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ANTIFUNGAL ACTIVITY AND PHYTOCHEMICAL PROFILE OF CHLOROFORM SOLUBLE FRACTION OF *DATURA METEL* FRUIT

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ABSTRACT

Datura metel is a weed of family Solanaceae. In the present study, dried and powdered fruits of this medicinal plant species were extracted in methanol for two weeks. After evaporating the solvent, the remaining gummy extract was mixed in water and fractionated using *n*-hexane to separate the non-polar fraction. The remaining aqueous phase was partitioned with chloroform in a separating funnel. The chloroform fraction was separated and evaporated on a rotary evaporator. The gummy biomass of this fraction obtained after complete evaporation of the solvent was dissolved in dimethylsulphoxide (DMSO) and its different concentrations (3.125 to 200 mg/ml) were prepared in malt extract broth. Antifungal activity of the extract was checked against a highly destructive soil-borne phytopathogen *Sclerotium rolfsii* isolated from bell pepper plants suffering from collar rot disease. Results revealed that there was 35–51% reduction in biomass of *S. rolfsii* due to different concentrations of the chloroform fraction. Twelve compounds were identified through GC-MS analysis of this fraction. The predominant were 1-hexacosanol (15.45%), 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester (14.15%), 1-octadecanol (12.81%), 1-octadecene (12.80%) and 1-eicosanol (8.12%), which could be responsible for antifungal activity against *S. rolfsii*.

Keywords: Bell pepper, *Datura metel* fruit, methanolic extract, natural fungicides, *Sclerotium rolfii.*

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INTRODUCTION

Datura metel L., family Solanaceae, is known for its numerous biological activities (Su *et al*., 2017). It grows as a wild plant in warmer regions all over the world. Having origin in Tropical America, the plant is also cultivated in various temperate and tropical regions (Mai *et al.,* 2017). The plant has been used in folk medicines for centuries to treat pain, insanity, cough, convulsion convulsions, asthma, and rheumatism (Fu *et al.,* 2017). Presence of tropane class of alkaloids in *D. metel* makes this plant to be used as mydriatic, anti-spasmodic and sedative agent (Nuhu, 2002). Main chemical constituents of *D. metel* are withanolide-type steroids (Yang *et al.,* 2010). Furthermore, some amide alkaloids and megastigmane sesquiterpenes have also been isolated from this plant (Kuang *et al*., 2008; Yang *et al*., 2010). The plant is known to have insecticidal activity against cotton bollworm *Helicoverpa armigera* (Singh and Singh, 2008), herbicidal activity against *Parthenium hysterophorus* (Javaid *et al.,* 2010), antifungal activity against *Macrophomina phaseolina, S. rolfsii* and *Colletotrichum gloeosporioides* (Javaid and Saddique, 2011; Karim *et al*., 2017; Jabeen *et al.,* 2022), antibacterial activity against *Bacillus cereus, Escherichia coli* and *Klebsiella pneumoniae* (Akharaiyi, 2011), antioxidant activity (Akharaiyi, 2011), and anticancer activity (Pan *et al*., 2007).

Sclerotium rolfsii is a serious soil-borne plant pathogen with extensive host range (Jabeen *et al.,* 2021). Above 500 plant species belonging to about 100 families are susceptible to this pathogen (Deepthi and Reddy, 2013). Mostly members of Leguminoseae, Cucurbitaceae, Solanaceae and Brassicaceae families are host of this fungus (Javaid and Khan, 2016; Wavare *et al.,* 2017; Sharf *et al.,* 2021). A number of strategies are being used to control this fungal pathogen including application of fungicides (Augusto *et al*., 2010; Khan and Javaid, 2015). However, this strategy is undesirable especially in food crops like bell pepper because of ill effects of synthetic fungicides on environment and health risks associated with the application of these agrochemicals (Westlund *et al*., 2018). Many recent studies have shown that extracts and pure compounds of plant species namely *Coronopus didymus, Chenopodium album, C. quinoa, Acacia nilotica* and *Melia azedarach* can be used as alternatives to synthetic fungicides for the control of *S. rolfsii* (Javaid and Iqbal, 2014; Sana *et al*., 2016, 2017; Ali *et al*. 2020; Khan *et al.,* 2020) and other fungal pathogens such as *Macrophomina phaseolina* (Khan and Javaid, 2020a; Banaras *et al.,* 2021). However, studies regarding antifungal activity of *D. metel* fruit against *S. rolfsii* are

rare. Therefore, the present study was carried out to investigate antifungal activity of chloroform fraction of methanolic fruit extract of *D. metel* against *S. rolfsii* and the identification of possible antifungal constituents through GC-MS analysis.

MATERIALS AND METHODS

Preparation of extract: Fruits of *D. metel* (5 kg) were collected from Lahore, Pakistan, dried and crushed, and dipped in methanol (10 l) for 10 days. After that, material was passed through doubled layered muslin cloth. Residues were re-extracted with methanol and filtered through a double layered muslin cloth again followed by filtration by a filter paper sheet. The solvent was evaporated under reduced pressure on a rotary evaporator at 45 °C to yield crude methanolic extract of *D. metel* fruit. Crude gummy methanolic extract was added to 300 ml distilled water and shaked well to homogenized the mixture. The mixture was transferred to a separating funnel and extracted successively with *n*-hexane (500 ml × 5) followed by chloroform (500 ml). Thereafter, chloroform was evaporated under vacuum in a rotary evaporator and 19.9 g of the chloroform sub-fraction were obtained (Javaid *et al.,* 2018). Chloroform fraction was selected on the basis of various previous studies where this fraction showed more antifungal activity as compared to *n*-hexane sub-fraction (Banaras *et al.,* 2020; Khan *et al.,* 2021).

Antifungal bioassay: Antifungal activity of chloroform sub-fraction was investigated *in vitro* against *S. rolfsii.* The fungus was isolated on malt extract agar medium from a bell pepper plant suffering from collar rot disease. The isolated fungus was identified on morphological basis especially on the basis of pattern of mycelial spreading and sclerotia formation. Koch's postulates confirmed the pathogenicity of the fungus.

For antifungal bioassays, 1.2 g of the chloroform sub-fraction was dissolved in 0.5 ml of DMSO followed by addition of 5.5 ml malt extract broth to prepare a 200 mg/ml solution. This stock solution was serially double diluted to make lower concentrations of 100, 50, 25, 12.50, 6.25 and 3.125 by adding malt extract broth. For control, 0.5 ml of DMSO were mixed with 5.5 ml malt extract broth and serially double diluted to get various concentrations of DMSO in control corresponding to various concentrations of DMSO in the extract treatments. Each treatment was replicated three times with 1 ml medium in each 10-ml volume test tube. Tubes were inoculated by taking standard droplets of 10 µl from fungal suspension. Test tubes were incubated for seven days at 25 °C. After seven days, fungal biomass was filtered, dried and weighed (Khan and Javaid, 2020b).

Percentage decrease in fungal biomass in each treatment over corresponding control treatment was calculated by applying the following formula:

$$\text{Decrease over control (\%)} = \frac{C - T}{C} \times 100$$

Where C and T represent fungal biomass in control and extract treatments, respectively.

GC-MS analysis: Chloroform sub-fraction was dissolved in chloroform to make it diluted, and filtered. The filtrates were subjected to GC-MS analysis for identification of the chemical constituents present in it. GC-MS was conducted on Shimadzu GC-2010 plus system coupled with an auto injector AOC-20i, an auto sampler AOC-20s and a gas chromatograph using helium as a carrier gas.

Statistical analysis: Data regarding antifungal activity of the chloroform sub-fraction was presented as means ± standard errors of three replicates. The data were analyzed by one-way analysis of variance (ANOVA) followed by LSD test for significance of difference in treatment means ($P \leq 0.05$). Computer software Statistix 8.1 was used for data analysis.

RESULTS AND DISCUSSION

Different concentrations of chloroform sub-fraction of fruit extract exhibited highly pronounced adverse effect on fungal growth causing 36–50% reduction in biomass of *S. rolfsii* (Fig. 1). Previous studies mostly showed antifungal activity of other parts of *D. metel* such as leaves, roots, seeds and flowers. Rinez *et al.* (2013) recorded 69% in radial growth of *Tichoderma viride* due to aqueous flower extract and 24–76% suppression in growth of *Fusarium oxysporum* f. sp. *melonis* due to various organic solvent leaf extracts of *D. metel.* A 1.5% seed extract of *D. metel* reduced the growth of *Colletotrichum gloeosporioides* by 80% (Karim *et al.,* 2017). Similarly, ethanolic extract of *D. metel* leaves caused 100% inhibition in spore germination of *Colletotrichum camelliae*, *Botryodiplodia theobromae* and *Pestalotiopsis theae* (Saha *et al.,* 2005).

Twelve compounds were identified in the chloroform fraction of methanolic fruit extract of *D. metel.* Molecular weight, molecular formulae and peak areas of these compounds are illustrated in Table 1 and their antifungal activities, if any, are presented in Table 2. The most abundant compound was 1-hexacosanol (15.45%) followed by 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester (14.15%), 1-octadecanol (12.81%),1-octadecene (12.80%), 1-eicosanol (8.12%), 1-heptadecene (7.90%), 1,3(15),10-bisabolatriene (7.61%),1,6,10- farnesatrien-3-ol (7.65%), 1-pentadecanol (5.80%), 1-pentadecene (2.68%), 1-tetradecanol (2.61%) and 1-dococene (1.10%).

Compounds identified in the chloroform soluble fraction of methanolic fruit extract generally belonged to alcohols, alkenes and esters. According to Tuney *et al*. (2006) antimicrobial activity of plants extracts may be due to volatile compounds such as terpenoid, volatile fatty acids, phenols, phytol (acyclic diterpene alcohol), alkenes and esters. Similarly, Kim and Park (2012) found significant antifungal activity of alcohols (citronellol and geraniol) isolated from *Acorus gramineus* against *Phytophthora cactorum, Fusarium circinatum* and *Cryponectria parasitica*. Manohar (2015) identified 21 bioactive components from *Geodorum densiflorum* through GC-MS where alcohols and esters had antimicrobial activity. Kolawole *et al*. (2015) identified 78 organic compounds in *Senna alata* through GC-MS and found that aldehydes, alkenes, fatty alcohols, acetic acid, ketones and esters had good antifungal activity. Pohl *et al*. (2011) reported that alkenes and fatty acids have antifungal activity. Alkenes inhibited the myrisoylation of proteins of cell membrane. They also inhibited b-oxidation, triacylglycerol and sphingolipid synthesis and topoisomerase activity. Kiruthika and Sornaraj (2011) identified the alkenes in the *D. metel* flower through GC-MS having antimicrobial activity.

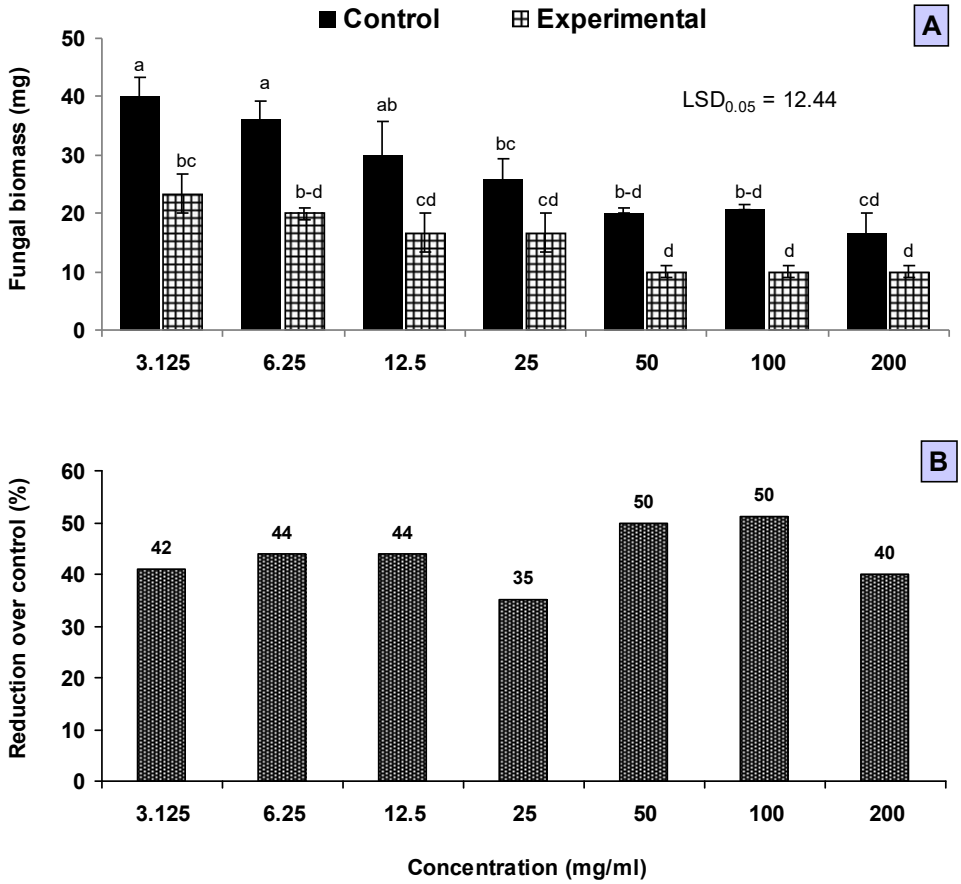


Fig. 1. Effect of chloroform sub-fraction of methanolic fruit extract of *Datura metel* on *in vitro* growth of *Sclerotium rolfsii*. A: Effect of chloroform sub-fraction on fungal biomass, B: Percentage inhibition in fungal biomass over corresponding control treatments due to different concentrations of chloroform sub-fraction. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

The most abundant compound in the present study was 1-hexacosanol. In previous studies, it also exhibited antimicrobial activity against many bacteria and yeast (Castilho *et al.,* 2012). Apart from its antifungal activity, this compound is also known to exhibit a variety of biological activities. Mosquito larvicidal activity of an insect repellent plant *Chromolaena odorata* is reported to be due to this compound (Gade *et al.,* 2017). This compound has also been isolated from *Euphorbia peplus* and is expected to be one of the major compounds in *n*-hexane fraction of methanolic extract having leishmanicidal activity (Amin *et al.,* 2017).

The second most predominant compound found in the present study was 1,2-benzenedicarboxylic acid bis (2-methylpropyl) ester. Previously, this compound has been identified in diverse groups of organisms with a

number of important properties. Klusaite *et al*. (2016) isolated this compound from bacterial strain 1410WF1-TSA30-2 and found that it had antibacterial activity against Gram- positive strains. Dong *et al*. (2016) identified it from an algal species *Cladophora fracta* and reported its algicidal property against *Gymnodinium breve* and *Heterosigma akashiwo*. It was among the dominant compounds of a flowering plant *Achillea pachycephala,* collected from Iran (Rahimmalek *et al.,* 2012). This compound has also been reported from seaweed *Ulva pertusa* with allelopathic activity against *Gymnodinium breve* (Wang *et al.,* 2008). 1,6,10-Farnesatrien-3-ol, also known as nerolidol, is a sesquiterpene alcohol naturally occurring in essential oils of many plants and flowers including lemon grass, tea tree, ginger, jasmine, neroli and others (Kaiser, 1993). Apart from exhibiting a number of other biological activities, it also possesses antifungal activity against a variety of fungal species infecting both human and plants (Chan *et al.,* 2016). It exhibited fungicidal activity against *Candida albicans* (Curvelo *et al.,* 2014). In a similar study, it stopped the growth of *Trichophyton mentagrophytes* at a 0.4 mg/ml concentration (Park *et al.,* 2009). Lee *et al*. (2007) described strong antifungal effects of this compound against *Microsporum gypseum* causing dermatophytosis, an infection of keratinized tissues such as hair, nail and skin. The compound is present in *cis*- and *trans*- forms. *Trans*-nerolidol, isolated from essential oil of *Lantana radula* showed strong fungistatic activity against a plant pathogenic fungus *Corynespora cassiicola* (Passos *et al.,* 2012). Likewise, essential oil of *Piper chaba* with *Trans*-nerolidol as main constituent showed antifungal activity against numerous phytopathogenic fungi namely *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Colletotrichum capsici* and *Phytophthora capsici* (Rahman *et al.,* 2011).

Table 1. Structures of various constituents isolated from chloroform fraction of methanolic fruit extract of *D. metel*.

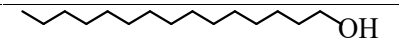
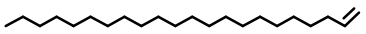
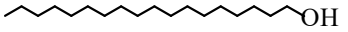
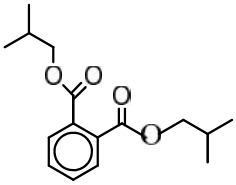
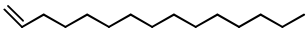
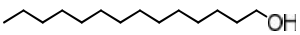
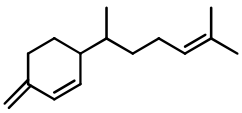
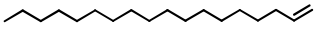
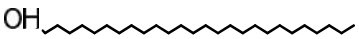
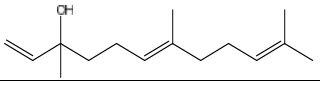
Sr. #	Names of the compounds	Molecular Formula	Molecular Weight	Peak Area(%)	Structures
1	1-Pentadecanol	$C_{15}H_{32}O$	228	5.80	
2	1-Dococene	$C_{22}H_{44}$	308	1.10	
3	1-Heptadecene	$C_{17}H_{34}$	238	7.90	
4	1-Octadecanol	$C_{18}H_{36}$	252	12.81	
5	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	14.15	
6	1-Pentadecene	$C_{15}H_{30}$	210	2.68	
7	1-Tetradecanol	$C_{14}H_{30}O$	214	2.61	
8	1,3(15),10-Bisabolatriene	$C_{15}H_{24}$	204	7.61	
9	1-Octadecene	$C_{18}H_{36}$	252	12.80	
10	1-Eicosanol	$C_{20}H_{42}O$	298	8.12	
11	1-Hexacosanol	$C_{26}H_{54}O$	382	15.45	
12	1,6,10- Farnesatrien-3-ol	$C_{15}H_{26}O$	222	7.65	

Table 2. Nature and properties of compounds identified from chloroform fraction of methanolic fruit extract of *Datura metel* through GC-MS analysis.

Comp. No.	Names of compounds	Nature	Property	Reference
1	1-Pentadecanol	Fatty alcohol	Antimicrobial	Kubo *et al*. (1994)
2	1-Docosene	Alkene	-	-
3	1-Heptadecene	Alkene	-	-
4	1-Octadecanol	Fatty alcohol	Antibacterial	Al-Hakami *et al*. (2013)
5	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	Ester	Antibacterial, algicidal, allelopathic	Wang *et al*. (2008); Dong *et al*. (2016); Klusaite *et al*. (2016)
6	1-Pentadecene	Alkene	-	-
7	1-Tetradecanol	Fatty alcohol	Therapeutic	Hasturk *et al*. (2009)
8	1,3(15),10-Bisabolatriene	Sesquiterpene	Antibacterial	Sharma *et al*. (2016)
9	1-Octadecene	Alkene	Antioxidant	Adeosun *et al*. (2013)
10	1-Eicosanol	Alcohol	-	-
11	1-Hexacosanol	Ceryl alcohol	Antimicrobial, larvicidal, leishmanicidal	Castilho *et al*. (2012); Amin *et al*. (2017); Gade *et al*. (2017)
12	1,6,10- Farnesatrien-3-ol	Sesquiterpene alcohol	Antifungal, antibacterial,	Chan *et al*. (2016)

Conclusion: The present study concludes that chloroform fraction of methanolic fruit extract of *D. metel* exhibited antifungal activity against *S. rolfsii* was possibly because of presence of 1,2-benzenedicarboxylic acid bis (2-methylpropyl) ester; 1,6,10- farnesatrien-3-ol; and 1-hexacosanol. This study will be helpful in preparation of natural products-based fungicides for the control of *S. rolfsii* by using the structures of potential antifungal compounds as analogues.

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