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Assessment of Environmental Factors on Secondary Metabolites and Toxicological Effects of *Datura metel* Leaves Extracts

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Abstract

This study evaluated the chemical composition, physicochemical properties, toxicological and environmental potentials of the *Datura metel (D. metel) leaves* extracts. Fresh leaves of *D. metel* were harvested at three different times of the day (morning, afternoon and evening), extraction done in succession using n-hexane and ethanol solvents respectively. Preliminary phytochemical screening, physicochemical analysis for specific gravity, pH, colour, total ash and moisture contents of the dried extracts were determined. The characterisation of the bioactive compounds using Gas Chromatography-Mass Spectrometry, showed the presence of different compounds at the three different times of the day, out of which phytol and 9,12,15-octadecatrienoic acid were present at all times. These results confirmed that some bioactive compounds present in this plant are dependent on environmental factor , time of harvest and the choice of solvent used while the presence of others are independent on these factors. Each extract was administered orally to Wistar rats for two weeks. There were significant difference ($p < 0.05$) in activities of AST (serum and heart), ALT (liver and serum) and ALP (Serum, liver and kidney), as well as the concentrations of albumin and protein in the liver and serum and urea in the serum of experimental rats given n-hexane and ethanolic extracts of *D. metel*. Generally it also suggest that the leaf extracts collected at three different times of the day had some significant toxicological effect, thus may not be totally safe for consumption at the dosage indicated since tissues membranes integrity of the Wistar rats were not potentially preserved.

Keywords: *Datura metel*; GC-MS; Toxicological studies;, Phytochemical; Photochemical reactions.

1.0 Introduction

Medicinal plants are plants that are used for the treatment of several diseases due to their pharmacological effects in human body. They possess bioactive compounds which are useful for healing numerous human diseases (Nostro *et al*., 2000). It was reported that over 50 % of all modern drugs are of natural products (Bharathi *et al*., 2010).

Datura metel with common name Devil's trumpet, thorn-apple and metel is a Nigerian indigenous medicinal plant that belongs to the family *Solanaceae*. It is widely used in phytomedicine to treat disease conditions such as gonorrhea, menstrual pain, insanity and insomnia. It is known for several activities such as anti-bacterial against burn-causing pathogens (Gnanamani *et al*., 2003), antifungal against phytopathogens (Kagale *et al*., 2004; Lal and Dhiman, 2011), anti-stress, analgesic, anticancer, and free radical scavenging activity (Satyavati *et al*., 1977; Duke and Ayensu, 1984; Alabri *et al.,* 2014). The *D. metel* leaves' crude extracts that were used for the treatment of various human aliments, possess antibacterial and antifungal activity which, also justify its use in the traditional medicine. In the flow of nutritional pyramid, animals obtained their basic food materials from plants and its derivatives. Higher plants employed a type of feeding system called autotrophic nutrition. This usually involves the synthesis of organic compounds from inorganic raw materials. Autotrophic organisms synthesizes organic molecules from inorganic material by photochemical processes which occurs in two folds via: (1) Photosynthesis, method employed by all green plants; this involves the synthesis of organic compound primarily sugars, from carbon dioxide and water using sunlight as the source of energy and chlorophyll (other related pigment) for trapping the solar energy. (2) Chemosynthesis, here the synthesis of organic compounds from carbon dioxide and water is driven by special methods of respiration involving the oxidation of diverse inorganic matter such as iron, ammonia and hydrogen sulphide; also used by certain bacteria (Robert, 1976).

The various chemical and photochemical reactions taking place in atmosphere typically depend upon temperature, composition, humidity and intensity of sunlight. Photochemical reactions take place in the atmosphere by the absorption of solar radiation in the ultraviolet (UV) region. Absorption of photons by chemical species gives rise to electronically excited molecules, which can bring about certain reactions which are not possible under normal laboratory conditions perhaps at higher temperatures and in the presence of chemical catalysts. The electronically excited molecules produced by the absorption of a photon may undergo any of the following changes: (a) reaction with other molecules on collision; (b) polymerization; (c) internal rearrangement; (d) dissociation and (e) de-excitation by fluorescence or de-activation by returning to the original state. Any of the first four changes mentioned above may serve as initiating chemical stage or a primary process. The overall photochemical reaction occurs in three steps, namely: (1) Absorption of radiation,(initiating phase) (2) Primary reactions (propagating phase), (3) Secondary reactions(terminating phase).(Dara and Mishra, 2010).

All parts of the plant contain varieties of phytochemicals that serve health benefit for humans. The phytochemicals that have been found in *D. metel* include quinine, phylobatannins, alkaloids, steroids, saponins, terpenoids, flavonoids, glycosides and tannins. These phytoconstituents were obtained from various parts of the plant, like the leaf (Donatus and Ephraim, 2009; Kutama *et al*., 2010), the root (Jamdhadel, 2010) and the shoot (Arshad, 2008; Akharaiyi, 2011; John De Britto and Herin, 2011).

In this research, we carried out successive extraction of the leaves of the plant harvested at three different times of the day and investigated the effect of sunlight on the formation of the phytochemical constituents stored in the leaves. We also evaluated the toxicological potential on Wistar rats.

2.0 Materials and Methods

2.1 Chemicals and Reagents

The assay kits for urea, albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were products of RANDOX Laboratory Limited, Cumlin, UK. All other reagents used were of analytical grade and supplied by Sigma-Aldrich Inc., St. Louis, USA.

2.2 Plant Material

Fresh *D. metel* leaves were harvested in Bubu area, Tanke, Ilorin, Nigeria at three different times of the day: morning (6:00 a.m.); afternoon (1:00 p.m.), and evening (6:00 p.m.). The plant identification was done at the Herbarium of the Department of Plant Biology, University of Ilorin with plant voucher specimen number, UILH/001/845 assigned.

2.2\.1 Preparation of Plants

The harvested leaf samples were washed with water, air-dried under shade for six days until a constant weight was recorded. The dried leaves were ground into powdery form and kept in an air tight plastic container for further analysis.

2.2.2 Plant Extraction

The extraction process was carried out successively using non-polar (n-hexane) and polar (ethanol) solvents successively. 250 g of the powdered samples were extracted cold in 700 mL n-hexane for 72 hours, decanted and then filtered using Whatman No. 1 filter paper (Sringfield, Maidstone, Kent and England). The residues were dried and 700 mL ethanol was added to it and then left for another 72 hours, after which it was also decanted and filtered. Both the n-hexane and ethanol extracts were concentrated using the rotary evaporator (Buchi Rotavapor R110 Laboratories-Technik Ag. CH-9230 FLAWIL/SCHWE12, made in Switzerland) at 40 ^{o}C and kept at a temperature of – 4 ^{o}C until it is required for further analysis.

2.3 Preliminary qualitative test to identify the constituents of *D. metel leaves crude extract*

The crude extracts of the plant were subjected to preliminary qualitative phytochemical screening to identify the presence of secondary metabolites, such as steroids, quinines, alkaloids, phlobatannins, flavonoids, saponins, terpenoids, glycosides and tannins, following standard procedures as presented in table 2.

2.4 Physicochemical Analysis

The physicochemical analysis was carried out to determine the physical (specific gravity, colour and pH) and chemical (total ash content and moisture content) characteristics following standard procedures (A.O.A.C, 1990).

2.5 GC-MS Analysis

The leave extracts of *D. metel* was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) Agilent 7890 A in order to identify the bioactive components present in the extracts. A microsyringe was used to inject 1 µL of n-hexane and ethanol extracts into the GC-MS at 250 ^{o}C for a period of approximately 45 mins. Compounds were separated in HP5MS column

fused with phenylmethylsiloxane, with length 30 m x 250 µm and film thickness was 0.25 µm. The carrier gas was helium gas operated at a flow rate of 1 mL per minute. The compounds were separated in the GC and detected using GC-FID detector and each signal further subjected to mass spectrometry for fragmentation of each compound (Mohammed and Imad, 2013; Kareem *et al*., 2015).

2.6 Experimental Animals

Twenty one (21) healthy adult male Wistar rats weighing 165.5 g were sourced from the Animal House in Biochemistry Department of the University of Ilorin, Ilorin - Nigeria. The experimental rats were housed in well ventilated plastic encloses with fine sawdust as beddings and fed on standard rodent feeds and allowed access to water *ad libitum*. The animals were acclimatized for two weeks (Weeks 1 and 2) before being subjected to the experiment.

2.6.1 Animal Grouping and Extracts Administration

All rats were maintained under standard laboratory conditions (12-h light/dark cycle, 25 ± 2 ºC). The animals were then randomly distributed into 7 groups, each with three rats. The dried extracts were reconstituted in double distilled water which served as vehicle of oral administration. 1 ml of each extract which is an equivalent of 200 mg/kg body weight was administered orally once daily, for two weeks (weeks 3 and 4). Group A is the control and Groups B-G were test groups as shows in table 1. An appropriate ethical clearance had been obtained.

2.7 Collection of Blood Samples and Preparation of Serum

After two weeks of administration of the two extracts, the animals were fasted overnight after the last treatment before they are anaesthetized with di-ethyl ether by inhalation in an improvised incinerator, after which they were sacrificed and blood samples were collected by cardiac puncture into sterile plain tubes for analysis. Blood samples were allowed to stand for 30 minutes, after which it was centrifuged at 1000 g for 10 minutes. The collected serum as supernatant after centrifugation were appropriately labeled and stored in a freezer at -4 ºC until required for analysis.

2.8 Isolation and Homogenization of Tissues

After sacrifice, the rats were dissected in order to isolate tissues of interest (liver, heart and kidney). The isolated tissues were cleaned with cotton wool to remove blood stains, 1 g weighed and immediately stored in ice cold 0.25 M sucrose solution. The isolated organs were subjected to homogenization in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were stored in the freezer at -4 ºC before being used for analysis.

2.9 Biochemical Parameters

2.9.1 Total Protein Determination

The protein content of the serum and homogenates was determined using the Biuret method as described by Gornal *et al*., 1949 and modified by Sulaiman and Adeyemi (2010).

2.9.2 Determination of other Biochemical Parameters

The concentrations of urea, creatinine and albumin (ALB), as well as the enzyme activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were done according to the methods described in the RANDOX Laboratory assay kits.

2.10 Statistical Analysis

All data were expressed as the mean of 3 replicates ± standard error of mean (S.E.M). Statistical evaluation of data was performed by Graph pad prism version 5.02 using one-way analysis of variance (ANOVA). Post-Hoc test analysis was done using the Turkey's Multiple Comparison Test. Values were considered statistically significant at $p < 0.05$, confidence level of 95 %

3.0 Results

Three crude extracts of *D. metel* were analysed for the presence of secondary metabolites, nine phytochemicals were tested for, and six were detected (table 2). Table 3 displayed phytochemical screening and environmental effects of six *D. metel* n-hexane and ethanolic extracts representing morning (6:00 a.m); afternoon (1:00 p.m.), and evening (6:00 p.m.) results. Six compounds were detected,; five appeared in both n-hexane and ethanolic extracts (terpenoids, glycosides, steroids, flavonoids, and saponins) while one detected only in ethanolic extracts (alkanoids).

Physical characteristics of the crude leaves extracts of *D metel* showed that all the plants are green in colour, less dense than water and acidic in nature irrespective of the time of harvests (table 4a). The chemical characteristics of the dried leaves of *D metel* showed that it contains high total ash contents (>70 %) and low moisture contents (<10 %) (table 4b).Table 5 displayed the results of GC-MS characterization and

Table 1 Grouping of Experimental Animals

Groups	Extracts Administered	Route/Frequency	Duration (weeks)
A	Distilled water, control (CTR) 1ml	Oral, Once daily	2
B	*Datura,*Ethanol, Morning (DEM)200 mg/kg	Oral, Once daily	2
C	*Datura,*n-hexane,Morning, (DHM) 200 mg/kg	Oral, Once daily	2
D	*Datura,*Ethanol, Afternoon, (DEA) 200 mg/kg	Oral, Once daily	2
E	*Datura*,n-hexane,Afternoon, (DHA) 200 mg/kg	Oral, Once daily	2
F	*Datura,* Ethanol, Evening, (DEE)200 mg/kg	Oral, Once daily	2
G	*Datura,*n-hexane Evening, (DHE) 200 mg/kg	Oral, Once daily	2

(A): Control; (B): Morning Ethanol D. metel; (C): Morning n-hexane D. metel; (D): Afternoon Ethanol D. metel; (E): Afternoon N-hexane D. metel; (F): Evening Ethanol D. metel; (G): Evening N-hexane D. metel

environmental variation of n-hexane(table 5a) and ethanolic (table 5b) extracts of *D metel* harvested at three different times of the day. Thirty-two (n-hexane extract) and Twenty –four (ethanolic extract) compounds were identified with differing numbers in the two different extracts and at different times of the day. Phytol, 9, 12, 15-octadecatrienoic acid, and Alpha-tocopherol, were compounds detected with abundance percentage greater than 20.

Tables 6 – 12 presented the biochemical results: table 6 displayed the effects of the 6 extracts on the organ/body weight ratio of the male Wistar rats. Values were expressed as mean of three replicates ± S.E.M and those with different superscripts alphabets down the groups are significantly different at ($p < 0.05$) while those with the same superscripts alphabets are not significantly different.

Effect of the six extracts on the activities of aspartate transaminase in the serum and heart of the Wistar rats. There was a significant difference ($p < 0.5$) among the groups C –G compared to group A (control) in the serum while groups B, E and F showed a significant difference compared to Group A in the heart of the Wistar rats, (table 7).

Table 8 presented the effects of the six extracts on alanine transaminase activities in the serum and liver of the Wistar rats. There was a significant difference ($p < 0.5$) among the groups B – G compared to group A (control) in the serum while groups B - F showed significant difference compared to Group A in the liver. Alkaline phosphatase activities in the serum, liver and kidney of the Wistar rats were presented in table 9. There was a significant difference ($p < 0.5$) among the groups B–G compared to group A (control) in the serum while groups D and G showed the significant difference compared to Group A in the liver and B, E, and F in the kidney compared to Group A of the Wistar rats.Table 10 presented results for total protein concentrations in the serum and liver of the Wistar rats. Groups B-G showed significant difference in the serum while groups B, C, D, E and G are significantly difference in the liver. Table 11 showed the effects of the six extracts on the urea concentrations in serum of the Wistar rats. There was a significant difference among groups B-G when compared to group A. Table 12 showed the effects of the six extracts on the albumin concentrations in the serum and liver of the Wistar rats. There was a significant difference among the groups B-F compared to A in the serum while groups B, C, F, and G were significantly difference from group A in the liver of the Wistar rats compared to group A.

4.0 Discussion

4.1 Qualitative Phytochemical Screening of Datura metel leave extracts

The phytochemical screening was conducted on six different crude extracts; three of n-hexane extracts and three of ethanolic extracts (morning, afternoon and evening) respectively. The tests revealed that the phytochemicals present in the n-hexane of both morning and afternoon are the same (5 constituents-Terpenoides, glycosides, steroids, flavonoids and saponins), likewise for ethanolic extracts of both morning and afternoon (4 constituents-Glycosides, alkaloids, flavonoids and saponins).

Table 2 Secondary metabolite components of *D. metel*

S/No.	Secondary metabolites / Test	Observation	Inference
1	Terpenoids (Lal and Dhiman, 2011)	Formation of a bluish green precipitate	Detected
2	Glycosides (Asquith,1986)	Lack of formation of rose-pink color in the ammoniacal layer	Detected
3	Steroids (Barile *et al*., 2007)	Formation of brown ring at the junction	Detected
4	Alkaloids (Kokate, 2000)	Formation of a red precipitate	Detected
5	Flavonoids (Harbone,2005)	Formation of dark yellow colour in NaOH, became colorless in diluted acid	Detected
6	Saponins (Lal and Dhiman, 2011)	Formation of emulsion(stable persistent frothy)	Detected
7	Tannins (Hertog, 1993)	Absence of brownish green or blue-black color	Not Detected
8	Phlobatannins (Kokate, 2000)	Lack of formation of red colored precipitate	Not detected
9	Quinines (Alabri *et al*., 2014)	Lack of formation of red color	Not detected

Table 3 Phytochemical screening and Environmental effects *D. metel* n-hexane and ethanolic extracts in the Morning, Afternoon and Evening

S/No.	Secondary metabolites	Morning		Afternoon		Evening	
		n-hexane	Ethanol	n-hexane	Ethanol	n-hexane	Ethanol
1	Terpenoids	+	-	+	-	-	+
2	Glycosides	+	+	+	+	+	+
3	Steroids	+	-	+	-	+	+
4	Alkaloids	-	+	-	+	-	+
5	Flavonoids	+	+	+	+	+	+
6	Saponins	+	+	+	+	+	+
7	Tannins	-	-	-	-	-	-
8	Phlobatannins	-	-	-	-	-	-
9	Quinines	-	-	-	-	-	-

Note: + = present; - = absent

Table 4a Physical characteristics of crude extracts of *D. metel* harvested in the Morning, Afternoon and Evening

S/No.	Chemical constituents	Morning		Afternoon		Evening	
		n-hexane	Ethanol	n-hexane	Ethanol	n-hexane	Ethanol
1	Specific gravity g/cm^2	0.6531	0.7980	0.6926	0.8022	0.7527	0.7059
2	Color	Green	Green	Green	Green	Green	Green
3	pH	5.805	5.029	5.366	4.933	5.951	4.848

Table 4b Chemical characteristics of dried leaves of *D. metel*

S/No.	Chemical constituents	Morning (%)	Afternoon (%)	Evening (%)
1	Total ash contents	73	72	72
2	Moisture contents	8.09	8.02	8.02

The phytochemical compositions in the evening samples of n-hexane (4 constituents- steroids, glycosides, flavonoids, saponins) and ethanolic (6 constituents- terpenoid, steroids, glycosides, alkanoids, flavonoids, saponins) extracts contains differing numbers of secondary metabolites as noted in the morning and afternoon samples. Comparing all the n-hexane and all the ethanolic extracts; glycosides, flavonoids and saponins are present at three different times of the day, but all constituents (terpenoids, glycosides, alkaloids, flavonoids, steroids and saponnins) are present in the evening ethanolic extract while tannins, phlobatannins and quinines) are absent in all the groups. Extracts of the plants harvested in the evening were slightly different due to the absence of terpenoids and alkaloids in the n-hexane extract and the presence of terpenoids and alkaloids in the ethanolic extract of *D. metel* (Table 3). Five secondary metabolites have been identified as present in either n-hexane or ethanolic extracts depending on the time of harvests. However, alkaloids appeared only in ethanolic extracts. This results is almost similar to the work presented by Sakthi *et al*., (2011) though they used ethanol and acethyl acetate as medium of extraction and identified tannins instead of saponnin detected from this work.

These six identified secondary metabolites have health benefits as reported by different researchers (Beltowski *et al*., 1998; Olaleye, 2007; Gachande and Khillare, 2013). Plants are rich in wide varieties of secondary metabolites such as tannis, tepenoids, alkaloids and flavonoids which have been found in vitro to have antimicrobial properties (Cowan, 1999). Flavonoids and tannins belong to phenolic compounds in plants with primary antioxidants or free radical scavengers. These identified compounds are said to exhibit antimicrobial properties against B.subtilis, E.coli, S.aureus, P.vulgaris and S.typhi (Gachande and Khillare, 2013). These compounds may be responsible for the potent antioxidant ascribed to D.metel. Saponnins have antihypertensive and cardioprotective properties (Olaleye, 2007). Glycosides are innate cardioactive remedy used in the treatment of congestive heart failure and cardiac rhythm disorder (Brian, *et al*., 1985). Together the presence of saponnins and glycosides in the leaf of *D.metel* extracts might play a significant role in the cardioprotective potential.

Glycosides are innate cardioactive remedy used in the treatment of congestive heart failure and cardiac rhythm disorder (Brian, *et al*., 1985). Together the presence of saponnins and glycosides in the leaf of *D.metel* extracts might play a significant role in the cardioprotective potential. On the other hands, these glycosides may be toxic as it inhibits active transport of Na^+ in cardiac muscle, leading to inhibition of translocases during electron transport chain and interfere with cellular respiration and resulting to death (Beltowski *et al*., 1998).

4.2 The Physicochemical properties of *D. metel* dried leaf and crude extracts

The physical analysis revealed that the colour of the six extracts was green, pH data recorded were 5.805, 5.366 and 5.951 for the n-hexane extracts while 5.029, 4.933 and 4.848 were recorded for the ethanolic extracts of *D. metel* harvested in the morning, afternoon and evening respectively. There were no significant difference in the mean data obtained, indicating that all the extracts were acidic though with ethanolic extracts being slightly more acidic. The specific gravity data; 0.6531, 0.6926, 0.7527 (g/cm^2) for the n-hexane extracts and 0.7980, 0.8022, 0.7059 (g/cm^2) for the ethanolic extracts of *D. metel* harvested in the morning, afternoon and evening respectively revealed that all extracts dissolved, they were diluted and are less dense than water (Table 4a).

The chemical analysis data showed that there was no significant difference in the ash (74, 72, 72) and moisture contents (8.04, 8.02, 8.02) of *D. metel* harvested at the three different times of the day, as they all have high ash contents and low moisture contents (Sulaiman *et al*., 2014) (Table 4b).

4.3 GC-MS characterization of Leaves of D.metel in n-hexane and ethanolic extracts

The GC-MS characterization results of D. *metel* n-hexane extracts revealed the presence of 17, 12 and 14 bioactive compounds in the morning, afternoon and evening respectively. The most abundant were 9, 12, 15-octadecatrienoic acid at all times of harvesting: morning (29.42 %), afternoon (41.14 %) and evening (30.94 %) respectively.

Followed by phytol in the morning (18.28 %), afternoon (22.14 %) and evening (16.47 %) accordingly, followed by n-Hexadecanoic acid which was detected only in the morning (18.70 %) and afternoon (18.96 %) respectively, (Table 5a).

The GC-MS characterization results of D. *metel* ethanolic extract revealed the presence of 17, 10 and 13 bioactive compounds in the morning, afternoon and evening respectably. There were five persistent bioactive compounds detected in ethanolic extract: Phenol,2,4-bis(1,1-dimethylethyl (1.33 %, 3.62 % and 0.78 %); phytol (34.42, 52 % and 22.46 %); 9,12,15-octadecatrienoic acid ([alpha-linolenic acid, n-3 fatty acids] 9.72 %, 3.64 % and 13.23 %) ; scopolamine (5.27 %, 15.96 % and 10.87 %) and

atropine (3.49 %, 11.65 % and 9.91 %) respectively. Abundant compounds (at > 11 %) are phytol 34.42 %, 52.00 %, 22.46 % (morning, afternoon and evening) respectively, alpha tocopherol 20.83 %,

Table 5a GC-MS characterization of *D.metel* n-hexane extracts in the Morning (M) , Afternoon (A) and Evening (E) times of the day

S/No.	RT(min)	Compound name	Molecular formula	n-hexane (% Abundance)		
				M	A	E
1	27.820	3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one	$C_{14}H_{20}O_2$		1.30	
2	27.836	Trans-β-lonone	$C_{10}H_{18}$	1.47		
3	28.920	2(4H)-Benzofuranone,5,6,7,7-tetrahydro-4,4,7 Trimethyl	$C_{14}H_{22}O$			3.80
4	28.873	2(4H)-Benzofuranone,5,6,7,7-tetrahydro-4,4,7 Trimethyl	$C_{11}H_{16}O_2$		3.20	
5	28.943	Phenol,2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	6.04		
6	35.887	Propiolicacid,3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)	$C_{13}H_{20}O_3$	1.02		
7	36.461	Tetradecanoic acid	$C_{14}H_{28}O_2$			1.93
8	37.436	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	1.43	1.26	1.53
10	37.510	2-pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}O$	1.79		
11	38.385	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$			1.66
12	38.959	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$			18.25
13	39.786*	Phytol	$C_{20}H_{40}O$	18.28	22.14	16.471.18
14	39.697*	9,12,15-octadecatrienoic acid	$C_{18}H_{30}O_2$	29.42	41.14**	30.94
15	38.959	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	18.70	18.96	
16	40.140*	Octadecanoic acid	$C_{17}H_{35}COOH$	6.55**	4.41	7.23
17	40.263	1-heptatriacotanol	$C_{37}H_{76}O$	0.97		
18	40.520	Tert-hexadecanethiol	$C_{16}H_{34}S$	1.25		
19	40.640	1,3,12-Nonadecatriene-5, 14-diol	$C_{19}H_{34}O_2$			3.15
20	40.601	5-Hydroxy-2,3,3-trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone	$C_{20}H_{28}O_5$		1.81	
21	40.624*	Benzeneacetic acid	$C_{22}H_{30}O_5$	2.09	1.84	
22	40.703	Oxiraneoctanoic acid, 3-octyl-,methyl ester, cis	$C_{19}H_{36}O_3$			2.59
23	40.990	7-methyl-z*-tetradecen-1-oacetate	$C_{17}H_{32}O_2$	0.93		
24	41.095	Ethyl iso-allocholate	$C_{26}H_{44}O_4$			2.28
25	41.740*	17-pentatriacontene	$C_{35}H_{17}$	2.02	1.44	4.33
26	41.280*	Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	3.32		1.93
27	42.093	Hexa-t-butyl selenatrisiletane	$C_{14}H_{54}SeSi_3$		1.34	
28	43.311	Tricyclo[20.8.0.0(7,16)triacontane,1(22),7(16)-diepoxy	$C_{30}H_{52}O_2$	1.6		
29	44.740*	Squalene	$C_{30}H_{50}$	3.05		3.89
30	44.720	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11.15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$		3.0	

** Average value; ** Addition of % of compound that appeared more than once in the same time of harvest*

24.70 % (morning and evening) and scopolamine 15.96 % and atropine 11.65 % (afternoon). Depending on the compound of interest, the best time to harvest D metel for phytol is afternoon; 9,12,15- octadecatrienoic acid is evening; scopolamine is afternoon and atropine is afternoon (Table 5b). This showed that the best time of harvest is that time of the day when the composition of the compound of interest is in abundance.

As presented in tables 5a & 5b; the GC –MS characterization for the extracts obtained from *D. metel* harvested at the three different atmospheric conditions: morning, afternoon and evening showed potential difference in effect of temperature and sunlight energy as well as humidity on the formation of bioactive compounds in the leaves. The explanation for this observation is that light and temperature fluctuate considerably both in the course of a day and from one season to another (Robert, 1996). During the day, light and temperature are usually well above their minimum value for plants living in the open but carbon dioxide is the limiting factor for photosynthesis. In the cool of the early morning or evening, light or temperature may become a limiting factor and thus significantly influence the rate of photosynthesis. The presence of Diisootyl and Di-n-octyl phthalates (1.82 and 32.14 %) in the morning and evening respectively of n-hexane *D. metel* extract

implies that the soil is highly polluted with synthetic plasticizer and for the concentration to have been as high as 32.14 % (table 5a) recorded, it

indicates that this plant can effectively bio remediate this compound successfully. The evening results of GC-MS showing total percentage abundance of 100 % (table 5a) confirmed that the compound was detected and that it is totally soluble in n-hexane, hence not available into ethanolic extract.

The availability of linoleic acid (18:2) at 3.64 % only in ethanolic extract of morning implies that the *D. metel* in ethanolic extracts (table 5b) will be a rich source of polyunsaturated fatty acids (PUFA) in that linoleic and linolenic acids are the two most essential fatty acids required in the diet. Upon ingestion, linoleic acid undergo elongation and desaturation reactions

to produce arachidonic acid (20:4) needed for maintenance of vascular membrane integrity, antiplatelets activity, anti-inflammatory and analgesic effect, cell body defense, growth and learning processing. However, if present in high concentration, may enhance lipid peroxidation in a favorable condition with subsequent generation of reactive oxygen/nitrogen intermediates (Harvey and Ferrier, 2011). The availability of linolenic acid (18:3), 9, 12, 15-octadecatrienoic acid in

Table 5b GC-MS characterization of *D.metel* ethanolic extract in the Morning , Afternoon and Evening times of the day

S/No.	RT(min)	Compound name	Molecular formula	Ethanolic		
				M	A	E
1	22.439	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	1.80		1.65
2	28.93*	Phenol,2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	1.33	3.62	0.778
3	30.515	Cyclopenta[1,3]cyclopropa[1,2]cyclohepten-3(3aH)-one,1,2,3b,6,7,8-hexahydro-6,6-dimethyl	$C_{13}H_{18}O$	0.88		
4	37.938	5H-cyclopropa[3,4]benz[1,2-]azulen-5-one,9,9a-bis(acetyloxy)-1,1a,1b,2,4a,7a, 7b,8,9,9a-decahydro-2,4a,7b trihydroxyl 1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4a,7b trihydroxyl	$C_{20}H_{28}O_5$		1.81	
5	38.032	5-Hydroxy-6-methyl-12,13-dioxa-tricylo[7,3,1,0(1,6)] tridecane-8-carboxylic acid, methyl ester	$C_{14}H_{22}O_5$		4.98	
6	38.810	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$		4.98	
7	39.715*	Phytol	$C_{20}H_{40}O$	34.42**	52.00**	22.46**
8	41.64*	9,12,15-octadecatrienoic acid	$C_{18}H_{30}O_2$	9.72**	3.64	13.23**
9	40.20	Tetracose-2,6,14,18,22-pentaene-10,11-diol,2,6,10,15,19,23-hexamethyl			2.74	
10	38.11	8-Azabicyclo(3.2.1)octan-3-ol,6-methoxy-8-methyl	$C_9H_{17}NO_2$	1.99		1.81
11	39.148	Ethyl iso-allocholate		0.91		
12	38.896*	n-hexadecanoic acid	$C_{16}H_{32}O_2$	4.51		6.14**
13	41.12	Hexadecanoic acid,1-(hydroxymethyl)-1,2-etanediyl ester	$C_{35}H_{68}O_5$	3.79		4.34
14	40.090	Octadecanoicacid,2-hydroxy-1,3-propanediyl ester	$C_{18}H_{30}O_2$	0.88		1.94
15	40.87*	Scopolamine	$C_{17}H_{23}NO_4$	5.27	15.96**	10.87**
16	40.216	1,9-Diooxacyclohexadeca-4,13-diene-2-10-dione,7,8,15,16-teramethyl	$C_{18}H_{28}O_4$	1.33		
17	40.497*	Atropine	$C_{17}H_{23}NO_3$	3.49	11.65**	9.91
18	40.585	Benzeneacetic acid	$C_{22}H_{30}O_5$	2.04		
19	41.292	17-Pentatriacontene	$C_{35}H_{70}$		1.48	
20	39.148	12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	$C_{14}H_{24}O_3$			0.79
21	41.708	7,8-Epoxylanostan-11-ol, 3-acetoxy	$C_{32}H_{35}O_4$			1.40
22	43.224	Trilinollein	$C_{57}H_{98}O$	1.33		
23	43.311	Linoleic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	3.64		
24	44.31*	Alpha Tocopherol	$C_{29}H_{50}O_2$	20.83		24.70**

**Average value; ** Addition of % of compound that appeared more than once in the same time of harvest*

Table 6 Effects of Ethanol and n-Hexane Extracts of *D. metel* leaves collected at three different times of the day on Organ /Body-Weight ratio

Group	Liver Organ's Weight	Organ: body weight ratio	Heart Organ's weight	Organ: body weight ratio	Kidney Organ's weight	Organ: body weight ratio
A	5.62± 0.62[a]	3.50 ± 0.52[a]	0.58± 0.06[a]	0.36± 0.05 [a]	1.15±0.09[a]	0.71 ± 0.09 [a]
B	5.56± 0.59[a]	3.47 ± 0.52[a]	0.58± 0.06[a]	0.38 ± 0.05[a]	1.1 ± 0.08[a]	0.70 ± 0.09[a]
C	5.49± 0.60[a]	3.44 ± 0.54[a]	0.58± 0.06[a]	0.36 ± 0.05[a]	1.1 ± 0.08[a]	0.70 ± 0.09[a]
D	5.47± 0.62[a]	3.46 ± 0.57[a]	0.58± 0.06[a]	0.36 ± 0.06[a]	1.1 ± 0.08[a]	0.71 ± 0.10[a]
E	5.44± 0.65[a]	3.46 ± 0.58[a]	0.59± 0.07[a]	0.37 ± 0.06[a]	1.1 ± 0.08[a]	0.72 ± 0.10[a]
F	5.50± 0.66[a]	3.48 ± 0.61[a]	0.60± 0.06[a]	0.38 ± 0.07[a]	1.1 ± 0.07[a]	0.73 ± 0.10[a]
G	5.48± 0.70[a]	3.44 ± 0.65[a]	0.60± 0.07[a]	0.38 ± 0.06[a]	1.1 ± 0.07[a]	0.73 ± 0.11[a]

*Values were expressed as mean of three replicates ± S.E.M and those with different superscript alphabets down the group are significantly different at ($p < 0.05$). (A): Control; (B): Morning Ethanol **D. metel**; (C): Morning N-hexane **D. metel;** (D): Afternoon Ethanol **D. metel**; (E): Afternoon N-hexane **D. metel**; (F): Evening Ethanol **D. metel**; (G): Evening N-hexane **D. metel.***

both the extracts at the three times of the day to as much as 41.14 % in the afternoon of n-hexane implies that *D. metal* is a rich source of omega 3 which account for up to 40 % of fatty acids contents of retinal photoreceptor membranes (William, 2007) and has other health benefits

Table 7 Effects of Ethanol and n-hexane extracts of *D. Metel* leaves collected at three different times of the day on Aspartate transaminase activity in Serum and Heart homogenate

Group	Aspartate Transaminase (AST, U/I)	
	Serum	Heart
A	99.28±0.81	110.44±20.39
B	100.87±12.62[a]	138.55±14.80[b]
C	81.88±02.28[b]	103.62±06.65[a]
D	46.09±06.53[c]	111.30±08.28[a]
E	43.19±15.64[c]	132.32±28.60[b]
F	41.89±18.45[c]	135.51±21.61[b]
G	28.70±10.04[d]	113.04±03.01[a]

[a] *Values were expressed as mean of three replicates ± S.E.M and those with different superscript alphabets down the group are significantly different at (p<0.05). (A): Control; (B): Morning Ethanol **D. metel**; (C): Morning N-hexane **D. metel;** (D): Afternoon Ethanol **D. metel**; (E): Afternoon N-hexane **D. metel**; (F): Evening Ethanol **D. metel**; (G): Evening N-hexane **D. metel***

Phytol, relatively constant in the extracts both n-hexane and ethanolic and at the three defined time of the day, though with varying percentage (tables 5a & 5b) Phytol is an acyclic diterpenes that serve as precursor for the production of synthetic vitamin E and vitamin K1. The phytol present only in the in both n-hexane and ethaolic extracts, reflects the natural phenomenon of transformation of products formed during the day under the influence of ultraviolet radiation (sunlight) absorbed by chlorophyll via photosynthesis and photochemical reactions. E-phytol is a product of rearrangement and cyclization of aliphatic phytol. Moreover, alpha tocopherol that is present only in the evening (24.70 %) and morning (20.83 %) ethanolic extracts perhaps is a product of successive sequential transformation of products formed at the day time (light phase) and utilization and storage in the cool as it occurs early in the morning or evenings (dark phase) (Robert,1976; Maeda and Dellapenna, 2007). Alpha-tocopherol with the high percentage abundance > 20 % both in the morning and evening, makes the leave of high-quality in providing antioxidant effect in lipid phase during cell body defense mechanism (Table 5b). Furthermore, the phytol and E-phytol have same molecular formula, $C_{20}H_{40}O$ with stereoisomerism.

In addition, the atropine and scopolamine may be a good source of cholinergic inhibitor, act on muscarinic receptors both in the central and peripheral nervous system with muscle relaxant properties and useful in organophosphate poisoning (Bania *et al.,* 2004) as well as anaesthesia since atropine has inhibitory effect on secretory cells. The classical findings in *D. metel* toxicity are dryness of the mouth due to decreased salivation, thirst, fever, flushing, urinary retention due to relaxation of detrusor muscle of the bladder wall, papillary dilation (myadriatic effect), tachycardia (due to unopposed action of sympathetic neuronal discharge), ataxia, hallucination, delirium resulting to coma, palpitation, cardiac and respiratory arrest (Barceloux, 2008). The presence of these compounds would make the extracts toxic at high concentrations. The results showed that the time of harvest and ecological factors affects the bioactive compounds present in the indigenous medicinal plant of *D. metel*.

Table 8 Effects of Ethanol and n-hexane extracts *of D. metel* leaves collected at three different times of the day on Alanine transaminase activity in Serum and Liver homogenate of Wistar rats

Group	Alanine Transaminase (ALT, U/I)	
	Serum	Liver
A	17.3±6.3[a]	86.6±2.8[a]
B	26.9±9.5[b]	91.5±6.4[b]
C	26.5±2.0[b]	90.7±3.2[b]
D	12.1±5.3[d]	91.3±3.3[b]
E	22.4±1.2[c]	94.6±1.0[b]
F	11.8±3.0[d]	94.6±2.7[b]
G	09.8±0.4[e]	81.7±2.9[a]

[a] *Values were expressed as mean of three replicates ± S.E.M and those with different superscript alphabets down the group are significantly different at (p<0.05). (A): Control; (B): Morning Ethanol **D. metel**; (C): Morning N-hexane **D. metel;** (D): Afternoon Ethanol **D. metel**; (E): Afternoon N-hexane **D. metel**; (F): Evening Ethanol **D. metel**; (G): Evening N-hexane **D. metel.***

Table 9 Effects of Ethanol and n-hexane extracts of *D. metel* leaves collected at three different times of the day on enzyme (alkaline phosphatase) activities in serum and homogenates of Kidney and Liver of the Wistar rats

Group	Alkaline phosphatase (ALP, U/I)		
	Serum	Liver	Kidney
A	6414.17 ± 132.40[a]	1435.17 ± 739.91[a]	694.43 ± 20.23[a]
B	7516.83 ± 178.00[b]	1207.90 ± 596.82[a]	433.47 ± 15.18[b]
C	3787.87 ± 572.10[c]	1393.10 ± 437.46[a]	614.47 ± 33.67[a]
D	4027.80 ± 29.16[c]	2129.63 ± 295.24[b]	694.43 ± 43.73[a]
E	5324.07 ± 176.70[d]	1136.37 ± 865.11[a]	770.17 ± 75.77[b]
F	4995.80 ± 831.26[c]	1292.07 ± 303.16[a]	711.27 ± 77.96[b]
G	4819.00 ± 229.62[c]	2289.57 ± 358.78[b]	627.10 ± 76.59[a]

*Values are expressed as mean of three replicates ± S.E.M and those with different superscript alphabets down the group are significantly different at (p<0.05). (A): Control; (B): Morning Ethanol **D. metel**; (C): Morning N-hexane **D. metel;** (D): Afternoon Ethanol **D. metel**; (E): Afternoon N-hexane **D. metel**; (F): Evening Ethanol **D. metel**; (G): Evening N-hexane **D. metel.***

4.4 Effects of Ethanol and n-hexane Extracts of *D. metel* collected at three different times of the day on Organ/ Body-Weight ratio

Table 6 showed that the effects of ethanolic and n-hexane extracts of *D. metel* collected at three different times of the day on Organ Body-Weight ratios revealed no significant difference (p>0.05) for heart, liver and kidney compared to control in all *D. metel* administered groups.Meanwhile, organ-body weight ratio is a useful marker of cellular swelling, atrophy or hypertrophy (Amresh *et al.,* 2008; Oladiji *et al*., 2009; Adeyemi *et al*., 2015).

4.5 Aspartate Transaminase (AST, E.C. 2.6.1.1) Activity (U/I) in the Serum and Heart of Wistar rats given Ethanol and n-hexane Extracts of *D. metel* collected at three different times of the day

Table 7 shows the AST Activity (U/I) in the serum of rats given n-hexane

and ethanolic extracts of *D. metel*. The AST activity in the serum of rats administered with n-hexane and ethanolic extracts of *D. metel* in Groups C - G (81.88, 46.09, 43.19, 41.89 and 28.70) were significantly ($p<0.05$) lower than the control group (99.28) while AST activity in group B (100.87) was increased in relation to the control group (99.28) shows significant difference ($p<0.05$)

Table 10 Effects of Ethanol and n-hexane extracts of *D. metel* leaves collected at three different times of the day on Serum and Liver total protein concentrations in Wistar rats

Group	Total protein(mg/ml)	
	Serum	Liver
A	10.8±4.0[a]	6.8±0.5[a]
B	9.0±2.2[b]	11.4±4.7[b]
C	14.4±4.9[c]	8.1±2.3[c]
D	4.7±0.7[d]	11.7±1.1[b]
E	4.9±1.6[d]	14.7±3.3[b]
F	8.9±0.5[b]	6.8±1.4[a]
G	7.7±3.1[b]	9.1±2.1[c]

*Values are expressed as mean of three replicates ± S.E.M and those with different superscript alphabets down the group are significantly different at ($p<0.05$). (A): Control; (B): Morning Ethanol **D. metel**; (C): Morning N-hexane **D. metel;** (D): Afternoon Ethanol **D. metel**; (E): Afternoon N-hexane **D. metel**; (F): Evening Ethanol **D. metel**; (G): Evening N-hexane **D. metel.***

The AST activity in the heart of rats administered with the two extracts in Groups C, D and G (103.62, 111.30 and 113.04) in relation to the control Group (110.44) shows no significant difference ($p>0.05$) (Table 14). However, the activity of AST increased significantly ($p<0.05$) in the heart of rats administered with n-hexane and Ethanol extracts of *D. metel* in Groups B, E and F (138.55, 132.32 and 135.51). The ALT and AST are 'markers' of liver damage and can thus be used to evaluate liver cytolysis (Pramyothin *et al.,* 2006; Sulaiman *et al*., 2014). The AST Activity in the serum and heart of rats administered with extracts in groups B, C, D and G (*Datura* Ethanol Morning, *Datura* N-hexane Morning, *Datura* Ethanol Afternoon and *Datura* N-hexane (Afternoon) in relation to the control group A shows no significant difference. The lack of significant difference shows that administration of the extracts has no adverse effect on the tissues parameters as reported by Sulaiman *et al*., 2016. However, the AST Activity significantly increased in the serum and low in the heart of rats administered with extracts in groups administered with n-hexane and Ethanol extracts of *D. metel* in groups B, E and F in relation to the control which suggests AST leakage from the heart into the serum, this depicts that the membrane of the tissue may have been compromised by the extracts, hence altered membrane permeability.

4.6 Alanine Aminotransferase Activity (ALT, E.C. 2.6.1.2) (U/I) in the Serum and Liver homogenate of Rats Administered Ethanol and n-hexane Extracts of *D. metel* collected at three different times of the day

Table 8 shows the ALT Activity (U/I) in the serum of experimental rats given n-hexane and ethanol extracts of *D. metel*. The ALT Activity in the serum of rats administered with extract in Group B (26.90) in relation to Group C (26.49) shows no significant difference ($p>0.05$). However, the ALT activity reduced significantly ($p<0.05$) in the serum of rats administered with extracts in Groups D, F and G (12.05, 11.79, 9.82) in relation to the control (17.32).

Similarly, the ALT Activity in the liver of rats administered with n-hexane and ethanol extracts of *D. metel.* The ALT activity in the liver of rats administered with the extracts in Group G (81.67) is not significantly different ($p<0.05$) from ALT activity recorded in the control Group A (86.55). However, the ALT activity was slightly higher ($p>0.05$) in the liver of rats given the two extracts in Groups B-F (91.49, 90.71. 91.25, 94.64, 94.58) in relation to the control (86.55).

The measurement of ALT levels in human serum has proved to be a valuable indicator of liver function in clinical settings (Huang *et al.,* 2006; Sulaiman *et al*., 2014). The ALT and AST are 'markers' of liver damage and can thus be used to measure liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Pramyothin *et al.,* 2006). The ALT activity in the serum of rats administered with extracts in Group B and C shows a higher enzyme activities, suggesting the possibility of enzyme leakage from the liver or other tissues into the serum and this may destabilize the liver's integrity while ALT activity was significantly reduced ($p<0.05$) in the serum of rats administered with extracts in groups D, F and G in relation to the control group A. There was no significance difference in the ALT activity in the liver of rats administered group G in relation to the control. The integrity of the liver was therefore preserved as against the slightly higher enzyme activities recorded in the other groups

Table 11 Effects of Ethanol and n – hexane extracts of *D. metel* leaves collected at three different times of the day on urea concentration in the serum of Wistar rats

Group	Urea concentration (g/dl) Serum
A	27.6±6.8[a]
B	20.6±4.1[b]
C	7.12±2.8[d]
D	18.2±2.6[b]
E	16.2±6.7[b]
F	9.6±3.9[c]
G	9.1±1.7[c]

*Values are expressed as mean of three replicates ± S.E.M and those with different superscript alphabets down the group are significantly different at ($p<0.05$). (A): Control; (B): Morning Ethanol **D. metel**; (C): Morning N-hexane **D. metel;** (D): Afternoon Ethanol **D. metel**; (E): Afternoon N-hexane **D. metel**; (F): Evening Ethanol **D. metel**; (G): Evening N-hexane **D. metel.***

4.7 Alkaline Phosphatase (ALP, U/I) Activities of the Serum and Selected Tissues of Rats Administered Ethanol and n-hexane Extracts of *D. metel* collected at three different times of the day

Table 9 showed the ALP Activity in the liver of rats administered with extracts in Groups B, C, E and F (1207,9, 1393.1, 1136.4 and 1292.1) revealed no significant difference ($p>0.05$) in relation to the control group A (1435) However, the ALP activity increased significantly ($p<0.05$) in the liver of rats given with the extracts in groups D (2129.6) and G (2289.6) in relation to the control (1435.2). Also, the ALP activity in the kidney (Table 8) of rats administered with extracts in groups C, D and G (614, 694, and 627) when compared with the control group A (694.4) shows no significant difference ($p>0.05$) (Table 8). However, the ALP activity was significantly

increased ($p<0.05$) in the kidney of rats administered with the two extracts in groups E (770.2) (Afternoon N-hexane ***D. metel***;) and F Evening Ethanol ***D. metel*** (711.3) ; while a significant reduction was recorded in rats in group B (433) Morning Ethanol in relation to the control (694.4).

ALP is present in all tissues throughout the body, but is particularly concentrated in the liver, bile duct, kidney, bone, intestinal mucosa and the placenta (Wolf, 1999). ALP is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1972) and is often used to assess the integrity of the plasma membrane and endoplasmic reticulum (Akanji *et al.,* 1993). The ALP activities in the liver and heart of rats administered with extracts in groups B, C, E and F show no significant difference. However, the increased ALP activity in the liver of rats administered with the extracts in groups D and G without a concomitant leakage into the serum may be due to increased synthesis of the enzyme.

Table 12 Effects of Ethanolic and n-hexane extracts of *D. metel* leaves collected at three different times of the day on albumin concentration in Wistar rats

Group	Albumin (g/dl)	
	Serum	Liver
A	0.16 ± 0.07^{a}	0.05 ± 0.01^{a}
B	0.19 ± 0.04^{b}	0.04 ± 0.02^{b}
C	0.25 ± 0.02^{c}	0.04 ± 0.01^{b}
D	0.19 ± 0.02^{b}	0.05 ± 0.01^{a}
E	0.13 ± 0.05^{d}	0.06 ± 0.02^{a}
F	0.04 ± 0.01^{e}	0.04 ± 0.01^{b}
G	0.17 ± 0.01^{a}	0.08 ± 0.03^{c}

*Values are expressed as mean of three replicates ± S.E.M and those with different superscript alphabets down the group are significantly different at ($p<0.05$). (A): Control; (B): Morning Ethanol **D. metel**; (C): Morning N-hexane **D. metel;** (D): Afternoon Ethanol **D. metel**; (E): Afternoon N-hexane **D. metel**; (F): Evening Ethanol **D. metel**; (G): Evening N-hexane **D. metel.***

4.8 Total Protein Concentration in the Serum and Liver of Rats Administered Ethanolic and n-hexane extracts of *D. metel* collected at three different times of the day

The protein concentration (mg/dl) in the liver and serum of rats given n-hexane and ethanolic extracts of *D. metel*.(Table 10). The protein concentration (mg/ml) in the serum of rats given the aqueous extracts in Groups B, D, E, F and G (9.0, 4.7, 4.9, 8.9 and 7.7) in relation to the control Group A (10.8) decrease significantly ($p<0.05$) while the protein concentration (mg/dl) in the serum of rats administered with extracts in Groups C (14.4) increase significantly ($p<0.05$) in relation to the control (10.8). However, the protein concentration (mg/dl) in the liver of rats administered with extracts in Groups B, D, E (11.4, 11.7 and 14.7) was significantly increased ($p<0.05$) when compared with the control (06.8). The concentration of total protein is a useful 'marker' of secretory, synthetic and excretory functioning of the liver (Yakubu *et al.,* 2009; Sulaiman *et al*., 2016).

4.9 Urea Concentration in the Serum of Rats Administered Ethanolic and n-hexane Extracts of *D. metel* collected at three different times of the day

Table 11 showed the urea concentration (g/dl) in the serum of rats administered with the two extracts in groups B-G (20.6, 7.2, 18.2, 16.2, 9.6 and 9.1) when compared with the control group A (27.6) were significantly reduced ($p < 0.05$). The significance of the enzyme urease includes: to serve as a virulence factor in human and animal infections of the urinary and gastrointestinal tracts, play role in recycling of nitrogenous wastes in the rumens of domestic livestock (Mobley, Island and Hausinger, 1995). However, in the serum of rats administered with extracts in Groups B-G,there is low urea concentration compared to control which shows no toxicity of the extracts

4.10 Albumin Concentration (g/dl) in the Serum and Liver of Rats Administered Ethanolic and n-hexane Extracts of *D. metel* collected at three different times of the day

Table 12, showed the albumin concentration (g/dl) in the serum of rats administered with n-hexane and ethanol extracts of *D. metel*. The albumin concentration (g/dl) in the serum of rats administered with extracts in Groups B, C, D, (0.19, 0.25, 0.19,) when compared with the control Group A (0.16) shows significant increased ($p<0.05$). However, the albumin concentration was significantly lower ($p<0.05$) in the serum of rats administered with extracts in Groups E and F (0.13, 0.04) when compared with the control (0.16). The albumin concentration (g/dl) in the liver of rats administered with the two extract in Group B, C and F (0.04, 0.04, 0.04) were significantly ($p<0.05$), lowered while in Group G, it is significantly ($p<0.05$), high (0.08) when compared with the control Group A (0.05).

Albumin is the major plasma protein. Molecular weight is approximately 66.3 kDa. It is synthesized exclusively in the liver by hepatocytes. Albumin is responsible for several physiological and biochemical processes in the body that tends to maintain homeostasis. Acute or chronic injury to liver cells alters the synthetic function including production of albumin. In acute and subacute liver disease, the change in hepatic function is usually transient due to high power of regeneration of cells via compensatory mechanism. However, a falling concentration in chronic liver disease suggests a clinically significant deterioration in liver function 'decompensation'. The concentration of albumin is a useful 'marker' of secretory, synthetic and excretory functioning of the liver and kidney (Yakubu *et al*., 2009).

This study shows that administration of *D. metel* n-hexane and ethanolic extracts modulates the concentration of albumin in the serum and liver by down-regulating and up-regulating the synthesis of albumin, therefore enhances availability of albumin in the plasma

5.0 Conclusion

In conclusion, the leaves of *D. metel* has been investigated in this study and confirmed to contain some bioactive compounds such as alkaloids, steroids, glycosides, flavonoids, terpenoids and saponins, which provides a promising baseline for the traditional medicinal property. There is possibility that *D. metel* plant extracts can serve as a potent source for the development of natural antibacterial, antimicrobial, anticholinergic, anti-inflammatory, antioxidant and anticancer agents as evident from GC-MS results. Thus, *D. metel* extracts may also be used to eliminate pathogenic organisms especially when collected in the evening and extracted with ethanol. Though, there is need to select harvesting time and extraction solvent based on the ailment of interest for treatment. These results confirmed that the bioactive compounds present in this plant are dependent on time of harvest and the choice of solvent used for extraction. Thus, from

this study, the best time to harvest is evening and choice of solvent that revealed all the six phytoconstituents was ethanol.

Conflict of Interest

Authors declare no conflict of interest.

Authors Contribution

Conception: MAOA

Design: MAOA and FAS

Execution: MAOA, FAS, OA, KOY, LOG, LAK, KBO, GOA, DA, and TO

Interpretation: MAOA, FAS, HAA, and AAM

Writing of the paper: MAOA, FAS, HAA, and AAM

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