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

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ABSTRACT

Buchholzia coriacea 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 19th 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; Sermankkami 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 70°C to 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](image_url)  
*GC-MS Chromatogram of the aqueous extract of Bulchholzia coriacea seed*
Table 1

**List of identified phytocompounds of aqueous extract of Buchholzia coriacea seed**

<table>
<thead>
<tr>
<th>S/N</th>
<th>RT (min)</th>
<th>Name of compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area</th>
<th>Nature of compound</th>
<th>Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.242</td>
<td>Phenol, 3,5-bis (1,1-dimethylbutyl)</td>
<td>C_{14}H_{22}O</td>
<td>206</td>
<td>1.13</td>
<td>Ester</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>2</td>
<td>19.435</td>
<td>Hexadecanohexa acid, 15 methyl-methyl ester</td>
<td>C_{18}H_{36}O_{2}</td>
<td>284</td>
<td>0.69</td>
<td>Fatty acid ester</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>3</td>
<td>20.119</td>
<td>n-Hexadecanoic acid</td>
<td>C_{16}H_{32}O_{2}</td>
<td>256</td>
<td>9.60</td>
<td>Palmitic acid</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>4</td>
<td>21.858</td>
<td>Cyclopropaneoctanoic, 2-hexyl-, methyl-, methyl ester</td>
<td>C_{16}H_{34}O_{2}</td>
<td>282</td>
<td>1.58</td>
<td>Ester</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
<tr>
<td>5</td>
<td>22.850</td>
<td>(Z)-Docos-13-enoic acid</td>
<td>C_{22}H_{42}O_{2}</td>
<td>338</td>
<td>76.63</td>
<td>Fatty acid</td>
<td><img src="image5" alt="Structure" /></td>
</tr>
<tr>
<td>6</td>
<td>27.525</td>
<td>9,12-Octadecadienyl chloride (Z,Z)</td>
<td>C_{18}H_{36}ClO</td>
<td>298</td>
<td>10.37</td>
<td>Linoleic acid</td>
<td><img src="image6" alt="Structure" /></td>
</tr>
</tbody>
</table>

Table 2

**Activity of phyto-components identified in the aqueous extracts of Buchholzia coriacea seed using GC-MS method.**

<table>
<thead>
<tr>
<th>S/N</th>
<th>Name of compound</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phenol, 3,5-bis(1,1-dimethylbutyl)</td>
<td><strong>Antimicrobial, antifungal</strong></td>
</tr>
<tr>
<td>2</td>
<td>n-Hexadecanoic acid</td>
<td><strong>Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor</strong></td>
</tr>
<tr>
<td>3</td>
<td>Hexadecanoic acid, 15-methyl-, methyl ester</td>
<td><strong>Antioxidant</strong></td>
</tr>
<tr>
<td>4</td>
<td>Cyclopropaneoctanoic, 2-hexyl-, methyl-, methyl ester</td>
<td><strong>Antimicrobial, antifungal</strong></td>
</tr>
<tr>
<td>5</td>
<td>(Z)-Docos-13-enoic acid</td>
<td><strong>Antioxidant</strong></td>
</tr>
<tr>
<td>6</td>
<td>9,12-Octadecadienoic chloride (Z,Z)</td>
<td><strong>Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, anti-eczema, anti-acne, 5-alpha reductase inhibitor, anti-androgenic, anti-arthritis, anti-cornary, insectifuge</strong></td>
</tr>
</tbody>
</table>
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, hypcholesterolemic, antiarthritic, antioconorany and antiandrogenic activities (Table 2). In a previous study, we confirmed that 9, 12, Octadecadienoyl 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 (Bodopro 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 antimicrobrial, antifungal, antioxidant, antiinflammatory, hypcholesterolemic, antiarthritic, antioconorany 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.

REFERENCE

8. Boopathi AC., Sivakumar R. Phytochemical screening studies on the leaves and stem of


