International Journal of Life science & Pharma Research

GC-MS ANALYSIS OF THE AQUEOUS EXTRACTS OF BUCHHOLZIA CORIACEA ENGL (CAPPARIDACEAE), SEEDS.

[1]OMOREGIE GLORIA OMOROWA, [2*]MACDONALD IDU AND [2]OVUAKPORIE-UVO OGHALE.

[1] Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria.
[2]Department of Plant Biology and Biotechnology, University of Benin, Benin, Edo, Nigeria.

ABSTRACT

Buchholzia coriacea E. is one of the medicinal plants of importance belonging to the family Capparidaceae. Investigations aimed at identifying and isolating the major chemical compounds in *Buchholzia coriacea* aqueous seed extracts using Gas chromatography-Mass spectrometry (GC-MS) method was carried out. Results showed that *Buchholzia coriacea* aqueous seed extract contains six (6) bioactive compounds namely; Phenol, 3,5-bis(1,1-dimethylethyl); Hexadecanoic acid, 15-methyl-, methyl ester; n-Hexadecanoic acid; Cyclopropaneoctanoic, 2-hexyl-, methyl-, methyl ester;(*Z*)-Docos-13-enoic acid and 9,12-Octadecadienoyl chloride (Z,Z)-. (*Z*)-Docos-13-enoic acid was the prevailing compound with Retention Time (RT) of 22.850 and a peak area of 76.63. Finding suggests that (*Z*)-Docos-13-enoic acid could be an anti-oxidant.

KEY WORDS: *Buchholzia coriacea,* Seed, Aqueous extract, GC-MS analysis.

INTRODUCTION

Herbal medicines already form the basis of therapeutic use in developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too (De N and Ifeoma E, 2002).Today natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action (Charles A et al 2011). Natural remedies from medicinal plants prove as safe and effective, as many plant species have been used in folklore medicine to treat various ailments (Balamurugan K et al, 2012). Medicinal plants are the richest bio-resources of folk medicines, traditional systems of medicine, food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs (Ncube NS et al, 2008). Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening (Boopathi AC and Sivakumar R, 2011). Traditional systems of medicines are prepared from a single plant or combinations of a number of plants. The natural constituents of plant are derived from any parts of plant like leaves, roots, bark, flowers, fruits and seeds i.e., active components can be present in any parts of the plant (Gordon MC and David JN, 2001).The most important bioactive secondary metabolites in plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga HO et al, 2005).These phyto-compounds from medicinal plants are important in pharmaceutical industry for drug development and preparation of therapeutic agents (Nishak et al, 2011). There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Turker AU and Usta C, 2008). Screening of active compounds from plants has lead to the invention of new medicinal drugs and they have an efficient protection against various diseases including cancer (Sheeja K and Kuttan G, 2007).

Buchholzia coriacea Engl (Capparidaceae), commonly referred to as musk tree is one of important medicinal plants named after R.W buchholz who first collected the plants in Cameroon in the late 19^{th} century (Keay RWJ, 1989). *B. coriacea* is a perennial, evergreen, under-storey tree of lowland rain forest with large, glossy, leathery leaves and conspicuous cream white flowers in racemes at the end of the branches (Adisa RA et al, 2010). Its local names include 'Uworo' (Yoruba), 'Owi' (Edo), 'Esson Bossi' (Central Africa), 'Uke' (Ibo) (Quattrochi-Umbeto FLS, 2007). The plant is easily recognized by the compound pinnate leaves and the long narrow angular fruits containing large, usually aligned seeds. The plant's fruit is about 5 inches long and 2-3 inches in diameter and resembles avocado pear, yellowish when ripe with a yellow flesh containing a few large, black seeds about 1 inch long (Koudogbo B et al, 1972). It has been reported to be used in the treatment of a variety of illnesses such as syphilis, gonorrhoea, and convulsion in children, earache, smallpox, headache, sinusitis, and nasal congestion (Burkill HM, 1985; Ajaiyeoba EO et al, 2001; 2003; Irvine FR, 1961; Ezekiel OO and Onyeoziri NF, 2009).

GC-MS is increasingly applied in recent years for medicinal plants analysis. Identification of pure compounds even at low concentration less than 1mg is made possible by gas chromatography mass spectrometry (Liebler DC et al, 1996).The combination of an ideal separation technique – Gas Chromatography (GC) with the best identification technique - Mass Spectrum (MS) made GC-MS, which is an ideal technique for qualitative and quantitative analysis of volatile and semi volatile compounds. This technique (GC-MS) has proved to be a valuable and important method in analyzing fatty acids, non-polar compounds, lipids, volatile essential oil and alkaloids (Betz JM et al, 1997; Sermankkani M and Thangapandian V, 2012).Taking into consideration the medicinal importance of *Buchholzia coriacea* E., the ethanol extract of the seeds were analyzed for the first time using GC-MS. The present study was aimed to identify the phytoconstituents which may be of therapeutic value present in the seed of *Buchholzia coriacea* using GC-MS analysis.

MATERIALS AND METHODS

Collection of plant sources

The seeds of *Buchholzia coriacea* were collected in the month of December 2012 from Benin City, Edo State. The seeds were authenticated by Professor MacDonald Idu of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State.

Preparation of plant extract

Seeds were cleaned, shade dried and pulverized to powder using a mechanical grinder. Required quantity of powder was weighed and transferred to stopper flask, and treated with distilled water until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days afterwards, the extract was filtered using a muslin cloth. The extract was collected and evaporated to dryness using vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

Gas chromatography-Mass spectrometry analysis

The Gas chromatography-Mass spectrometry (GC-MS) analysis of the extracts was performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm diameter, and 0.25mm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51ml/min. injector and mass transfer line temperature were set at 200 and 240°C respectively. The oven temperature was programmed from 70to 220°C at 10°C/min, held isothermal for 1min and finally raised to 300°C. AT 10°C/min. 2 ml of water solution of the samples was manually injected in the split less mode, with a split ratio of 1:40 and with mass scan of 50-600 amu. Total running time of GC-MS is 35 min. The relative percentage of each extract constituents were expressed as a percentage with peak area normalization. Interpretation of mass spectrum of plant extracts were conducted using the database of National Institute of Standard and Technology (NIST) library having more than 62,000 spectral patterns. The spectrum of the compounds was compared with the spectrum of National Institute of Standard and Technology (NIST) library database.

RESULTS AND DISCUSSION

The GC-MS chromatogram of aqueous seed extract of *B. coriacea* showed the presence of six (6) bioactive compounds (Figure 1).The interpretation of mass spectrum was done by National Institute standard and Technology (NIST) database that contained more than 62000 patterns. The X-axis represents the retention time of each compound identified in minutes while the Y-axis represents the intensity/ the presence of various compounds with a corresponding percentage of peaks area at different retention Active compounds of aqueous extract of *B. coriacea* seeds. Table 1 shows the name of the compounds, molecular formula, molecular weight, peak area %, retention time (RT),nature of compound and structure of identified compounds of seeds of *B. coriacea.* While Table 2 shows the reported biological activity of the identified compounds in the plant's aqueous seed extract.

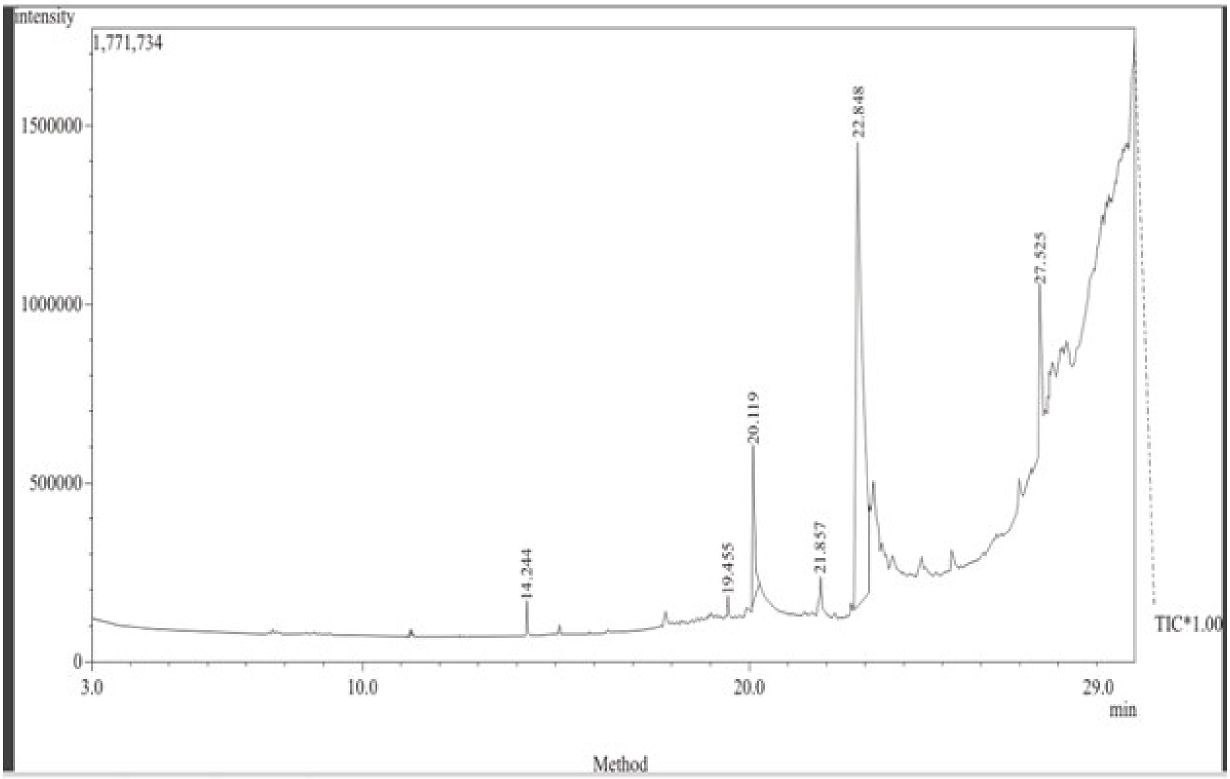


Figure 1
GC-SM Chromatogram of the aqueous extract of Bulchholzia coriacea seed

Table 1
List of identified phytocompounds of aqueous extract of Buchholzia coriaceaseed

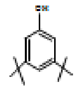
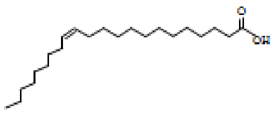
S/N	RT (min)	Name of compound	Molecular formula	Molecular weight	Peak area	Nature ofcompound	Structures
1	14.242	Phenol, 3,5-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	206	1.13	Ester	
2	19.455	Hexadecanohexaic acid, 15 methyl-methyl ester	$C_{18}H_{36}O_2$	284	0.69	Fatty acid ester	
3	20.119	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.60	Palmitic acid	
4	21.858	Cyclopropaneoctanoic, 2-hexyl-, methyl-, methyl ester	$C_{18}H_{34}O_2$	282	1.58	Ester	
5	22.850	(Z)-Docos-13-enoic acid	$C_{22}H_{42}O_2$	338	76.63	Fatty acid	
6	27.525	9,12-Octadecadienoyl chloride (Z,Z)-	C_{18} $H_{31}CLO$	298	10.37	Linoleic acid	

Table 2
Activity of phyto-components identified in the aqueous extracts of Bulchholzia coriacea seed using GC-MS method.

S/N	Name of compound	Activity
1	Phenol, 3,5-bis(1,1-dimethylethyl)	Antimicrobial, antifungal
2	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant,antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor
3	Hexadecanoic acid, 15-methyl-, methyl ester	Antioxidant
4	Cyclopropaneoctanoic, 2-hexyl-, methyl-, methyl ester	Antimicrobial, antifungal
5	(*Z*)-Docos-13-enoic acid	Antioxidant
6	9, 12-Octadecadienoyl chloride (Z,Z)-	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective,nematicide, insectifuge, antihistaminic, anti-eczemic, anti-acne, 5-alphareductase inhibitor, anti-androgenic,anti-arthritic, anti-coronary, insectifuge.

Gas chromatography combined with mass spectrometry (GC-MS) provides more precise information for qualitative analysis (Cong Z et al, 2007). Several phytochemical screening studies have been carried out in different parts of the world using GC-MS (Abirami P and Rajendran A, 2011;Gopalakrishnan S et al, 2011; Sangeetha J and Vijayalakshmi K, 2011; Vanitha V et al, 2011; Wu L et al, 1010;). Thus, there is a growing awareness in correlating the phytochemical compounds with their biological activities (Fernie AR et al, 2004; Robertson DG, 2005; Selvamangai G and Bhaskar A, 2012; Sumner LW et al, 2003).The compounds that were detected are Phenol, 3,5-bis(1,1-dimethylethyl), Hexadecanoic acid, 15-methyl-, methyl ester, n-Hexadecanoic acid, Cyclopropaneoctanoic, 2-hexyl-, methyl-, methyl ester, (*Z*)-Docos-13-enoic acid, 9,12 Octadecadienoyl chloride (Z,Z)- (Figure 1, Table 1). These possess antimicrobial, antifungal, antioxidant, antiflammatory, hypocholesterolemic, antiarthritic, anticoronary and antiandrogenic activities (Table 2).In a previous study, we confirmed that 9, 12, Octadecadienoic acid (ZZ)- has the property of anti-inflammatory and antiarthritic (Jones PJ 2002; Lalitharani S et al, 2009). n- Hexadecanoic acid has antioxidant, antimicrobial activities and larvicidal effect (Bodoprost J and Rosemeyer H, 2007; Falodun A et al, 2009).Thus *B. coriacea* seed is found to possess significant phytocompounds.

CONCLUSION

In conclusion, the seed of *B. coriacea* could be a potential source for useful drugs like antimicrobial, antifungal, antioxidant, antiflammatory, hypocholesterolemic, antiarthritic, anticoronary and antiandrogenic activities. These pharmacological activities provide inspiration for further investigation in the discovery of novel herbal drugs. This study may enhance the traditional usage however; further analysis is required for the pharmacological activity of specific compound of *B. coriacea*, which may lead to the development of novel drug for the treatment of specific disease. Thus the GC-MS analysis is the first step towards understanding the nature of active principles in *B. coriacea*.

REFERENCES

1. Abirami P. and Rajendran A. GC-MS determination of bioactive compounds of *Indigofera aspalathoides*. J. Nat Prod Plant Res. 2011; 1(4):126-130.
2. Adisa RA., Chouldhary MI. and Olorunsogo OO. Hypoglycemic activity of *Buchhozia coriacea* seeds in streptozotocin-induced diabetic rat and mice. Exp Toxicol Pathol. 2010;63(7-8): 619-625.
3. Ajaiyeoba EO., Onocha PA. and Olarenwaju OT. In-vitro antihelmintic properties of *Buchholzia coricea* and *Gynandropsis gynandra* extracts. Pharm. Bio. 2001; 39(3): 217-222.
4. Ajaiyeoba EO., Onocha PA., Nwozo SO. and Sama W. Antibacterial activities and preliminary phytochemical screening of four medicinal plants. J. Appl.Sci. 2003; 7(3): 4228-4338.
5. Balamurugan K., Nishanthini A. and Mohan VR. GC-MS analysis of *Polycarpaea corymbosa*(L.) Lam whole plant. Asian Pacific J. TropBiomed. 2012; S1289– S1292.
6. Betz JM., Gay ML., Mossoba MM., Adams S. and Portz BS. Chiral gas chromatographic determination of ephedrine-type alkaloids in dietary supplements containing Má Huáng.J. Assoc Analytical Chem Intl Official.1997; 80(2):303-15.
7. Bodoprost J. and Rosemeyer H. Analysis of phenacylester derivatives of fatty acids from human skin surface sebum by reversed-phase HPTLC: chromatographic mobility as a function of physico-chemical properties. Intl J. Mole Sci. 2007; 8:1111-1124.
8. Boopathi AC., Sivakumar R. Phytochemical screening studies on the leaves and stem of *Andrographis neesiana* wight – an endemic medicinal plant from India. World Appl. Sci. J.2011; 12(3):307-311.
9. Burkill HM. The useful plants of west tropical Africa. Vol. 1 Families A-D. Kew. Royal Botanic Garden. 1985: 960
10. Charles A., Leo Stanly A, Joseph M. and Alex Ramani V. GC-MS analysis of bioactivecomponents on the bark extract of *Alseodaphne semecarpifolia* Nees (Lauraceae). Asian J. Plant Sci Res.2011; 1(4):25–32.
11. Cong Z., Meiling QI., Qinglong S., Shan Z. and Ruonong Fu. Analysis of the volatile compounds in *Ligusticum chuanxiong* Hort using HS-SPME-GCMS. J. Pharm Biomed Analysis. 2007; 44: 464-470.
12. De N. and Ifeoma E. Antimicrobial effects of components of the bark extracts of neem (*Azadirachta indica* A. juss). J. Technol. Dev.2002; 8: 23-28.
13. Edeoga HO., Okwu DE.and Mbaebie BO.Phytochemical constituents of some Nigerian medicinal plants. Afr J. Biotechnl.2005; 4: 685-688.
14. Ezekiel OO. and Onyeoziri NF. Preliminary studies on the antimicrobial properties of *Buhholzia coricea* (Wonderful kola.) Afr. J. Biotechnol. 2009; 8(3):472-474.
15. Falodun A., Siraj R. and Choudhary MI. GC-MS analysis of insecticidal leaf essential oil of *Pyrenacantha staudtii* Hutch and Dalz (Icacinaceae). Trop.J.Pharm. Res.2009; 8:139-143.
16. Fernie AR., Trethewey RN., Krotzky AJ. and Willmitzer L. Innovation - Metabolite profiling: from diagnostics to system biology. Nature Reviews Molecular Cell Biology. 2004; 5:763–769.
17. Gopalakrishnan S., Saroja K. and Dulcy EJ. GC-MS analysis of the methanolic extract of the leaves of *Dipteracanthus patulus* (Jacq.) Nees. J. Chem Pharm Res. 2011; 3(3):477–480.
18. Gordon MC. and David JN. Natural product drug discovery in the next millennium. J. Pharm Biol. 2001; 20: 201-202.
19. Irvine FR. Woody plant of Ghana with special reference to their uses. London Oxford University Press. 1961:863.
20. Jones PJ. GC-MS Analysis of Ethanol Extract of *Wattakaka Volubilis*.Canadian Med. Assoc. Journal. 2002; 166:1555-1563.
21. Keay RWJ. Trees of Nigeria, Clarendon Press, Oxford. 1989:42-44.

22. Koudogbo B., Delaveau P. and Adjanohoun E. Study of an African Capparidaceae, *Buchholzia Coriacea* Engler. Ann Pharm Fr. 1972; 30(2):93-98.
23. Lalitharani S., Mohan VR., Regini GS. and Kalidass C.GC-MS analysis of ethanol extract of *Pathos scandens* L. leaf. J. Herb. Medi. Toxicol.200; 3: 159-160.
24. Liebler DC., Burr JA., Philips L. and Ham AJL. Gas chromatography - mass spectrometryanalysis of vitamin E and its oxidation products. Analyt Biochem. 1996; 236: 27-34.
25. Ncube NS., Afolayan AJ. and Okoh AI., Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. Afr J. Biotechl. 2008; 7:1797-1806.
26. Nisha K., Darshana M., Madhu G. and Bhupendra MK. GC-MS analysis and anti-microbialactivity of *Psidium guajava* (leaves) grown in Malva region of India. Intl J. Drug Dev Res. 2011; 3(4):237– 245.
27. Quattrochi-Umbeto FLS. CRC World Dictionary of Plant Names- Common Names, Scientific Names, Eponyms, Synonyms and Entomology. CRC Press, London. 2007: 367-368.
28. Robertson DG. Metabonomics in toxicology: A review. Toxicol Sci. 2005; 85:809–822.
29. Sangeetha J. and Vijayalakshmi K. Determination of bioactive components of ethyl acetate fraction of *Punica granatum* rind extract. Intl J. Pharm Sci Drug Res. 2011; 3(2):116– 122.
30. Selvamangai G. and Bhaskar A. GC-MS analysis of phytocomponents in the methanolic extract of *Eupatorium triplinerve.*Asian Pacific J. Trop Biomed. 2012; S1329–S1332.
31. Sermakkani M and Thangapandian V. GC-MS analysis of *Cassia italica* leaf methanol extract. Asian J. Pharm Clinic Res. 2012; 5(2): 90-94.
32. Sheeja K. and Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumour growth *in vivo* by *Andrographispaniculata* extract and andrographolide.Immunopharmacol Immunotoxicol. 2007; 29:81-93.
33. Sumner LW., Mendes P. and Dixon RA. Plant metabolomics: largescale phytochemistry in the functional genomics era. Phytochem. 2003; 62(6):817–836.
34. Turker AU. and Usta C. Biological screening of some Turkish medicinal plants for antimicrobial and toxicity studies. J. Nat Prod. 2008; 22:136-146.
35. Vanitha V., Umadevi KJ.and Vijayalakshmi K. Determination of bioactive components of *Annona squamosa* L leaf by GC- MS analysis. Intl J. Pharm Sci Drug Res. 2011; 3(4):309–312.
36. Wu L., Gao H., Wang X., Ye J., Lu J. and Liang Y. Analysis of chemical composition of *Chrysanthemum indicum* flowers by GC/MS and HPLC. J Med. Plants Res. 2010; 4(5):421– 426.