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Antimicrobial effect of crude extracts of fresh and dry leaves of *Datura metel* L.

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Abstract
Datura metel, is native of southern China is commonly called horn of plenty, devil's trumpet, angel's trumpet or thornapple. It is shrubby, sprawling, short-lived, tender perennial. The aim of this work is to investigate antimicrobial activities of different crude extracts from dry and fresh leaves of *Datura metel*. Different organic solvents including methanol, chloroform, hexane, ethyl acetate and butanol were used to prepare the crude extract from the fresh and dry leaves. Antimicrobial activities of different crude extracts from dry and fresh leaves of *Datura metel* were determined by DPPH method and agar agar diffusion method with minor modification. *In vitro* phytochemical screening for all the crude extract from both dry and fresh leaves was tested and found positive results for alkaloid, flavonoid, saponin and tannin compound. However, all the crude extract did not show positive result for steroids and triterpenoid compounds. The antioxidant activity results of both fresh and dry crude extracts show that when gradually increasing the samples concentration, there was an increase in the absorbance. Late leaf spot (LLS) (causal agent *Phaeoisariopsis personata*) and rust (causal agent *Puccinia arachidis*) are economically important foliar diseases of groundnut and have global significance. Aqueous leaf extract of *Datura metel* known for high antifungal activity against *P. personata* completely inhibited the germination of uredospores. Extracts of *Datura metel* (25g/L) applied as a prophylactic spray reduce the frequency of LLS lesions and rust pustules by 65-74%. In field studies, a programme of four sprays of *D. metel* leaf extract at 45, 60, 75 and 90 days after showing (DAS) was partially effective against the foliar diseases (LLS and rust) upto 95 DAS. The increase in pod yield by this treatment was comparable with the sustained application of chlorothalonil. Further validation of integrated applications of *D. metel* extract and chlorothalonil may reduce the dependency on fungicides in groundnut cultivation.

Keywords: *Datura metel*, methanol, chloroform, antimicrobial, antifungal, antibacterial

Introduction
Over the past decade, there has been a resurgence of interest in the investigation of natural materials as a source of potential drug substance. Plants have great potential for the treatment and management of some diseases and have been used in many countries for the treatment of different diseases. The medical value of plants lies in their bioactive phytochemicals constituents that produce definite physiological actions in the human body, plants and other animals. The chemical constituents include flavonoid, alkaloid, essential oils, saponins, tannins, etc. many medicinal plants contain some chemical constituents that can cause some harmful effects on human as well as plants if given in large quantity.
Datura metel L. is a member of the plant family Solanaceae. The name of Datura comes from Sanskrit Dustura (Dorman and Deans, 2002) [1] or Dahatura. There are any different species in the Datura genus. *Datura metel* is a herbaceous leafy plant and can grow upto 3ft in height. Its leaves are covered with short and soft greyish hairs. The leaves are alternate and can be 10-20cm long and 5-18cm wide. It is widely found in Asia, Africa, England, India and other tropical and subtropical regions (Kokate *et al*., 2008; Harbone, 1999) [3]. The fruit can contain upto 300 or more seeds. Splitting open when it is ripened to release the numerous seeds. In many parts of India especially in northern part, *Datura metel* is found growing as a weed in abundant farmlands, dumpsites but is sometimes cultivated. Some parts of the plant can be used for many purposes in several ways mostly for its psychoactive activities. This could make the different parts of *Datura metel* to be abused by some youths who are more users and are prone to smoking and drug abuse.

Major chemical constituent of *Datura metel* are scopolamine, daturadiol, hyoscyamine, fastudine, allatoin, niacin, vitamin c, tropine, notratropine, meteolodine, hyoscine, fastusic acid, etc (Dr JLN Shastry)

Materials and Methods

1. Collection of plants leaves

Datura metel leaves were collected from Gaya town in the month of December 2017.the samples were packed instantly in polythene bags to avoid decomposition of some bioactive compounds. The leaves were thoroughly washed with water and rinsed with distilled water and dried in shade for two weeks and finally dried in thermostatic oven at a temperature not exceeding 30 degree centigrade for 20 hours. The dried leaves were pulverized in a sterile electric blender to obtain a fine blender and stored in airtight glass container protected from sunlight. The other parts consisting of fresh samples (150gm) were cut into small pieces for the extraction process.

2. Extraction procedure for dry leaf powder samples

The dry leaf powder samples (150gm) were extracted with methanol solvent (350mL) for 3 days using Soxhlet extractor until complete extraction. After extraction, the sample was filtered with filter paper (Whatmann 41). The methanol solvent was evaporated using a rotatory evaporator under pressure for 30 min resulting in semi solid crude extract (9.31gm). the dry methanol crude extract (0.34gm) was transferred into test tube for antioxidant activity, antimicrobial and phytochemical screening. The methanol crude extract (9.0gm) was suspended in water (100mL) and shaken until the crude extract dissolved. The solution was transferred into a separately funnel and extracted successively and separately with 30mL and 20mL of hexane, chloroform, ethyl acetate and butanol respectively. After extraction all crude extract were put inside the fume hood for the solvent to evaporate. After the solvent was completely evaporated the hexane crude extracts (0.22gm), chloroform crude extract (0.12gm), ethyl acetate crude extract (0.15gm), butanol crude extract (0.36gm), and residual methanol fraction (0.44gm) were obtained.

3. Extraction procedure for fresh leaf samples

The small pieces of fresh leaf samples (200gm) were extracted using the maceration method with methanol solvent (300mL) for 3 days. After complete extraction, the sample was filtered with filter paper and solvent was evaporated using a rotatory evaporator under pressure for 30 min resulting in a semi sold crude extract (5.58gm). About (0.34gm) of methanol crude extract was transferred in a test tube for a different study. The methanol crude extract was suspended in water and then extracted successively and separately with hexane, chloroform, ethyl acetate and butanol. After extraction, all crude extracts were put inside the fume hood for few days. After the solvent evaporates the hexane crude extract (1.68gm), chloroform (0.11gm), ethyl acetate (0.32gm) and butanol (0.29gm) and residual methanol fractions (0.21gm) were obtained.

Observations

1. Antibacterial activity assay

The antibacterial potential test was carried out using the agar disc diffusion method (Kolkate, 2000). Negative controls were prepared by using the same solvents employed to dissolve the samples. Inhibition zones were measured and compared with the standard reference antibiotic amoxicillin. Each extract was subjected to serial dilution by using dimethyl sulphoxide (DMSO) as a solvent to give 2mg/ml, 1 mg/ml, 0.5mg/ml, and 0.25mg/ml solutions. The concentration of amoxicillin standard used for this study was at 1mg/ml. each prepared concentrations of the different extracts was tested for its antimicrobial activity against one-gram (+) bacteria (S. aureus) and three-gram (-) bacteria (E. coli, K. pneumoniae and P. aeruginosa) on nutrient agar plates using the disc division method. Whatman no 1 sterile filter paper discs (6mm diameter) were impregnated with methanol extracts and placed on the incubated agar. The concentration of amoxicillin standard used for this study was at 1mg/ml. all the plants were incubated at 37 degree Celsius for 24 hours. The evaluation of antibacterial activity was measured showing the diameter of the zones of inhibition of the tested bacteria. Each method in this experiment was replicated three times.

2. Antifungal activity

The antifungal activity against the test fungal agents was determined according to the poisoned food technique of Grover and Moore (1962). In fact, PDA medium was prepared and sterilized at 150°C for 30 min in autoclave. Appropriate quantities of aqueous extracts (1.5, 3, 5 and 6.25 ml) and distilled water were added to this medium (40 ml), cooled to 45 to 50°C, to get 1, 2, 3 and 4% (w/v) concentrations of leaf and flower aqueous extracts. The control medium received the same quantity (1.5, 3, 5 and 6.25 ml) of sterile distilled water. Stock solution of organic extracts (5 ml) prepared above at 3000, 6000 and 9000 ppm were added to PDA medium. Control received the same quantity (5 ml) of diluted methanol used as control for all bioassays with organic extracts. The plant extracts were thoroughly mixed with the medium. Ten millilitres of each medium were poured in each 9 cm diameter sterilized Petri plate. After solidification, mycelial plugs of 5 mm diameter were taken with a pre-sterilized cork borer from 5 to 7 days old culture of test fungus and were placed in each Petri plate. Each treatment was replicated thrice. Plates were incubated in an incubator at 25 ± 2°C for 3 to 7 days. Fungal radial growth was measured by averaging the two diameters taken from each colony. Percentage growth inhibition of the fungal colonies was calculated by applying the following formula (Khanh *et al*., 2005):

Growth/inhibition (%) = [(Growth in control – growth in treatment)/ growth in control] *100

3. Phytochemical screening

Biochemical screening results showed that alkaloids, flavonoids, saponins and tannins were present in the fresh and dry leaves crude extract of *Datura metel*. But all the crude extract from both leaf samples did not show any colour change for triterpenoids and steroids test. However, methanol crude extracts from fresh and dry leaves showed negative test for steroids, tannins and triterpenoids but showed positive test for alkaloids, saponins and flavonoids. Both hexane crude extracts and chemicals such as alkaloids, steroids, flavonoids and triterpenoids were absent except for saponins and tannins. Ethyl acetate crude extract from both leaves showed positive test for alkaloids and saponins. The chloroform crude extract from both leaves showed positive

test for alkaloids saponins and tannins. The butanol crude extract, a group of chemical constituents such as saponins, tannins, steroids and triterpenoids were absent except alkaloids and flavonoids (Table1).

Table 1: Biochemical analysis of hexane, ethyl acetate, chloroform, butanol and methanol crude extract from the fresh and dry leaves of *Datura metel*

Inference	Extracts	biochemicals					
		alkaloids	steroids	flavonoids	saponins	tannins	triterpenoids
Hexane extract	Fresh leaves	-	-	-	+	+	-
	Dry leaves	-	-	-	+	+	-
Chloroform extract	Fresh leaves	+	-	-	+	+	-
	Dry leaves	+	-	-	+	+	-
Ethyl acetate extract	Fresh leaves	+	-	-	+	-	-
	Dry leaves	+	-	-	+	-	-
Butanol extract	Fresh leaves	+	-	+	-	-	-
	Dry leaves	+	-	+	-	-	-
Methanol extract	Fresh leaves	+	-	+	+	-	-
	Dry leaves	+	-	+	+	-	-
+ =presence, - =absence.							

Discussion

Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer. (Hossain and Nagooru, 2011, Suresh and Nagarajan, 2009) [7, 8]. All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant crude extracts (Gonzalez-Guevara *et al*., 2004) [9]. The biochemical screening of hexane, ethyl acetate, chloroform, butanol and methanol crude extracts from fresh and dry powder leaf samples of *D. metel* used in this study revealed that the crude extracts contained alkaloids, flavonoids, saponins and tannins (Table 1). The phytochemical screening of methanol fresh and dry leaf crude extracts studied showed the presence of active chemical constituents such as alkaloids, flavonoids and saponins (Table 1). Saponins were also present in other dry leaf crude extracts of *D. metel*. The most effective bioactive compounds alkaloids and flavonoids were found in polar methanol and butanol crude extracts. Tannins are another active compound found to be present in hexane and chloroform extracts. Therefore, the detected different bioactive compounds in different crude extracts from dry and fresh leaves of *D. metel* may be responsible for the antioxidant and antibacterial activities. Several reports are available on flavonoid groups which exhibited high potential biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-allergic reactions (Anyasor *et al*., 2010, Chao *et al*., 2002, Igbinosa *et al*., 2009, Thitilertdecha *et al*., 2008) [10, 11, 12, 13]. Saponins are also bioactive constituent which involved in plant defence system because of their antimicrobial activity (Barile *et al*., 2007, Ayoola *et al*., 2008) [14, 15]. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers (Barile *et al*., 2007, Ayoola *et al*., 2008, Akharaiyi, 2011, Varahalarao and Kaladhar, 2012, Sekar *et al*., 2012) [14, 15, 16, 17, 18].

The antimicrobial activity of the fresh and dry plant crude extracts was estimated using standard conventional methods against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. The dry methanol crude extract of *D. metel* and its fractions revealed comparatively small antibacterial potential against gram-positive and gram-negative bacteria at the concentrations of 2 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml with their respective zones of inhibition of 0–11 mm (Table 2). However, the fresh methanol crude extract of *D. metel* and its fractions revealed a moderate antibacterial potential against the employed bacterial strains and all working concentrations with their respective zones of inhibition of 0–17 mm (Table 2). The methanol fresh crude extract showed moderate antibacterial potential against *S. aureus*, *E. coli* and *P. aeruginosa* bacteria, at the concentrations of 2 mg/ml, 1 mg/ml and 0.5 mg/ml (Table 2).

Table 2: Antimicrobial activity of different crude extracts of *D. metel* against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus*.

Crude Extract	Concentration	*E. coli*[a] (mm)		*S. aureus* (mm)		*P. aeruginosa* (mm)		*K. pneumoniae* (mm)	
		Fresh leaves	Dry leaves	Fresh leaves	Dry leaves	Fresh leaves	Dry leaves	Fresh leaves	Dry leaves
Hexane	2 mg/ml	11 ± 0.11	7 ± 0.30	12 ± 0.20	8 ± 0.33	16 ± 0.22	6 ± 0.17	nd	nd
	1 mg/ml	15 ± 0.23	10 ± 0.44	nd	nd	13 ± 0.51	8 ± 0.41	7 ± 0.32	7 ± 0.27
	0.5 mg/ml	9 ± 0.18	8 ± 0.35	nd	nd	12 ± 0.27	6 ± 0.28	8 ± 0.18	8 ± 0.10
	0.25 mg/ml	8 ± 0.44	8 ± 0.28	10 ± 0.31	7 ± 0.32	nd	nd	nd	nd
	Standard	30 ± 0.22	30 ± 0.10	26 ± 0.13	26 ± 0.34	7 ± 0.54	7 ± 0.23	8 ± 0.41	8 ± 0.28
	2 mg/ml	12 ± 0.43	6 ± 0.33	16 ± 0.20	12 ± 0.21	14 ± 0.09	6 ± 0.10	6 ± 0.22	6 ± 0.10
Ethyl acetate	1 mg/ml	11 ± 0.08	11 ± 0.30	11 ± 0.16	10 ± 0.21	13 ± 0.22	8 ± 0.34	7 ± 0.54	7 ± 0.17
	0.5 mg/ml	9 ± 0.23	7 ± 0.25	7 ± 0.15	8 ± 0.31	12 ± 0.41	6 ± 0.24	nd	nd
	0.25 mg/ml	8 ± 0.12	6 ± 0.21	nd	nd	7 ± 0.12	7 ± 0.55	9 ± 0.20	9 ± 0.32
	Standard	30 ± 0.11	30 ± 0.23	20 ± 0.52	20 ± 0.22	7 ± 0.41	7 ± 0.56	7 ± 0.29	7 ± 0.08
	2 mg/ml	Nd	nd	nd	nd	10 ± 0.52	8 ± 0.21	8 ± 0.09	8 ± 0.09
Chloroform	1 mg/ml	13 ± 0.41	11 ± 0.25	12 ± 0.37	16 ± 0.32	8 ± 0.41	6 ± 0.22	7 ± 0.22	7 ± 0.22
	0.5 mg/ml	9 ± 0.23	8 ± 0.27	nd	nd	6 ± 0.41	6 ± 0.41	7 ± 0.12	7 ± 0.12
	0.25 mg/ml	8 ± 0.30	8 ± 0.37	11 ± 0.20	8 ± 0.26	nd	nd	7 ± 0.45	7 ± 0.14

	Standard	30 ± 0.31	30 ± 0.25	8 ± 0.45	8 ± 0.23	8 ± 0.41	8 ± 0.59	8 ± 0.05	8 ± 0.15
	2 mg/ml	17 ± 0.22	7 ± 0.23	12 ± 0.33	6 ± 0.34	10 ± 0.61	6 ± 0.21	7 ± 0.17	7 ± 0.29
Butanol	1 mg/ml	12 ± 0.17	7 ± 0.28	9 ± 0.12	7 ± 0.34	8 ± 0.29	7 ± 0.49	8 ± 0.23	8 ± 0.54
	0.5 mg/ml	9 ± 0.20	6 ± 0.28	9 ± 0.09	9 ± 0.31	8 ± 0.37	8 ± 0.18	nd	nd
	0.25 mg/ml	9 ± 0.55	9 ± 0.39	8 ± 0.22	8 ± 0.12	7 ± 0.49	7 ± 0.23	nd	nd
	Standard	10 ± 0.22	10 ± 0.37	7 ± 0.61	7 ± 0.27	8 ± 0.12	8 ± 0.34	9 ± 0.11	9 ± 0.19
Methanol	2 mg/ml	16 ± 0.38	8 ± 0.12	16 ± 0.37	6 ± 0.44	17 ± 0.08	8 ± 0.17	6 ± 0.15	6 ± 0.39
	1 mg/ml	12 ± 0.19	6 ± 0.44	12 ± 0.55	6 ± 0.31	14 ± 0.12	8 ± 0.23	6 ± 0.28	6 ± 0.43
	0.5 mg/ml	13 ± 0.26	7 ± 0.25	10 ± 0.13	7 ± 0.33	8 ± 0.71	8 ± 0.12	7 ± 0.03	7 ± 0.33
	0.25 mg/ml	8 ± 0.13	8 ± 0.56	8 ± 0.22	8 ± 0.34	7 ± 0.12	7 ± 0.42	8 ± 0.61	8 ± 0.10
	Standard	10 ± 0.22	10 ± 0.24	7 ± 0.33	7 ± 0.17	11 ± 0.09	11 ± 0.32	7 ± 0.13	7 ± 0.32

nd = Not detected.

A Values are represented as the mean ± S.D. of three experiments

Conclusion

The present antimicrobial study of different crude extracts of *D. metel* showed that the methanol crude extract from fresh leaves shows highest activity against the employed bacteria. Similarly the methanol crude extract from fresh leaves showed the highest antioxidant activity. Phytochemical screening showed that the antioxidant and antibacterial activities of the crude extracts of *D. metel* depend on the presence of phytochemicals such as alkaloids, steroids, flavonoids and tannins. This plant crude extracts could serve as potential sources of new antimicrobial and antioxidant agents. Further research is needed towards isolation and identification of active principles present in the extracts which could be used for pharmaceutical use.

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