Fungicidal activity of seeds of *Abrus precatorius* L., *Datura metel* L., and *Diploknema butyracea* (Roxb.) H.J. Lam against phytopathogenic fungi

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Abstract: This study aimed to investigate the antifungal activity of seeds of *Abrus precatorius* L., *Datura metel* L. and *Diploknema butyracea* (Roxb.) H.J. Lam against common phytopathogenic fungi. The powdered seeds were extracted by Soxhlet extraction method using methanol as a solvent. Antifungal activity was performed by poison agar technique with different concentrations ranging from 5 to 20% of seed extracts against two fungal pathogens - *Fusarium oxysporum* f. sp. *cubense* R4 (*Foc*R4) and *Rhizoctonia* spp. Among three plant seed methanolic extracts at 5% concentration, *D. metel* L. seeds showed significant inhibition with 69.81±3.30% percentage inhibition of diameter growth (PIDG) against *Foc*R4 on 8th day and 77.51±3.00% PIDG *Rhizoctonia* spp. on 5th day. All the plant seed extracts showed complete inhibition against mycelial growth at ≤10% concentration. UV-visible spectroscopy spectra showed the peaks at 238 nm and 280 nm wavelengths for the seed extracts of *A. precatorius* L., and *D. metel* L., respectively while *D. butyracea* (Roxb.) H.J. Lam showed the peaks centered at 206 nm and 262 nm which were assumed to represent the antifungal compounds. The antifungal activity of the plant extracts could be attributed by the phytochemicals present in crude seed extracts. Our results indicate that seeds of the examined three plants are potential biofungicides which could substitute chemical fungicides.

Keywords: *Abrus precatorius; Datura metel; Diploknema butyracea*; *Antifungal activity*; *Fusarium*; *Rhizoctonia*.

Introduction

Plant disease caused by pathogens such as viruses, bacteria, and fungi pose a significant threat to agricultural production. The prevalence of these diseases affects the plant population resulting in yield loss ranging from 80-98%[1]. Phyto-pathogenic fungi cause major plant diseases occupying nearly 80% of the total damage in agricultural yields[2]. To combat these fungal diseases, chemical fungicides are applied resulting in detrimental effects on the human health and environment, kill the beneficial organism and develop resistance against the chemical fungicides[3]. A huge amount of fungicides are consumed in Nepal to control pests and diseases. According to a report published by the Plant Quarantine and Pesticide Management Center of the Government of Nepal, 407887.76 Kg of fungicide was imported in 2020/2021 in Nepal[4]. The annual consumption of fungicide occupies 50% where the farmers applied the fungicides haphazardly leading to the environmental pollution and health impact[4,5,6]. Instead of chemical fungicides, application of plant-based metabolites with the fungicidal property is the appropriate alternative approach to control the plant fungal pathogens[3,7]. Plants have rich sources of bioactive compounds such as phenolics, flavonoids, alkaloids which exhibit antimicrobial properties and act as a defensive molecule against the plant pathogens

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thereby reducing disease without risks to animals' health and the environment[8,9]. It has been reported that nearly 10% of the plant species worldwide exhibit pesticidal activities so identifying and harnessing the potential of these botanicals is crucial for sustainable agricultural practices[3,8]. Plants such as Neem (*Azadirachta indica*, A. Juss), Garlic (*Allium sativum* Linn.), Eucalyptus (*Eucalyptus globulus* Labill), Turmeric (*Curcuma Longa*, Linn.), Tobacco (*Nicotiana tabacum*, Linn.), Ginger (*Zingiber officinale*, Rosc.) Chinaberry (*Melia azedarach*), Thorn Apple (*Datura stramonium* Linn.) and Black pepper (*Piper nigrum* Linn.) are rich sources of antimicrobial metabolites like phenols, phenolic acids, quinones, flavones, flavonoids, flavanols, tannins, and coumarins. These constituents have potent pesticidal compounds, as evidenced by both in vivo and in vitro studies demonstrating their efficacy[10,11]. Recent studies have reported the antimicrobial and antifungal efficacy of plant extracts against phytopathogenic fungi and associated fungal diseases[12,13,14]. The plant extracts from *Andrographis paniculata* Wall, *Backhousia citriodora* F. Muell., *Clinacanthus nutans* (Burm. f.) Lindau, *Ficus deltoidea* Jack, *Phaleria macrocarpa* Boerl., and *Piper betle* L. inhibit the growth of phyto-pathogenic fungi including *Fusarium oxysporum*, *Rhizoctonia solani*, and *Ganoderma boninense*[7].

Nepal has a rich heritage of using medicinal and aromatic plants (MAPs) not only for therapeutic purposes but also in various other roles including pesticide and fungicides[15]. The plant species like *A. precatorius* L., *D. metel* L. and *D. butyracea* (Roxb.) H.J. Lam which have been traditionally applied for medical use due to their pharmacological properties, may also be potential fungicide because of their antifungal components[16,17]. These plants contain bioactive compounds such as alkaloids, flavonoids, and phenolic compounds, which have demonstrated inhibitory effects against a wide range of microorganisms, including fungi[18,19]. Fungicidal plant extracts serve as excellent alternatives to synthetic chemicals in agriculture, offering economic, effective, and eco-friendly disease management solutions. Considering the negative impact on agriculture and adverse effect on farmers' health due to synthetic fungicides, the use of botanical and plant-based fungicides has been increased for controlling fungal diseases[20,21]. Therefore, searching for more efficient plant-based fungicides which are environment friendly and non-hazardous to humans and animals, is an important task for researchers.

In this context, the seeds of these plants may have more potent antifungal constituents which can be applied to control fungal diseases in agricultural settings to support sustainable agricultural practices. Identifying new plant based potential fungicides not only reduce the huge application of synthetic fungicides but help to conserve biodiversity as useful plant species are propagated and protected. In this study, antifungal activity of three different plant seeds was evaluated against two fungal pathogen - *Fusarium* spp. and *Rhizoctonia* spp. and possible antifungal agents were suggested using UV-vis spectroscopic analysis. This study provides the firm foundation that the seeds of *A. precatorius* L., *D. metel* L. and *D. butyracea* (Roxb.) H.J. Lam could be applied for development of biofungicides.

Materials and methods

Collection and extraction of plants parts

The plant seed of *A. precatorius* L. and *D. metel* L. were collected from local market in Kathmandu while *D. butyracea* (Roxb.) H.J. Lam from Makwanpur district in Nepal. All the three plant seeds were identified by Botanist at the Research Centre for Applied Science and Technology (RECAST), Tribhuvan University. As shown in Table 1, the plant seeds have potential for antifungal activities. All the seeds were washed, dried and grinded with the help of a mixer. About 30 g of powdered seeds were extracted in 300 mL of methanol in Soxhlet apparatus at 60 °C. The extract was concentrated by removing the solvent using the rotary evaporator. The final extracts were collected and stored at 4 °C in the refrigerator for future use[25]. All the chemicals including methanol (Qualigens, India) and Potato Dextrose Agar (Himedia, India) used in this study were of analytical grade.

Fungal culture

The fungal cultures *F. oxysporum* f. sp. *cubense* R4 (*Foc*R4), and *Rhizoctonia* spp. were obtained from the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Malaysia. These fungal cultures were sub-cultured and maintained on Potato Dextrose Agar (PDA).

Table 1. Name of the plants, family, parts used and its functions.

Scientific name of plant	Local name	Family	Parts for extraction	Functions	Ref.
Abrus precatorius L.	*Rati gedi*	Leguminaceae	seeds	Antifungal	22
Datura metel L.	*Kalo Dhaturo*	Solanaceae	seeds	Antifungal, fatal poison,	23,24
Diploknema butyracea (Roxb.) H.J. Lam	*Chiuri*	Sapotaceae	seeds	Antimicrobial, Antifungal, antioxidant	18

Antifungal screening of plant seed extracts

The stock solutions of plant seed extracts were prepared by dissolving in the methanol as the previous method[26]. Poison agar technique was used to screen antifungal activity of plant extracts. The filter sterilized plant seed extracts were added to autoclave sterilized molten PDA medium so as to maintain final concentrations of 5, 10, 15, and 20% (w/v), separately. The test fungal mycelia with 4 mm diameter from parent plate were plugged with the sterilized cork-borer and placed at the center of plant seed extract incorporated PDA plates. The plates were incubated at room temperature (26±2°C) until the mycelia growth of negative control plates (without plant seed extracts) covered the whole petri plate (d = 80 mm). The colony diameter was measured daily and percentage inhibition of diameter growth (PIDG) values was calculated by using formula[27] as given below.

$$\text{PIDG} = \frac{D1-D2}{D1} X\ 100\%$$

where, D1= Diameter growth of mycelia in control plates; D2= Diameter growth of mycelia in treatment plates

UV analysis

The crude plant seed extract is the mixture of several compounds. In order to examine the peaks representing different compounds present in the seed extracts, the samples were scanned in UV-Vis Spectrophotometer (Shimadzu 1900-i) at a range of 190 – 600 nm. The peaks at different wavelengths were assumed to represent individual compounds. The peaks were assigned to specific compounds or groups of compounds based on available literature.

Data analysis

The experimental data were entered in MS Excel and expressed as the mean ± standard deviation (SD) of three replicate measurements.

Results and discussion

Mycelial growth inhibition

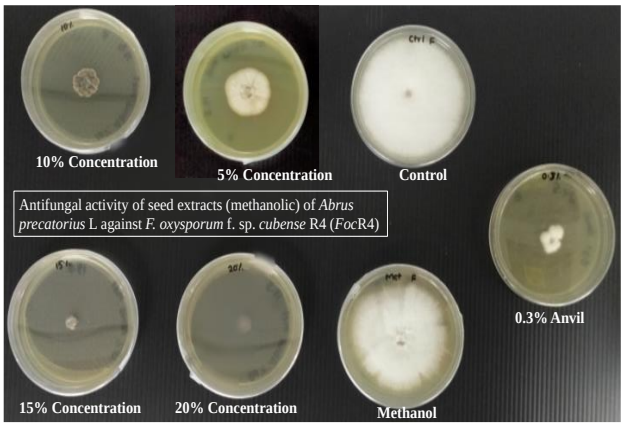

Figure 1: **Antifungal activity assay by Poison agar technique. The photographs showed *Fusarium oxysporum* f. sp. *cubense* R4 (*Foc*R4) growth on control (no inhibitory substance added), methanol and 0.3% Anvil incorporated PDA plates as indicated. Left side plates, as indicated, were incorporated with 5 to 20% concentrations of methanolic extracts from *A. precatorius* L. seeds. The diameter of visible mycelial growth of the fungi was measured to evaluate inhibitory actions.**

The methanolic seed extracts of all the three plants - *A. precatorius* L., *D. metel* L. and *D. butyracea* (Roxb.) H.J. Lam exhibited antifungal activities against both the *Foc*R4 and *Rhizoctonia* spp. (Fig. 1).

The incubation period for *Foc*R4 and *Rhizoctonia* spp. was recorded on 8 and 5 days, respectively as the mycelial growth covered the full 80 mm petri plate for the given time period when cultured in absence of any inhibitors or seed extracts. So, the test plates were also incubated for the same time periods to record the inhibition due to the extracts. The different fungal species have different growth rates. In general, our results showed that all three tested plant seeds have fungicidal potential at 10% or above concentrations.

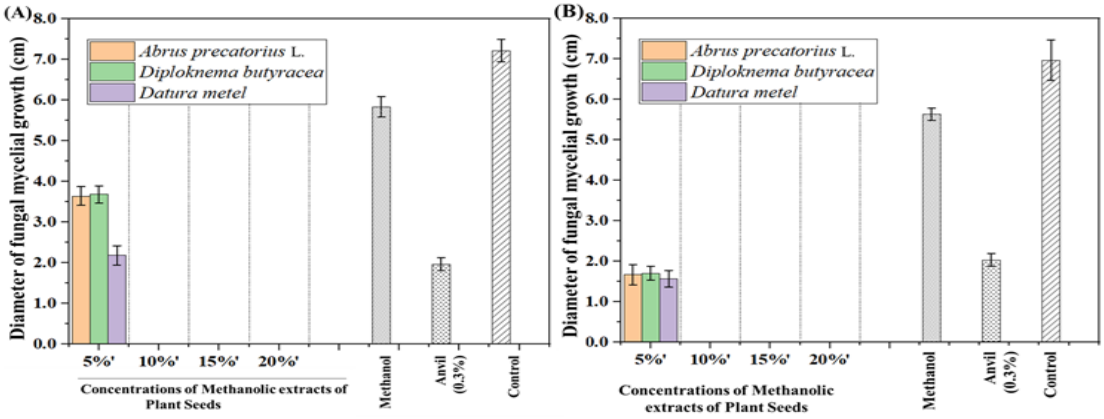

Figure 2: **Diameter of mycelial growth of (A) *F. oxysporum* f. sp. *cubense* R4 and (B) *Rhizoctonia* spp. at different concentrations of methanolic extracts of seeds of *A. precatorius* L., *D. butyracea* (Roxb.) H.J. Lam and *D. metel* L., 'Methanol' was extraction control, 'Anvil (0.3%)' was used as positive control, while 'control' represented the fungal growth in absence any inhibitory substances. Error bars represented the standard deviation from mean diameter (cm) of mycelial mass. No mycelial growth was obtained at or above 10% concentrations.**

As shown in Fig. 2, *Foc*R4 showed mycelial growth with diameter of 7.22 ± 0.28 cm and *Rhizoctonia* spp. with 6.97 ± 0.50 cm (control) when incubated for 8 and 5 days, respectively on PDA plates. Since methanol was used as solvent during extraction from seeds of the plants, the fungal growth was also evaluated at presence of methanol, which showed slight inhibition with the mycelial growth diameter of 5.83 ± 0.25 and 5.6 ± 0.15 cm for *Foc*R4 and *Rhizoctonia* spp., respectively. Although methanol could have inhibitory effects on plant pathogenic fungi depending upon the concentration, application method and specific fungal species[28], there was no significant inhibition of mycelial growth of both test fungal species in this study. We applied a common fungicidal chemical – Anvil at 0.3% w/v concentration as positive control, in which radial fungal growth was minimum with diameter of 1.97 ± 0.15 and 2.03 ± 0.15 cm for *Foc*R4 and *Rhizoctonia* spp., respectively. Anvil is an effective chemical fungicide against plant pathogenic fungi like *Fusarium* spp. and *Rhizoctonia* spp. Anvil ceases fungal growth and reproduction, thereby controlling the spread of these pathogens in crops[29]. When different concentrations of three different plant seed extracts were applied, the visible mycelial growth was obtained only at 5% concentrations, while no fungal growth was noticed in the case of 10%, 15% and 20% concentrations of all three plant seed extracts (Fig. 2). This indicated minimum fungicidal concentration for all plant seed extracts against both the fungal species ranged below or equal to 10%. However, previous studies have reported lower concentrations for example 500 µg/mL[29] of seed extract and 3.5%[19] of leaves and stem extract of *D. metel* showing inhibition against various fungi including *Rhizoctonia solani*. At 5% concentrations of seed extracts of all three plants, mycelial growth of the both fungi were reduced by more than half compared to control. Comparatively less growth was observed for *Rhizoctonia* spp. in presence of extracts of all the plants' seeds. Among three plants, minimum growth of both fungal species was obtained on the PDA plates that were incubated with seed extracts of *Datura metel* L.

Differing levels of inhibition by plant extracts can be attributed to the variations in their antifungal properties,

both in terms of quality and quantity[31], for instance minimum inhibitory concentration (MIC) of all parts of *D. metel* with different solvents was found 625.0 µg mL^{-1} against *Aspergillus fumigatus*, *A. flavus* and *A. niger*[3].

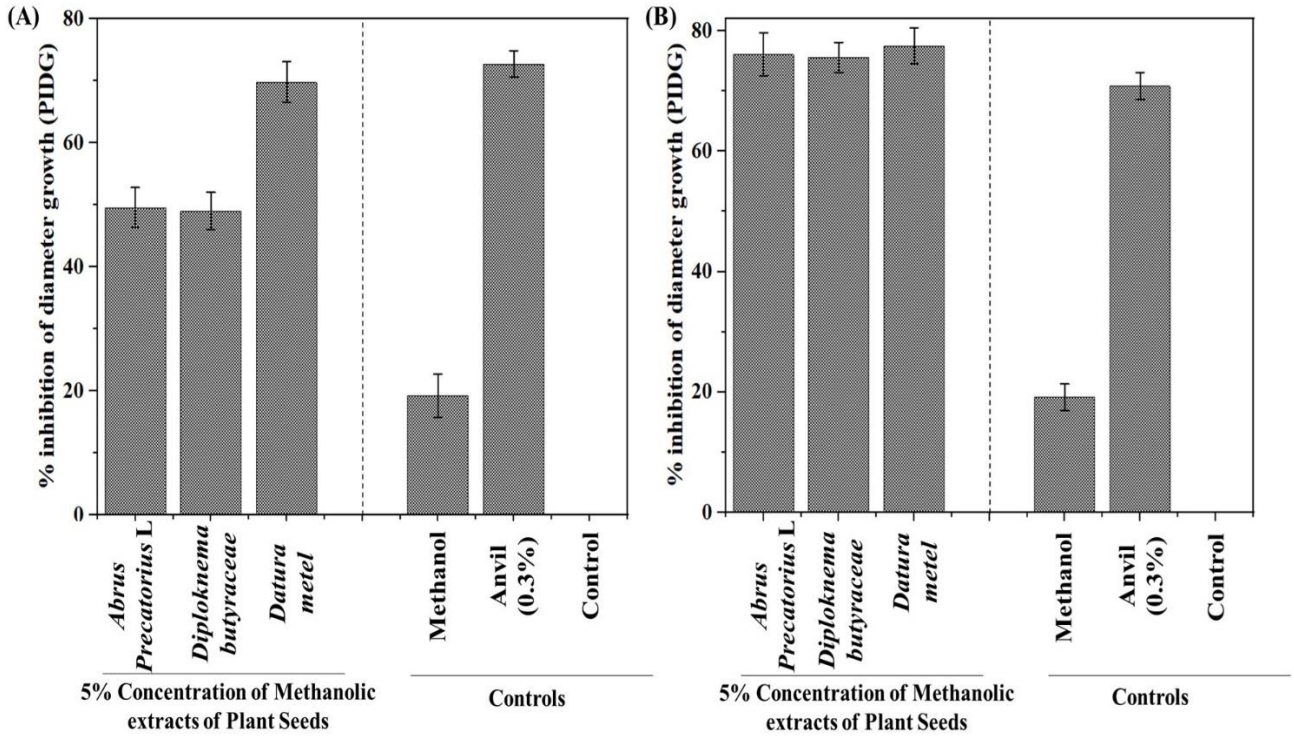

Figure 3: Percentage inhibition of diameter growth (PIDG) exhibited by methanolic extracts of seeds of the *A. precatorius* L., *D. butyracea* (Roxb.) H.J. Lam and *D. metel* L. against (A) *F. oxysporum* f. sp. *cubense* R4, and (B) *Rhizoctonia* spp. 'Methanol' was extraction control, 'Anvil (0.3%)' was used as positive control, while 'control' was negative control. Error bars represented the standard deviation from mean PIDG.

Fungicidal activity as percentage inhibition of diameter growth (PIDG)

Since mycelial growth of both fungi was completely inhibited by 10% and above concentrations of seed extracts of all three plants, the percentage inhibition of diameter growth (PIDG) using mycelial growth measurements was calculated only for 5% concentrations of plant seed extracts as shown in Fig. 3. PIDG quantifies the inhibitory efficacy against radial growth fungal pathogens on agar medium plate. Higher PIDG values signify greater inhibition of fungal growth and thus stronger fungicidal activity[7]. In this study, Anvil (0.3%) a positive control showed maximum PIDG (72.77±2.12% and 70.81±2.19% against *Foc*R4 and *Rhizoctonia* spp., respectively) while methanol as extraction control showed <20% PIDG (19.21 ± 3.48% and 19.14 ± 2.19% against *Foc*R4 and *Rhizoctonia* spp., respectively) in three replicate experiments. Control observations validated that the experimental data was reliable.

At 5% concentrations of methanolic extracts of seeds, *A. precatorius* L. and *D. butyracea* (Roxb.) H.J. Lam exhibited on average nearly 50% PIDG (49.58 ± 3.19% and 49.03 ± 3.00%, respectively) against *Foc*R4. However, *D. metel* L. seeds showed more prominent inhibition of 69.81±3.30% against the same fungi which roughly equivalent to PIDG of 0.3% Anvil (Fig. 3(A)). Similarly, in case of *Rhizoctonia* spp., 5% concentration of methanolic seed extracts of all plants exhibited even better PIDG, greater than positive control (0.3% Anvil). *A. precatorius* L., *D. butyracea* (Roxb.) H.J. Lam and *D. metel* L. showed PIDG of 76.08±3.61%, 75.60±2.49% and 77.51±3.00%, respectively against *Rhizoctonia* spp. (Fig. 3(B)).

Wide range of antifungal activity of *Datura* spp. has been reported against fungal phytopathogens like white-rot

fungus *Trametes versicolor*, brown rot fungus *Rhodonia placenta* (Fr.)[32] and *Sclerotium rolfsii*[33]. Similarly, in corroboration with our findings, *A. precatorius* L. has also been reported for its promising activity against wide range of phytopathogenic fungi like *C. albicans*, *C. tropicalis*, *C. krusei*, *Aspergillus fumigates*, A. *flavus*[34], *Fusarium solani* and *Alternaria solani*[35] due to presence of flavonoids, alkaloids, glycosides, steroids and saponins[36,37]. In our study, we showed that seed extract of *D. butyracea* (Roxb.) H.J. Lam was fungicidal. In agreement to these findings, previous studies have not only reported its activity against phytopathogenic fungi like *Rhizoctonia bataticola*, *Rhizoctonia solani* and *Sclerotium rolfsii*[38], but also against *Candida albicans* and human pathogenic bacteria[39] probably due to presence of phenols, vitamin C, lycopene, carotene were present in the seed and pulp of *D. butyracea*[40].

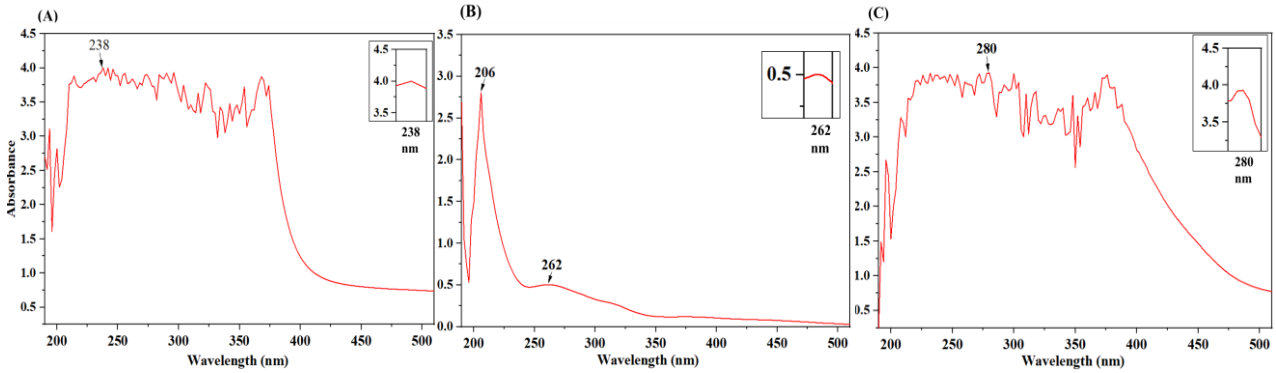

Figure 4: UV-Vis Spectrophotometric spectra of methanolic extract of seeds of (A) *A. precatorius* L., (B) *D. butyracea* (Roxb.) H.J. Lam and (C) *D. metel* L. The representative absorbance peaks were indicated by specific wavelength. The insights in each figures showed a magnified presentation of key peaks.

Possible antifungal compounds shown by UV-Vis spectrophotometric scanning

UV-Vis spectrophotometric scanning is an analytical technique to detect, identify and quantify different compounds in a mixture. This method plays a significant role in the plant extraction process where various compounds such as flavonoids, alkaloids and polyphenols may be present in plants[41]. The UV-Vis spectrophotometric scan spectra in the range of 190 - 600 nm for the methanolic extracts of seeds of three plants - *A. precatorius* L., *D. butyracea* (Roxb.) H.J. Lam and *D. metel* L. obtained in this study are presented in Fig. 4 (A), (B), and (C). The UV- Vis scanning provided presumptive predictions of different compounds which may be antifungal agents present in plant seed extracts. In this study, UV-Vis of *A. precatorius* L. shows the spectra at 238 nm (Fig. 4 (A)). The absorbance peak found in this range 230-285 nm could be the flavonoids and its derivatives[42]. The study carried out by Bhagat et al., (2020) found the UV spectra of seed extract of *A. precatorius* L. at 223 nm, 270.46 nm and 400 nm in ethanol and petroleum ether that indicate the presence of abrin and lecithin[43]. Abrin is the highly toxic protein found in the seeds of *A. precatorius* L. which inhibit the protein synthesis in eucaryotic cells through inactivation of the 60S ribosomal subunit[44].

UV-Vis spectra of *D. butyracea* (Roxb.) H.J. Lam showed prominent peaks at 206 nm and 262 nm (Fig. 4(B)). The saponins[45] from defatted cake; phenolic compounds, vitamin C, lycopene, carotene[40] from seeds and alkaloid, terpenoid, anthraquinones, tannin, cardiac glycoside, flavonoid, carbohydrate, and polyphenol[39] from different parts of *D. butyracea* were shown to exhibit the antifungal activity against several phytopathogenic fungal species. Therefore, we assume that the peak shown by *D. butyracea* (Roxb.) H.J. Lam seeds in this study might be similar compounds.

Methanolic extract of *D. metel* L. seeds showed the peak at 280 nm spectra in UV-Vis spectra (Fig. 4(C)). Since previous studies on UV analysis of extracts from this plant reported the presence of scopolamine and atropine[16,46-48], it is speculated that these compounds were key agents

showing promising antifungal activity in this study too. However, several other chemical compounds identified from seeds of *D. metel* include squalene, 9-Octadecenoic acid (Z), methyl ester, n-Hexadecanoic acid[49]. Since UV-vis analysis is just presumptive in identifying the specific compounds, further instrumental analysis like mass spectroscopy is warranted to confirm the compounds and determine its quantity.

Thus, our study clearly indicates presence of bioactive compounds in methanolic extracts of seeds the tested plants showing antifungal activity which suggests these plants can be potent biofungicides. The application of plant based fungicides in agriculture not only reduces hazards to humans but also beneficial to soil health and organic agricultural production contributing to sustainable agriculture, food security and a safe environment.

Conclusion

Three plants studied for antifungal activity showed the inhibition against selected fungal pathogens. However, *D. metel* L. had shown the highest inhibition against both fungi. Therefore, *D. metel* L. can be a potent biofungicide to control disease caused by *Fusarium* spp. and *Rhizoctonia* spp. The findings from the above experiments provide the evidence these plant extracts could serve as sustainable alternatives to chemical fungicides. The current results generate potential solutions to control pathogenic fungi as well as aligns to reduce the use of synthetic chemical pesticides in agriculture. Thus, this study could lead to more eco-friendly fungal disease management strategies especially in Nepal where agriculture plays a vital role in the economy and community livelihoods.

Author's Contribution

EM and DRJ designed the study. EM conducted the lab experiments, analyzed the data and drafted the original manuscript. MYW supervised, provided the resources, reviewed and funded the research work. UT assisted in the lab experiment and interpreted the data. DRJ and RA supervised the research, revised and edited the manuscript.

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References

1. Nazarov, P., Baleev, D., Ivanova, M., Sokolova, L. and Karakozova, M. 2020. Infectious plant diseases: Etiology, current status, problems and prospects in plant protection. *Acta Naturae*. **12**: 46–59.
2. Peng, Y., Li, S. J., Yan, J., Tang, Y., Cheng, J. P., Gao, A. J., Yao, X., Ruan, J. J. & Xu, B. L. 2021. Research progress on phytopathogenic fungi and their role as biocontrol agents. *Frontiers in Microbiology*. **12**: 670135.
3. Shuping, D. S. S. and Eloff, J. N. 2017. The use of plants to protect plants and food against fungal pathogens: A review. *African Journal of Traditional, Complementary and Alternative Medicines*. **14**(4): 120-127.
4. Plant Quarantine and Pesticide Management Center (PQPMC). 2021. Pesticide statistics (*in Nepali*). P: 2. Pesticide Registration and Management Division, Ministry of Agricultural Development, Kathmandu, Nepal.
5. Diwakar, J., Prasai, T., Pant, S. R. and Jayana, B. L. 2008. Study on major pesticides and fertilizers used in Nepal. *Scientific world*. **6**(6): 76-80.
6. Ghimire, K. and GC, A. 2018. Trend of pesticide use in Nepal. *Journal of the Plant Protection Society Nepal*. **5**: 32-42.
7. Wong, M. Y., Hamid, S., Shah, N. A. I., Rahim, N. M., Kasim, Z. Z. and Razak, N. H. A. 2020. Antifungal potential of six herbal plants against selected plant fungal pathogens. *Pertanika Journal of Tropical Agricultural Science*. **43**(2): 107-117.
8. Pandey, P. A. 2023. Review on: common botanicals in Nepal on management of crop diseases. *Journal of Applied Biotechnology & Bioengineering*. **10**(6): 186-192.
9. Zhou, X., Zeng, M., Huang, F., Qin, G., Song, Z. and Liu, F. 2023. The potential role of plant secondary metabolites on antifungal and immunomodulatory effect. *Applied Microbiology and Biotechnology*. **107**(14): 4471-4492.
10. Khameneh B., Iranshahy M., Soheili V., Bazzaz BSF. 2019. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance and Infection Control*. **8**: 118.

11. Šovljanski, O., Kljakić, A.C. and Tomić, A. 2023. Antibacterial and antifungal potential of plant secondary metabolites. In: Mérillon, JM., Ramawat, K.G. (eds) Plant specialized metabolites. Pp: 1-43. Reference Series in Phytochemistry. *Springer, Cham*.
12. Saroj, A., Chanotiya, C. S., Maurya, R., Pragadheesh, V. S., Yadav, A. and Samad, A. 2019. Antifungal action of *Lippia alba* essential oil in *Rhizoctonia solani* disease management. *SN Applied Sciences*. **1**: 1-12.
13. Masoud, S. A. and Hamed, S. 2022. Antifungal activity of cassia plant extract and silica nanoparticals (NPs) against *Fusarium oxysporum* and *Rhizoctonia solani*. *Egyptian Journal of Chemistry*. **65**(6): 573-583.
14. Soliman, S. A., Hafez, E. E., Al-Kolaibe, A. M., Abdel Razik, E. S. S., Abd-Ellatif, S., Ibrahim, A. A., Kabeil S. S. A. and Elshafie, H. S. 2022. Biochemical characterization, antifungal activity, and relative gene expression of two Mentha essential oils controlling *Fusarium oxysporum*. the causal agent of *Lycopersicon esculentum* root rot. *Plants*. **11**(2): 189.
15. Bhattarai, K.R. and Subedi, S.C. 2023. Important medicinal and aromatic plants – Nepal. In: Medicinal and aromatic plants of the world. Pp: 1-7. Encyclopedia of Life Support Systems (EOLSS).
16. Steenkamp, P. A., Harding, N. M., Van Heerden, F. R. and Van Wyk, B. E. 2004. Fatal Datura poisoning: Identification of atropine and scopolamine hyoscyamine and scopolamine by high performance liquid chromatography/photodiode array /mass spectrometry. *Forensic Science International*. **145**(1): 31-39.
17. Ganesan, K., Nair, S. K. P., Sinaga, M. and Gani, S. B. 2016. A Review on the phytoconstituents and pharmacological actions in the medicinal plants of Bedabuna forest, Jimma zone, South West Ethiopia reported effect on experimental models. *European Journal of Biomedical and Pharmaceutical Sciences*. **3**(1): 62-83.
18. VasudhaUdupa, A., Gowda, B., Kumarswammy, B. E. and Shivanna, M. B. 2021. The antimicrobial and antioxidant property, GC–MS analysis of non-edible oil-seed cakes of Neem, Madhuca, and Simarouba. *Bulletin of the National Research Centre*. **45**(1): 1-14.
19. Hanif, S., Jabeen, K., Akhtar, N. and Iqbal, S. 2022. GC-MS analysis & antifungal activity of *Datura metel* L. against *Rhizoctonia solani* Kuhn. *Anais da Academia Brasileira de Ciências*. **94**(1): e20200851.
20. Santra, H. K. and Banerjee, D. 2020. Natural products as fungicide and their role in crop protection. *Natural Bioactive Products in Sustainable Agriculture*. **2020**:131-219.
21. Kafle, S., Vaidya, A., Pradhan, B., Jørs, E. and Onta, S. 2021. Factors associated with practice of chemical pesticide use and acute poisoning experienced by farmers in Chitwan district, Nepal. *International Journal of Environmental Research and Public Health*. **18**(8): 4194.
22. Mobin, L., Saeed, S. A., Ali, R., Saeed, S. G. and Ahmed, R. 2017. Antibacterial and antifungal activities of the polyphenolic fractions isolated from the seed coat of *Abrus precatorius* and *Caesalpinia crista*. *Natural Product Research.* **32**(23): 2835-2839.
23. Asma, B. S., Palanimurugan, A., Dhanalakshmi, A. and Thangadurai, S. 2022. Synthesis and characterization of favored transition metal ions using *Datura metel* seed extracts and its better antimicrobial activity. *Materials Today: Proceedings*. **68**: 515-522.
24. Rajesh, D. and Sharma, G. L. 2002. Studies on antimycotic properties of *Datura metel*. *Journal of Ethnopharmacology*. **80**(2-3): 193–197.
25. Pratheeba, T., Vivekanandhan, P., Faeza, A. N. and Natarajan, D. 2019. Chemical constituents and larvicidal efficacy of *Naringi crenulata* (Rutaceae) plant extracts and bioassay guided fractions against *Culex quinquefasciatus* mosquito (Diptera: Culicidae). *Biocatalysis and Agricultural Biotechnology*. **19**: 101137.
26. Dadang. L. and Ohsawa, K. 2001. Efficacy of plant extracts for reducing larval populations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) and cabbage webworm, *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae), and evaluation of cabbage damage. *Applied Entomology and Zoology*. **36**(1): 143–149.
27. Lee. K. W., Omar, D., Cheng, G. L. E., Nasehi, A. and Wong, M. Y. 2018. Characterization of phenazine and phenazine-1-carboxylic acid isolated from *Pseudomonas aeruginosa* UPMP3 and their antifungal activities against *Ganoderma boninense*. *Pertanika Journal of Tropical Agricultural Science*. **41**(4):1795-1809.
28. Iranbakhsh, A., Ebadi, M. and Bayat, M. 2010. The inhibitory effects of plant methanolic extract of *Datura stramonium* L. and leaf explant callus against bacteria and fungi. *Global Veterinaria*. **4**(2): 149-155.
29. Jambhulkar, P. P., Sharma, M., Lakshman, D. and Sharma, P. 2015. Natural mechanisms of soil suppressiveness against diseases caused by *Fusarium*, *Rhizoctonia*, *Pythium*, and *Phytophthora*. In: Organic amendments and soil suppressiveness in plant disease Management (eds. Meghvansi, M. K., and Varma A.). Soil Biology Series. *Springer*. **46**: 95-123
30. Bel Hadj Salah, K., Tahrani, L., Alamoudi, S., Al-Quwaie, D. A. H., Harzallah-Skhiri, F. and Aouni, M. 2022. In vitro antibacterial, antidermatophyte and anticandida potentials of different organic extracts and total alcaloids of Indian thorn apple (*Datura metel* L.) from Tunisia. *Applied Ecology & Environmental Research*. **20**(2): 1585-1598.
31. Kurucheve, V., Gerard, E. J. and Jayaraj, J. 1997. Screening of higher plant for fungi toxicity against *Rhizoctonia solani* in vitro. *Indian Phytopathology*. **50**(2): 235-241.

32. Vega-Ceja, J. E., Jiménez-Amezcua, R. M., Anzaldo-Hernández, J., Silva-Guzmán, J. A., Torres-Rendón, J. G., Lomelí-Ramírez, M. G. and García-Enriquez, S. 2022. Antifungal activity of *Datura stramonium* L. extractives against xylophagous fungi. *Forests*. **13**(8): 1222.
33. Jabeen, N., Khan, I. H. and Javaid, A. 2022. Fungicidal potential of leaf extract of *Datura metel* L. to control *Sclerotium rolfsii* Sacc. *Allelopathy Journal*. **56**(1): 59-68.
34. Elumalai, E. K., Sivamani, P., Thirumalai, T., Vinothkumar, P., Sivaraj, A. and David, E. 2009. In vitro antifungal activities of the aqueous and methanol extract of *Abrus precatorius* Linn (Fabaceae) seeds. *Pharmacologyonline*. **2**: 536-543.
35. Yasmin, N., Ulabdin, Z., Shahid, M., Sheikh, M. A., Manzoor, A. and Jamil, A. 2015. Biosorption Characteristics and Novel Antifungal Activity of *Abrus precatorius* Seed Extract. *Asian Journal of Chemistry*. **27**(4): 1388-1390.
36. Prashith Kekuda T. R., Vinayaka, K. S., Soumya, K. V., Ashwini, S. K. and Kiran, R. 2010. Antibacterial and antifungal activity of methanolic extract of *Abrus pulchellus* wall and *Abrus precatorius* Linn-A comparative study. *International Journal of Toxicological and Pharmacological Research*. **2**(1): 26-29.
37. Sharma, B. and Kumar, V. 2018. Determination of antifungal potential of secondary metabolites from *Jatropha curcas* and *Abrus precatorius*. *Seeds*. **1**(68.50): 95-100.
38. Saha, S., Walia, S., Kumar, J., Dhingra, S. and Parmar, B. S. 2010. Screening for feeding deterrent and insect growth regulatory activity of triterpenic saponins from *Diploknema butyracea* and *Sapindus mukorossi*. *Journal of Agricultural and Food Chemistry*. **58**(1): 434-440.
39. Chhetry, A. K., Dhakal, S., Chaudhary, L., Karki, K., Khadka, R. B., Chaudhary, G. P., Bastola, T., Poudel, A., Aryal, P. and Pandey, J. 2022. Study of antibacterial activity of root bark, leaves, and pericarp extracts of *Diploknema butyracea* and evaluation of prospective antioxidant activity. *Journal of Tropical Medicine*. **2022:** 6814901.
40. Dawadi, P., Siddiqui, M. A., Belbase, S., Syangtan, G., Kronenberg, B., Rana, K., Ercişli, S., Chaudhary, R., Joshi, D. R. and Bhatt, L. R. 2023. Characterizing Nutritional, Antioxidant and Antimicrobial Values of *Diploknema butyracea* (Roxburgh) HJ Lam from the Chepang Community, Makwanpur, Nepal. *Nepal Journal of Biotechnology*. **11**(2): 65-74.
41. Guemari, F., Laouini, S. E., Rebiai, A., Bouafia, A., Meneceur, S., Tliba, A., Majrashi, K. A., Alshareef, S., A., Menaa, F. and Barhoum, A. 2022. UV-visible spectroscopic technique-data mining tool as a reliable, fast, and cost-effective method for the prediction of total polyphenol contents: validation in a bunch of medicinal plant extracts. *Applied Sciences*. **12**(19): 9430.
42. Vanitha, A. 2020. Chemical constituents from ethanol extract of *Abrus precatorius* by using GCMS techniques. *Scholar: National School of Leadership*. **9**(1.4): 1-10.
43. Bhagat, D. S., Sontakke, S. K., Gurnule, W. B. and Shejul, S. K. 2020. Ultrasonic-assisted extraction of active ingredients from *Abrus precatorius* seeds for study of antimicrobial activity. *Alochana Chakra Journal.* **9**(5): 717-724.
44. Carlini, C. R. and Grossi-de-Sa, M. F. 2002. Plant toxic proteins with insecticidal properties—A review on the potentialities as bioinsecticides. *Toxicon*. **40**: 1515-1539.
45. Lalitha, T. and Venkataraman, L. V. 1991. Antifungal activity and mode of action of saponins from *Madhuca butyracea* Macb. *Indian Journal of Experimental Biology*. **29**(6): 558-562.
46. Tejaswini, P., Pradeep, H. R., Khare, D. and Math, A. 2023. Determination of total alkaloid content in unpurified Dhattura beeja (*Datura metel* L. seeds) and purified Dhattura beeja (*D. metel* L. seeds) by UV-spectroscopic method. *Journal of Drug Research in Ayurvedic Sciences*. **8**(2): 181-189.
47. Jakabová, S., Vincze, L., Farkas, Á., Kilár, F., Boros, B. and Felinger, A. 2012. Determination of tropane alkaloids atropine and scopolamine by liquid chromatography–mass spectrometry in plant organs of Datura species. J*ournal of Chromatography A*. **1232**: 295–301.
48. Céspedes-Méndez, C., Iturriaga-Vásquez, P. and Hormazábal, E. 2021. Secondary metabolites and biological profiles of Datura genus. *Journal of the Chilean Chemical Society*. **66**(2): 5183-5189.
49. Al-Snafi, A. E. 2017. Medical importance of *Datura fastuosa* (syn: *Datura metel*) and *Datura stramonium* - A review. *IOSR Journal of Pharmacy.* **7**(2):43-58.