Chemical Constituents and Antimicrobial Activities of Combretumpaniculatum

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ABSTRACT

Combretumpaniculatum(combretaceae), has been used widely in ethno medicine in the treatment of chronic diarrhea and dysentery, flatulence, vomiting, colic, and enlarge spleen and liver. The leaf and root of Combretumpaniculatum were pulverized and extracted by serial exhaustive method with solvents: chloroform, ethyl acetate and ethanol in order of increasing polarity, the chemical composition and the antimicrobial activities were investigated using Gas Chromatography- Mass Spectrometry (GC-MS) and Agar-well Diffusion methods respectively. The GC-MS analysis carried out on the leaves and roots extracts of Combretumpaniculatum afforded fifty compounds with the prevailing compounds as Thiirane, Urea, 1,5-Heptadiene, Carbonyl sulfide, Hydrazine, Propanamide, Guanidine, Isobutylamine, Acetic acid, Hexanoic acid, Inositol, Ethane, 5-Chlorovaleric acid and 1-Propanol. These active constituents have been reported to be responsible for many biological activities. The antibacterial analysis of the extracts revealed high activity against the tested pathogens except Pseudomonas aeruginosa staphylococcus aureus (ethylacetate leaf and root extract) and candida albicans (ethanol root). The Minimum Inhibitory Concentration of the extracts were recorded as low as 0.0078mg/ml, the results obtained confirmed that the extracts were highly bactericidal and fungicidal at low concentration. The plant Combretumpaniculatum therefore can be explored in the development of potent antibiotics.

Key Words: *Combretumpaniculatum, Gas Chromatography-Mass Spectrometry, Antimicrobial, Agar-well diffusion, Serial exhaustive method.*

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1. INTRODUCTION

Ethno pharmacologist, botanists, microbiologists, and natural product chemist are surveying the earth for phytochemicals which could be developed for treatment of infectious diseases (Zirihi *et al*., 2005). Recently, the search for specific plant components that convey health benefits has widened to encompass the vast range of 'non-nutritive' compounds present in plant foods, and their potential to improve health. Evidence is growing that most plant constituents, belonging to the group termed **"**bioactive compounds**"**, which may help to promote optimal health and to reduce the risk of chronic diseases such as cancer, coronary heart disease, stroke and perhaps Alzheimer's disease, (Edeoga *et al*., 2005). According to Azmir*et al.,* (2013), the history of plants used for mankind is as old as the start of human kind. At first people used plant for nutritional purposes but after the discovery of medicinal properties, this natural flora became useful sources of disease cure and health improvement across various communities. Vinatoru reported in 2001 that Egyptians papyruses show that coriander and castor oil were useful for medicinal applications, cosmetics and preservatives through thousands of recipes. In 19th century Romanian

pharmacopoeia introduced herbal products and in 1904 the first institute of medicinal herbs was established in Clujcity (in Romania), (Vinatoru, 2001).

C*ombretumpaniculatum* (called forest frame in English, Alo/Gusa in Tiv) is a shrub with leaves 3cm, deep root system with vivid scarlet flowers attaining 15 m length widely spread in tropical Africa along Benue valley. C*ombretumpaniculatum* (combretaceae), has been used widely in ethno medicine in the treatment of chronic diarrhoea and dysentery, flatulence, vomiting, colic, and enlarge spleen and liver (Cheng *et al;* 2003).

The aim of this research is to ascertain the chemical constituents and determine the antimicrobial activities of the crude extract of the leaves and root of *Combretumpaniculatum*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The The leaves and the root of Combretumpaniculatum plant were collected in the forest of Asogo, Usher, Shiakpev,ofYooyo ward in Katsina-ala Local Government Area of Benue state, Nigeria, and were properly identified in the Department of Biological Sciences, Federal University Wukari. The leaves were collected by cutting down the stem of the tree and the root by digging the ground before collecting it. The leaves were critically examined to be free of disease and defect of any sort. Only healthy plant parts were used for the analysis. The plant material were air dried and pulverized using electric Blender.

2.2 Extraction by Serial Exhaustive Extraction method

250 g of the pulverized sample (leaves) was soaked in about 1250ml of ethanol and 200 g in 750ml of ethanol for the root for 72 hours. The extracts was first filtered using a filtered cloth of which the filtrate obtained were further filtered under reduced pressure using Whatman no. 1 filter paper, evaporated and concentrated into solid extracts using rotatory evaporator, kept under room temperature overnight to remove all solvent. This process was repeated for ethyl acetate sequentially in order of increasing polarity. The extracts were kept refrigerated until required for analysis.

2.3 Gas chromatography-mass spectroscopy (GC-MS)

GC-MS analysis was carried out on a GC claries 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver. 5.2.0

2.4 Sensitivity test of crude extract by Agar Well diffusion method

The agar well diffusion method was used (Nostroetal., 2002). The crude extracts of Combretumpaniculatum were diluted with Dimethyl Sulphoxide (DMSO). Bacteria from 24 hours slants were suspended in saline solution (0.9% w/v Nacl) individually till the turbidity matches with that of MacFarland 0.5 solution (mixture of 9.9ml of 1% w/v solution of $BaCl_2$). The standardized innocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton Agar plates with the aid a sterile swab stick. The prepared plates were allowed to dry by keeping them half open and face downwards for 30 minutes. With the aid of sterile cork borer (6mm in diameter), four appropriately labeled wells were punched into each agar, 0.05ml of each extract was used. DMSO was used as negative control while flovid and Augmentin were used as positive control. The plates were kept for 30 minutes in the bench for the diffusion of the crude extracts to take place before incubation. After, these plates containing bacterial cultures were lifted gently and placed in the incubator at 37^{o}C for 24 hours. After incubation, the plates were examined and the inhibition zones were measured using a ruler in mm. the observed inhibition zones were recorded appropriately in mm for the various diameter zone of inhibition. (Bryant,1972 and Cruckshank et al., 1975). Triplicate plates were prepared for each extract and controls.

While for the fungi, Sabouraud dextrose agar was used and the incubation period was 48 hours. The zones of inhibition of the antifungal activities were also determined.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method outlined by Onyeagba et al., (2004). Serial dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB) and potato Dextro Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth (MHB) and Potato Dextro Broth (PDB). Each tube containing the broth and the extract was incubated with standardized organisms. A tube contains sterile broth (MHB and PDB) without any organism been used as a control. All test tubes were then incubated at 37^{o} C for 24 h. after incubation period; the tubes were examined for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without any visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

2.6 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The results from the Minimum Inhibitory Concentration (MIC) were used to determine the MBC and MFC. A sterile wire loop was dipped into the tubes that did not show turbidity in the MIC test. This was then streaked unto a freshly prepared sterile nutrient agar plates. The plates were incubated at 37^{o}C for 24 h. after the incubation period; the plates were then examined for the presence of growth. This was done to determine if the antimicrobial effect of the extract was bactericidal or fungicidal.

3. RESULTS AND DISCUSSIONS

3.1 Antimicrobial Analysis

The result of anti-bacterial activity is presented in table 5.1. The activities of the crude extracts of ethanol and ethyl acetate obtained from extract of leaves and root of C. paniculatum were tested on six clinical isolates; Staphylococcus aureus, klebsiellaaerogenes, E-Coli, Pseudomonas aeruginosa, Salmonella typhimurium and Shigelladysentrieae. Ethyl acetate root extracts demonstrated the highest activity for candida albicans test (45.00 mm zone diameter of inhibition), follow by ethanol leaves on the same organism and ethylacetateon Aspergillusniger (with 40.00mm zone of inhibition).Ethanol root do not show any inhibition on candida albicans (00.00mm). This implies that the leaf and root extracts which are unable to inhibit the growth of bacteria and fungi. The result also showed that almost all the extracts inhibited bacteria against pathogens such as Staphylococcus aureus, klebsiellaaerogenes, E-Coli, Pseudomonas aeruginosa, Salmonellatyphimurium,Shigelladysentrieae. Ethanol leaf, root and ethyl acetate leaf which do not show any inhibition on Staphylococcus aureus, ethyl acetate leaf, root and ethanol root do not show any inhibition on pseudomonas aeruginosa, as well as the leaf of ethyl acetate crude extract which show no inhibition on salmonella spp. Extract that does not inhibit or exhibit anti-bacterial activity on certain bacteria, have zero zone in inhibitory (00,00mm)..

Hence, from the result obtain from both antifungal and anti-bacterial test, it implies that the leaves and root of Combretumpaniculatum can actually be used to cure diseases associated with various diseases such as diarrhea, stomach cramps/pain, nausea, cause by Staphylococcus aureus, klebsiellaaerogenes, E-Coli, Pseudomonas aeruginosa, Salmonell spp., Shigella, candida albicans, and Aspergillusniger.

The crude extract of ethanol leaves inhibited the subculture at dilution tube 0.25 for MIC and 0.0313 for MBC and MFC, also viable growth on subculture at 0.125,0.0313 for MIC and 0.0156, 0.0039 for MBC and MFC. The result confirmed that the ethanol leaf extracts were highly bacteriacidal at very low concentration.

The crude extract of ethyl acetate root also shows the inhibition of the subculture at dilution tube 0.125 for MIC, 0.0156 and 0.078 for MBC and MFC and viable growth on subculture at 0.0625, 0.0313 and 0.0156 for the MIC and 0.0078, 0.0039 for MBC and MFC also shown in table 4.5. The result confirmed that the ethylacetate extracts were very highly fungicidal at very low concentration.

3.2 Gas Chromatography And Mass Spectrophotoscopy (GC-MS) Analysis

The GC-MS analysis carried out on the leaves and roots of Combretumpaniculatum indicates fifty compounds. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) are presented in (Table 4.8, 4.9, 4.10 and 4.11) above. The prevailing compounds were; Thiirane, Urea, 1,5-

Heptadiene, Carbonyl sulfide, Hydrazine, Propanamide, Guanidine, Isobutylamine, Acetic acid, Hexanoic acid, Inositol, Ethane, 5-Chlorovaleric acid, 1-Propanol. The investigation based on the analysis concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities so that these might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may create a new way to treat many incurable diseases.

Some of the antimicrobial and pharmacological activities exhibited by these compounds have reported. Guanidine is used to mimic the activities of polymoxin B and its derivatives act as both potent antibiotic and effective permeabilizers of the outer membranes of Gram-negative bacteria (Li etal.,1998). Some of the compound rival polymyxin B in Gram-negatives are also active Gram-positive organisms. They serve as antibiotics to inhibit bacterial growth (Matsuzaki et al., 1995). Inositol is an antimicrobial agent which to inhibit Gram-positive bacteria such as bacillus subtilis, E.coli etc, hence possesses antitumor, anti-inflammatory, antibacterial, antifungal, antiviral, antiprotozoal, antiulcer, hepatoprotective and hepacurative effects (Cafarchia et al., 2002). Chlorovaleric acid efficiently inhibits bacterial growth of pathogens and it significantly increased the outer plasma membrane permeability resulting in the loss of barrier function even including slight leakage of nucleotide. It is also a natural bioactive compound present in the livestock (Albayrak et al., 2010, Alberto et al., 2006). Thiirane is used as antibiofilm (Schwaz et al., 2016). Hexanoic acid a fatty acid exhibited patterns of inhibition against oral bacteria (Petshow et al., 1996). Propanamide has been reported to inhibit Candida albicans, biofilm associated infections. (Bholi et al., 2005).

4. CONCLUSIONS

The GC-MS analysis showed the presence of fifty compounds from the ethyl acetate root extract of *Combretumpaniculatum*. The antimicrobial analysis of the crude extract also showed excellent activity against the tested pathogens. Therefore, this research is in agreement with traditional use of the plant for the treatment of chronic diarrhoea and dysentery, flatulence, vomiting, colic, and enlarges spleen and liver.

5. CONFLICT OF INTEREST

The authors have declared no conflict of interest.

Table 5.1: Result of Anti-bacterial activity of *Combretumpaniculatum* crude extracts

Test Organism	ELE BR		ERE BR	EALE	EARE		
Vol.used	0.50	0.50		0.50		0.50	
Staphylococcus aureus	00.00	00.00		00.00		20.00	
klebsiella aerogenes		00.00	18.00		20.00		25.00
E-Coli		25.00	30.00		15.00		22.00
Pseudomonas aeruginosa		25.00	00.00		00.00		00.00
Salmonella		30.00	25.00		00.00		40.00
Shigella	20.00	30.00		20.00		40.00	
Positive(+) control streptomycin							
Negative(-) control	00.00		00.00		00.00		00.00

Key: ELE= Ethanol leaf extract, ERE= Ethanol root extract, EALE= Ethyl acetate leaf extract, EARE= Ethyl acetate root extract, MZI= mean zone of inhibition

Table 2: Result of Antifungal activity of *Combretumpaniculatum* crude extracts

Test	ELE		ERE		EALE	EARE
Organism	BR		BR	BR		BR
MZI MZI	MZI		MZI			
Vol.used	0.50	0.50		0.50		0.50
Candida albican	40.00	00.00	25.00		45.00	
Aspergillusniger	30.00	10.00		20.00		40.00

Key: ELE= Ethanol leaf extract, ERE= Ethanol root extract, EALE= Ethyl acetate leaf extract, EARE= Ethyl acetate root extract, MZI= mean zone of inhibition.

Table 3: Result of Minimum Inhibitory Concentration (MIC) Activity of Crude Extracts.

Presence or absence of					
Turbidity on test organism S.a		E.c	S.l	C.a	A.s
Ethanol leaf					
MIC mg/ml	0.25	0.0625	0.0625	0.0078	0.0313
MBC mg/ml	0.5	0.125	0.125	0.0156	0.0625
Ethylacetate root					
MIC mg/ml	0.125	0.0625	0.0156	0.0078	0.0156
MBC mg/ml	0.25	0.125	0.0313	0.0156	0.0313

Key: += no growth on subculture (MIC), -= no growth on subculture (MBC), ++= visible growth in media and control. Tube 9 = Media and Culture Control.S.a= Staphylococcus aureus, E.c= E.coli, S.l= Salmonella spp,C.a= Candida Albican, A.s=Aspergillusniger

Table 4: Result obtained from Gas Chromatographic-Mass Spectroscopic analysis of ethyl acetate leaf extract (EALE), of Combretumpaniculatum (C.P)

Peak	R.TIME	Area%	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)
1	68.086	1.88, 1.46, 1.851	Thiirane	C_2H_4S	60.114
2	69.650	1.18, 1.16	Urea	CH_4N_2O	60.056
3	74.443	3.89	1,5-Heptadiene	C_9H_{16}	124.227
4	75.318	1.39	Carbonyl sulfide	COS	60.07
5	77.301	1.47, 1.25, 1.31,2.17	Hydrazine	N_2H_4	32.046
6	78.084	1.25,1.25,1.20,3.56 ,2.93,4.33,1,36,3.0 5,1.51,&2.28	Propanamide	C_3H_7ON	73.095
7	79 .133	1.75,1.81,1.64,1.82 ,3.65,2.03,1.58,1.5 6,1.86,2.64,1.19,1. 97,2.45	Guanidine	CH_5N_3	59.072
8	80.124	1.28,2.66,3.36,1.98 ,1.45	Isobutylamine	$C_4H_{11}N$	73.139
9	86.785	2.65,1.31	Acetic acid	CH_3COOH	60.052
10	90.975	1.20,1.23	Hexanoic acid	$C_6H_{12}O_2$	117.152
11	92.674	1.61	Inositol	$C_6H_{12}O_6$	180.156
12	95.304	2.24	Ethane	C_2H_6	32.055
13	95.609	1.96	5-Chlorovaleric acid	$C_5H_9ClO_2$	136.575

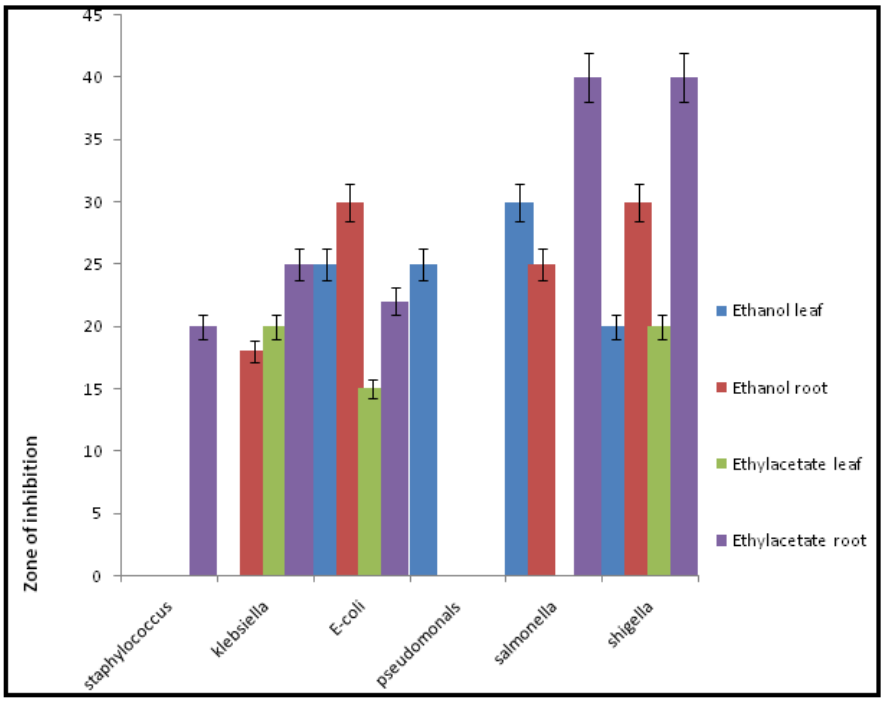

Figure 1: Histogram of anti-bacterial activity of Combretumpaniculatum extract

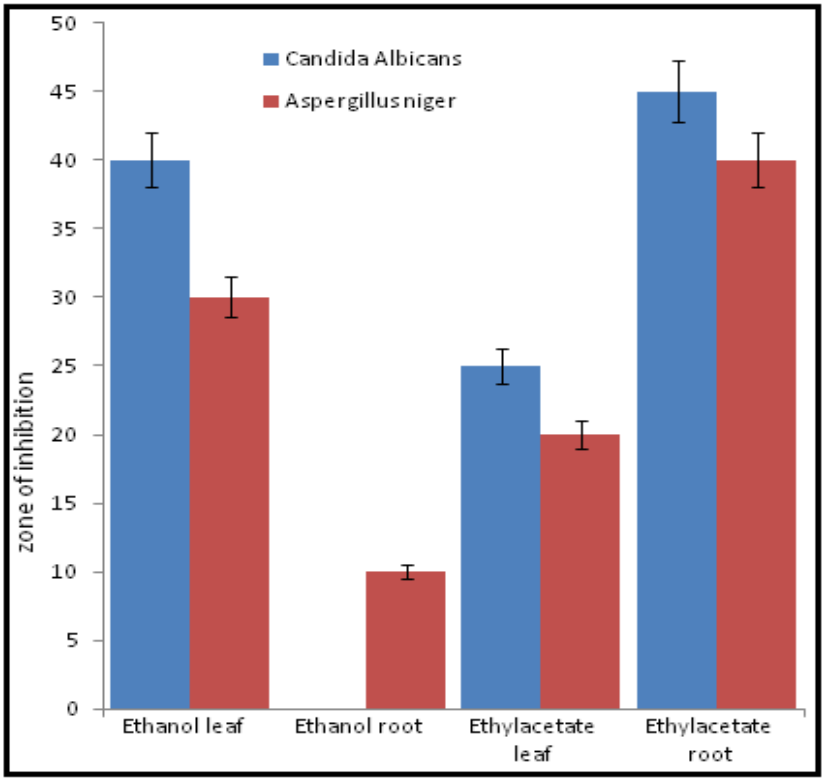

Figure 2: Histogram of antifungal activity of Combretumpaniculatum of crude extract

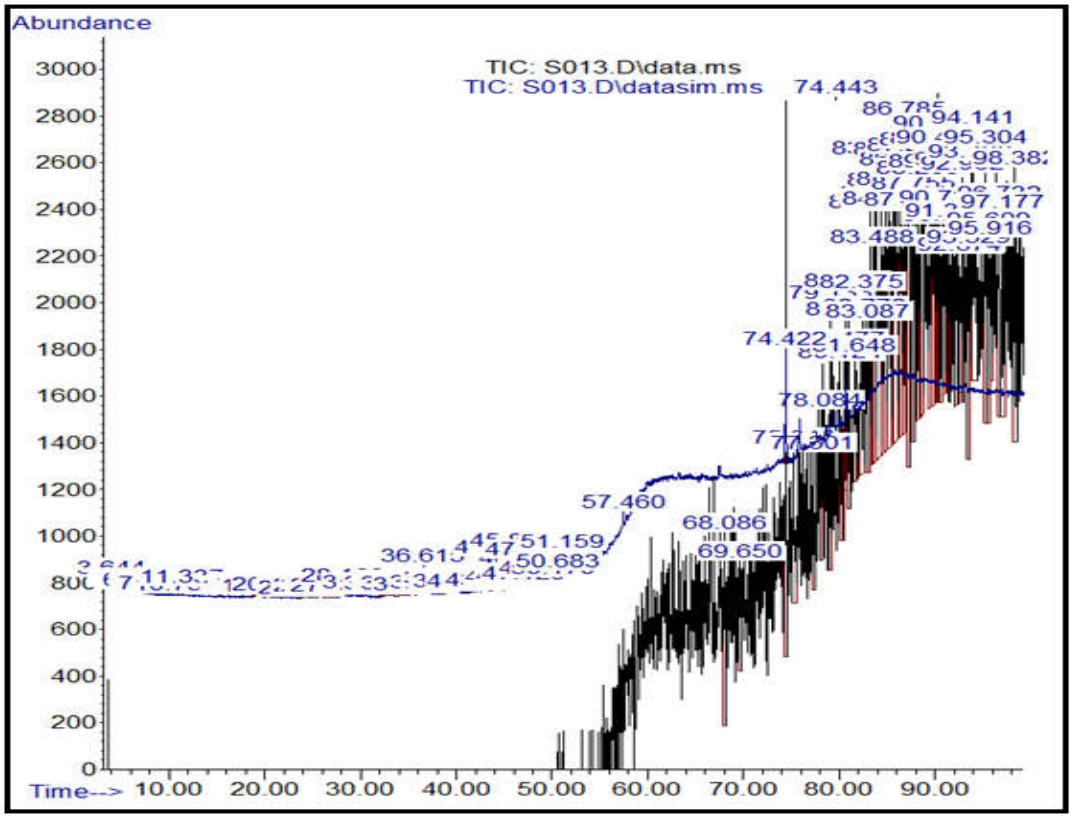

Figure 4: GC-MS spectrum of ethyl acetate root extract of Combretumpaniculatum

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