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Phytochemical analysis and antimicrobial activity of crude extract of *Datura metel* leaves

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ABSTRACT

The aim of this work was to investigate and compare the phytochemical screening and 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 extracts from the fresh and dry leaves. Antimicrobial activities of different crude extracts from dry and fresh leaves of Datura metel were determined by agar disc diffusion method with minor modification. In vitro phytochemical screening for all crude extracts from both dry and fresh leaves was tested and shown positive result for alkaloid, flavonoid, saponin and tannin compounds. However, all the crude extracts did not show positive results for steroids and triterpenoid compounds. The methanol crude extract and its derived fractions from dry and fresh leaves showed small and moderate antibacterial potential with one gram positive (Staphylococcus aureus) and three gram negative (Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa) bacteria in the range of 0–17%. In conclusion, all organic crude extracts from both fresh and dry leaves could be used as potential sources of new antimicrobial properties.

Keywords: *Datura metel*, Different organic solvents, Soxhlet extractor, Phytochemical screening, Antimicrobial activity

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INTRODUCTION

The study and use herbal medicine has increasingly become a less toxic source of medicinal of plants used for the treatment of many diseases. Native Americans traditionally used about 2500 of the approximately 20,000 plant species that are native to North America. About 80% of the population worldwide use traditional medicine, which has compounds derived from medicinal plant [1, 2, 7, 8, 24]. *Datura metel* leave is classified in the plant family solanaceae. A perennial herbaceous plant, belonging to the solanaceae family can reach a height of 1.5m. Leaves are simple, alternate, dark green, broadly ovate, shallowly lobed and glabrous. Flowers are large, solitary, and trumpet-shaped with a sweet fragrance usually appreciated in the mornings and evenings, with a wide range of colours, ranging from white to yellow and light to dark purple. The flowers are hermaphrodite and are pollinated by insects. The fruit is in the form of a capsule covered with short spines. A variety of phytochemicals have been found to occur in *Datura metel*. These phytoconstituents comprises alkaloids, flavonoids, phenols, tannins, saponins and sterols. The phytoconstituents of *Datura* were analysed from various parts of the plant like the leaf root andshoot. The plant finds application in the treatment of diarrhea and skin diseases [2, 3, 4]. It is used in the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation skin-ulcers and wounds. It is also used in the treatment of burns. It is used to calm cough and to treat laryngitis and Treacheries. Antibacterial studies were done on *Datura metel*. Plant extracts have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infections caused by disease causing pathogens [5].

Several reports have been carried out with antimicrobial activity against bacteria, bacterial pathogens and fungi [6, 7]. Moreover, scientific studies and the results on antimicrobial and phytochemical screening on ethanol and hydro alcoholic crude extracts of this plant have been reported earlier. But our study has been planned to determine the

antioxidant activity and phytochemical compounds of different organic crude extracts from both dry and fresh leaves *Datura metel.* The antimicrobial activity of different crude extracts from both fresh and dry leaves against some selective pathogenic bacteria locally available for possible development of new drugs for the prevention and treatment of infectious diseases caused by bacterial pathogens. Therefore, the aim of this present work is to investigate the phytochemical screening and antimicrobial activities of different crude extracts from dry and fresh leaves of *Datura metel* [8, 9].

MATERIALS AND METHODS

Materials

The chemicals used in this present study such as hexane, chloroform, ethyl acetate and acetic anhydride and butanol were purchased from Sigma–Aldrich, Germany. Methanol was obtained from Emsure, Germany. Ammonia was obtained from Appli Chem, Germany. Sodium hydroxide and sulphuric acid were obtained from Ohilip Harris, England. Filter papers that were used in the disc were purchased from Whatman, GE Healthcare companies, China. The bacterial strains such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E.Coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were obtained from Federal Medical Center, Katsina, Nigeria. The UV spectroscopy (UV-1800 Shimadzu spectrophotometer, Japan) was used for measuring the absorbance of the samples.

Plant sample

The leaves of sample *Datura metel* were collected and identified in Biological garden of Department of Biology, Umaru Musa Yar'adua University, Katsina, Nigeria. The samples were packed instantly in polyethylene bags to avoid decomposition of some bioactive compounds.

Preparation of samples

The leaf samples were washed carefully with water to remove dust and foreign materials. Then the washed leaves were divided in two parts. One part of the leaf samples (200 gm) were dried under shade at temperature (25 °C) for 7 days. The other parts consisting of fresh samples (200 gm) were cut into small pieces for the extraction process. After drying the leaf samples (150 gm) were ground into a powder form using a grinder for 30 s.

Extraction procedure for dry leaf powder samples

The dry leaf powder samples (150 gm) were extracted with methanol solvent (350 ml) 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 rotary evaporator under pressure for 30 min resulting in a semi solid crude extract (9.31 g). The dry methanol crude extract (0.34 g) was transferred into test tube for antioxidant activity, antimicrobial and phytochemical screening. The methanol crude extract (9.0 gm) was suspended in water (100 ml) and shaken until the crude extract dissolved. The solution was transferred into a separatory funnel and extracted successively and separately with 30 ml and 20 ml of hexane, chloroform, ethyl acetate and butanol, respectively. After extraction all crude extracts were put inside the fume hood for the solvents to evaporate. After the solvent was completely evaporated the hexane crude extracts (0.22 g), chloroform crude extracts (0.12 g), ethyl acetate crude extracts (0.15 g) butanol crude extracts (0.36 g) and residual methanol fractions (0.44 g) were obtained.

Extraction procedure for fresh leaf samples

The small pieces of fresh leaf samples (200 gm) were extracted using the maceration method with methanol solvent (300 ml) for 3 days. After complete extraction, the sample was filtered with filter paper and the solvent was evaporated using a rotary evaporator under pressure for 30 min resulting in a semi solid crude extract (5.58 g). About (0.34 g) 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 extracts (1.68 g), chloroform (0.11 g), ethyl acetate (0.32 g) and butanol (0.29 g) and residual methanol fractions (0.21 g) were obtained.

Preliminary phytochemicals screening

The stock solution was prepared from each of the crude extracts such as hexane, chloroform, ethyl acetate, butanol and methanol extracts (100 mg); and was dissolved in 10 ml of its own mother solvents. The obtained stock solutions were subjected to preliminary phytochemical screening.

Test for alkaloids

The dry powder samples (1 gm) were taken in a test tube and an ammonia solution (3 ml) was added to it. They were allowed to stand for few minutes. Then chloroform (10 ml) was added to the test tube samples which was

shaken and then filtered to remove the powder samples. The chloroform was evaporated using a water bath and Mayer's reagent (2 ml) was added. A cream colored precipitate was immediately produced which indicates the presence of alkaloids.

Test for flavonoids
A few drops of diluted sodium hydroxide solution were added to the stock solution of *Datura metel* (0.5 ml). An intense yellow colour appeared in the plant crude extract, which became colourless upon the addition of a few drops of diluted H_2SO_4 acid. This shows the presence of flavonoids.

Test for saponins
The stock solution from each crude extract of (0.5 ml) was diluted with distilled water (20 ml) and then the test tube was shaken by hand for 15 min. The formation of a foam layer on the top of the test tube showed the presence of saponins.

Test for steroids
The powder samples of (1g) were dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall sides. The colour of the upper layer turned red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for tannins
The stock crude extract solution (0.5 ml) was dissolved in chloroform (5 ml) and added acetic anhydride (1 ml). Finally sulphuric acid (1 ml) was added carefully to the solution along the wall sides of the vessel. A green colour was formed, showing the presence of tannins.

Test for triterpenoids
The dry crude plant extract (5 mg) was dissolved in chloroform (2 ml) and then acetic anhydride (1 ml) was added to it. One millilitre of concentrated sulphuric acid was added to the solution. The formation of reddish violet colour shows the presence of triterpenoids.

Antibacterial activity assay
The antibacterial potential test was carried out using the agar disc diffusion method [13]. 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 2 mg/ml, 1 mg/ml, 0.5 mg/ml, and 0.25 mg/ml solutions. The concentration of amoxicillin standard used for this study was at 1 mg/ml. Each prepared concentration 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 disc diffusion method. Whatman No. 1 sterile filter paper discs (6 mm diameter) were impregnated with methanol extracts or subfractions of *Datura metel* and placed on the inoculated agar. The concentration of amoxicillin standard used for this study was at 1 mg/ml. All the plates were incubated at 37 °C for 24 h. Evaluation of antibacterial activity was measured showing the diameter of the zones of inhibition against the tested bacteria. Each method in this experiment was replicated three times.

RESULTS

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

Inference	Extracts	Phytochemicals					
		Alkaloids	Flavanoids	Saponins	Steroids	Tannins	Triterpenoids
Butanol extract	Fresh leaves	−	−	+	-	+	−
	Dry leaves	−	−	+	-	+	−
Chloroform extract	Fresh leaves	+	−	+	-	+	−
	Dry leaves	+	−	+	-	+	−
Ethyl acetate extract	Fresh leaves	+	−	+	-	+	−
	Dry leaves	+	−	+	-	−	−
Hexane extract	Fresh leaves	+	+	−	-	−	−
	Dry leaves	+	+	−	-	−	−
Methanol extract	Fresh leaves	+	+	+	-	−	−
	Dry leaves	+	+	+		−	−

+ = Presence; − = absence

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. pneumonia* (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
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	9±0.51	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	nd	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
	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
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	nd	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	nd	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.

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 [1,2, 8,7 9, 10]. All secondary metabolite components displayed antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant crude extracts [11]. The biochemical screening of hexane, ethyl acetate, chloroform, butanol and methanol crude extracts from fresh and dry powder leaf samples of *Datura 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 *Datura 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*Datura metel*bioactive compounds in different crude extracts from dry and fresh leaves of *Datura metel*may be responsible for the 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 [12, 13, 14, 15]. Saponins are also bioactive constituent which involved in plant defense system because of their antimicrobial activity. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers [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 *Datura 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 *Datura 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). However, all crude extracts from dry samples showed small activity against all

employed bacterial strains. Butanol crude extract from fresh samples showed moderate activity against *S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae* at concentrations 2 mg/ml and 1 mg/ml but dry crude extract samples showed small potential at most of the concentrations against all bacterial strains. However, the bacterium *K. pneumoniae* did not show any potential activity at the concentration of 0.5 mg/ml and 0.25 mg/ml. The chloroform crude extracts from fresh and dry samples did not show any activity against *E. coli* and *S. aureus* at the concentration of 2 mg/ml. *P. aeruginosa* and *K. pneumoniae* with chloroform crude extracts showed small activity at all concentrations. The hexane and ethyl acetate subfractions showed moderate antibacterial potential against most of the tested bacteria. But, the hexane crude extract did not show any activity against *P. aeruginosa* and *K. pneumoniae* tested bacterial strains at the concentration 0.25 mg/ml. The ethyl acetate crude extracts also did not show any activity against *S. aureus* and *K. pneumoniae* tested bacterial strains at the concentrations of 1 mg/ml and 0.5 mg/ml and 2 mg/ml and 0.25 mg/ml. The control inhibits the growth of all the tested bacteria. Generally, the antimicrobial activity of plant crude extracts depends on the dose and the type of bacterial strains employed. Also these antibacterial actions could be related to their chemical components in the crude extracts [8, 18, 19, 20]. The bioactive compounds such as tannins and flavonoids components were present in the crude extracts. However, these bioactive compounds were inducing the antioxidant and antimicrobial activities. The amount of active components in the crude extract may be diluted or increased their concentrations by fractionation [20, 21] because they have the ability to inactivate microbial activity, enzymes, cell envelope transport proteins, and so forth [22,23,24]. Further studies are required for the isolation and identification of individual active compounds and also *in vivo* studies are needed for better understanding of their mechanism of action as antimicrobials.

CONCLUSION

The present antimicrobial study of different crude extracts of showed that *Datura metel* methanol crude extract from fresh leaves and chloroform crude extract from dry leave shows highest activity against the employed bacteria. Phytochemical screening showed that the antibacterial activities of the crude extracts of *Datura 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 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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