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Phytochemical Screening and Antibacterial Activity of *Datura Metel* L. Leaves Extracts

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Abstract

Microbial diseases are one of the major health problems in many countries, because development of microbial resistance to antibiotics.Search for potential antimicrobial agents is one of the several attempts to find effective and affordable ways to control these diseases. Moreover, the side-effects which associated by synthetic antibiotics renders the natural products currently of much research interest in view of their biological activities due to their long and safe uses. The aim of the present study was to screen for phytoconstituent, and to investigate the *in vitro* antibacterial activity of fractions (hexane, chloroform, ethyl acetate and aqueous) from *Daturametel* leaves against four pathogenic bacteria isolates, namely, *Staphylococcus aureus, Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*, by using agar well diffusion method. For the antibacterial bioassay, three concentrations (0.25, 0.50, and 1 mg/mL) of each fraction solutions were prepared. The phytochemical screening of *D. metel* leaves revealed the presence of some bioactive principles such as alkaloids, terpenoids, steroids,

flavonoids, phenolic compounds and tannins. The antibacterial activity among the fractions was extremely broad against all test organisms. In general, the aqueous fraction is the most sensitive, while the hexane fraction is more resistance by organisms. The highest concentration of aqueous fraction (1 mg/mL) showed maximum zone of inhibition (21 mm) against *E. coli*,and showed lowest zone of inhibition (4 mm) against *K. pneumonia*, whereas, ethyl acetate (1 mg/mL) showed zone of inhibition (17 mm) against *E. coli*. The antibacterial activities of *D. metel* may be due to presence such phytochemicals and this may warrant further research to determine the bioactive compound(s).

Keywords: Daturametel; Phytochemical screening; Antibacterial activity

1. Introduction

The growing bacterial resistance against commercial standard and reserve antibiotics and the search for new active substances with antibacterial activity against pathogenic bacteria is of increasing importance. Because of the side effects and the resistance that pathogenic microorganisms built against antibiotics, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Service, 1995; Mundt et al., 2003). In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions, this situation forced scientists to search for new

antimicrobial substances. Many of the spices and herbs used today have been valued for their antimicrobial effects and medicinal powers in addition their flavor and fragrance qualities, many scientists recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Ceylan and Fung, 2004; Davidson et al., 2005). The effects of plant materials typically result from the secondary products present in the plant; it is usually not attributed to a single compound but a combination of the metabolites. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Rathish and Sumitra, 2005). *Datura metel L (*Solanaceae) is used in Sudanese traditional medicinal to cure diseases such as asthma, cough, convulsion and insanity. The leaves and seeds are widely used as anesthetic, antispasmodic, antitussive, and bronchodilator and it is used in the treatment of catarrh, diarrhea, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin ulcers and wounds, also used in the treatment of burns, to calm cough and to treat laryngitis and trachitis (Okwu and Morah, 2007). This study seeks to ascertain the usefulness of *D. metel* in the treatment of infectious conditions caused by common pathogenic bacteria. The present study has been planned to screen phytochemicals and evaluate the antibacterial activity of *D. metel* against certain pathogenic bacteria namely; *S. aureus, K. pneumoniae, P. aeruginosa* and *E. coli* for possible potential as drugs for the prevention and treatment of infectious diseases caused by bacterial pathogens.

2. Materials and Methods

2.1 Plant material

The plant (*Datura metel L.*) was collected randomly from local area of Al Fashir Valley and identified of the National Herbarium; University of Al Fashir wherea voucher specimen has been deposited for future reference. The leaves were separated, washed with distilled water to remove dust and other foreign particles and left on a clean surface to dry in the shade for 10-15 days. The dried material was grinded to fine powder using blender grinder and stored in air tight bottles. The Powdered material was used further for phytochemical screening and preparation of extracts.

2.2 Preparation of plant extract

The powdered leaves of the plant 100 g were extracted successively in the Soxhlet apparatus with hexane, chloroform, ethyl acetate and methanol.The prepared extracts were evaporated to dryness under reduced pressure usinga rotary evaporator to yield 2.15g hexane extract, 1.92 g chloroform extract, 1.22g ethyl acetate extract and 8.43g methanolic extract.

2.3 Phytochemical analysis

2.3.1 Test for Alkaloids

The powdered sample 0.5 g was taken in a test tube and 3 mL ammonia solution was added and allowed to stand for few minutes, then 5 mL chloroform was addedand shaken, then filtered to remove the powder samples. The chloroform was evaporated using a water bath and Mayer's reagent was added.A creamy precipitate produced (Kokate, 2000).

2.3.2 Test for Terpenoids and Steroids

The crude plant extract0.5g was treated with 0.5 mL of acetic anhydride and 0.5 mL of chloroform. Then concentrated sulphuric acid was added slowly. Red violet colour was observed (Evans and Evan's, 2002).

2.3.3 Test for Flavonoids

A few drops of diluted sodium hydroxide solution were added to 0.5 mL methanolic extract. An intense yellow colour appeared which became colourless upon the addition of a few drops of diluted H_2SO_4 acid (Siddiqui and Ali, 1997).

2.3.4 Test for Phenolic Compounds (Ferric chloride test)

The crude plant extract 0.5 g was diluted in 5 mL of distilled water and filtered. To the filtrate, 5% Ferric chloride was added. Dark green colour was observed (Iyengar, 1995).

2.3.5 Test for Tannins

To 0.5 mL of extract solution, one ml of water and 1- 2 drops of ferric chloride solution wad added. A dark blue colour was observed (Parekh et al., 2005).

2.3.6 Antibacterial activity assay

The antibacterial potential test was carried out using the agar disc diffusion method (Evans and Evan's, 2002).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 antibacterial activity against one Gram positive bacteria (*S. aureus*) and three Gram negative 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 the prepared extracts of placed on the inoculated agar. All the plates were incubated at 37 ^{O}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.

3. Results And Discussion

Phytochemical screening of thehexane, chloroform, ethyl acetate andmethanolic extracts of the dried powdered leaves of *D. metel*revealed arepresence of triterpenes and steroids, alkaloids, flavonoids and Phenolic Compounds and the results were compatible with the published literature (Table 1).

Table 1 Results of Phytochemical screening of *D. metel* leaf extracts

	Phytochemicals			
Extract	Trierpenes and steroids	Alkaloids	Flavonoids	phenolics
Hexane	+	-	-	-
Chloroform	+	+	+	+
ethyl acetate	+	+	+	+
Methanol	+	+	+	+

+ =Presence, - =absence.

The detected secondary metabolites are known to possess certain activities such as antimicrobial, antioxidant and antitumor amongst other (De, 2004). The promising results prompted as to proceed with antibacterial testing of the prepared hexane, chloroform, ethyl acetate and methanol extracts.

Table 2 Antibacterial Activity of *D. metel L*. Leaf Extracts

Extracts	**Conc. (ppm)**	***E.coli* (mm)**	***S.aureus* (mm)**	***P.eruginosa* (mm)**	***K.pneumonia* (mm)**
Hexane	1	7± 0.30	6± 0.33	5± 0.17	6±0.10
	0.5	4± 0.44	nd	4± 0.41	3±0.27
	0.25	Nd	nd	2±0.28	nd
	standard	30± 0.10	26± 0.34	7± 0.23	8± 0.28
Chloroform	1	3± 0.25	5± 0.32	6± 0.22	5± 0.22
	0.5	2± 0.27	2± 0.26	4± 0.41	3± 0.12
	0.25	1±0.20	nd	nd	1± 0.14
	standard	25± 0.25	6± 0.23	6± 0.59	6± 0.15
Ethyl acetate	1	17±0.30	5±0.21	6± 034	5± 0.32
	0.5	10±0.30	3±0.31	5± 0.55	2±0.17
	0.25	4± 0.21	nd	2± 0.24	nd

Methanolic	standard	28± 0.23	18± 22	5± 0.56	5± 0.088
	1	21± 0.10	9± 0.42	6± 0.15	4± 0.37
	0.5	9± 0.42	7± 0.29	5± 0.21	4± 0.41
	0.25	7± 0.25	6± 0.33	4± 0.12	5± 0.33
	standard	10± 0.22	9± 0.17	9± 0.32	8± 0.32

Nd=Not detected, Values are represented as the mean ± S.D. of inhibition zone diameter of three experiments

The *D. metel* crude extracts were tested using standard conventional methods against*S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumonia*. The methanolic extract showed comparative antibacterial potential against Gram-positive and Gram-negative bacteria at the concentrations of 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL with their respective zones of inhibition of0 –30 mm. The methanolic and ethyl acetate extracts showed moderate antibacterial activity against most of the tested bacteria,but the ethyl acetate extracts did not show any activity against*S. aureus* and *K. pneumoniae* at the concentrations of 0.25 mg/mL. The chloroform extract of samples did not show any activity against*P. aeruginosa* and *S. aureus* at the concentration of

0.25mg/mL. *E. coli* and *K. pneumoniae* were less susceptible to the chloroform extracts at all concentrations. The hexane extract did not show activity against *E. coli, S. aureus* and *K. pneumoniae* tested bacterial strains at the concentration 0.25 mg/mL. The control inhibited the growth of all tested bacteria.Further studies are designed for the isolation and identification of individual compounds of the active methanolic extract.

4. Conclusion

The study of antibacterial activity of *D. metel* L. extracts showed that the methanol extract of the leaves extracted promising activity against bacterial human pathogens when compared to ethyl acetate extract. Phytochemical screening revered presence of phytochemical compounds such as alkaloids, terpenoid, flavonoids, tannins and phenolic compounds. The results also confirmed that scientific studies carried out on medicinal plants having traditional uses might warrant fruitful results and could serve as useful source of new antibacterial agents.

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