International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.1, pp 478-483, Jan-Mar 2011

Antibacterial, Phytochemical and Antioxidant activities of *Datura metel*

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Abstract: The antibacterial efficacy of crude aqueous and ethanol extracts leaf, stem bark and roots of *Datura metel* at 20mg/ml were assayed against eight clinical bacterial isolates (*Streptococcus β hemolytic, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus* and *Streptococcus dysenteriae*) by well in agar method The leaf and stem bark of the plant was antagonistic against the test bacteria species with inhibitory zones of between 35-12 mm while the highest inhibitory zone displayed by one of the reference drug (streptomycin) was 45 mm at 10mg/ml concentration. Among the tested bacterial isolates *Staph. aureus* was the most inhibited majorly with the ethanol extract. The aqueous extract displayed inhibitory zones of between 22-10 mm while the crude ethanol extract exhibited inhibitory affinity between 32-12 mm. The MIC of the plant extracts on the susceptible bacterial isolates was at 20mg/ml and MBC affinity was at 20-10mg/ml. The aqueous extracts of the plants was more sufficient in the extraction of the substances with phytochemical and antioxidant activities than ethanol extract. The phytochemical compounds common among the crude aqueous and ethanol extracts of the plant parts were saponins, flavonoids, alkaloids, glycocides and phenol. The aqueous extract of the plant displayed antioxidant activity of between 49.30-23.82% while the ethanol extract was between 25.51-3.41%. The general results observed consider the plant as natural source of antioxidants and phytochemical quality for antimicrobial effectiveness.
Keywords: Clinical bacteria, extracts, antibacterial, photochemical, antioxidant, Nigeria.

INTRODUCTION

Medicinal plants are used for the ailment of several microbial and non-microbial originated diseases due to their valuable effects in health care. The affordability, reliability, availability and low toxicity of medicinal plants in therapeutic use has made them popular and acceptable by all religions for implementation in medical health care all over the world. Plants are indeed the first material used in alternative medicine type of remedy against many diseases. Several plants have therapeutic and pharmaceutical effects, for antimicrobial, antioxidant, anti-infectious and anti-tumour activities[1,2]. Herbal medicine has been widely used as an integral part of primary health care in many countries[3]. Medicinal plants may constitute a reservoir of new antimicrobial substances to be discovered. About 80% of developing countries, citizens used traditional medicine based on plant products. Thus many studies have been conducted on medicinal plants. They are screened for antimicrobial activities, their properties and efficacy. The development of some modern drugs could not have been possible in the absence of the bioactive constituents of plants. Due to some antibacterial ineffectiveness, there is increase in population of patients that attend medical centers in developing countries. The intractable problem of antibiotic resistance has led to the resurgence of interest in herbal products as sources of novel compounds to suppress or eradicate the ever increasing problems of re-emergence of newer diseases.

In this study, we have carried out the preliminary study of antibacterial, phytochemical and antioxidant

activities of the leaf, stem-bark and roots of *Datura metel* .

MATERIALS AND METHODS

Apparently healthy leaf, stem bark and roots of *Datura metel* were collected from a forest in Akure, Ondo State, Nigeria and identified in Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria. The plant parts were dried for 3 weeks and then ground to powder with a mechanical grinder. The powders obtained were extracted separately with ethanol and water at room temperature (25 ± 2^0C). The resulting crude extracts were filtered and evaporated in shaker water bath maintained at 55-65^{0}C. The obtained semi-dried crude extracts were contained in plastic containers and labeled appropriately as AE (aqueous extract) and EE (ethanol extract).

The antibacterial screening of the various extracts was assessed against clinical bacterial strains isolated from human urine, feaces and septic wounds. The bacterial isolates include: *Streptococcus β hemolytic* (urine), *Pseudomonas aeruginosa* (wound), *Escherichia coli* (urine), *Staph. aureus* (wound), *Klebsiella pneumoniae* (urine), *Bacillus cereus* (wound) *Shigella dysenteriae* (feaces) *Campylobacter jejunum* (feaces) and *Salmonella typhi* (feaces). Nutrient agar (Lab M) and nutrient broth (Lab M) were used for the sub culturing of the bacterial isolates. Mueller-Hinton agar (Hi-media) was used for the bacterial sensitivity screening.

The antibacterial screening of the crude extracts were evaluated by agar well diffusion[4]. The crude extracts were reconstituted in 5% V/V aqueous dimethyl sulphoxide (DMSO) at concentration of 20mg/ml. The inocula of the test bacterial isolates were prepared from 24h broth culture. The absorbance was read at 530nm and adjusted with sterile distilled water to match that of 0.5Mc Farland standard solution. From the prepared bacterial suspension, other dilutions were prepared to give a final concentration of 10^6. 1ml each of the bacterial suspension was obtained with sterile syringe and needle and spread plated with Mueller-Hinton agar. The plates were allowed to stand for 1.5h for the test bacterial isolates to be fully embedded and properly established in the seeded medium. With a sterile cork borer (Gallenkamp), well of equal depth (Δ = 5mm diameter) were dug with a previously sterilized No 4 cork borer. Each well was aseptically filled up with the respective extracts avoiding splashes and overfilling. The plates were incubated at 37^{0}C for 24 – 48h. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the well. The halos diameter as an index of the degree of sensitivity, were measured with a transparent plastic ruler. Sterile 5% aqueous DMSO was used as negative control while methicillin and streptomycin (10mg/ml) were used as the positive control. The minimum inhibition concentrations (MIC) of the leaf extracts was only determined because they showed higher inhibitory activities than bark and roots of the studied plant. 1ml of the extracts concentrations at 1.25-20mg/ml were mixed with 8ml of Mueller-Hinton broth, 1ml of 24h culture of the test bacterial organisms (1.0×10^6 cell/ml) was inoculated into each test tube of the different concentrations and mixed thoroughly. The test tubes were then incubated at 37^{0}C for 24h. The tube containing the lowest dilution of the extract with no detectable bacterial growth by the naked eye was considered as the point of minimum inhibitory concentration (MIC). One milliliter each of the MIC positive tubes were pour plated with freshly prepared nutrient agar. The plates were incubated for possible growth at 37^{0}C for 96h. Plates without growth were considered as bactericidal concentrations and those with growth as bacteriostatic concentrations of the extracts. All experiments were carried out in triplicates.

The phytochemical was determined using chemical methods and by adopting standard protocols to identify the constituents as described by[5, 6].

Antioxidant assay was by the criteria of [7, 8]. Spots with antioxidant activity inhibit free radical effect of DPPH thus leading to deep yellow colour on a purple background on TLC plates. The antioxidant activity of the various spots was monitored based on the colour changes observed with time in seconds on the TLC plates.

Table 1: Phytochemicals of the tested plants

Secondary metabolites	***Datura metal***	
	AE	EE
Saponins	+	+
Flavonoids	+	+
Tannins	+	-
Glycocides	+	+
Phenols	+	+
Alkaloids	+	+
Steroids	+	-
Terpenoids	+	-

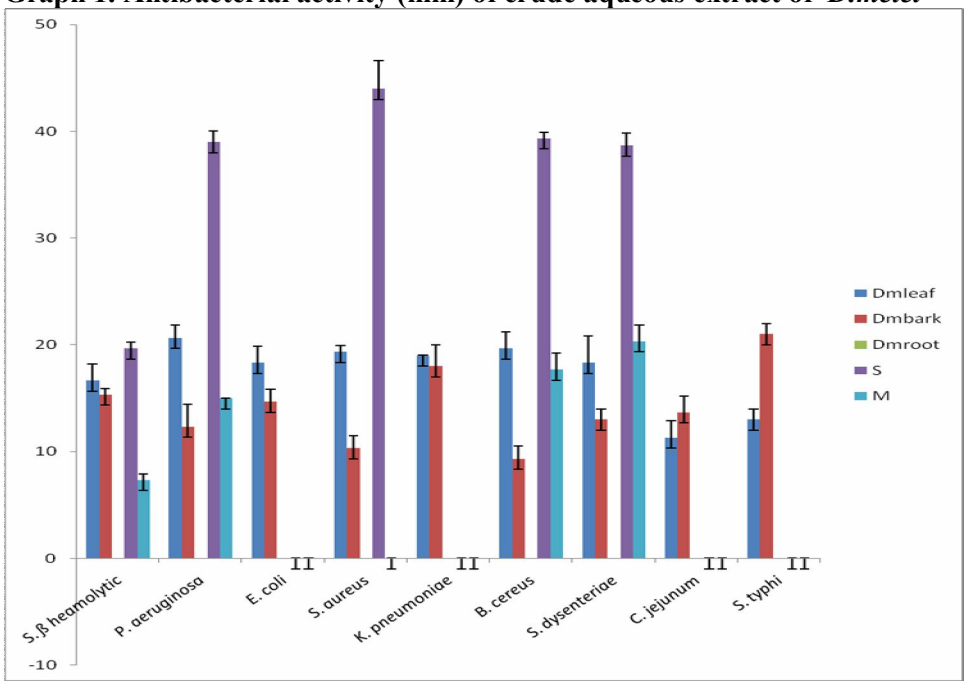
AE: aqueous extract, EE: ethanol extract, -: absent, +: present

Table 2: Free radical scavenging activity of the plant.

Plants extracts		**Radical scavenging activity (%)**		
		Leaves	Bark	Root
Datura metel				
	AE	49.80	23.82	0.00
	EE	25.51	3.41	0.00

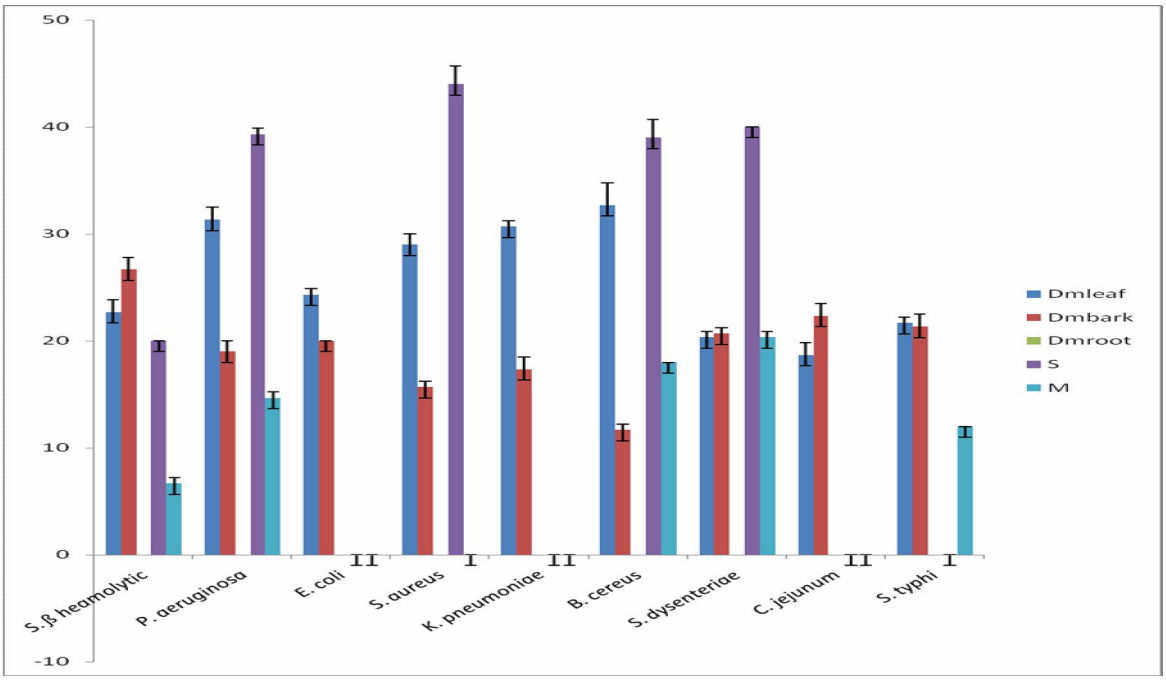
AE :aqueous extract, EE: ethamol extract

Graph 1. Antibacterial activity (mm) of crude aqueous extract of *D.metel*



0 to 45 Zone of inhibition in mm; Volume per: 20mg/ml(plant extract), 10mg/ml(antibiotic), Borer size used: 4mm

Graph 2. Antibacterial activity (mm) of crude ethanol of *Datura metel*



0 to 45 Zone of inhibition in mm; Volume per: 20mg/ml (plant extract), 10mg/ml(antibiotic), Borer size used: 4mm

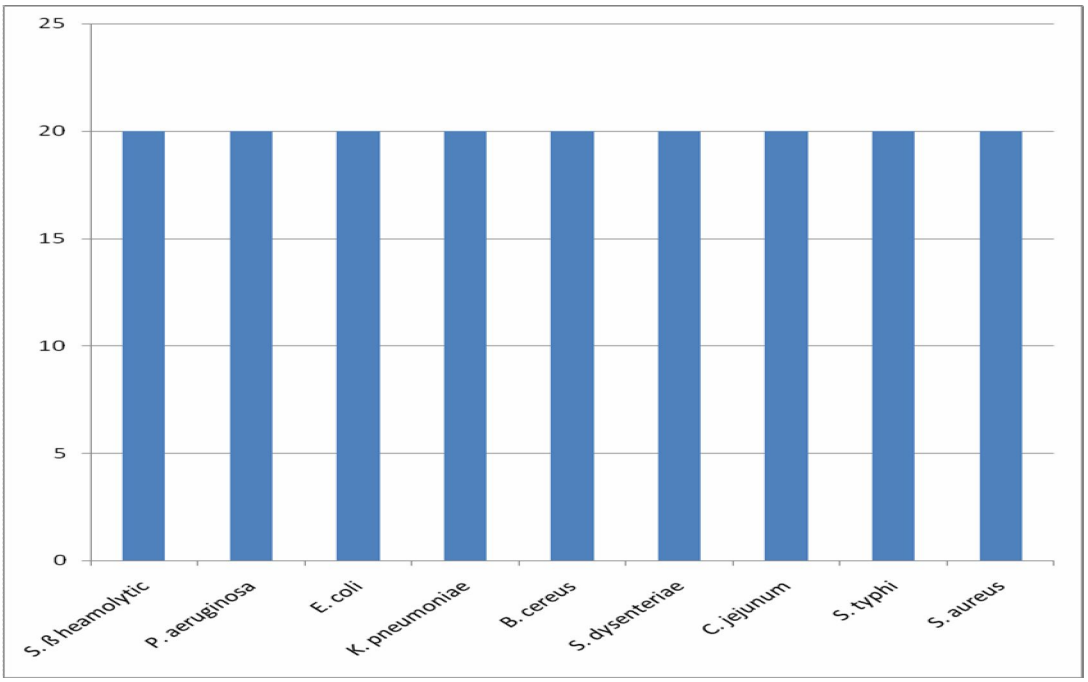
RESULTS AND DISCUSSION

The leaf and stem bark extracts of *D. metel* inhibited the tested bacterial isolates. The highest inhibition exerted on the bacteria species by the aqueous extract of the leaf and stem bark was 22 mm, ethanol leaf extract (33mm) and stem bark extract (26 mm) in contrast to 45 mm displayed by streptomycin (positive control antibiotic). Meanwhile, *P. aeruginosa* and *S. typhi* were the most inhibited while *C. jejunum* and *B. cereus* were the least inhibited by the aqueous extract (Graph 1). The ethanol extracts of the plant leaf demonstrated more inhibitory potency on the isolates with *P. aeruginosa, K. pneumonia* and *B. cereus* inhibited with zones above 30mm. The ethanol leaf extract least inhibited *C. jejunum* (19 mm) and the stem bark extract least inhibited *B. cereus* with zone of 11mm. However, eight of the test bacteria species were inhibited above 20 mm with leaf ethanol extract while the stem bark inhibited only four of the test bacteria above 20 mm (Graph 2). All the test organisms were susceptible to the plant aqueous and ethanol extracts with various degree of sensitivity ranging between 9-33 mm, whereas, *E. coli*, *K. pneumonia*, *C. jejunum* and *S. typhi* were resistant to both streptomycin and methicillin (positive control). The *Staph. aureus* isolated and used in this study was identified to be methicillin resistant strain as proved in the bioassay. This observation with the screening bacteria activities of the employed plants has added to the existing plants known to inhibit methicillin resistant *Staph. aureus* strain.

On the general note, the root extracts of the plants showed no antibacterial activity while the leaf extracts exhibited more therapeutic effect on the tested isolates. The significant antibacterial activity of the employed plant leaf and stem bark extracts were comparable to the reference antibiotics Methicillin and Streptomicin (10mg/ml).

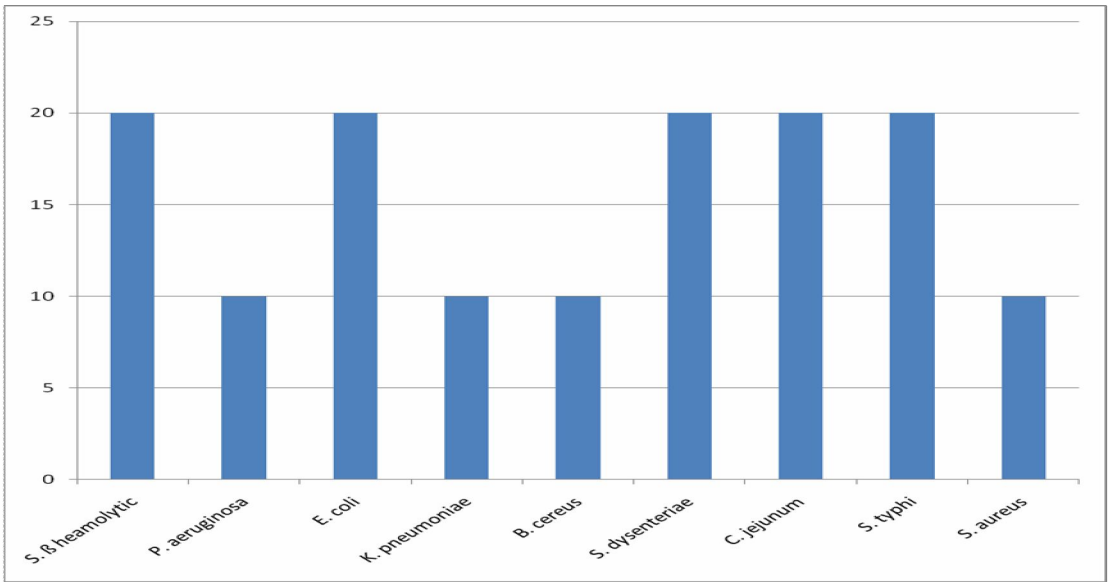
The Gram negative isolates (*P. aeruginosa*, *E. coli*, *K. Pneumoniae*, *C. jejunum* and *S. typhi*) in this study were less susceptible to the plants extracts than the Gram positive bacterial isolates (*S. β hemolytic*, *Staph. aureus*, *S. dysenteriae* and *B. cereus*. The less susceptibility of the Gram negative isolates to the plants extracts have not proved the bacteria resistant but could mean that they need higher grade solvents for extraction or may necessitate higher concentrations than used in this study for more therapeutic activity.

Graph 3. Minimum inhibitory concentration (mg/ml) of *Datura metel* leaf extract



0 to 20mg/ml extract concentration; Volume per tube: 1ml, test tube size used: 10ml

Graph 4. Minimum bactericidal concentration (mg/ml) of *Datura metel* leaf extract



0 to 20mg/ml extract concentration; Volume per plate: 1ml, Petri plate size used: 20ml

The envisage problem in the traditional use of medicinal plants is the quantity of extract required to enhance effective therapy. Graph 3 explains the minimum inhibitory aspect of the employed plant. The MIC result obtained, ascertain 20 mg/ml affinity, while graph 4 summarizes the minimum bactericidal action at 10 – 20 mg/ml. The MIC results pointed out the least concentration for therapeutic activity while the MBC results established possibly the concentrations at which the leaf crude extracts of the studied plant were bactericidal and bacteriostatic for therapeutic evaluations on the test bacterial isolates. On the basis of the contents of the compounds possessing antibacterial properties, the order of potency is leaf followed by stem bark and root which could not display any potency.

The antibacterial activities of the studied plant extracts were comparable to the reference antibiotic (positive control) used. Therefore, this study offers a scientific basis for the use of the plant extracts for the treatment of infections that could be caused by the strains of the test bacterial organisms.

From the plant, saponin, flavonoids, tannins, phenols and alkakoids glycocides, steroids and terpenoids were identified as phytochemical compounds present. Meanwhile, saponins, flavonoids glycocides, phenols and alkaloids were common to the test plant ethanol and aqueous extracts. Steroid, terpenoids and tannins were absent in the ethanol extract. Table 1 highlights the phytochemical yield of the plant aqueous and ethanol extracts. However, the aqueous extracts contained more of the identified phytochemical compounds than the ethanol extracts. Majority of the phytochemical compound identified in the aqueous extract have been reported to be highly of therapeutic importance. This finding is in agreement with. The antioxidant activity determined also showed that aqueous extract was more efficient in the extract of the substances with antioxidant than ethanol.

Antioxidant activities were higher in the plant leaf than bark. The aqueous extract of the leaf and stem bark shown antioxidant effect of 48.80 and 23.82% respectively, while the ethanol extract was respectively 25.51 and 3.41%. As observed in Table 2, the root of the considered plant showed no antioxidant activity as free radical scavenging was absent. The promising result obtained has subjected this plant extract to further analyses to screen for its toxicity and side effect for possible perfect therapeutic value.

REFERENCES

1. Akroum S., Satta, D. and Lalauoui, K., Antibacterial, antioxidant, cytotoxic activities and phytochemical screening of some Algerian plants, Euro. J. Sci. Res 2009, 31(2), 289-295.
2. Amjad K., Dhia, S.K. and Abeer, K., 'Genetic relationship among salvia species and antimicrobial activity of their crude extracts against pathogenic bacteria'. Asian Jour, Plant Sci, 2005, 4(5), 544-549.
3. Akinyemi., K.O. Oladapo, O. Okwara, C.E. Ibe, C. and Fasure, K.A., Screening of crude extracts of six medicinal plants used in South-West Nigeria unorthodox medicine for anti-methicilin resistant-Staphylococcus aureus activity. Compl. Alt. Med, 2005, 5, 5-8.
4. Nair, R. and Chando, S., Anticandida activity of *Punica granatum* exhibited in different solvents. Pharm. Biol, 2005, 42: 21-25.
5. Trease, G.E and Evans, W.C., 'Pharmacognsy' 11th edn. Brailliar Tiridel Canada. Macmillian Publishers, 1989.
6. Harbone, J.B., 'Phytochemical methods London. Chapman and Hall Ltd. 1973, pp. 49-188.
7. Cuendet, M. Hostetmann, K. and Potterat, O., 'Iridiod glucosides with freee radical scavenging properties from Fragrea bluma' Helvetica Chemica Acta 1999, 80, 1144-1152.
8. Cuendet M, Potterat O, Salvi A, Testa B, Hostetmann K. 'A stilbene and dihydrochalcones with radical scavenging activities from *Loiseleuria procumbens*' Phytochemistry, 2000, 54, 871-874.

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