NISEB Journal Vol. 18, No. 3. September, 2018 1595-6938/2018
Printed in Nigeria (2018) Society for Experimental Biology of Nigeria
http://www.ojs.klobexjournals.com/index.php/nisebj

Phytochemical and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Ethanol Leaf Extract of *Cordia millenii*

Ijeoma Jane Nnadozie, Omolola Olajide, Adeniyi Richard Adewole and Joy Okpuzor*
Department of Cell Biology and Genetics,
University of Lagos, Lagos. Nigeria

Abstract
Medicinal plants are known to possess phytochemical constituents which have been demonstrated by in-depth scientific research to be efficacious in the treatment and management of various diseases. The aim of the study was to identify and characterize the phytochemical and bioactive components present in the crude extract of Cordia millenii leaves. The air-dried leaves were pulverized and subjected to cold extraction for 72 hours. The filtrate was subsequently concentrated to yield an aqueous ethanolic crude extract. The crude extract was then subjected to both qualitative and quantitative phytochemical screening as well as Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The phytochemical screening of the crude extract of C. millenii revealed the presence of total flavonoid content (60.71 ± 0.16%) and phenolic content (53.91 ± 0.11%) as most abundant compounds. Also, the GC-MS analysis revealed different types of high and low molecular weight chemical entities with varying quantities present in the extract. These chemical compounds are considered biologically and pharmacologically important, most notable were phytol (98%), hexadecanoic acid (93%) and vitamin E (90%). The present study provides critical evidence that the extract possesses major bioactive compounds that were identified and characterized spectroscopically, some of which have been implicated in anti-oxidant and anti-inflammatory activities.

Keywords: Phytochemical analysis, GC-MS, *Cordia millenii*, anti-oxidant and anti-inflammatory activities

Introduction
Plants have been widely used to treat various ailments, since ancient times. Due to the accessibility, availability and affordability of medicinal plants, about 80% of the population of developing countries use medicinal plants and plant products in treating major medical problems [1]. One of the important medicinal values inherent in plants resides in the bioactive phytochemical constituents that produce definite physiological actions on the human and animal body [2]. Phytochemicals are non-nutritive compounds (secondary metabolites) found in plants. They work with nutrients and dietary fibre to protect against diseases and they contribute to flavour and colour found in plants [3]. They can be classified into sub-groups according to their chemical structure, which include terpenoids (e.g. carotenoids), phytosterols, polyphenols (e.g. tannins, flavonoids, phenolic acids) and glucosinolates [4]. Phytochemicals have proven to be beneficial to the body performing such functions including promoting the function of the immune system, acting as antibacterial or antiviral agents, reducing inflammation, prevention of cancer and cardiovascular diseases [5].
Cordia millenii is a plant that belongs to the Boraginaceae Family, commonly known as African *Cordia.* In Nigeria, its Igbo name is "okwe", "omo" in Yoruba and "waawankurmii" in Hausa [6]. Its origin and geographic distribution occurs from Sierra Leone, East to Western Kenya and Tanzania, south to DR Congo and Northern Angola. The tree is commonly planted in West Africa. In Nigeria, *Cordia millenii* seed powder mixed with palm oil is applied externally to ringworm and itching skin and is located mostly in the south eastern part of Nigeria especially Nsukka [7]. The leaf decoction of the plant is taken to dispel worms, treat asthma, cough and cold. The flowers provide nectar and pollen for honey bees [8]. The decoction of the bark is used as remedy for fever, general weakness of the body, stomach-ache and as gargles. Given these bioactivities and little or no information in literature on the therapeutic significance, the present study was designed to characterize the phytochemical profile of *Cordia millenii*.

Materials and Methods
Plant Sample
The leaves of *Cordia millenii* were purchased from a traditional herbal practitioner in Mushin Local Government of Lagos State and authenticated by Dr. A.B. Kadiri of the Department of Botany, University of Lagos. Voucher specimen (LUH7608) was deposited at the Herbarium for future reference.
Extraction of crude extract
The leaves of the plant were rinsed with water, air-dried and powdered to fine grade using Binatone electric blender (BLG-402). A total of 150g of powdered material were subjected to cold maceration extraction in 1.5L of 70% Ethanol while vigorously shaking at regular intervals at room temperature for 72 hours [9; 10]. The

**Corresponding Author's Email: ijeomajanennadozie@yahoo.com*

filtrate was collected and lyophilized to obtain 15.3g of the crude ethanol extract. The percentage extraction yield was determined to be 10.2% in accordance to [11].

Qualitative and Quantitative Phytochemical screening

The freshly prepared crude ethanolic extract of leaves was subjected to qualitative and quantitative chemical tests using standard procedures as described by [12; 13; 14; 15]

Gas Chromatography- Mass Spectrometry (GC-MS) Analysis

The GC–MS analysis of bioactive compounds from the extracts of the ethanol leaf extract of *C millenii* leaves was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA). Gas Chromatography- Mass Spectrometry (GC-MS) analysis were carried out at the Central Research Laboratory (CRL) of the University of Lagos, Akoka using 7890A model Gas chromatography system coupled to a VL/MSD 5975C mass spectrometer (GC-MS Agilent Technologies, USA), the instrument made use of the following conditions: Column HP5MS fused silica capillary column [30m (length) x 0.32mm (diameter) x 0.25µm (film thickness)] composed of 100% dimethyl polysiloxane. Helium gas (99.9999%) was used as the carrier gas at a constant flow rate of 1ml/min and an injection volume of 1µl was employed with injector temperature at 250^{o}C and pressure at 8.802 psi. The oven temperature was programmed initially from 80^{o}C (held for 2 mins) with an increase of 5^{o} C /min to 120^{o}C/min, then 10^{o}C / min to 300^{0} C/ min to hold for 6min. The total GC running time for the extract was 30 mins. The relative percentage amount of each component was calculated, by comparing its average peak area to the total area. The interpretation on mass spectrum of GC-MS was done using the databases of National Institute of Standards and Technology (NIST) version 2.0 g year 2011 library. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library to ascertain the name, molecular weight and structure of the components of the plant extract.

Results and Discussion

The qualitative phytochemical analysis of *C. millenii* revealed the presence of active secondary metabolites such as tannins, saponins, flavonoids, terpenoid, alkaloids, cardicalycosies and phenol **Table 1**, no reducing sugar was present. The results are almost in agreement with the results reported by [10] and [16]. The phytochemicals disclosed the quantities of flavonoid (60.7g/100g) and phenol (53.9g/100g) were significantly higher than alkaloids (0.13g/100g) as shown in **Table 2**. It is likely that these bioactive phyto-components may be responsible for many of the pharmacological and therapeutic activities exhibited by the plant in the prevention and treatment of diseases as reviewed.

Table 1: Qualitative phytochemical screening of ethanolic extracts of the leaves of *Cordia millenii*

S/N	Phytochemicals	Test	Observations	Results
1	Phenol	Ferric chloride test	Dark green coloration	+
2	Tannin	Ferric chloride test	Blue- black precipitation	+
3	Terpenoid	Chloroform+ H_2SO_4	Reddish-brown coloration	+
4	Phlobatanins	% 1 HCL	Red Precipitation	+
5	Saponin	Froth test	Persistence of frothing upon vigorous shaking	+
6	Alkaloids	Dragendoff's reagents	Blue- black precipitation	+
7	Flavonoid	NH_3 + H_2SO_4	Yellow coloration	+
8	Steroid	Libermann Burchard's test	Violet to blue or green	+
9	Reducing Sugar	Fehling's test	Absence of Red Precipitation	–
10	Cardiac glycoside	Keller-Kiliani test	Greenish ring above brown ring	+

Table 2: Quantitative analysis of *Cordia millenii* extract

Compound	Cordia millenii ethanolic leaf extract (mg/100g)
Phenol	53.905 ± 0.115
Tannin	38.955 ± 0.055
Saponin	0.31 ± 0
Alkaloid	0.135 ± 0.005
Flavonoid	60.705 ± 0.165

Values are expressed as Mean± SEM

Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection [17]. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations and degree of polymerization. Functional hydroxyl groups in flavonoids mediate their antioxidant properties by scavenging free radicals and/or by chelating metal ions [18; 19]. It has been shown that flavonoids are known to inhibit the initiation and promotion of tumour cells [20] as well as reduction of coronary heart disease associated with intake of flavonoids [21]. In the current study, the presence of phenolic compounds was observed to be significant in *C. millenii* ethanol extract. Phenols are chemical components that occur ubiquitously as natural colour pigment responsible for the colour of fruits of plants. Phenolic compounds essentially represent a host of natural antioxidant, anti-inflammatory, antidiabetic, hepato protective, anticancer and anti-microbial properties found in medicinal plants [22; 23]. Trace quantities of alkaloids were also found in the extract and are known to mainly consist of nitrogen atoms naturally occurring in plants. Numerous reports available on alkaloids have demonstrated their usefulness in exhibiting potential pharmacological properties such as antimalarial e.g. quinine, anticancer e.g. homoharringtonine [24], antibacterial e.g. chelerythrine [25], and anti-hyperglycemic activities e.g. piperine [26]. Other alkaloids possess psychotropic (e.g., psilocin) and stimulant activities (e.g., cocaine, caffeine, and nicotine) and have been used as recreational drugs [26]. Presence of flavonoids, phenol and alkaloids gives an insight into the value of *C. millenii* to be used as a basic medicinal agent for antibacterial, anti-cancer, anti-inflammatory and anti-oxidant activities. The knowledge of its antimicrobial and antioxidant promoting activities has been reported by [10].

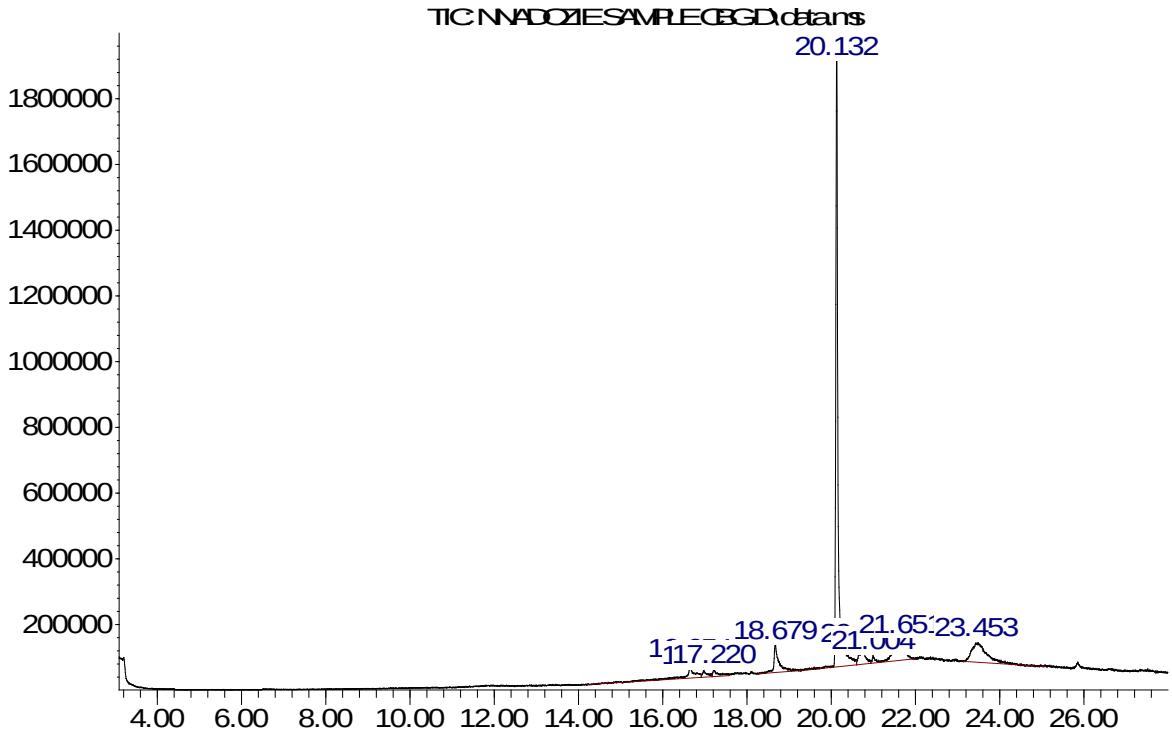

Figure 1: GC-MS chromatogram of *Cordia millenii* ethanol extract

The bioactive compounds present in *Cordia millenii* were identified and characterized using Gas Chromatography Mass Spectrometry (GC-MS) and the chromatograms with their corresponding peaks are shown in **Figure 1**. The GC- MS analysis of the plant extract identified 9 compounds. The active compounds with their molecular formula, molecular weight (MW), retention time (RT), area percentage composition and quality of the extract are presented in **Table 3**. Important natural antioxidant compounds such as Linoleic acid, palmitic acid, oleic acids, marganic acid, phytol, phtalic acid and Vitamin E were identified with a percentage

Table 3: The GC-MS analysis of phyto-components in *Cordia millenii* ethanol extract

S/N	Name of compound	Molecular Formular	Molecular Wt (g.mol-1)	Retention time (RT)	% composition	Quality
1	Tridecanedial	$C_{13}H_{24}O_2$	213.0	16.654	4.80	25
2	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	253.6	16.980	1.30	35
3	1,6-Octadiene, 3,7-dimethyl	$C_{10}H_{18}$	138.2	17.220	1.62	27
4	Hexadecanoic acid, ethyl ester	$C_{16}H_{36}O_2$	284.4	18.679	6.26	93
5	Phytol	$C_{20}H_{40}O$	296.5.	20.133	58.32	98
6	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	292.4	20.751	4.22	55
7	Heptadecanoic acid (Heptadecylheptadecanoate)	$C_{34}H_{68}O_2$	508.9	21.002	0.94	20
8	1-Tridecene	$C_{13}H_{26}$	182.3	21.649	10.05	80
9	Vitamin E	$C_{29}H_{50}O_2$	430.7	23.451	12.49	92

and quality analytical grade ranging from 18- 99% (**Table 3**). Phytol and vitamin E had the highest quality percentage 98% and 92% respectively. Phytol is an acyclic diterpene alcohol that could be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1 and plays an active role as a natural antioxidant [27]. Vitamin E is a known compound whose biological activities were reported by [28] as an antioxidant needed to protect the endometrial environment from oxidative damage and in disease conditions including cancer, ageing, arthritis and cataracts. According to [29], a combination of palmitic acid and linoleic acid demonstrates antioxidant properties. The compounds identified in the present study possess antioxidant properties that support previous studies and thus serve as potent compounds for therapeutic drug development [16]. This study puts into perspective the usefulness of *Cordia millenii* as a natural anti-oxidant with anti-inflammatory properties that could serve for therapeutic drug development. This will therefore necessitate the need to explore a detailed pharmacological and biosynthetic activity of *Cordia millenii*.

References

1) Pierre W, Esther N, Nkeng-Efouet PA, Nguelefack TB, Albert K: Reproductive Effects of *Ficus asperifolia* (Moraceae) In Female Rats. *Afr Health Sci* **9**(1): 49 – 53 .2009.
2) Beegum GJ, Beevy SS, Sugunan VS: Qualitative Phytochemical Screening and GC-MS Analysis of *Boerhavia diffusa* L. *Inter J Emerg Technol Adv Eng* **4**(7):318-324. 2014.
3) Molefe-Khamanga DM, Mooketsi NA, Matsabisa MG, Kensley, RM: Qualitative Phytochemical Studies of Solvent Extracts from *Myrothamnus flabellifolius*. *Online Int J Med Pl Res* **1**(1): 1-5. 2012.
4) Campos-Vega R, Oomah BD: Chemistry and classification of phytochemicals. In: Tiwari, B. K., Brunton, N. P. and Brennan, C. S. (Eds.) Handbook of Plant Food Phytochemicals: Sources, Stability and Extraction, John Wiley and Sons Ltd, Oxford. pp. 6-48. 2013.
5) James DB, Abu EA, Wurochekke AU, Orji GN: Phytochemical and antimicrobial investigation of the aqueous and methanolic extracts of *Ximenia americana*. *J. Med. Sci* **7**(2): 284-288. 2007.
6) Keay RWJ, Onochie CFA, Stanfield DP: Nigeria Trees. Department of Forest Research Publishers Ibadan. vol 1, pp 38-265.1964.
7) Udeozo IP, Eboatu AN, Arinze RU, Okoye HN: Some fire characteristics of fifty-two Nigerian Timbers. *Anachem J* **5**(1): 920-927. 2011.
8) Udeozo IP, Ejikeme CM, Eboatu AN, Arinze RU, Kelle HI: An Assay of Characteristics, Chemical Constituents and Functional Group Analysis of *Cordia millenii*: A Tropical Timber. *Int J Life Sci Res* **4**(2):29-36. 2016.

9) Oladimeji OS, Lawal OA, Ogundimu, EO: Anti-Oxidants Levels in Female Rats Administered Pro-Fertitility Ethanolic Leave Extract *Byrsocarpus coccineus.*. *Eur Sci J* **10**(15): 1857 – 7881. 2014.
10) NGA N, Vandi D, Flora M.A, *et al.*: Preliminary Screening of *Cordia millenii* and their Antimicrobial and Antioxidant Activities. *Int J Res Stud Biosci* **3**(12): 39-46. 2015.
11) Zhang S, Bi H, Liu, C: Extraction of bioactive components from *Rhodiola sachalinensis* under ultrahigh hydrostatic pressure. *Sep. Purif. Technol.* **57**: 275-280. 2007.
12) Harbourne, JB: Phytochemical Methods. A guild to modern techniques of plant analysis. 3rd ed. Chapman and Hall, London, UK. 302 pp .1998.
13) Trease GE, Evans, WC: Pharmacognosy. 15th ed. W.B. Saunders, London. 406pp. 2002
14) Sofowora, LA: Medicinal plants and traditional medicine in Africa. 2nd ed. Spectrum Books Limited, Nigeria. 289pp. 2008
15) Mir A, Sawhney SS, Jassal MMS: Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale. Wudpecker J Pham Phamacol* **2**(1): 001 – 005. 2013
16) Ezeonu CS, Ejikeme CM: Qualitative and Quantitative determination of phytochemical contents of indigenous Nigeria softwoods. *New J of sci*. **2016**: 1-9. 2016
17) Dixon RA, Dey PM, Lamb CJ: Phytoalexins: enzymology and molecular biology. *Adv Enzymol Relat Areas Mol Biol* **55**: 1–136.1983.
18) Kumar S, Pandey AK: Chemistry and biological activities of flavonoids: An Overview. *Sci. World J* **2013**:1-16.2013
19) Kumar, S, Mishra, A and Pandey, AK: Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using in vitro models. *BMC Complement Altern Med* **13**:120. 2013.
20) Kim SY, Kim JH, Kim SK, MJ Oh, Jung MY: Antioxidant activities of selected oriental herb extracts. *J Am Oil Chem Soc* **71**(6): 633–640. 1994.
21) Hertog MGL, Feskens EJM, Hollman PCH, Katan JB, Kromhout D: Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study," *The Lancet*: **342**(8878):1007–1011.1993.
22) Sulaiman S, Ibrahim D, Kassim J, Sheh-Hong L: Antimicrobial and antioxidant activities of condensed tannin from Rhizophora apiculate barks. *J Chem Pharm Res* **3**(4): 436-444. 2011
23) Kumar S, Pandey AK: Phenolic content, reducing power and membrane protective activities of *Solanum xanthocarpum* root extracts. *Vegetos*. **26**: 301–307. 2013.
24) Kittakoop P, Mahidol C, Ruchirawat S: Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Curr Top Med Chem*. **14**(2): 239–252. 2014.
25) Cushnie TPT, Cushnie B, Lamb, AJ: Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int J Antimicrob Agents*. **44**(5):377–386. 2014.
26) Qiu S, Sun H, Zhang AH, et al: Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chin J Nat Med*. **12**(6): 401–406 .2014.
27) Santos CC, Salvadori MS, Mota CV, et al: Antinociceptive and Antioxidant Activities of Phytol *In Vivo* and *In Vitro* Models. *Neurosci J* **2013**:01-09.2013
28) Cicek N, Eryilmaz OG, Sarikaya E, Gulerman C, Yasemin G: Vitamin E effect on controlled ovarian stimulation of unexplained infertile women. *J Assist Reprod Genet.* **29**:325–328. 2012
29) Cho KH, Hong JH, Lee KT: Monoacylglycerol (MAG)- oleic acid has stronger antioxidant, anti-atherosclerotic and protein glycation inhibitory activates than MAG- palmitic acid. *J Med Food*. **13**(1): 99-107. 2010