

Gas Chromatography-Mass Spectrometry and Infra-Red Studies of Bioactive Phytoorganic Components of *Combretum dolichopentalum* Leaves

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Abstract

Gas chromatography-Mass spectrometry analysis of *Combretum dolichopentalum* showed presence of 24 bioactive photo organic compounds which includes: Nitrocyclohexane, Phenol-2,6-bis(1,1-dimethyl)-4-methyl, methyl carbamate, 2,7 Dimethyl-1-octanol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecane, Heptadecanoic acid, Octadecanoic acid, Tetratetracotane, 1-Fluorodecane, Eicosanoic acid, (E)-2,4,5-Trimethoxypropenyl benzene, 2(4H)-Benzofuranone-5,6,7,7a-tetrahydro-4,4,7a-trimethyl-amino caproic lactam, 4-t-butyl-2-(1-methyl-2-nitroethyl) cyclohexanone, 6,10-Dimethyl-2-undecanoate, Ethyl octadecanoate, 9-Hexadecanoic acid, 2-Buthyl-1-octanol. The presence of these bioactive compounds may be the underlying scientific evidence for the many therapeutic effect of the plant extract.

Keywords: Gas chromatography-Mass spectrometry; Infra-red; Bioactive compounds; Therapeutic effect

Introduction

Plants are used as a source for many potent drugs [1], as plants synthesize lower molecular weight organic compounds which possess various biological activities [2-4]. A large number of medicinal plants and their purified constituents have shown therapeutic activities [5], and have proved as safe and effective therapeutic agents. Many plant species have been used in folklore medicine to treat various ailments [6]. The development of pharmaceuticals begins with identification of active principles, detailed biological assays and dosage formulations followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drug [7].

Combretum dolichopentalum is used traditionally for curing several ailments like stomach ache, gastro intestinal disorders (such as dysentery, passage of bloody stool and diarrhoea) and stomach ulcer in and around Ogwa in Imo State of Nigeria. Also in Ezinihitte Mbaise, Imo State, *C. dolichopentalum* is taken by women after parturition for reconditioning of the uterus [8]. Aqueous extracts of the leaves of *C. dolichopentalum* have antioxidant activities [9]. This study is a pioneer in identifying the organic compounds of *C. dolichopentalum* using GC-MS analysis.

Materials and Methods

Sample collection and preparation

Combretum dolichopentalum belong to the family of Combretaceae. Fresh leaves of *C. dolichopentalum* were harvested from a farm in Obinze in Owerri West Local Government Area of Imo State, Nigeria (N 5° 23'41.1" and E 6° 57' 14.0"). The sample specimen was deposited with voucher IMSUH12 at Imo State University herbarium. Fresh leaves of the plant were plucked from their stems, washed with distilled water and allowed to dry at room temperature (21-25°C). The dried samples were pulverized (using electric blender) and stored in an airtight container kept in a dark cupboard.

Preparation of samples for Gas Chromatography-Mass Spectrum (GC-MS) analysis

Fifty grams of the sample were soaked in absolute ethanol for 48

hours in various portions and repeatedly extracted with ethanol. The combined and concentrated ethanol extract were re-extracted using chloroform to obtain chloroform soluble portion and stored in sample bottle for GC-MS analysis.

GC-MS analysis was carried out using GC-MS QP 2010 Plus Shimadzu Japan equipment. An aliquot of 1 µl of the chloroform soluble portion of the sample was injected into the column with injector temperature at 230°C and carrier gas pressure of 100 kpa. The column has a length of 30 m with a diameter of 0.25 mm and a flow rate of 50 ml/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5 kV and sampling rate of 0.2 seconds. The Mass Spectrometer was also equipped with a computer fed Mass Spectra data bank. The Mass spectrum yielded a spectrum of compounds which was compared with the spectrum of National Institute of Standard and Technology (NIST) database with over 62,000 spectral patterns [10,11]. The identity of the spectra above 95% was used to ascertain the name, molecular weight and structure of the components in the leaves of *C. dolichopentalum*.

Column chromatographic separation of plant sample

Eight hundred grams of the pulverized samples were soaked in 95% ethanol for 48 hours and filtered. The filtrates were concentrated using rotary evaporator regulated at 40°C to obtain the ethanol extract. The crude ethanol extracts were partitioned between chloroform and water to afford chloroform soluble fractions. Ten grams of the chloroform soluble fractions were subjected to column chromatography over silica gel. The column was eluted as described by Abdelgadir et al. [12] with minor modifications using 100 ml petroleum ether, followed by petroleum ether/chloroform mixture and labelled as follows; (a) 100 ml petroleum ether A_{CD}, (b) 90/10 B_{CD}, (c) 80/20 C_{CD} (d) 70/30 D_{CD}, (e) 60/40 E_{CD}, (f) 50/50 F_{CD}, (g) 40/60 G_{CD}, (h) 30/70 H_{CD} (i) 20/80 I_{CD} (j) 10/90 J_{CD}. Further elution using chloroform and methanol mixture afforded the fraction 90/10 K_{CD}, 80/20 L_{CD} 70/30 M_{CD}, 60/40 N_{CD}, 50/50 O_{CD}, 40/60 P_{CD}, 30/70 Q_{CD}, 20/80 R_{CD} 10/90 S_{CD}.

Thin layer chromatography of column eluents

The eluents from column chromatography were subjected to thin layer chromatography following the method of Watanable et al. [13] with minor modifications using silica gel 60 G and iodine vapour for development. This was to select the pure eluents which appeared as a

spot from the impure eluents that appeared as more than one spots. Fraction F_{CD} was obtained using petroleum ether/chloroform mixture (3:1) with a Ratio of front (Rf) value of 0.82. Fraction I_{CD} was obtained with Rf value of 0.83 which appeared as a spot. Fraction D_{CD} had Rf value of 0.84, G_{CD} with Rf value of 0.85, J_{CD} with Rf value of 0.86, K_{CD} with Rf value of 0.89 and M_{CD} with Rf value of 0.64.

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis

Fourier transform infra-red spectroscopic analysis of the sample was carried out according to the method of Hashimoto et al. and Geethu et al. [14,15]. One gram of the dried plant sample was mixed with 0.5 g of potassium bromate (KBr) and 1.0 ml of nujol oil (a solvent for preparation of sample by Buck 530 IR-spectrophotometer) was added with the aid of a syringe to form a paste. The paste was introduced into the instrument sample mould and allowed to scan at a wavelength of 600-4000 nm to obtain the spectra wavelength. The eluents from the column chromatography were subjected to FTIR analysis; here 1.0 ml of the nujol oil was added to 1.0 ml of the chloroform fraction.

Results and Discussion

Identification of the chemical constituents of plants is important for the discovery of new therapeutic agents [16], and GC-MS is an important tool for this process. Gas chromatography mass spectrometer identifies the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compounds [17]. The chemical characterization of active fraction of tested *C. dolichopentalum* leaves showed a mixture of fatty acids, nitrocyclohexane, furans, alkanes, organic esters, modified alkanones, alcohols, and eicosanoic acids.

A total of 24 peaks (Figure 1) were observed when the chloroform soluble portion of ethanol extract of *C. dolichopentalum* leaves was subjected to GC- MS (Table 1). Some of the bioactive compounds include; hexadecanoic acid, Cyclohexamine, Caprolactam, Phenol-2,6-bis(1,1-dimethylethyl)-4, methyl carbamate, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol), Heptadecanoic acid.

Hexadecanoic acid, a fatty acid with potential antimicrobial and anti-diarrhoeal activities [18], is reported to cause growth inhibition and apoptosis induction in human gastric cancer cells [19]. Cyclohexamine belong to an aliphatic amine class. It is used as an intermediate in the synthesis of other organic compounds such as sulphenamide; a base reagent used as accelerators for vulcanization. Cyclohexamine is also used as a building block for pharmaceuticals, e.g., mucolytics, analgesics, and bronchodilators [20].

Phenol-2,6-bis(1,1-dimethylethyl)-4, methyl carbamate could be used to synthesize Phenol-4-[2-(aminomethyl)-4-thiazolyl]-2,6-bis(1,1-dimethylethyl) monohydrochloride which is used for the treatment of Huntington's disease [21].

The identified hexadecanoic acid ethyl ester has antioxidant activities which are important in biological processes [22,23]. While, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol) has antimicrobial and anti-inflammatory activities [23]. Octadecanoic acid (stearic acid) is less likely to be incorporated into cholesterol esters, and thus was found to be associated with lowered LDL cholesterol when compared to other saturated fatty acids in epidemiologic and clinical studies [24]. It is also used to produce dietary supplements [25].

Heptadecanoic acid or margaric acid, is a saturated fatty acid which occurs as a trace component of fat and milk fat of ruminants [26,27].

Ethnobotanical database compounds such as furan, phenols,

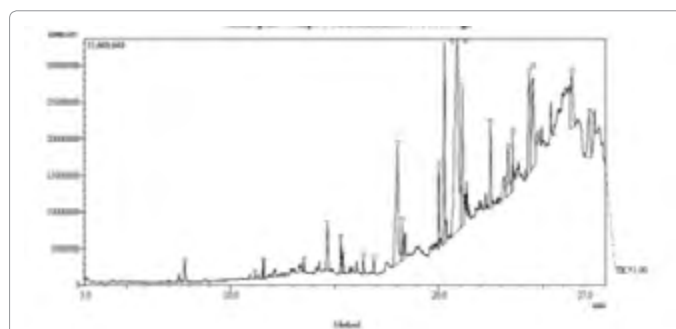


Figure 1: GC-MS profile of chloroform soluble portion of leaves of *C. dolichopentalum*.

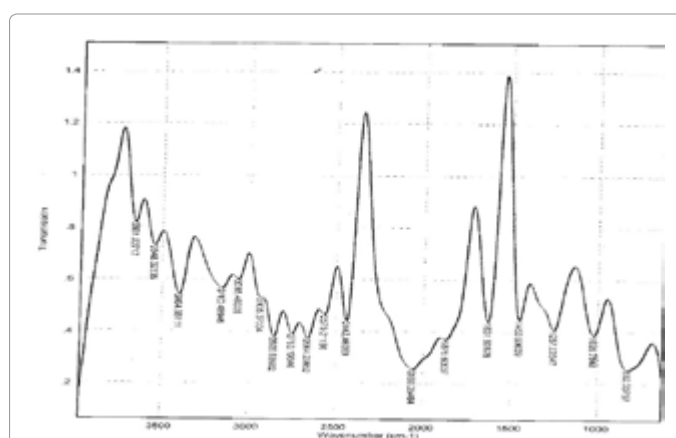


Figure 2: FTIR spectra of pulverized *C. dolichopentalum* leaves showing absorption peaks of different functional groups.

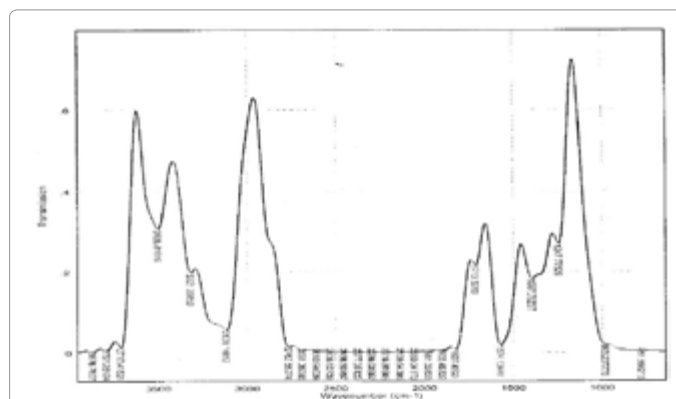


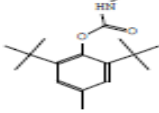
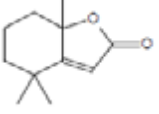
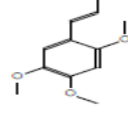


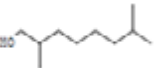
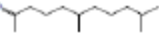

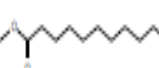


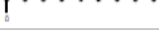


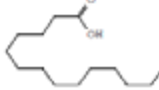


Figure 3: FTIR spectra of eluate, Dcd (4-t-Butyl-2-(1-methyl-2-nitroethyl) cyclohexanone) of *C. dolichopentalum* leaves.

flavonoids and fatty acid esters possess antioxidant, antibacterial, antimicrobial, anti-inflammatory, antiproliferative, anticancer, antitumor, antidiabetic, antiarthritic, antimalarial and automatic nerve activities [28,29]. Thus, the presence of these compounds in *C. dolichopentalum* implies a possibility of exhibiting afore mentioned activities as found in plants such as *Litsea glutinosa* [30], *Suaeda maritime* [31], *Alpinia hainanensis* and *Alpinia katsumadai* [32], and *Macrotyloma uniflorum* [33].

The spectra wavelength observed in *C. dolichopentalum* served as a characteristic medium to elucidate the inherent functional

S No	Molecular Weight	Molecular Weight	Molecular Formula	Molecular Structure
1	Nitrocyclohexane	129	C ₆ H ₁₁ NO ₂	
2	Amino caproic lactam	113	C ₆ H ₁₁ NO	
3	Phenol-2,6-bis (1,1-dimethyl)-4 -methyl, methyl carbamate	277	C ₁₇ H ₂₇ NO ₂	
4	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	180	C ₁₁ H ₁₆ O ₂	
5	1,2,4-trimethoxy-5-prop-1-enylbenzene	208	C ₁₂ H ₁₆ O ₃	
6	4-t-Butyl-2-(1-methyl-2-nitroethyl) cyclohexanone cyclohexanone	241	C ₁₅ H ₂₃ NO ₃	
7	2,7- dimethyl-1-octanol	158	C ₁₀ H ₂₂ O	
8	1-Tridecyne	180	C ₁₀ H ₂₂ O	
9	6,10-dimethyl-2-undecanone	198	C ₁₃ H ₂₆ O	
10	Hexadecane	226	C ₁₆ H ₃₄	
11	Methyl 14-methylpentadecanoate	270	C ₁₇ H ₃₄ O ₂	
12	Heptedecanoic acid	270	C ₁₇ H ₃₄ O ₂	
13	Ethyl octadecanoate	312	C ₂₀ H ₄₀ O ₂	
14	Methyl trans-9-octadecanoate	296	C ₁₉ H ₃₆ O ₂	
15	3,7,11,15-tetramethyl-2-hexadecen-1-ol	296	C ₂₀ H ₄₀ O	
16	11-Hexadecanoic acid	254	C ₁₆ H ₃₀ O ₂	
17	Octadecanoic acid	284	C ₁₈ H ₃₆ O ₂	


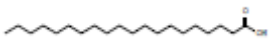

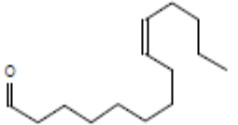
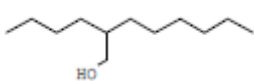
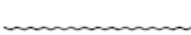

18	1-Fluorodecane	160	$C_{10}H_{12}F$	
19	Eicosanoic acid	312	$C_{20}H_{40}O_2$	
20	Eicosane	282	$C_{20}H_{42}$	
21	9-tetradecenal	210	$C_{14}H_{26}O$	
22	2-Butyl-1-octanol	186	$C_{12}H_{26}O$	
23	Cyclododecane epoxide	182	$C_{12}H_{22}O$	
24	Tetratetracotane	618	$C_{44}H_{90}$	

Table 1: Molecular weights, formula and structures of compounds identified from the GC-MS of ethanol extract of *C. dolichopentalum*.

S No	Wavelength (cm ⁻¹)	Functional Group	Compounds
1	833	C-Cl	Chloro compound C-Cl stretch
2	1027	R-O-P	Ether CO stretch
3	1257	RNH ₃	1 ^o amine NH stretch
4	1454	CH ₃	Methyl CH stretch
5	1632	RNH ₂	2 ^o amine NH stretch
6	1876	C-O-C	Cyclic ester CO stretch
7	2060	RCOOH	Carboxylic acid COO stretch
8	2442	R-C=N	Nitriles CN anti-symmetric stretch
9	2570	CH ₂ SH	Thiol SH stretch
10	2664	CH ₂ SH	Thiol SH stretch
11	2753	CH ₂	Methylene CH stretch
12	2856	CH ₂	Methylene CH stretch
13	2935	R-S-C=N	Thiocyanate SCN anti-symmetric stretch
14	3065	RCHOH	1 ^o alcohol OH stretch
15	3160	RCHOH	1 ^o alcohol OH stretch
16	3405	RNH ₂	2 ^o amine NH stretch
17	3548	R ₂ CHOH	3 ^o alcohol OH stretch
18	3662	R ₃ N	3 ^o amine NH stretch

Table 2: FTIR spectra characteristics of crude ethanol extract of *C. dolichopentalum*.

group and organic compounds in the plant [15,34]. The Infra-Red of the pulverized leaves (Table 2) showed different peaks, indicating transitions between vibration levels of different molecules. The peak value at 833 cm⁻¹, was assigned C-Cl stretch of chlorine compound and 1027 cm⁻¹ was assigned CO stretch of ether compounds (Figure 2). The medium bands at 1257 cm⁻¹, 1632 cm⁻¹, 3405 cm⁻¹ were thus assigned NH stretch of amine compounds. The weak band at 1876 cm⁻¹, and 2060 cm⁻¹ were assigned CO stretch of unsaturated ester and carboxylic compounds. Also, 1454 cm⁻¹, 2753 cm⁻¹, 2856 cm⁻¹, 2570 cm⁻¹ and 2664 cm⁻¹ peaks were assigned CH and SH symmetric stretch of methylene and thiol compounds. The broad band at 3065 cm⁻¹, 3160 cm⁻¹, and

3548 cm⁻¹ were assigned OH stretch of primary (1^o) and tertiary (3^o) alcohol compounds [15,35].

The Infra-Red of the seven eluates are shown in Figures 3-9. The interpretation of the IR spectra of the eluates using the GC-MS spectra indicated Dcd eluate (Figure 3) as 4-t-Butyl-2(1-methyl-2-nitroethyl) cyclohexanone, with the R_f value of 0.84, molecular weight- 234 and an empirical formula C₁₃H₂₃NO₃. N-H stretch occurs in the range 3500-3300 cm⁻¹. Eluate Fcd (Figure 4) as flourodecane an alkane which IR spectrum are usually simple with few peaks. C-H stretch occur around 3000 cm⁻¹ and alkanes (except strained ring compounds) absorption

always occurs to the right of 3000 cm^{-1} . Gcd eluate (Figure 5) was identified as phenol-2, 6- bis (1,1-dimethyl ethyl)-4, methyl carbamate with the empirical formular $\text{C}_{17}\text{H}_{27}\text{NO}_2$. Icd (Figure 6) and Kcd (Figure 7) eluates were identified as pentadecane ($\text{C}_{15}\text{H}_{32}$) and hexadecane ($\text{C}_{16}\text{H}_{34}$) respectively. Eluates Jcd (Figure 8) and Mcd (Figure 9) as 2, 7-dimethyl-1-octanol ($\text{C}_{10}\text{H}_{22}\text{O}$) and 2-Butyl-1-octanol ($\text{C}_{12}\text{H}_{26}\text{O}$) respectively [10,11,36-38].

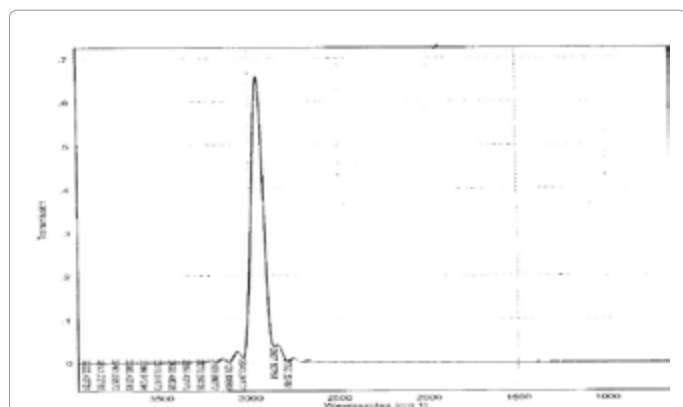


Figure 4: FTIR spectra of eluate Fcd (1-Fluorodecane) of *C. dolichopentalum* leaves.

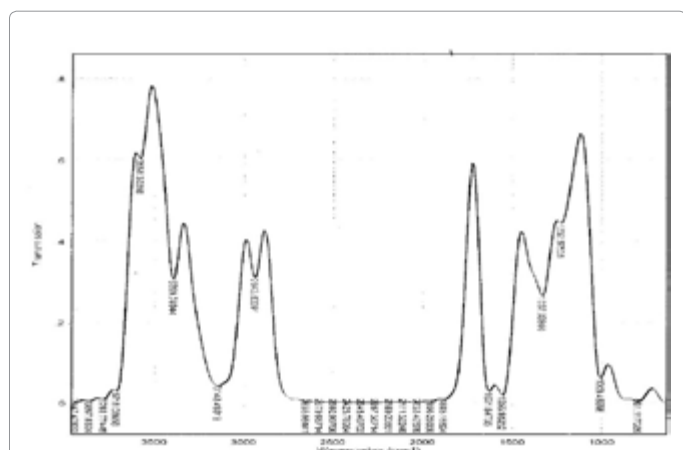


Figure 5: FTIR spectra of eluate Gcd (Phenol-2,6-bis - (1,1-dimethylethyl) - 4 - methyl methyl carbamate) of *C. dolichopentalum* leaves.

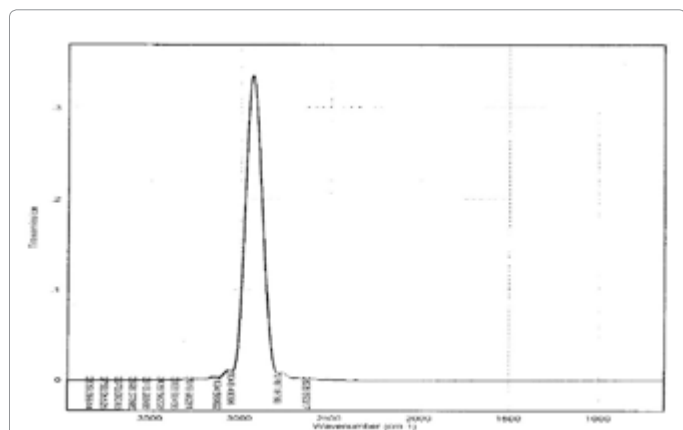


Figure 6: FTIR spectra of eluate Icd (Eicosane) of *C. dolichopentalum* leaves.

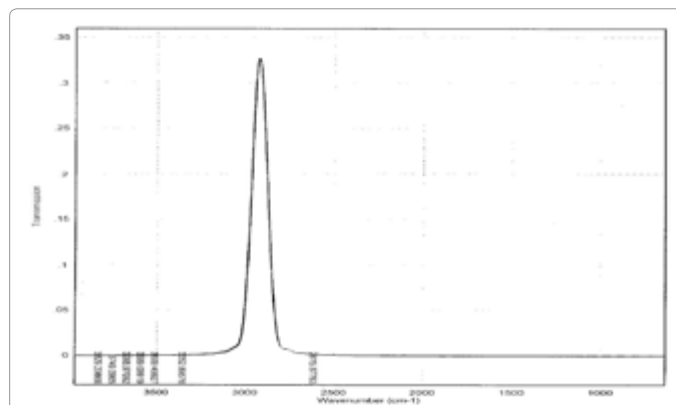


Figure 7: FTIR spectra of eluate Jcd (2,7-Dimethyl-1-octanol), of *C. dolichopentalum*.

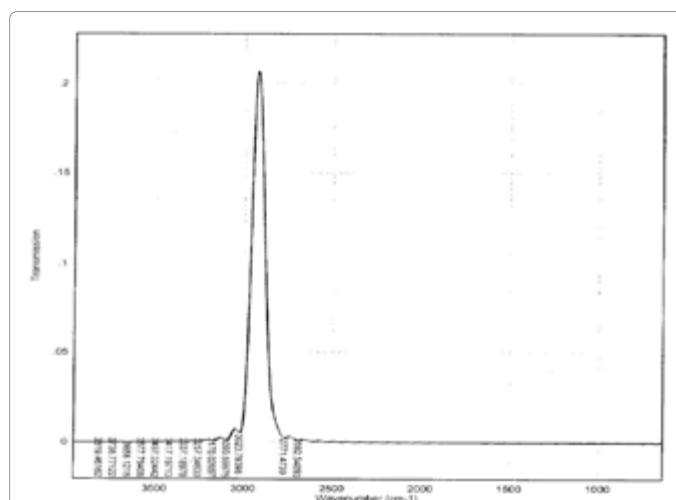


Figure 8: FTIR spectra of eluate Kcd (Hexadecane) of *C. dolichopentalum* leaves.

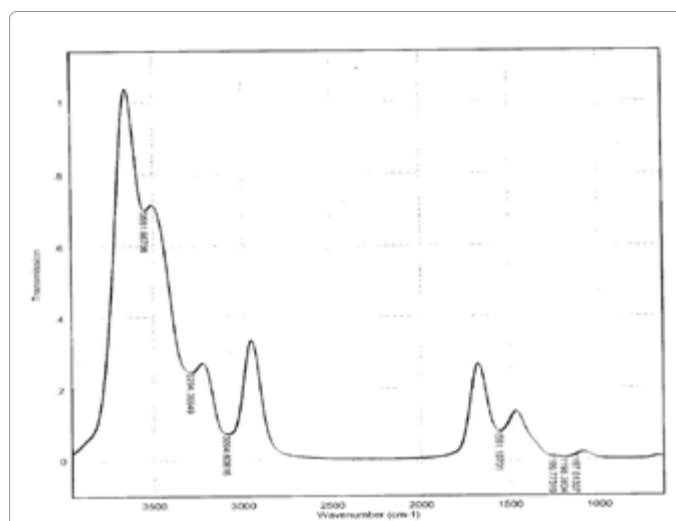


Figure 9: FTIR spectra of eluate Mcd (2-Butyl-1-octanol) of *C. dolichopentalum* leaves.

The present study showed that leaves of *C. dolichopentalum* contain a variety of biologically active phytoorganic compounds by using GC-MS and IR spectroscopy as pharmacognostic tool for the identification of plant constituents. The beneficial roles of these bioactive phytoorganic constituents can be harnessed and used in the pharmaceutical and food industries for the production of drugs and raw materials for industrial purposes.

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