J.*, 14(1): 98-102.

Kumari, M.E.V. N. and V. Krishnan. 2016. Antimicrobial aActivity of *Alternanthera Sessilis* (L.) R. BR. Ex. DC and *Alternanthera Philoxeroides* (Mart). Griseb. *World. J. Res. Rev*., 3(3): 78-81.

McKee, M.L., L.O. Zheng, E.C. Sullivan, R.A. Kehoe, B.M. Doyle Prestwich, J.J. Mackrill and F.O. McCarthy. 2020. Synthesis and evaluation of novel ellipticines and derivatives as inhibitors of *Phytophthora infestans*. *Pathogens*, 9(7): 558.

Mohr, F.B., C. Lermen, Z.C. Gazim, J.E. Goncalves and O. Alverton. 2017. Antifungal activity, yield, and composition of *Ocimum gratissimum* essential oil. *Genet. Mol*., 16(1): 1-10.

Mushatq, S., M.S. Haider, A. Ali, S. Javed, I. Khokhar and I. Mukhtar. 2012. *In vitro* comparative screening of antibacterial and antifungal activities of some common weeds extracts. *Pak J. Weed. Sci. Res.*, 18(1): 15-25.

Pamila, U.A. and S. Karpagam. 2017. Antimicrobial activity of *Alternanthera bettzickiana* (regel) g. Nicholson and its phytochemical contents. *Int. J. Pharm. Sci. Res.*, 8(6): 2594-2599.

Pamila, U.A. and S. Karpagam. 2017. GC-MS analysis of ethanolic extract of *Alternanthera philoxeroides* and *Alternanthera bettzickiana* from India. *World Res. J. Biol. Biol. Sci.*, 4(2017): 2-4.

Patil, R.N. and S.P. Rothe. 2016. Antifungal activity of some fodder plants leaf extract. *Int. J. Adv. Res. Sci. Eng. Technol.*, 3(11): 205-208.

Pulipati, S., P.N.S. Babu, B. Sree, U.E. Kumar, S.K. Shaheela, J.M. Krishna and T. Chakradhar. 2016. Phytochemical analysis and antimicrobial investigations of ethanolic leaf extract of *Alternanthera philoxeroides* (Mart.) Griseb. *WJPPS.*, 5(2): 1122-1129.

Qadir, M.A., S.K. Shahzadi, A. Bashir, A. Munir and S. Shahzad. 2017. Evaluation of phenolic compounds and antioxidant and antimicrobial activities of some common herbs. *Int. J. Anal. Chem.*, 2017: 1-6 https://doi.org/10.1155/2017/3475738.

Rafiq, M., A. Javaid and A. Shoaib. 2021. Antifungal activity of methanolic leaf extract of *Carthamus oxycantha* against *Rhizoctonia solani*. *Pak. J. Bot*., 53(3): 1133-1139, 2021. http://dx.doi.org/10.30848/PJB2021-3(17)

Raghavendra, T.P.K.H. 2017. Antifungal activity of *Helichrysum buddleioides* DC. against seed borne fungi. *EC. Microbiol.*, 6(2): 54-59.

Saklani, S., M. Gahlot, A. Kumar, R. Singh, R. Patial and P. Kashyap. 2011. Antimicrobial activity of extracts of the medicinal plant *Coleus forskohlii*. *Int. J. Drug. Res. Technol.*, 1(1): 52-59.

Waghmare, S.J., A. Dhodi and D.H. Sarnobat. 2019. Evaluation of local isolates of *Trichoderma* spp. against *Macrophomina phaseolina*. *Int. J. Uni. Sci. Technol.*, 5(1): 41-45.

Zheng, G., S. Luo, S. Li, J. Hua, W. Li and S. Li. 2018. Specialized metabolites from *Ageratina adenophora* and their inhibitory activities against pathogenic fungi. *Phytochemistry*, 148(2018): 57-62.

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