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IN-VITRO ANTIMICROBIAL ASSAY AND PHYTOCHEMICAL SCREENING OF THE BIOACTIVE COMPONENTS OF *Datura metel* (GEGEMU) ON SELECTED CLINICAL ISOLATES

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Abstract: *Datura metel* is a medicinal plant used as phytomedicine to treat traditionally a wide range of health complications. This study explored the *in vitro* antimicrobial activities and phytochemical screening of ethanolic extract of *D. metel* seed and its fractions (chloroform, n-hexane and aqueous) on selected clinical isolates. The antimicrobial efficacy was assayed using agar well diffusion method. The Minimum Inhibitory Concentration (MIC) was done using the doubling macro broth dilution method and the tubes with no visible turbidity were used for the Minimum Lethal Concentration (MLC). Both qualitative and quantitative phytochemical screenings were determined with reference to the standard laboratory procedure and spectrophotometry methods respectively. The structural elucidation of the bioactive compounds was carried out using Gas Chromatography-Mass Spectrometry (GC-MS). The results showed the antimicrobial efficacies of *D. metel* seed extract against four selected clinical isolates *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Candida albicans*. *E. coli* showed the highest level of susceptibility to all the tested extractants while *C. albicans* showed the least. There was no visible turbidity at 25 to 100 mg/ml against all the bacterial isolates whereas *C. albicans* showed visible turbidity at 25 mg/ml for chloroform and n-hexane extracts for the Minimum Inhibitory Concentration (MIC). MLC (Minimum Lethal Concentration) of the extracts of *D. metel* was observed at 50 to 100 mg/ml of extract for both crude (ethanol) and aqueous solvents. Phytochemical screening indicated the presence of saponin, steroids, coumarin, flavonoids, glycosides, terpenoids and alkaloids. A total number of 6 bioactive compounds which include hydroxyl ethyl vinyl sulfide, dimethyldiaziridine, ethane diazo, flourooctane, urethane and propanenitrile were determined by comparing the GC-MS spectra. Flourooctane showed the highest peak in terms of abundance at the 5th minute while urethane showed the lowest abundance at the 6th minute. In conclusion all extractants used showed appreciable antimicrobial efficacies and this indicates that the seed extract can be used as an alternative source for new antimicrobial agents and may also serve as a good source of antioxidant due to the high level of flavonoids present.

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Introduction

Antimicrobial drugs are crucial in reducing the global burden of infectious diseases (Bhatia and Narain, 2010). However, the fast rise and spread of multidrug resistant (MDR) strains in pathogenic bacteria has become a huge public health threat because there are fewer, if not no effective antimicrobials available for treating pathogenic bacterial-caused infections (Giamarellou, 2010). As a result, given the evidence of the rapid global spread of resistant clinical isolates, the need for novel antimicrobials is critical. However, given the quick and widespread establishment of resistance to newly introduced antimicrobial drugs in the past, even new antibiotic families are likely to have a short lifespan (Marasini *et al*., 2015). A large variety of medicinal plants have been identified as important sources of natural antibacterial substances that may be beneficial in the treatment of these difficult bacterial diseases. Antibiotic resistance is on the rise, and novel antimicrobials are in short supply (Theuretzbacher and Mouton, 2011; Walsh and Toleman, 2012). The need for new, effective, and affordable drugs to treat microbial infections is a key concern in global health care, particularly in developing nations, where infectious diseases account for up to half of all deaths (Awouafack *et al*., 2013; Srivastava *et al*., 2013). In light of this, the discovery of alternative medication classes to treat infectious diseases is critical (Srivastava *et al*., 2013).

Nigeria is home to a diverse range of plant species that contain a wide range of bioactive compounds (Fennell *et al*., 2004; McGaw *et al*., 2008). In many rural parts of the world, the lack of access to Western primary health care services has aided the continued use of

traditional medicine for human treatment. Even in areas where orthodox pharmaceuticals are readily available, a considerable portion of the population continues to use herbal therapies in addition to or instead of conventional medicines (McGaw *et al*., 2008).

Datura metel is a medicinal plant that has long been used as a phytomedicine to treat a variety of health issues (Berkov *et al*., 2006). This plant can be investigated further due to its vast variety of traditional uses and the presence of a wide range of chemical compounds in various portions of the plant. *Datura metel* is a blooming plant that can reach a height of 3 feet. *Datura metel* has leaves that are 10-20 cm long and 5-18 cm wide. It has grey hairs on its leaves that are short and silky.

Datura species have a lot of alkaloids in their leaves and seeds; include atropine, scopolamine, and hyoscyamine (Berkov *et al*., 2006). Flavonoids, phenols, tannins, saponins, aminoacids, and sterols are among the phytoconstituents present in *Datura metel* (Donatus and Ephraim, 2009). Several scientific investigations on ethanol and hydro-alcoholic crude extract antioxidant and phytochemical screening have been published (Okwu and Morah, 2007; Okoli *et al*., 2006). Given the vast potential of *D. metel* as sources of antimicrobial drugs, the goal of this study was to determine the antimicrobial activities of different *Datura metel* extractants *in vitro* against the most common clinical microbial pathogens and to determine the structure of the bioactive compounds present in the most effective extract.

Materials and Methods

Sample Collection and Identification

The plant part, which is the seed of *Datura metel*, was taken from the local environment along a running river in Offa, Offa Local Government Area of Kwara State, Nigeria, based on traditional medicinal history. The collected fruits were taken to the Herbarium Unit of the Department of Life Science at the University of Ilorin in Kwara State, Nigeria, where the plant was identified and validated with the voucher number **UILH/001/845/2021**.

Preparation of Plant Extracts

The plant part sample (fruits) was cleaned under running tap water and then air-dried for a month in the shade at room temperature (25 ^{o}C) with continual monitoring. Using a clean home grinder, the seeds were removed from the fruit and processed into a fine powder. The weight of the pulverized powder was collected, and a cold percolation process was used to make the plant extract. Two hundred and fifty (250) grams of fine powder from plant powder was dissolved in 2500 mL absolute ethanol for seven (7) days at room temperature. The supernatant was filtered and kept in a glass container using Whatman filter paper. The filtrates were then evaporated under reduced pressure at 50 ^{o}C using a rotary evaporator to give the crude extract after the extraction. For later usage, the crude extract was collected in a vial. The yield was determined as a percentage (% yield) (Manandhar *et al*., 2019).

$$\text{Percentage Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \text{ X } 100 \qquad (1)$$

Solvent-solvent partitioning

The prepared solution was effectively separated using increasing polarity solvents such as n-hexane (200 ml), chloroform (200 ml), and distilled water (100 ml). All three fractions were dried in a water bath at a low temperature of 50 ^{o}C before being stored in airtight containers for subsequent analysis (Muhit *et al*., 2010).

Collection of Microbial Culture

Four clinical isolates; *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Candida albicans* were procured from the Department of Medical Microbiology Laboratory Unit of University of Ilorin Teaching Hospital (UITH) around Kwara State, Nigeria. The isolates were confirmed using series of morphological and conventional biochemical tests to identify the selected microorganisms (Cheesbrough, 2012). The isolates were maintained on freshly prepared agar slants and kept in a refrigerator at 4 ^{o}C until required.

Identification of the clinical isolates

Cultural examination

On blood agar, the morphological properties of colonies were investigated. After 24 hours of incubation at 37 ^{o}C, the shape, color, edge and texture were recorded.

Microscopic examination

To analyze the Gram stain under a light microscope, a single colony of each isolate was put on a clean slide (Atlas *et al*., 2000).

Biochemical tests

Biochemical assays were performed on the probable isolates, as described by Cheesbrough (2012).

Preparation of Media

All the media used were prepared according to the manufacturers' instructions by weighing the required quantities of powder (in grams) and dissolving in equivalent volume of distilled water (in ml). The media were dissolved completely on hot plate while stirring with a sterile rod to prevent burning at the bottom of the conical flask and also achieving homogenized solution. The media were then sterilized by autoclaving at 121 ^{o}C for 15 minutes (Cheesbrough, 2012).

Preparation of McFarland Standard

Barium sulphate standard suspension was used as the turbidity standard. 1 % v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water, 1 % w/v solution of barium chloride was also prepared by dissolving 0.5 g of dehydrated barium chloride ($BaCl_2$-$2H_2O$) in 50 ml of distilled water and 0.05 ml of the barium chloride solution was added to 9.95 ml of the sulphuric acid solution and thoroughly mixed (Cheesbrough, 2012). The turbid solution that was formed was then transferred into a test tube as the standard for comparison (0.5 McFarland standards) that corresponds to approximately $1.0x10^{-5}$ cfu/ml) (Chessbrough, 2012).

Inoculums Standardization

The previously prepared overnight broth culture of each isolate was used as inoculum by diluting with sterile saline solution. The sterile normal saline was prepared by weighing 0.5 g of NaCl and dissolved in 100 ml of sterile distilled water and 0.1 ml of each overnight broth culture of the test organisms were dispensed into separate test tubes containing sterile normal saline. The suspension was adjusted to match the 0.5 McFarland standard which had a similar appearance of an overnight broth culture (El-Mahmood and Amey, 2001). This served as the standard inoculum which was used for the antibacterial testing and for the determination of MIC and MLC of the extracts.

Preparation of Antibiotic Dilution

The antibiotic ciprofloxacin and fluconazole were purchased from a known pharmacy in Ilorin, Kwara State, Nigeria and reconstituted by dissolving 3 g of the ciprofloxacin and fluconazole powder in 100 ml of distilled water so as to obtain a concentration of 30 mg/ml. The prepared dilution of the antibiotics was used for subsequent antimicrobial test as positive control (Garba *et al*., 2011).

Antimicrobial Assay of Seed Extracts of *D. metel*

The antibacterial activity of the seed extracts was conducted using the method described by Al-Mahmood (2009). The stock was prepared by dissolving 0.6 g of each crude extracts of Datura in 6 ml of DMSO (dimethylsulfoxide) in different vials (bottles) to make a stock solution of 100,000 µg/ml (100 mg/ml). Mueller Hinton Agar plate was lawn cultured with standardized bacterial culture broth while Potato Dextrose Agar was used for the inoculation of *Candida albicans*. Seven wells of 6 mm were bored in the inoculated media containing the bacterial and fungal inoculums with the help of sterile cork-borer (6 mm). Each well was filled with five different concentrations from the stock solution of the extracts at concentrations (10, 50, 100, 150, and 200) of each solvent extract from the seed were introduced into their respective wells. 100 µl of DMSO was introduced into the sixth well to serve as negative control while 100 µl of 30 mg/ml of ciprofloxacin was introduced into the seventh well to serve as a positive control for the bacterial isolates and 100 µl of fluconazole was used as the positive control for the fungal isolate. The inoculated plates were left to stand for about 30 minutes to allow diffusion of extract before incubating at 37 ºC for 24 hours for the bacterial isolates and the fungal isolate was incubated at 37 ºC for 3 days. After incubation, the plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm using a transparent meter rule. Each of the experiment was conducted in duplicate and the mean results were taken for the test organisms (Kaniz *et al*., 2012).

Determination of Minimum Inhibitory Concentration, Minimum Lethal Concentration for *D. metel*

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibited visible growth of microorganisms after overnight incubation (Andrews, 2002). The doubling macro dilution broth method was used to determine the MIC. Two (2) ml of the reconstituted crude extract at a concentration of 100,000 µg/ml (100 mg/ml) was added to 2 ml sterile Nutrient Broth for the bacterial isolates, 2 ml of the reconstituted crude extract was added to 2 ml of Potato Dextrose Broth for the fungal isolate and the tubes were mixed together to homogenize. Two (2) ml of this extract concentration from the previously mixed tube was transferred to another test-tube and this dilution continued until the 6th test-tube was reached, giving extract concentrations ranging from 500 – 3.125 mg/ml in different test tubes and 0.1 ml of a 24 hour culture of bacteria and 3 day culture of fungus previously adjusted to 0.5 McFarland standard was inoculated into each of the test tubes and the contents were thoroughly mixed. A test tube containing the broth and extract was used as positive control while a test tube containing the broth and bacterial/fungal inoculum was used as negative control. The inoculated culture tubes were incubated at 37 ºC and were observed for growth after 24 hours for the bacterial isolates and 3 days for the fungal isolate. The lowest concentration of extract showing no visible growth when compared with the control was considered as the MIC as demonstrated by the method Andrews (2002). The Minimum Lethal Concentration (MLC) is the lowest concentration of antimicrobial agent that prevented the growth of an organism. Aliquot (0.1 ml) from the tubes that showed no visible bacterial/fungal growth from the determination of Minimum Inhibitory Concentration was inoculated on a sterile Mueller Hinton Agar for 24 hours at 37 ºC for

the bacterial isolate while the fungal isolate was inoculated on sterile Potato Dextrose Agar at 37 ºC. The lowest concentration in which no growth occurred was taken as the Minimum Lethal Concentration (MLC) as demonstrated by Andrews (2002).

Phytochemical screening of Datura extracts

Phytochemical examinations were carried out for all the extracts as per the methods of Prashant *et* al. (2011).

GC-MS analysis for bioactive compounds of *D. metel*

Phytochemical analysis of the bioactive compounds of the extract of *D. metel* was carried out using GC-MS analyzer (BRUKER SCION 436-GC SQ). Extract was dissolved in ethanol (high-performance liquid chromatography grade) and filtered through WhatmanTM FILTER DEVICE (0.2 µm). Helium (99.99 %) was used as carrier gas, at a flow rate of 1 ml/minute in split mode. RESTEK Rtx®-5 (Crossbond® 5 % diphenyl/95 % dimethyl polysiloxane) with 30 m length, 0.25 µm df, and 0.25 mm ID column was used for separation of phytochemicals and 1 µL of sample was injected to column. The injector temperature was 150 ºC. The temperature of oven starts at 70 ºC and hold for 2 minutes and it was raised at a rate of 7 ºC per minute up to 320 ºC, held for 1 minute. Temperature of ion sources was maintained at 250 ºC. The mass spectrum was obtained by electron ionization at 70 eV and detector operates in scan mode 30 – 500 Da atomic units. Total running time was 15 minutes including 3 minute solvent delay (Sharma *et al.,* 2018).

Results

The cultural characteristics, conventional biochemical tests and Gram's staining of the clinical isolates (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Candida albicans*) on blood agar in Table 1 showed that all isolates possessed circular and entire edge with respect to shape and edge, although *Escherichia coli* and *Klebsiella pneumoniae* were grey in colour whereas *Staphylococcus aureus* and *Candida albicans* were observed to be cream in colour, while the textural characteristics showed that *E. coli* and *S. aureus* were non mucoid whereas *K. pneumoniae* and *C. albicans* were mucoid in nature.

The chosen clinical isolates' conventional biochemical tests and Gram staining findings indicated that all were positive for catalase and negative for oxidase, with the exception of *S. aureus*, which was positive for coagulase and *E. coli*, which was negative for citrate. Indole and methyl red tests were positive for *E. coli* and *C. albicans*, while urease and Vogues Proskauer tests were positive for *K. pneumoniae* and *S. aureus*. All of the isolates tested positive for glucose, maltose, and sucrose, with the exception of *C. albicans*, which was lactose negative. Fructose was shown to be negative in *E. coli* and *K. pneumoniae*, whereas it was found to be positive in *S. aureus* and *C. albicans*. Two (2) of the isolates were Gram's negative *E. coli* and *K. pneumoniae*, while two were Gram's positive *S. aureus* and *C. albicans*.

The percentage yield recorded in Figure 1 indicated that the aqueous extract from the crude extract using solvent-solvent partitioning showed the highest percentage yield with 57.70% yield while chloroform showed the lowest percentage with 4.00% yield.

The mean values ± standard deviations of duplicate results for zones of inhibitions obtained using agar well diffusion method from different solvents are shown in Table 2. All solvent extracts showed appreciable antimicrobial activity with the diameters of zones of inhibition ranging from 0.00 ± 0.00 mm to 13.50 ± 0.71 mm in diameter. *Escherichia coli* had the highest susceptibility values for all solvents while *Klebsiella pneumoniae* showed the lowest susceptibility values to the ethanolic and solvents-solvent extracts of *D. metel.* The standard antibiotics; ciprofloxacin for the bacterial isolates and fluconazole for the fungal isolate used as standard antibiotics maintained preferable inhibition zones compared to the solvents extracts at 100 µl concentration while the DMSO showed no zones of inhibition against all tested isolates. The antimicrobial efficacy decreased with decreasing concentrations and showed minimal or no antimicrobial effects at concentration lesser than 100 µl. This indicated that amongst the isolates tested, *E. coli* had the highest zone of inhibition across all solvents used while *K. pneumoniae* had the lowest.

Table 3 shows the extract's Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC). All bacterial isolates showed no obvious turbidity against the solvents at concentrations of 25 mg/ml, although mild turbidity was noticed in the potato dextrose broth used for *C. albicans* at 25 mg/ml for chloroform and n-hexane extracts. As a result, the MICs for both extracts were found to be 50 mg/ml. In addition, the negative control tubes without culture exhibited no turbidity, but all of the positive control tubes with culture showed substantial turbidity. To estimate the MLC, these concentrations were plated out on previously solidified culture medium including nutrient agar and potato dextrose agar (Minimum Lethal Concentration).

The MLC (Minimum Lethal Concentration) of *D. metel* extracts was found to be 50 mg/ml for both crude (ethanol) and aqueous solvents, whereas all isolates grew at 50 mg/ml for chloroform and n-hexane extracts.

The Table 4 is a representation of the qualitative phytochemical screening of *D. metel*. The table indicated that seven phytochemicals were present in

the sample including saponin, steroids, coumarin, flavonoids, glycosides, terpenoids and alkaloids while six were not.

The mean values of the quantitative phytochemical screening of *D. metel* are displayed in the Figure 2. The result showed that flavonoids had the highest value with 121.03 mg/100g while alkaloids had the lowest value with 0.27 mg/100g.

A total number of 6 bioactive compounds were analyzed by comparing the GC-MS spectra with NIST MS library data base. The peaks of different compounds identified from the ethanolic extract of *D. metel* are presented in Figure 3. The major compounds identified by matching the spectra with the NIST library data base were hydroxyl ethyl vinyl sulfide, dimethyldiaziridine, ehane diazo, flucoronate, urethane, propnanenitrile etc. it was observed that the flucoronate showed the highest peak in terms of abundance at the 5th minute while urethane showed the lowest abundance at the 6th minute. The structural representation including the molecular structure, weight and other parameters with respect to NIST library comparison are presented in Table 5.

Table 1: Basic Cultural Characteristics, Conventional Biochemical Tests and Gram Reaction for Confirmation of Clinical Isolates on Blood Agar

Clinical Isolates	Morphological Characteristics				Conventional Biochemical Tests								Sugar Fermentation					Microscopy			Probable Isolates
	Colour	Shape	Edge	Texture	Ca	O	Co	Ci	U	I	MR	VP	G	F	M	S	L	Gram Stain	Cell Argmnt.	Motility	
E. coli	Grey	Circular	Entire	Non Mucoid	+	–	–	–	–	+	+	–	+	–	+	+	+	–ve	Singly	Non Motile	*E. coli*
K. pneumoniae	Grey	Circular	Entire	Mucoid	+	–	–	+	+	–	–	+	+	–	+	+	+	–ve	Pairs	Non Motile	*K. pneumoniae*
S. aureus	Cream	Circular	Entire	Non Mucoid	+	–	+	+	+	–	–	+	+	+	+	+	+	+ve	Clusters	Non Motile	*S. aureus*
C. albicans	Cream	Circular	Entire	Mucoid	+	–	–	+	–	+	+	–	+	+	+	+	–	+ve	Budding	Non Motile	*C. albicans*

Key; Ca = Catalase, O = Oxidase, Co = Coagulase, Ci = Citrate, U = Urease, I = Indole, MR = Methyl Red, VP = Vogues Proskauer, G = Glucose, F = Fructose, S = Sucrose, L = Lactose, –ve = Negative, +ve = Positive

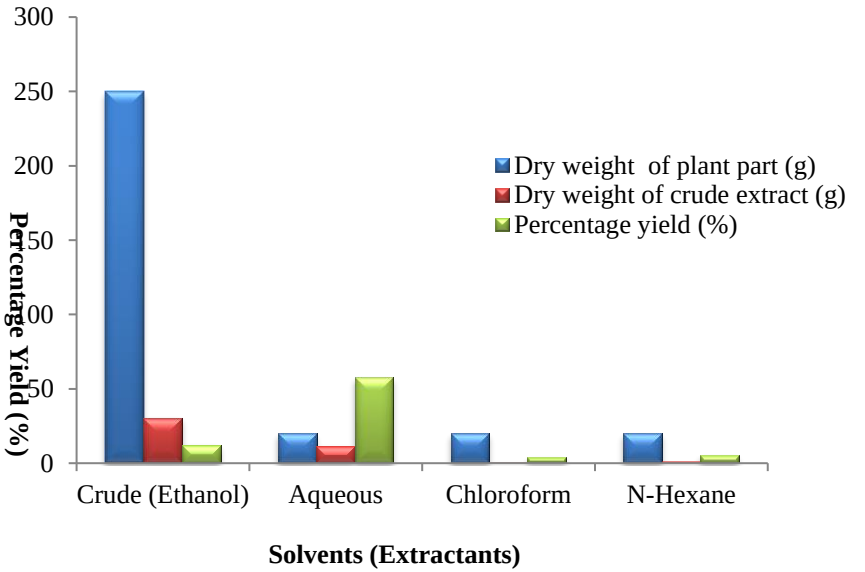


Figure 1: Percentage Yield (%) of the Crude Extract and Solvent-Solvent Partitioning Resulting from the Extraction of *D. metel* using Different Extractants

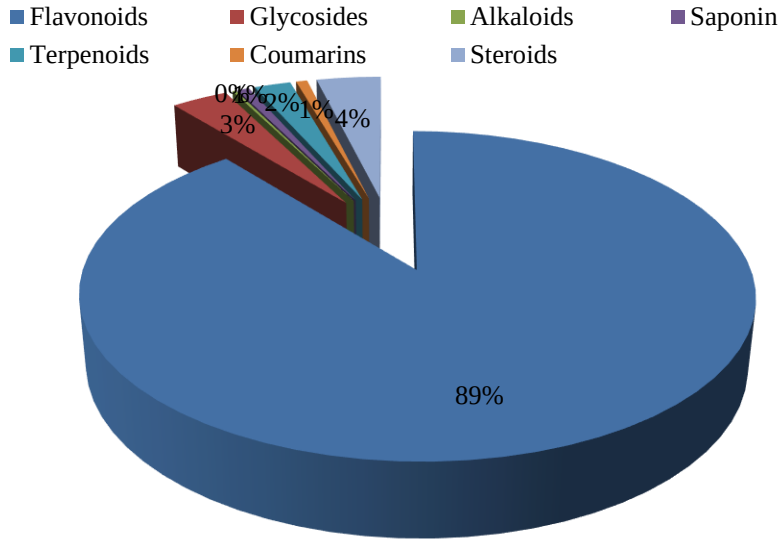


Figure 2: Mean ± SD of Duplicate Values of Quantitative Phytochemicals Analysis of *D. metel* Ethanolic Extract

Table 2: Antimicrobial Assay of Different Solvent Extracts of Seeds of *D. metel* and the Standard Antibiotics used on Selected Clinical Isolates

Concentrations (µl)	Extractants	Inhibition Zones in Diameter (mm)			
		Escherichia coli	***Klebsiella pneumoniae***	***Staphylococcus aureus***	***Candid albicans***
200	Crude (Ethanol)	13.50 ± 0.71^{b}	9.50 ± 0.71^{a}	10.50 ± 0.71^{b}	11.50 ± 0.71^{b}
	Aqueous	10.50 ± 0.71^{a}	8.50 ± 0.71^{a}	8.50 ± 0.71^{a}	10.50 ± 0.71^{b}
	Chloroform	11.00 ± 0.00^{a}	7.50 ± 1.41^{a}	9.00 ± 0.00^{ab}	7.50 ± 0.71^{a}
	N-Hexane	10.50 ± 0.71^{a}	7.50 ± 1.41^{a}	8.50 ± 0.71^{a}	8.00 ± 0.00^{a}
150	Crude (Ethanol)	10.00 ± 0.00^{c}	5.00 ± 1.41^{a}	9.50 ± 0.71^{c}	9.50 ± 0.71^{d}
	Aqueous	7.50 ± 0.71^{b}	5.50 ± 0.71^{a}	7.00 ± 1.41^{ab}	8.00 ± 1.41^{c}
	Chloroform	9.00 ± 1.41^{c}	7.50 ± 1.41^{b}	7.50 ± 0.71^{b}	7.00 ± 0.00^{b}
	N-Hexane	5.50 ±0.71^{a}	5.50 ± 0.71^{a}	6.00 ± 1.41^{a}	5.00 ± 0.00^{a}
100	Crude (Ethanol)	7.50 ± 0.71^{b}	5.00 ± 1.41^{b}	7.50 ± 0.71^{b}	8.50 ± 0.71^{c}
	Aqueous	4.50 ± 0.71^{a}	3.50 ± 0.71^{a}	3.00 ± 1.41^{a}	4.50 ± 0.71^{ab}
	Chloroform	6.50 ± 0.71^{b}	6.50 ± 0.71^{c}	6.50 ± 0.71^{b}	6.00 ± 0.00^{b}
	N-Hexane	3.00 ± 0.00^{a}	3.00 ± 0.00^{a}	2.50 ± 0.71^{a}	3.50 ± 0.71^{a}
50	Crude (Ethanol)	4.50 ± 0.71^{b}	2.50 ± 0.71^{b}	2.50 ± 0.71^{b}	3.00 ± 0.00^{b}
	Aqueous	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Chloroform	4.00 ± 0.00^{b}	4.00 ± 0.00^{c}	4.50 ± 0.71^{c}	3.00 ± 0.00^{b}
	N-Hexane	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
10	Crude (Ethanol)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Aqueous	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Chloroform	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	N-Hexane	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Ciprofloxacin (100 µl)		12.50 ± 1.41^{c}	10.50 ± 0.71^{c}	9.50 ± 0.71^{c}	
Fluconazole (100 µl)					8.50 ± 0.71^{c}
DMSO (100 µl)		0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Key; DMSO = Dimethyl Sulfoxide

The Mean Values with Different Alphabets are Significantly Different at Alpha (α) level significance i.e. α = 0.05 (95%)

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of Different Extractants of *D. metel* against Selected Clinically Significant Isolates

	Concentrations of *D. metel* extract (mg/ml)	
Solvents/Organisms	**MIC**	**MLC**
Crude (Ethanol) Extract		
E. coli	25	50
K. pneumoniae	25	50
S. aureus	25	50
C. albicans	25	50
Aqueous Extract		
E. coli	25	50
K. pneumoniae	25	50
S. aureus	25	50
C. albicans	25	50
Chloroform Extract		
E. coli	25	100
K. pneumoniae	25	100
S. aureus	25	100
C. albicans	50	100
N-Hexane Extract		
E. coli	25	100
K. pneumoniae	25	100
S. aureus	25	100
C. albicans	50	100

Table 4: Qualitative Phytochemical Analysis of Ethanolic Extract of *D. metel*

Phytochemicals	**Reactions**
Saponin	+
Tannin	–
Phelonic	–
Steroids	+
Coumarin	+
Flavonoids	+
Glycosides	+
Terpenoids	+
Triterpenes	–
Athocyanin	–
Phlobatamin	–
Amino Acids	–
Alkaloids	+

Key; – = Not Present, + = Present

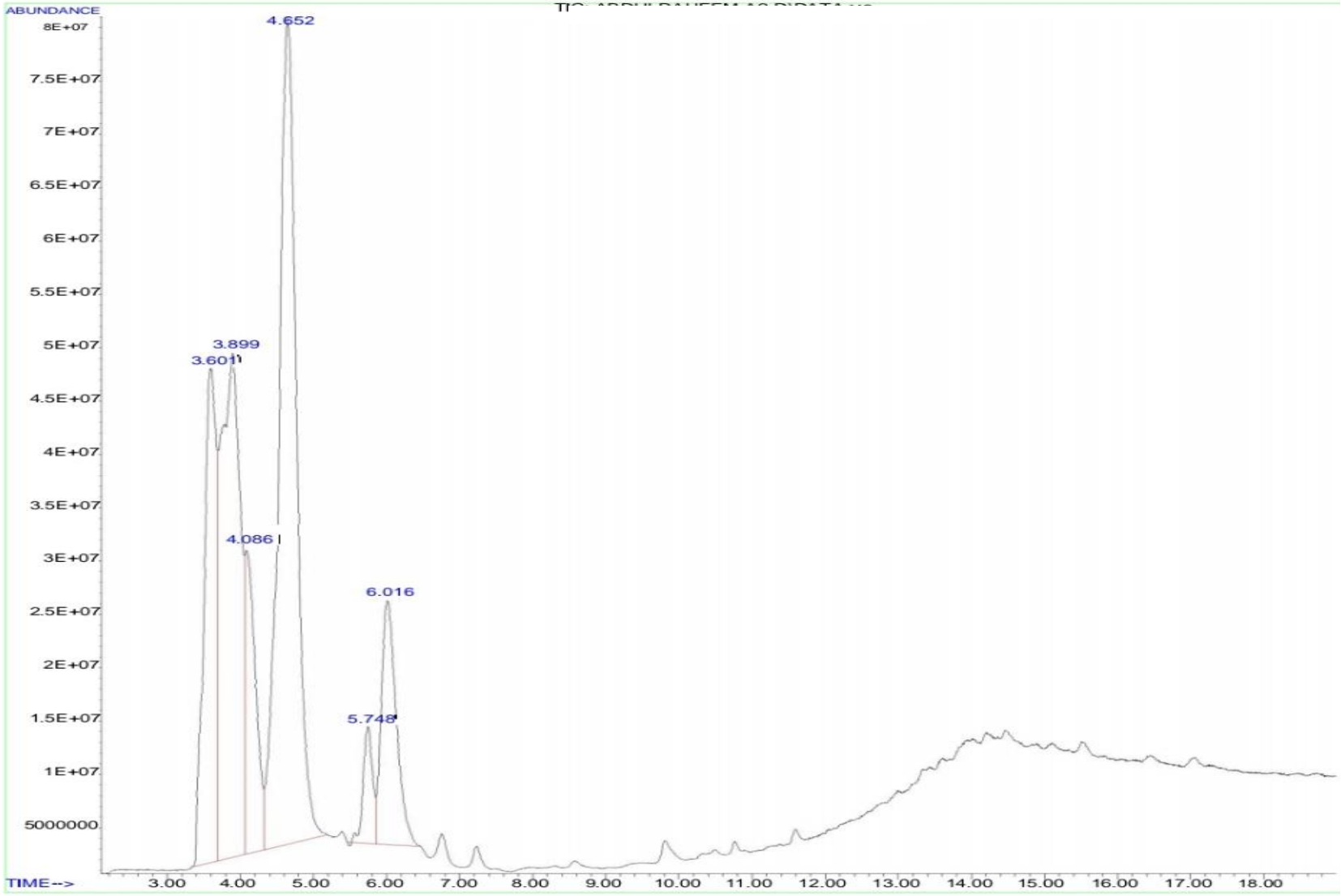
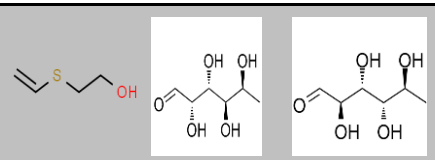
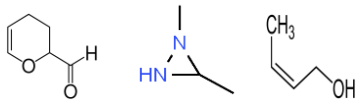
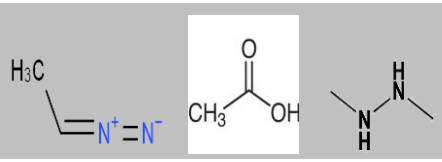
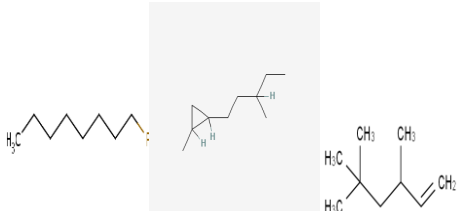
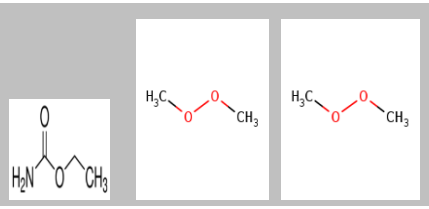
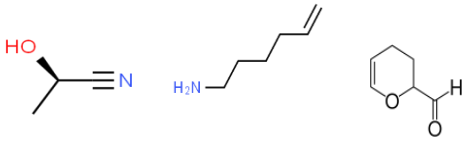


Figure 3: Graph Showing Different Peaks of the Bioactive Compounds of Ethanolic Extract of *D. metel* Screened by GC-MS Analysis

Table 5: NIST Library of Gas Chromatography – Mass Spectrometry of Ethanolic Extract of *D. metel*

PK#	RT	AREA %	LIBRARY/ ID	MOLECULAR WEIGHT	MOLECULAR STRUCTURE	MOLECULAR FORMULAR	REF #	CAS#	QUAL
1	3.601	15.62	2-Hydroxyethyl vinyl sulfide	104.170		C_4H_8OS	4737	003090-56-0	36
			D-Fucose	164.156		$C_6H_{12}O_5$	33884	003615-37-0	32
			Alpha-1-rhamnopyranose	164.156		$C_6H_{12}O_5$	33896	035810-56-1	23
2	3.901	25.41	1,3-Dimethyldiaziridine	72.109		$C_3H_8N_2$	635	026177-36-6	9
			2-Propenal	56.060		C_3H_4O	165	000107-02-8	9
			2-Buten-1-ol, (z)-	72.110		C_4H_8O	680	004088-60-2	9
3	4.089	8.46	Ethane, diazo-	*56.068*		$C_2H_4N_2$	161	001117-96-0	3
			Acetic acid	60.050		$C_2H_4O_2$	260	000064-19-7	2
			Hydrazine, 1,2-dimethl-	60.100		$C_2H_8N_2$	278	000540-73-8	2
4	4.652	38.07	1-Flourooctane	132.220		$C_8H_{17}F$	14487	000463-11-6	59
			Cyclopropane, 1-methyl-2-(3-methyl pentyl	140.270		$C_{10}H_{20}$	18069	062238-07-7	40
			1-Hexene, 3,5,5-trimethyl-	126.243		C_9H_{18}	11571	004316-65-8	37
5	5.746	2.85	Urethane	89.093		$C_3H_7NO_2$	2151	000051-79-6	74
			Peroxide, dimethyl	62.067		$C_2H_6O_2$	330	000690-02-8	9
			Peroxide, dimethyl	62.067		$C_2H_6O_2$	328	000690-02-8	9
6	6.015	9.58	Propanenitrile, 2-hydroxy-	71.078		C_3H_5NO	602	000078-97-7	9
			Hex-5-anylamine	99.174		$C_6H_{13}N$	3529	034825-70-2	9
			2-Propenal	56.060		C_3H_4O	167	000107-02-8	5

Discussion

Medicinal herbs are extremely essential in the traditional treatment of a variety of ailments (Ng *et al*., 2012). The bioactive components that are responsible for certain biological functions differ between species. Secondary metabolites serve a key role in the creation of novel therapeutic agents in modern medicine (Abreu *et al*., 2012). The advent of antibiotic-resistant pathogenic microorganisms necessitated the creation of more selective and effective medications with fewer adverse effects. As a result, there is a need to investigate the antimicrobial activities of *Datura metel* extracts in various solvents against clinical isolates.

This present study was conducted to investigate the antimicrobial efficacies of the seed extracts of *D. metel* against four clinical isolates utilizing solvents such as ethanol (crude), aqueous, chloroform, and n-hexane. The zones of inhibition were quantified and compared to the antimicrobials ciprofloxacin and fluconazole, which are both typical antimicrobials. The clinical isolates on blood agar were confirmed using thorough cultural features and biochemical testing. The findings of standard biochemical assays and Gram staining of the chosen clinical isolates revealed the biochemical results of the isolates due to the presence of specific enzymes with two of the organisms being Gram positive (*S. aureus* and *C. albicans*) and the remaining two reacting as Gram negative as a result of the type of cell wall individual organism possessed.

The aqueous extract recoded the maximum percentage yield (57.70 %), while chloroform resulted in minimum percentage yield (4.00 %), this must have resulted from the poor extraction efficiency of chloroform when compared to aqueous as solvents used for extraction. This result is similar to the study conducted by Dixon and Jeena (2016), where six different solvents were also used with n-hexane and chloroform also showing the least percentage yields.

The antibacterial action of beneficial phytochemicals in higher plants has long been known. The solvents used to extract phytoconstituents may have an impact on their efficacy. Water and ethanol extractions are well-known in Ayurveda due to their benign properties and edibility. Mukhtar *et al*. (2012) investigated the antibacterial activity of aqueous and ethanolic medicinal plant extracts. Many researchers found similar effects of ethanolic extract in their studies (Abkhoo and Jahani, 2017; Bitchagno *et al*., 2017; Sharma *et al*., 2010). Plant extracts have separate modes of action from antibiotics already in use, implying that cross-resistance with currently used drugs is minimal (Dubey *et al*., 2015). The mean values ± standard deviations of duplicate results for zones of inhibitions obtained using agar well diffusion method from different solvents extracts indicated appreciable antimicrobial activity with the diameters of zones of inhibition ranging from 0.00 ± 0.00 mm to 13.50 ± 0.71 mm in diameter with *Escherichia coli* having the highest susceptibility values for all solvents while *Klebsiella pneumoniae* showed the lowest susceptibility values to the ethanolic and solvents-solvent extract of *D. metel.* The standard antibiotics; ciprofloxacin for the bacterial isolates and fluconazole for fungal isolate used as standard antibiotics still maintained preferable inhibitory zones when compared to the solvents extracts at 100 µl concentration while the DMSO showed no zones of inhibition against all tested isolates. The antimicrobial efficacy decreased with decreasing concentrations and showed minimal or no antimicrobial effects at concentration lesser than 100 µl. This indicated that amongst the isolates tested, *E. coli* recorded the highest zone of inhibition across all solvents used while *K. pneumoniae* recorded the lowest. The results in this current study is closer to the result obtained by Anitha *et al*. (2014) where he used three different extraction solvents which are acetone, chloroform and aqueous on *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* at different concentrations using the leaf extract of *D. metel*.

The seed extracts are believed to have a broad range antibacterial and antifungal activities against both Gram positive and Gram negative bacteria, as well as yeast, based on the results of MIC and zone of inhibition values and their comparison to those of mainstream antibiotics. Antibacterial and antifungal activities increased with increasing concentrations of all extracts in all microorganisms tested, as evidenced by the results. The MIC (Minimum Inhibitory Concentration) of the extract with no apparent turbidity for the concentrations was reported at 25 mg/ml for all bacterial isolates against the solvents, however the MIC for *C. albicans* was observed at 50 mg/ml for chloroform and n-hexane extracts. The MLC of *D. metel* extracts indicated that there was no observable growth at concentrations ranging from 50 to 100 mg/ml for both crude (ethanol) and aqueous solvents, however all isolates demonstrated observable growth at 50 mg/ml for chloroform and n-hexane extracts.

The secondary metabolites produced by plants have been the recent point of focus in this research due to their effectiveness against resistant microorganisms. The study also showed that the seed contains phytochemicals such as saponin, steroids, coumarin, flavonoids, glycosides, terpenoids, and alkaloids. The mean values ± SD of the quantitative

phytochemical screening of *D. metel* indicated that flavonoids had the highest value with 121.03 mg/100g while alkaloids had the lowest value with 0.27 mg/100g. The antibacterial properties of seed extracts may be due to the presence of a high quantity of flavonoids compared to other phytochemicals.

The compounds identified from the ethanolic extract of *D. metel* by matching the spectra with the NIST library data base were hydroxyl ethyl vinyl sulfide, dimethyldiaziridine, ethane diazo, flourooctane, urethane, propanenitrile. It was observed that the flourooctane showed the highest peak at the fifth minute while urethane showed the lowest at the sixth minute. The antibacterial activity of the ethanolic extract of *D. metel* was thought to be related to the presence of the bioactive component 2 hydroxyethyl vinyl sulfide in the seed extract. This study thus showed that *D. metel* showed significant antimicrobial efficacies against the selected clinical isolates and also possess bioactive compounds which can be used in the treatment of certain infections.

Conclusion

The ethalonic extract demonstrated the highest degree of activity against the chosen isolates in this research of the antibacterial efficacies of several extractants of *D. metel* on selected clinical isolates. The high quantity of flavonoids contained in the plant seed extract indicated that the seed might be a strong source of antioxidants, according to the phytochemical data. It's also possible that the seed extract of *D. metel* contains bioactive components that might be useful in the treatment of some illnesses.

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