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Phytochemical studies of various polarities leave crude extracts of Omani *Datura metel* L. and evaluation of their antimicrobial potential

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PEER REVIEW

Peer reviewer

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Comments

The present study on biochemical screening of various leaves crude extracts of *D. metel* is giving the valuable brief and scientific information about this plant.

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ABSTRACT

Objective: To identify the chemical constituents and evaluate antimicrobial potential of various crude extracts from leaves of *Datura metel* grown in Oman.

Methods: The leaf samples were collected from the University of Nizwa and extracted with methanol by using Soxhlet extractor. The isolated crude extract was defatted with distilled water and extracted with solvents of different polarities including hexane, chloroform, ethyl acetate and butanol. Chemical compositions of the crude extracts were analyzed by gas chromatography-mass spectrometer and their antimicrobial potential was evaluated by agar disc diffusion method against one Gram positive bacteria *Staphylococcus aureus* and two Gram negative bacteria *Escherichia coli* and *Pseudomonus aeruginosa*.

Results: The crude extracts were composed of different organic compounds such as alkaloids, hydrocarbons, aromatic hydrocarbons, organic acids, terpenoids, vitamin etc. The methanol and its fractionated crude extracts showed antimicrobial potential with inhibition zone in the range of 0-13 mm.

Conclusions: The selective crude extract from the leaves of *Datura metel* could be used as natural antibiotics.

KEYWORDS

Datura metel, Soxhlet extractor, Crude extracts, Gas chromatography-mass spectrometry analysis, Antimicrobial activity

1. Introduction

Since ancient times, different parts of medicinal plants have been used to cure specific ailments. The raw materials of medicinal and aromatic plants, crude extracts and essential oils contain various active secondary metabolites like tannins, terpenoids, alkaloids, flavonoids, phenols, steroids and glycosides[1]. Crude extracts of all plants especially medicinal plants are potential sources of antimicrobial, antifungal and antibacterial agents[2-4]. The resistance of bacterial strains against antibiotics makes many scientists and researchers recently pay attention to the crude extracts and biologically active pure compounds isolated from plant species used in herbal remedies[5-9].

Datura metel L. (*D. metel*) is one of the most important herbal plants used worldwide for the treatment of different diseases[10-12]. *D. metel* is a flowering plant belonging to family Solanaceae. The maximum height of this plant is about 1.5 m. Its leaves are completely different; the leaves are simple, alternate, dark green, broadly ovate, shallowly lobed and glabrous. The leaves are traditionally used for treatment of diarrhoea, gonorrhoea and bronchitis[6-11,13-15]. They are also used for the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful

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menstruation, skin ulcers and wounds[16-19]. Recently, the paste of leaves has been used in the treatment of burns[16]. In Nigeria, this plant is traditionally used to cure diseases such as asthma, cough, convulsion and insanity[19]. The Nigeria nationals are also using the leaves and seeds as anesthetic, antispasmodic, antitussive, bronchodilator and hallucinogenic[18]. The main chemical constituents of *D. metel* reported in the literature are alkaloids[20-22]. The main target of the present work was to isolate and identify chemical constituents and to evaluate the antimicrobial potential of various crude extracts of the leaves of *D. metel* which was native to Sultanate of Oman.

2. Materials and methods

2.1. Chemicals

Methanol, hexane, chloroform, ethyl acetate, butanol and amoxicillin were from Sigma-Aldrich Company Limited, Germany. Dichloromethane and acetone were from British Drug Houses, UK. Filter paper discs were obtained from Whatmann, China. Bacterial strains, including *Staphylococcus aureus* ATCC 29213 (*S. aureus*), *Escherichia coli* ATCC 9637 (*E. coli*), and *Pseudomonas aeruginosa* ATCC 9027 (*P. aeruginosa*) were collected from Nizwa Hospital, Nizwa, Sultanate of Oman.

2.2. Plant materials

The leaf samples of *D. metel* were collected from the University of Nizwa campus. After collection the samples were transported to the Natural Product Laboratory and kept at room temperature for cleaning, drying and extracting. Then fresh leaf samples were separated from the ones affected by fungus and bacteria.

2.3. Preparation of plant material

The unaffected leaf samples of *D. metel* were washed with water to remove the dust and foreign particles. After washing, the leaf samples were dried at room temperature for 3 weeks and ground into fine powder.

2.4. Extraction

The leaf powder sample (85 g) was extracted with methanol (300 mL) by using Soxhlet extractor until complete extraction. The methanol was completely evaporated by using rotary evaporator to give methanol free crude extract. The crude extract (33.7 g) was defatted with water (200 mL) and fractionated by using hexane, chloroform, ethyl acetate and butanol to give hexane, chloroform, ethyl acetate, butanol and residual hydro alcoholic fractions.

2.5. Antimicrobial assay

All crude extracts from the leaves of *D. metel* were tested for their antimicrobial activity by using three bacterial strains such as *S. aureus*, *E. coli* and *P. aeruginosa* grown on nutrient agar plates by using disc diffusion technique. Four concentrations 0.25, 0.50, 1.00 and 2.00 mg/mL were prepared for each crude extracts by using dimethyl sulphoxide as a solvent. The positive control amoxicillin was also prepared with the same solvent. Filter paper discs of 5 mm in diameter were impregnated with each concentration and placed on the agar plate which was inoculated with standard bacteria strains. All the sample plates were incubated at 37 °C for 24 h. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone against the tasted bacteria. Each experiment was done in triplicate.

2.6. Gas chromatography-mass spectrometry (GC-MS) analysis

The different polarities of crude extracts of *D. metel* were analysed by using a Perkin Elmer GC (Model Perkin Elmer Clarus 500, USA) coupled with a Perkin Elmer Clarus 600 C MS. The special fused silica capillary column was used for the analysis of crude extracts with specification of 30 m×0.25 mm ID, film thickness 0.25 μm. For the detection, separation and ionization by an electron ionization system with ionization energy 70 eV was performed. Helium was used as a carrier gas at constant flow rate of 1 mL/min. The temperatures of mass transfer line and injector were set at 220 and 300 °C, respectively. The oven temperature was initially set at 50 to 150 °C at a rate of 3 °C/min, then held for 10 min and finally raised to 300 °C at a rate of 10 °C/min. The crude samples was diluted with dichloromethane solvent (1:100, v/v) and filtered. The clean plant crude extracts (1 μL) were taken in a Hamilton syringe and injected into injector with split mode. The split ratio was 1:120. The percentage of chemical constituents in the plant crude was expressed as a percentage by peak area. Based on the retention time, the chemical constituents were identified and characterized in various crude extracts. The mass chromatograms of the crude extracts were matched with standards existing computer library (Mainlab, Replib and Tutorial data of GC–MS systems)[9-17].

3. Results

3.1. Analysis of different crude extracts

Ten different chemical constituents were identified in hexane crude extract from the leaves of *D. metel*, which are representing 5.54% of the total crude extract. The identified chemical constituents are listed in Table 1 according to their retention time. The different chemical constituents and chromatogram of hexane crude extract are showed in Figure 1 and Table 1. The chemical constituents are hexanoic acid (1.80%), heptanoic acid (0.31%), octanoic acid (0.08%), nonoic acid (0.21%), nonoicacid,z-oxo-ME (0.10%), eugenol (0.29%), neophytadiene (0.64%), hydrocarbon (1.59%), and vitamin E (0.52%).

Twenty five different chemical constituents were identified in ethyl acetate crude extract by GC-MS. The identified different chemical constituents represented 79.66% of the total extract. The list of chemical constituents and chromatogram were present in ethyl acetate crude extract (Figure 2 and Table 1), and the chemical constituents are as follows: pentanoic acid (0.93%), dihydroxy acetone (0.11%), hexanoic acid (18.40%), heptanoic acid (6.04%), nonanoic acid-2-oxo-mo (1.50%), octanoic acid (2.54%), glycocyantidine (0.04%), nonanoic acid-ME (0.11%), nonanoic acid (6.69%), propanedioic acid (0.09%), decanoic acid (0.26%), β-endesmol (0.47%), neophytadiene (12.02%), (2E)-3.7.11.15-tetramethyl-2-hexadeacane (0.85%), hexadecanoic acid (1.14%), phytol (2.44%), α-linolenic acid-ME(z,z,z) (1.77%), squalene

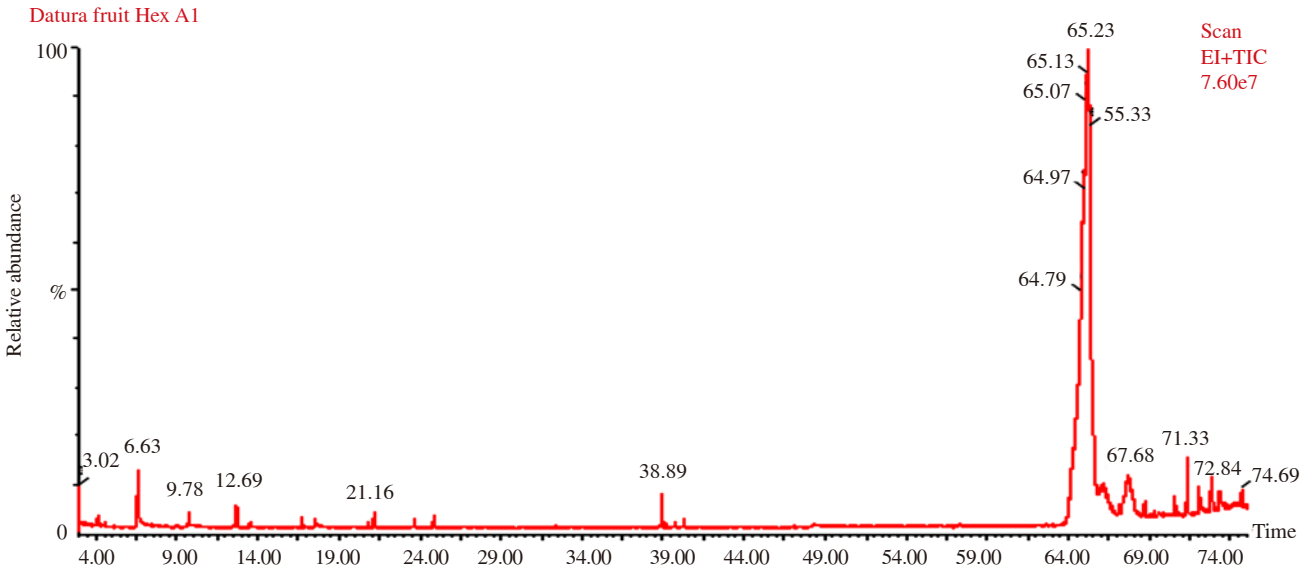

Figure 1. Chemical constituents of hexane crude extract of *D. Metel*.

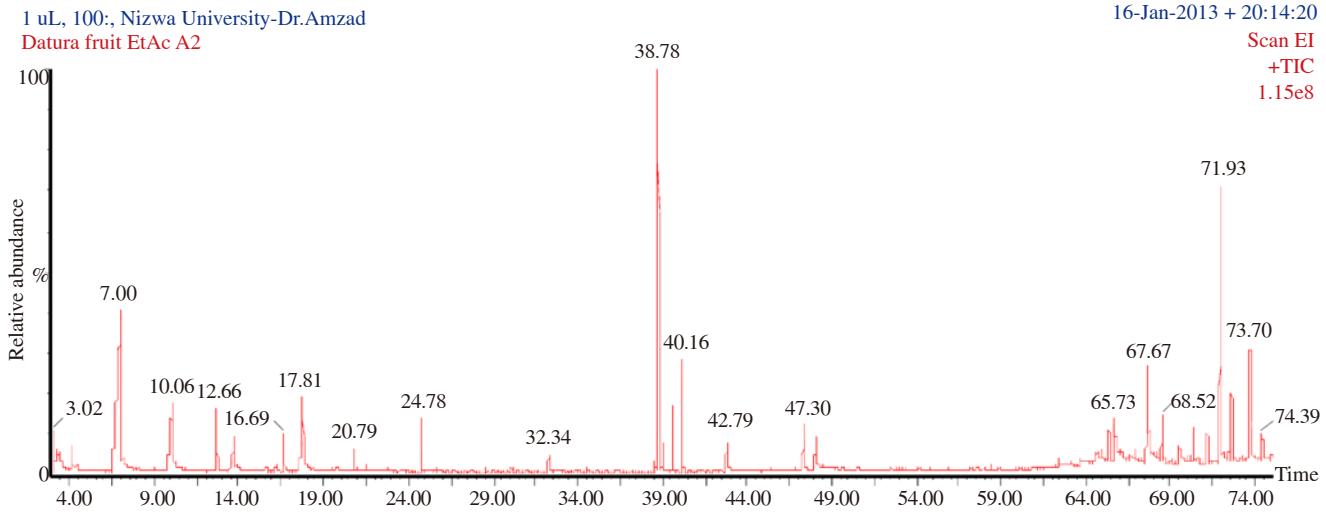

Figure 2. Chemical constituents of ethyl acetate crude extract of *D. metel*.

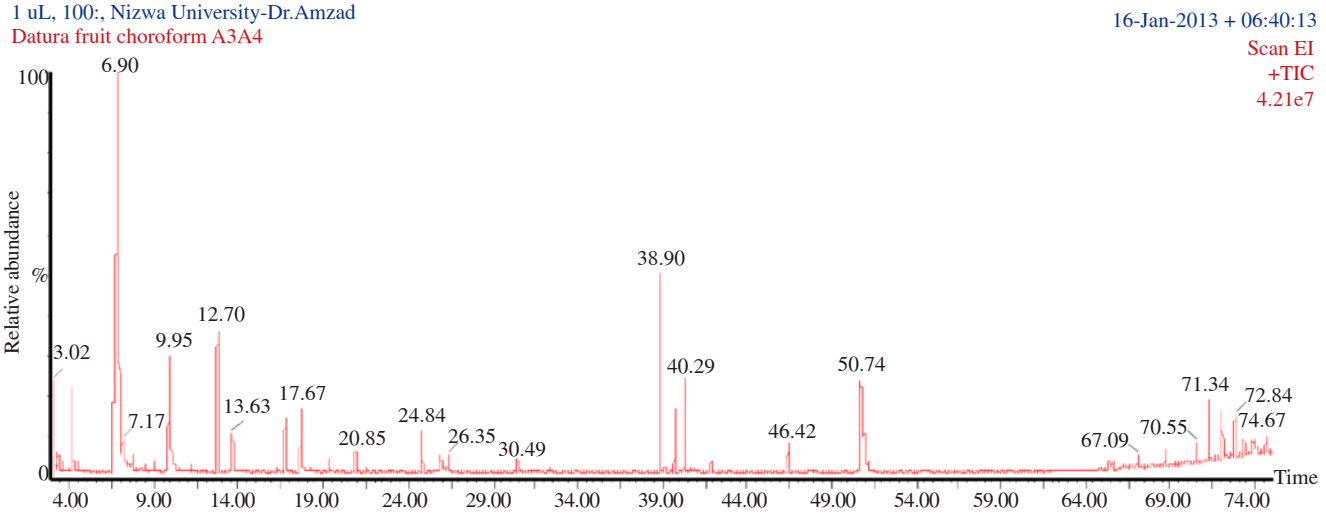

Figure 3. Chemical constituents of chloroform crude extract of *D. metel*.

(0.97%), hydrocarbon (6.70%), vitamin E (2a-tocphynol) (10.65%), and campesterol (5.94%). The chloroform crude extract from leaf samples of *D. metel* was analyzed and identified by using GC-MS, and the extract contained twenty one different chemical constituents representing 87.34% of the total extract. The chemical constituents that were characterized in chloroform crude extract (Figure 3 and Table 1) are as hexanoic acid (39.67%), heptanoic acid (6.97%), hyoscyamine (10.88%), neophytadiene (6.61%) and nonoic acid (3.89%).

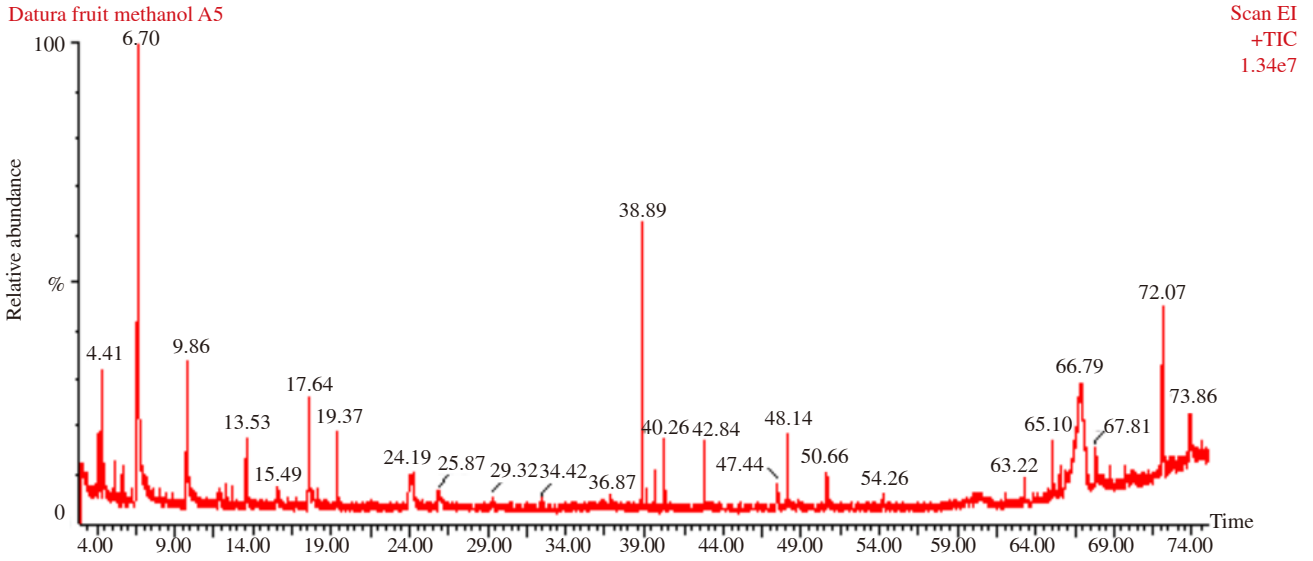

Figure 4. Chemical constituents of methanol crude extract of *D. metel*.

Table 1
Chemical constituents of different crude extracts from the leave of *D. metel*.

Compound Name		Retention time (min)	MW	Peak area (%)
Hexane crude extract	Hexanoic acid	6.64	116	1.80
	Heptanoic acid	9.78	130	0.31
	Octanoic acid	13.53	144	0.08
	Nonoic acid	17.58	150	0.21
	Nonoicacid,z-oxo-ME	20.84	155	0.10
	Eugenol	21.16	164	0.29
	Neophytadiene	38.89	278	0.64
	Hydrocarbon	70.55	278	0.41
	Hydrocarbon	71.34	370	1.18
	Vitamin E	72.09	430	0.52
Chloroform crude extract	Pentanoic acid	4.19	102	2.57
	Hexanoic acid	6.88	116	39.67
	Heptanoic acid	9.95	130	6.97
	Octanoic acid	13.63	144	1.78
	Nonoic acid	19.37	158	3.89
	2-methyl-4-vinylphenol	20.85	150	0.46
	Nonoic acid,2-oxo-ME	24.84	186	0.69
	Tridecane,4-8-dimethyl	25.85	212	1.19
	Levoglucosan	26.35	162	1.75
	Neophytadiene	39.72	222	6.61
	5-10-etoloxy-2,3,7,8-tetrahydro-1H,6H,dipyrolo(1,2-a:1:2-d)pyrazine	46.42	250	0.34
	Anhydroatropine	50.74	271	1.01
	Hyoscyamine	65.38	289	10.88
	Squalene	67.09	410	0.36
	Hydrocarbon	68.68	420	0.41
	Hydrocarbon	70.55	420	0.72
	Hydrocarbon	71.33	420	0.83
	D- -tocophenol	71.33	428	2.63
	Hydrocarbon	72.08	434	2.09
	Hydrocarbon	72.83	435	1.74
	Hydrocarbon	73.37	436	0.75
Methanol crude extract	Pentanoic acid	4.15	102	1.08
	Hexanoic acid-ME	5.18	130	0.52
	Octanol	5.68	128	0.66
	Hexanoic acid	6.70	116	27.93
	Heptanoic acid	9.86	130	6.11
	Pyranone	12.34	144	0.48
	Octonoic acid	13.53	144	2.20
	Nonoic acid	17.46	158	4.57

MW: Molecular weight.

Table 1, continued
Chemical constituents of different crude extracts from the leave of *D. metel*.

Compound Name		Retention time (min)	MW	Peak area (%)
	4-vinyl guaiacol	19.36	150	2.14
	Levoglncosan	25.84	162	0.26
	Neophytadiene	38.89	278	7.30
	Plamitic acid	40.84	256	1.91
	Phytol	47.44	296	1.09
	-linolenic acid(z,z,z)	48.14	292	2.31
	Hyoscyamine	50.66	289	1.49
	Squalene	65.55	410	0.69
	Vitamine E	72.02	430	6.21
Ethyl acetate crude extract	Pentanoic acid	4.19	102	0.93
	Dihydroxy acetone	4.40	90	0.11
	Hexanoic acid	6.99	116	18.40
	Heptanoic acid	10.06	130	6.04
	Nonanoic acid-2-oxo-mo	12.66	99	1.50
	Octanoic acid	1.73	144	2.54
	Glycocyantidine	15.43	99	0.04
	Nonanoic acid-ME	15.63	172	0.11
	Nonanoic acid	17.81	158	6.69
	Propanedioic acid	18.44	146	0.09
	Decanoic acid	21.53	172	0.26
	β-endesmol	32.34	222	0.47
	Neophytadiene	38.73	278	12.02
	(2E)-3.7.11.15-tetramethyl-2-hexadeacane	39.01	280	0.85
	Hexadecanoic acid	42.79	256	1.14
	Phytol	47.30	296	2.44
	-linolenic acid-ME(z,z,z)	48.10	292	1.77
	Squalene	65.39	410	0.97
	Hydrocarbon	66.94	420	0.37
	Hydrocarbon	68.52	420	1.43
	Hydrocarbon	70.38	420	1.17
	Hydrocarbon	71.16	420	1.11
	Vitamin E(2a-tocphynol)	71.93	430	10.65
	Hydrocarbon	72.66	434	2.62
	Campesterol	73.70	400	5.94

MW: Molecular weight.

Finally, the methanol crude extract was analyzed and identified by using GC-MS, and it contained seventeen different chemical constituent representing 66.95% of the total extract from leaf samples of *D. metel*. The chemical constituents founded in

the crude extract (Figure 4 and Table 1) were pentanoic acid (1.08%), hexanoic acid-ME (0.52%), octanol (0.66%), hexanoic acid (27.93%), heptanoic acid (6.11%), pyranone (0.48%), octanoic acid (2.20%), nonoic acid (4.57%), 4-vinyl guaiacol (2.14%), levoglucosan (0.26%), neophytadiene (7.30%), plamitic acid (1.91%), phytol (1.09%), a-linolenic acid(z,z,z) (2.31%), hyoscyamine (1.49%), squalene (0.69%), and vitamin E (6.21%).

3.2. Antimicrobial activity

Antibacterial potential of the prepared crude extracts was evaluated against three bacteria strains and the inhibition of bacterial growth was measured. Different crude extracts at different concentrations exhibited antibacterial potential against *S. aureus*, *E. coli* and *P. aeruginosa*. The antimicrobial results are presented in Table 2. All crude extracts from the leaves showed small potential against one Gram-positive and two Gram-negative bacteria at different concentrations with zones of inhibition in a range of 0-13 mm. Methanol crude extract showed small antibacterial potential against *E. coli* at all concentrations. However, the chloroform extracts did not show any antibacterial potential against *S. aureus* and *P. aeruginosa* at all concentrations. All the crude extracts from leaves showed moderate potential against *E. coil* at all working concentrations. The hexane and ethyl acetate crude extracts also showed moderate potential against *P. aeruginosa* but the remaining crude extracts did not show any potential against this bacteria. The ethyl acetate crude extract showed moderate activity against *S. aureus* at the concentration of 2 and 1 mg/mL. Butanol also showed activity against *S. aureus* at the concentrations 2.00, 1.00 and 0.50 mg/mL. However, the hexane and chloroform crude extracts did not show any activity against *P. aeruginosa* at any concentrations.

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

Crude extract	Concentration (mg/mL)	*E. coli*[a] (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)
Hexane	2.00	9.00±0.15	nd	9.00±0.17
	1.00	7.00±0.29	nd	7.00±0.21
	0.50	6.00±0.48	nd	7.00±0.11
	0.25	6.00±0.13	nd	6.00±0.24
	Standard	33.00±0.20	nd	dn
Chloroform	2.00	13.00±0.43	nd	dn
	1.00	8.00±0.06	nd	dn
	0.50	7.00±0.12	nd	dn
	0.25	6.00±0.17	nd	dn
	Standard	32.00±0.13	nd	dn
Ethyl acetate	2.00	7.00±0.22	8.00±0.90	7.00±0.19
	1.00	7.00±0.16	6.00±0.45	6.50±0.27
	0.50	6.00±0.18	dn	6.00±0.13
	0.25	6.00±0.23	dn	6.00±0.65
	Standard	28.00±0.31	dn	6.00±0.33
Butanol	2.00	9.00±0.13	9.00±0.20	dn
	1.00	9.00±0.07	7.00±0.16	dn
	0.50	8.00±0.28	6.00±0.26	dn
	0.25	7.00±0.43	dn	dn
	Standard	30.00±0.12	dn	dn
Methanol	2.00	8.00±0.18	7.00±0.38	dn
	1.00	8.00±0.12	dn	dn
	0.50	6.00±0.20	dn	dn
	0.25	6.00±0.11	dn	dn
	Standard	32.00±0.21	28.00±0.12	dn

nd: Not detected. Standard: Amoxicillin.
[a]: Values are represented as mean±SD of three experiments.

4. Discussion

Different types of bioactive compounds are present in foodstuffs of both plant and animal origin. Epidemiological studies show a positive relationship between dietary intake of whole grains, fruits, vegetables, fish and fermented milk products and health status. All of these foodstuffs contain known bioactive compounds such as dietary fibres, phytosterols, carotenoids, peptides, bioactive lipids, alkaloid, flavonoids and probiotics[2-4]. The whole plant of *D. metel* contains high concentration of different alkaloids which increased gradually with maturity of the plant[17-25]. The yield of extraction by using different polarities solvents from the leaf of *D. metel* is different. The highest percentage of recovery yield is methanol and the lowest is ethyl acetate. According to the chromatogram, hexane crude extracts contain some bioactive compounds such as octanoic acid, eugenol and vitamin E which are widely used for preparation of different medicines[15-24]. The bioactive compounds, hexanoic acid (39.67%) and hyoscyamine (10.88%) isolated and identified from chloroform crude extracts have very strong antimicrobial property[14-18]. However, the activities of other compounds in the crude extract are still not known. The ethyl acetate and methanol crude extracts also contain lots of bioactive compounds which are traditionally and pharmaceutically used to prepare the medicine[22,23]. The detected compounds in the leaf crude extracts of *D. metel* may be responsible for the antioxidant and antibacterial potential. Several reports showed strong potential activities of the plant like antioxidant, anti-inflammatory, antimicrobial, antiangionic, anticancer and anti-alergic activities[21-23]. The antimicrobial potential of the plant crude extracts was measured by agar disc method against the mentioned three bacterial strains. The results obtained in this study are almost similar to antioxidant and antimicrobial activity of *D. metel* crude extracts reported by other authors[11,13,14].

No previous studies reported leaves extracts of *D. metel* grown in the Sultanate of Oman. In this study, a good number of bioactive compounds were isolated and identified from different polarities crude extracts. The methanol extract and its fractions possess moderate antimicrobial potential, and this may be due to the presence of alkaloids, normal hydrocarbons, cyclic aromatic hydrocarbons, organic acids, terpenoids and vitamin.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

D. metel is the most important herbal plant used worldwide

for the treatment of different diseases. *D. metel* is a flowering plant belonging to family *Solanaceae*. Traditionally, it is also used for the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin-ulcers and wounds. Currently, no work has been done on this species by the researcher.

Research frontiers

The aim of this study is to prepare various crude extracts using solvents with different polarities for qualitative evaluation of their chemical constituents by GC-MS, and study the antimicrobial potential of these crude extracts.

Related reports

Several report available showed strong potential activities of the plant like antioxidant, anti-inflammatory, antimicrobial, antiangionic, anticancer and anti-alergic properties.

Innovations and breakthroughs

The experimental data generated in this study is new information and data will be useful to the scientific community.

Applications

D. metel is used worldwide as a medicine. According to the present paper, there are so many bioactive compounds that can be used to prepare medicine.

Peer review

The present study on biochemical screening of various leaves crude extracts of *D. metel* is giving the valuable brief and scientific information about this plant.

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