



MICROBIOLOGY

GC-MS analysis & antifungal activity of *Datura metel* L. against *Rhizoctonia solani* Kuhn

SHAHNAZ HANIF, KHAJISTA JABEEN, NAUREEN AKHTAR & SUMERA IQBAL

Abstract: The current study was designed to evaluate the antifungal properties of *Datura metel* L. against *Rhizoctonia solani* Kuhn. To achieve this objective, six concentrations of leaves & stem methanol extract of *D. metel* viz. 1%, 1.5%, 2%, 2.5%, 3% & 3.5% were tested against *R. solani* *in vitro*. Leaf extract of *D. metel* was found more effective as its 3.5% concentration caused 75% retardation in test fungal growth as compared to the stem extract. *D. metel* methanolic leaf extract was fractionated between *n*-butanol, *n*-hexane, chloroform & ethyl acetate & bioactivities of isolated fractions were tested against *R. solani*. The chloroform fraction was found highly effective, as its concentrations 0.1% & 0.01% caused 27% & 21% growth inhibition respectively. So, this particular chloroform fraction was further analyzed to identify various chemical constituents through GC-MS (Gas chromatography mass spectroscopic) analysis. Twelve phyto-constituents viz. eugenol, 2-pentadecanone 6,10,14 trimethyl, pentadecanoic acid, pentadecanoic acid, 1 4-methyl- methyl ester, phytol, 9,12,15-octadecatrienoic acid, heptacosane, *n*-hexadecanoic, 6-octadecanoic acid, 9, 12 octadecanoic acid, dodecanoic & tetradecanoic acids were identified. So, the present study concluded that the presence of these bioactive constituents make *D. metel* as an effective antifungal agent against *R. solani*.

Key words: Damping-off, *in vitro*, phyto-constituents, GC-MS.

INTRODUCTION

Among the destructive pathogens, *Rhizoctonia solani* Kuhn. is the worst pathogen that causes foot rot & seedling damping off disease in tomatoes (Gondal et al. 2019). *Rhizoctonia Solani*, belongs to class Basidiomycetes, is considered as a universal pathogen that cause diseases in many plants including important crops like soybean, rice, corn, beans, sugarcane, sorghum, potato, spinach, melon, lettuce & tomato (Kuramae et al. 2003, Ferrucho et al. 2012, Uppala & Zhou 2018). This fungus is present in soil as vegetative mycelium & develops special structures known as sclerotia that persist free or rooted in plant debris causing pre or post emerging damping off disease worldwide

(Lakshman et al. 2008, Bartholomaeus et al. 2017). *Rhizoctonia solani* is also called as necrotrophic soil born plant pathogen, due to destruction of many economically significant crops of the world (Verwaaijen et al. 2017). Tomato (*Solanum lycopersicum* L.) family Solanaceae is considered as a significant crop worldwide among the vegetables. Tomatoes contain important minerals, vitamins, sugars & the most important anti-oxidant lycopene (Khalid 2013). In Pakistan tomatoes are grown in an area of 62536 hectares with a production of approximately 587111 tons with maximum yield of 10.5 tons per hectare (FAO 2016). Due to the attack of several viral & soil borne microbial pathogens, the yield per hectare of tomato is badly affected. In nurseries where young vulnerable transplants

are produced damping-off caused by *R. solani*, causes substantial losses (Arakawa & Inagaki 2014).

Damping-off is a mutual problem in almost all fields & greenhouse crops. Different approaches are applied to control this phytopathogenic fungus includes cultural, biological & chemical practices. *Rhizoctonia* can be controlled by cultural means like crop rotation; which destroys the sclerotia. One of the important steps to resist damping off disease is, the use of sclerotia free certified seeds in the soil. Organic matter mulching in soil is also considered as a significant eco-friendly method to control the disease (Mishra et al. 2012). Majority of fungicides like mefenoxam, etridiazole & fosetyl-aluminum can be used to halt the disease. The application of fungicides like Moncut & Quadris produce significant results to control the *R. solani* infection in soils (Wharton et al. 2007). Fumigation with metham & sodium can also be used to control the disease (Muriung et al. 2014). The excessive use of fungicides affects soil productivity, soil texture & growth of soil microorganisms (Rani et al. 2017).

Management of phytopathogenic diseases by nontoxic anti-fungal agents like extracts of plants is an environment friendly alternative over harmful pesticide (Mangang & Chhetry 2012, Tumen et al. 2013). The use of plant materials as natural constituents could be a substitute to fungicides (Tsair-Bor & Shang-Tzen 2008). Plant extracts are usually comprise of many secondary metabolites like terpenoids, saponins, alkaloids phenolic, & flavonoids glycosides possess strong antifungal effects (Bobby et al. 2012). Several higher plants & their constituents have been reported to control a number of pathogens that cause severe plant diseases (Elzaki et al. 2012, Hanif et al. 2017, Munawwar et al. 2018, Khan et al. 2018, Bashir et al. 2019).

Datura metel the member of Solanaceae family is famous due to its herbicidal, anti-bacterial & anti-fungal activity (Monira & Munan 2012). This plant contains tropane, withanolide, trigloyl esters of tropine, calystegines & pseudotropine alkaloids (Ghani 2003, Dabur et al. 2004). Therefore, this study was planned to assess the phytochemical potential of *D. metel* against the target pathogenic fungus, *R. solani*.

MATERIALS & METHODS

Collection of *Datura metel* plants

Stem & leaves of the test plant, *D. metel* were collected from Lahore. The plant material was washed under running tap & blot dried. Dust & insect free stem & leaf materials were surface sterilized with 1% sodium hypochlorite for 2 minutes followed by several washing with sterilized water. Surface disinfected test plant materials were dried in an electric oven at 40 °C overnight & kept in labeled sealed bags.

Culturing of *rhizoctonia solani*

R. solani was isolated from disease plant of tomato by a specific method; the sclerotia were scratched from surface of tomato dipped in spirit for few second & then soaked in distilled water. After that these sclerotia were put on filter paper for maximum absorption of moisture. The sterilized sclerotia were placed in the Petri dishes having MEA & were kept in incubator at temperature 22°C. After two days, the fine filaments of mycelia were originated from sclerotia. Pure culture of test fungus was kept in refrigerator at temperature of 4°C (Hanif et al. 2017).

Antifungal assays

Dried plant material fifty grams were soaked separately in 250 mL methanol at room temperature for three days. After that the test

plant materials were filtered with sterilized muslin cloth & evaporated at 35 °C till the final volume of 3.5 g extracts (in the form of thick paste) were attained. Aqueous 20% (W/V) stock solutions of crude plant extracts were prepared by adding an adequate amount of distilled water & stored at 4 °C. Malt Extract Agar (MEA) 2% was prepared in conical flask. Medium was sterilized by autoclaving for 20 min at 121 °C. Six concentrations of *D. metel* methanolic extract viz. 1%, 1.5%, 2%, 2.5%, 3% & 3.5% were made by adding 3.5, 5.2, 7.0, 8.8, 10.5 & 12.3 mL of stock solutions to 56.5, 54.8, 53, 51.2, 49.5 & 47.7 mL sterilized water respectively. Within control treatment, plant extracts was lacking. In each concentration Chloromycetin @ 50 mg per 100 mL was added to inhibit bacterial growth. Three replicates were prepared for every treatment. From one week old culture of the selected test fungus *R. solani*, all the flasks were inoculated with 5 mm mycelial discs by using sterilized cork borer. All these flasks were incubated at 28 °C for one week. After seven days readings were taken by filtering the media with pre weighed, sterilized & labeled filter paper. The filtered biomass was dried in oven at 120 °C & weighed till constant weight. Percentage growth inhibition was calculated by using following formula:

$$\text{Fungal growth inhibition (\%)} = \frac{\text{Growth in treatment} - \text{Growth in control}}{\text{Growth in control}} \times 100$$

Fractionation bioassay

Fifty grams dried *D. metel* plant material was dissolved in 150 mL methanol. The extract was separated with four different organic solvents viz. chloroform, *n*-butanol, *n*-hexane & ethyl acetate using separating funnel (Sherazi et al. 2016). The isolated organic fractions were subjected for *in vitro* antifungal activity by using the procedure of (Jabeen et al. 2013). For each isolated fraction 20% stock solution was made by making its two different concentrations i.e.,

0.10% & 0.01%. Antibacterial capsules were mixed to prevent any kind of bacterial growth. Distilled water was considered as a control & it had no plant extract. For each treatment three replicates were prepared and inoculation was done with the culture of test fungus. After one week, the readings was taken. The dried biomass of fungus was weighed & percentage growth inhibition was calculated by using following formula:

$$\text{Fungal growth inhibition (\%)} = \frac{\text{Growth in treatment} - \text{Growth in control}}{\text{Growth in control}} \times 100$$

GC-MS analysis

Chloroform fraction was found to be the most effectual among all the fractions. Fifty grams dried material of *D. metel* was dipped in 150 mL methanol. The extract was then filtered with the help of nylon membrane filter having pore size (0.22 & analyzed for Gas chromatography & Mass Spectrometry analysis (GC-MS). With Hewlett Packard 5890 series gas chromatograph having 5973N mass selective detector, GC-MS analysis of the test sample was done. The gas chromatography was interfaced to a mass spectrometer prepared by capillary column of definite specification (30 x 0.25µm ID x 0.25µm Df) & with an Elite-5MS (5% diphenyl / 95% dimethyl poly saline). The initial oven temperature was 50 °C for 2 min, increased at 20 degree/min to 280 °C, & held for 10 min. 70 Ev Electron impact mode was generated for ionization in the GC-MS analysis. The injection was done in the split-less mode with injection port at 250 degree centigrade temperature. Data acquirement was accompanied in the MS scan mode with the range of 40-650 m/z. The test components were recognized by comparison of their mass spectra with those of mass spectral libraries (NIST & Wiley) to determine its name, structure & molecular weight (Yusoff et al. 2017).

RESULTS & DISCUSSION

In present study *D. metel* leaf & stem methanolic extracts were evaluated to control the pathogen of stressful damping-off disease. The methanolic extract of leaves significantly suppressed the test fungus biomass. Maximum 75% reduction in the test fungus growth was detected in 3.5% concentration. The 3% concentration also retarded the test fungal growth up to 69% (Figure 1a). Stem methanolic extract also possessed antifungal effect but it was lower as compared to leaf methanolic extract (Figure 1b). Earlier Bajwa et al. (2008) observed that extracts of *D.*

metel significantly controlled chickpea blight pathogen, *Ascochyta rabiei*. The plant extracts of *D. metel* also inhibited the spore germination of *Aspergillus flavus* & *A. niger* (Khan & Nasreen 2010). Hanif et al. (2017) also reported that methanolic extract of *Akbizzia lebbeck* significantly suppressed the growth of *R. solani*.

Organic solvent fractions, chloroform, *n*-hexane *n*-butanol & ethyl acetate, were isolated from *D. metel* leaf methanolic extract. *In vitro* experiment was carried out with these isolated fractions. The results with chloroform fractions were found best as compared to other fractions as shown in (Figure 2) that reduced

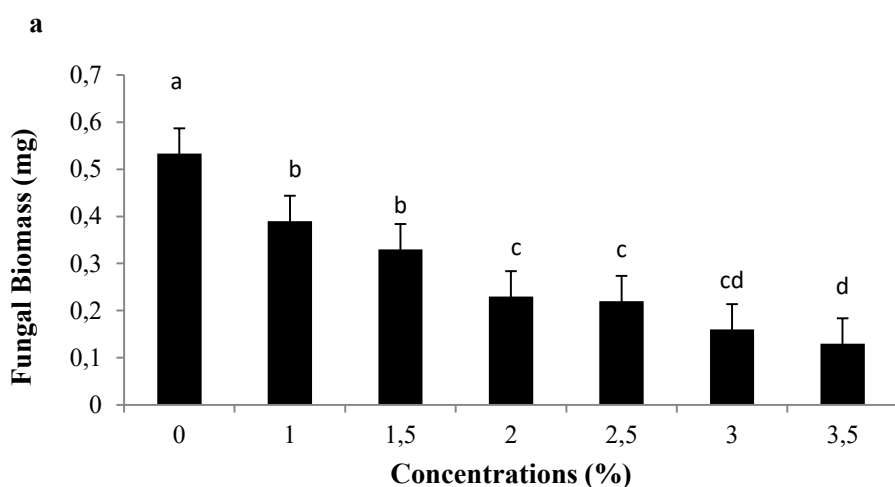


Figure 1a. Effect of *Datura metel* leaf extract on growth of test fungus *Rhizoctonia solani*. Vertical bars indicated standard error of means of replicates. Value having letters showed significant variations analyzed through LSD Testing.

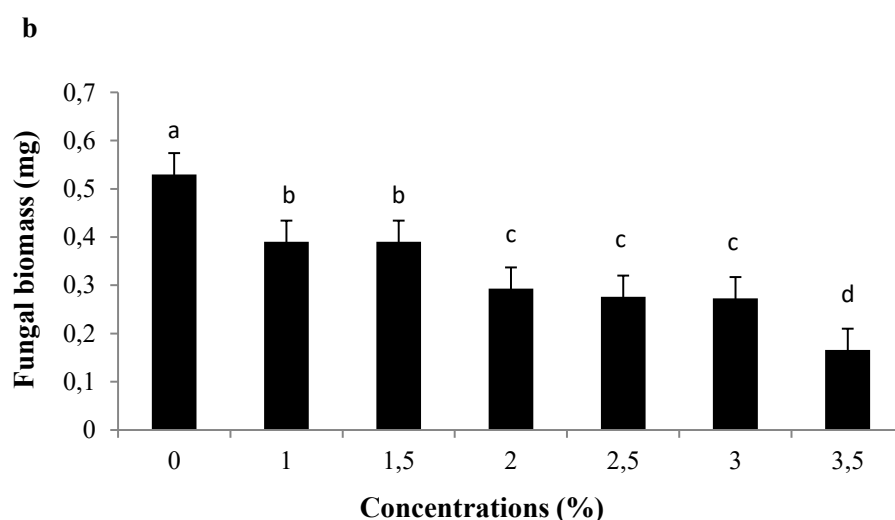


Figure 1b. Effect of *Datura metel* stem extract on growth of test fungus *Rhizoctonia solani*. Vertical bars indicated standard error of means of replicates. Values having letters showed insignificant variations analyzed through LSD Testing.

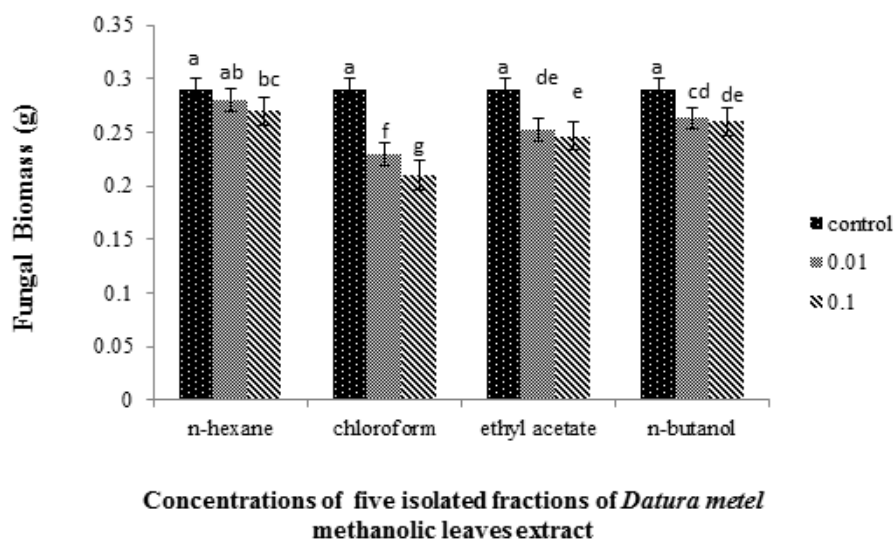


Figure 2. Influence of organic fractions of *Datura metel* leaf with *in vitro* growth of fungus *Rhizoctonia solani* Vertical bars indicated standard error of means of replicates. Values having letters showed insignificant variations analyzed through LSD Testing.

21% - 27% test fungus biomass. Similar activity of chloroform fraction of *D. metel* was observed against *Colletotrichum gloeosporioides* causing anthracnose disease in mango Karim et al. (2017). Difference in antifungal activity of organic fractions may be acknowledged to the polarity & non-polarity of the solvents. It is quite possible that antifungal potential depends on the nature of solvents (Rauf & Javaid 2013) as they have differential solubility for different phytochemicals. *D. metel* contain various phytochemicals which are responsible for antifungal potential against phytopathogenic *Aspergillus flavus*, *A. fumigatus* & *A. niger* (Sharma 2002). *D. metel* extracts also found effectual in retarding the growth of *Macrophomina phaseolina* the cause of charcoal rot disease in corn, soya bean (Javaid & Saddique 2012).

Gas chromatography – Mass Spectrometry analysis with chloroform fraction of *D. metel* was carried out & twelve compounds were identified (Figure 3, Table 1). Some major chemicals like dodecanoic acid, 6-octadecanoic acid, 9, 12-octadecanoic acid, *n*-hexadecanoic acid & tetradecanoic acid with peak area 34.54%, 14.842%, 13.35%, 10.133% & 8.448% respectively along with some minor constituents like phytol

(6.312%), 9,12,15-octadecatrienoic acid (3.599%), heptacosane (3.593%), eugenol (1.570%), pentadecanoic acid (1.455%), 2-pentadecanone 6,10,14 trimethyl (1.136%) & pentadecanoic acid 1 4-methyl- methyl ester (1.019%) were identified. Most of the identified compounds were saturated fatty acids & alkane hydrocarbons. Some were aliphatic diterpenoid alkene, sesquiterpenoids & aromatic (allylbenzene) in nature. In *D. metel*, *n*-hexadecanoic acid was present, which is a methyl ester & it might be responsible for retardation of fungal growth of the test fungi. In the present study, Dodecanoic acid (lauric acid) & 9, 12-octadecadienoic acid was also identified. Walters et al. (2003) reported the antifungal activity of lauric acid against phytopathogenic fungi. The antifungal as well as antibacterial activity of 9, 12-octadecadienoic acid derivatives was assessed by Kapoor & Mishra (2014). It has also been reported that saturated fatty acid possessed antifungal potential against *R. solani*, *Fusarium avenaceum*, *Aspergillus niger*, *Candida albicans*, *Blumeria graminis*, *Cucumerinum lagenarium*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Myrothecium verrucaria* & *Pythium ultimum* (Carolina et al. 2011).

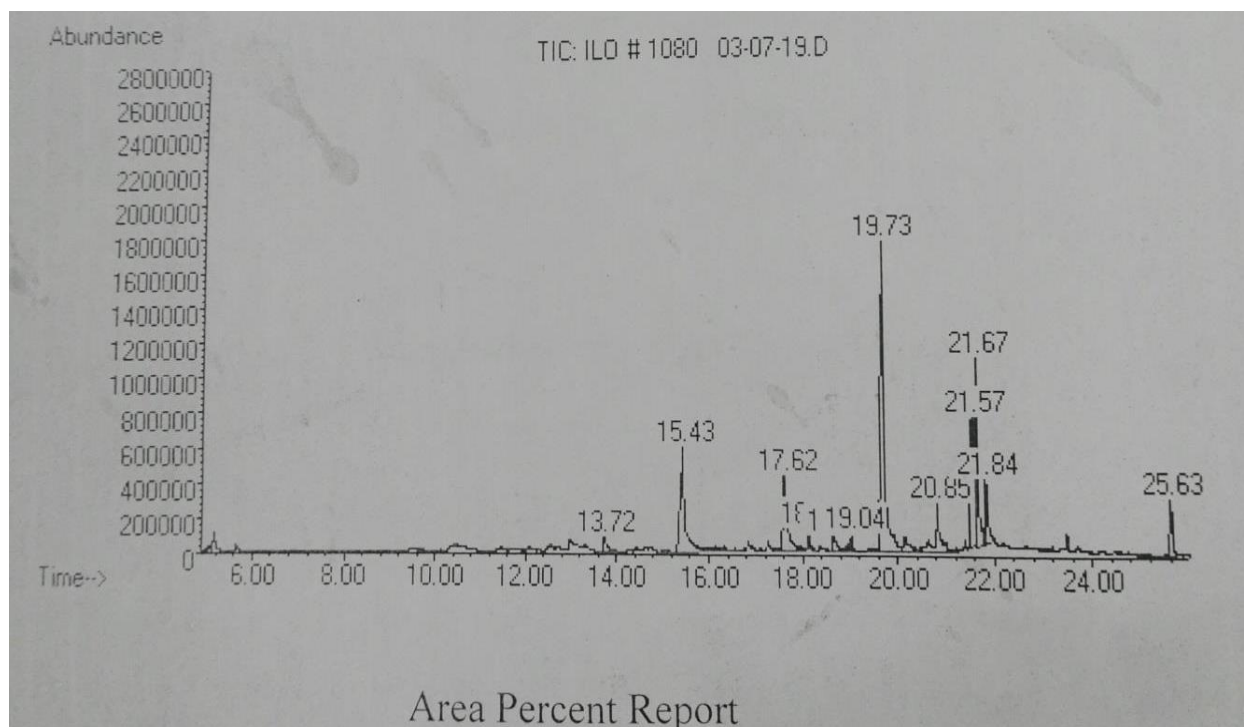


Figure 3. Chromatogram with chloroform extract of *Datura metel* leaf.

Derbalah et al. (2012) suggested that tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, phytol, linalool, 1, 8 cineole & 9, 12, 15 octadecanoic acid were found effective against *Sclerotium rolfsii*. Heptacosane, a volatile oil, was also reported to be hold antifungal as well as antioxidant properties (Kether et al. 2012). Eugenol (4-allyl-2-methoxyphenol) is a naturally present phenolic component also has antifungal potential (Ghosh et al. 2005, Wang et al. 2010).

On the basis of these findings, the present study concluded that *Datura metel* has significant antifungal potential against *Rhizoctonia solani* due to the presence of high proportion of a variety of antifungal phytochemicals. These findings can be used in future investigation for the development of new potential antifungal compounds.

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How to cite

HANIF S, JABEEN K, AKHTAR N & IQBAL S. 2022. GC-MS analysis & antifungal activity of *Datura metel* L. against *Rhizoctonia solani* Kuhn. *An Acad Bras Cienc* 94: e20200851. DOI 10.1590/0001-376520220200851.

Manuscript received on June 15, 2020;
accepted for publication on October 25, 2020

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