Antioxidant

Taiwo Olayemi Elufioye, Olubunmi O. Mada

INTRODUCTION

Herbal medicine predates the other forms of health care used by humans and it has evolved alongside development of modern civilization. Herbal medicines in most developing countries have played a central role in health care since time immemorial. In most developing countries, herbs rather than drugs are often used in health care. Herbal medicines are 'finished, labeled medicinal products that contain as active ingredients, above ground or underground parts of plants or other plant materials, or combinations thereof, whether in the crude state or as plant preparations. Plant materials in this case include juices, gums, resins, fatty oils, essential oils and any other substances of this nature. Herbal formulations in several dosage forms have been claimed to be very beneficial to human health. They are known to be effective in improving blood circulation, purifying the kidney and reducing the development of kidney stones. Other benefits include improving digestion, reducing blood pressure, assisting in the elimination of bad cholesterol, preventing the development of diabetes and improving the immune system and memory. Bitters are traditionally alcoholic preparations flavoured with botanical matter such that the end result is characterized by a bitter, sour, or bittersweet flavor. Medicinal herbal bitters contain blended ingredients in a water or alcohol (tincture) base. Originally sold as a digestive aid because of their ability to increase the production of saliva and digestive juices, bitters became popular in Europe in the 1600s. They generally have been reported to prevent kidney and bladder infections, help to regulate blood pressure and dilate arteries, facilitate digestion, prevent disorders like ulcers, gastritis, insomnia, stress and depression and prevent overweight and excess body fat. Phytochemical analysis has shown that bitters contain complex carbohydrate, alkaloids, vitamins and minerals that have antioxidant, antiviral and antispasmodic properties. It has also been shown that these ingredients work together to reduce inflammation, control pain,
RELAX MUSCLES AND IMPROVE DIGESTION AND ELIMINATION. BITTERS CAN ALSO BE EFFECTIVE AS APPETITE STIMULANT.

PAX HERBAL BITTERS IS A TINCTURE OF DIFFERENT HERBAL INGREDIENTS AND HAS NET VOLUME OF 190 mL, 6.42 FL; CHARACTERIZED BY DARK BROWNISH COLOUR WITH STRONG BITTER TASTE, AROMATIC ODOUR AND 100% MOISTURE CONTENT. THE BITTER, ACCORDING TO MANUFACTURER’S CLAIM, WAS FORMULATED TO PROMOTE BLOOD CIRCULATION, PREVENTS KIDNEY STONES ASSOCIATED TO DIGESTION, ACTIVATES BILE FLOW, AND INCREASES IMMUNITY OF THE BODY AGAINST BACTERIA AND FUNGI INFECTIONS. THIS PRODUCT, THOUGH WIDELY ACCEPTED AND USED HAS NO REPORT ON ITS CHEMICAL CHARACTERIZATION. IN THIS STUDY, WE PROFILED THE METABOLITES PRESENT IN DIFFERENT EXTRACTS OF PAX HERBAL WITH THE VIEW TO CORRELATING THEM WITH ITS CLAIMED PHARMACOLOGICAL ACTIONS.

MATERIALS AND METHODS

EXTRACTION

The Pax Herbal bitters® syrup (10 x 190 mL) were exhaustively extracted with n-hexane, dichloromethane (DCM) and methanol separately for 24 h. The different extracts were concentrated at 40°C using Rotary evaporator and stored for subsequent analysis.

QUALITATIVE PHYTOCHEMICAL STUDY

Analysis for various phytoconstituents in the formulation was carried using standard method.13 The presence of alkaloids, saponins, flavonoids, cardiac glycosides, and tannins were evaluated.

DPPH ANTIOXIDANT ASSAY

The radical scavenging ability of the bitters (crude) as well as DCM and methanolic extracts were determined using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate).10

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

The total phenolic content of the bitters, DCM and methanolic extracts were determined using the folin-ciocalteu’s phenol reagent.11

DETERMINATION OF TOTAL FLAVONOIDS CONTENT (TFC)

This was carried out based on the aluminium chloride colorimetric assay method.12 Quercetin at varying concentrations was used as standard.

GC-MS ANALYSIS OF N-HEXANE FRACTION

GC – MS analysis was carried out using GC – MSD 5975 Agilent instrument. Column thickness, length, and internal diameter were 0.25 μm, 30 meters and 0.32 mm respectively. Helium was used as carrier gas at a flow rate of 10 mL/min. The column temperature was initially kept at 80°C and increased to 290°C at a rate of 10°C /min. The injector temperature was 250°C and split ratio was adjusted at 1:100. The injection volume was 2 μL in ethyl acetate and detector was Mass Selective Detector. The relative percentage peak area of each compound was calculated by dividing its average peak area with the total area of all compounds present. Detected peaks were interpreted by comparing with the National Institute of Standards and Technology (NIST) library data (Ver.2.0-Year 2005) to ascertain the names and molecular weights of the components of the test samples.13

FTIR ANALYSIS

FTIR analysis of the various extracts was done on Perkin Elmer Spectrophotometer system in the mid IR region of 400-4000 cm⁻¹ with 16 scan speeds.14

UV-VIS SPECTROSCOPY ANALYSIS

UV-Visible spectra for various extracts was performed on a PerkinElmer lambda 25 UV-VIS spectrophotometer equipped with 1.0 cm quartz cells.14

RESULTS

PERCENTAGE YIELD

The solvent-solvent extraction gave a yield of 0.0579 % (w/v) for n-Hexane extract, 0.08 % (w/v) for DCM, 0.0784 % (w/v) for DCM residue, 0.237 % (w/v) for methanol extract and 0.0353 % (w/v) for aqueous (Table 1).

PHYTOCHEMICAL SCREENING

The result from the qualitative phytochemical screening carried out on the crude bitter is as presented in Table 2.

DPPH RADICAL SCAVENGING ANTIOXIDANT ASSAY

The DPPH radical scavenging antioxidant activity result indicating the highest activity in the DCM extract is represented in Figure 1.

TOTAL PHENOLIC CONTENT (TPC)

The Total phenolic composition of the extracts is shown in Figure 2. Methanol extract had highest phenolic content of 20 mg/g followed by DCM extract (5.39 mg/g) and crude extract (4.43 mg/g) using Garlic as the standard.

TOTAL FLAVONOIDS CONTENT (TFC)

Total flavonoids content is represented in Figure 3.

GC-MS

The GC-MS analysis of the hexane fraction showing various peaks is presented in Figure 4 with the interpretation when compared with a standard library data shown in Table 3. The correlation of some of the identified compounds with reported biological activities is also presented in Table 4.

FTIR ANALYSIS

The FTIR peaks of the various samples including the crude drug, methanol, hexane, DCM extracts and DCM residue are presented in Figures 6-10 while the identified functional groups are reported in Table 5.

UV ANALYSIS

The UV spectra of the hexane, DCM and crude extracts are as shown in Figures 11-13.

DISCUSSION

BITTERS ARE COMPOSED OF COMPLEX MIXTURE OF COMPOUNDS WITH A WIDE RANGE OF MOLECULAR STRUCTURES. THIS IS BECAUSE BITTERS ARE USUALLY MADE AS AQUEOUS OR ALCOHOLIC EXTRACTS OF SEVERAL MEDICINAL PLANTS. THIS COMPLEXITY MAKES COMPLETE CHARACTERIZATION OF THE CHEMICAL COMPOSITION DIFFICULT. NOTWITHSTANDING, THE COMPOSITIONAL DATA OF BITTERS IS IMPORTANT FOR A BETTER UNDERSTANDING OF THE VERY MANY PHARMACOLOGICAL CLAIMS ATTRIBUTED TO THEM. FEW STUDIES HAVE CONTRIBUTED TOWARD THE CURRENT KNOWLEDGE OF MEDICINAL BITTERS. SOME OF THESE INVOLVE ASSESSMENT OF TOXICITY, EFFICACY, QUALITY EVALUATION, PHYSICOCHEMICAL ANALYSIS, FORMULATION STUDIES, AND EFFECT ON BIOCHEMICAL PARAMETERS. THERE ARE ONLY FEW CHARACTERIZATION STUDIES FOR INSTANCE YOYO BITTERS.

Table 1: Percentage (%) yield of various extracts/fractions of Pax herbal bitters.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight (g)</th>
<th>(w/v) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>1.10</td>
<td>0.0579</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>1.52</td>
<td>0.08</td>
</tr>
<tr>
<td>DCM residue</td>
<td>1.49</td>
<td>0.0784</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.51</td>
<td>0.237</td>
</tr>
<tr>
<td>Aqueous</td>
<td>0.67</td>
<td>0.0353</td>
</tr>
</tbody>
</table>
Table 2: Phytochemical screening of crude bitters.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff</td>
<td>Formation of reddish black precipitate indicates the presence of alkaloids.</td>
<td>present</td>
</tr>
<tr>
<td></td>
<td>Wagner</td>
<td>The formation of brownish precipitate indicates the presence of alkaloids.</td>
<td>present</td>
</tr>
<tr>
<td></td>
<td>Mayer</td>
<td>The formation of a white creamy precipitate shows and indicates presence of alkaloids.</td>
<td>present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>General test</td>
<td>The presence of pinkish red colour that was developed within 2-3 min.</td>
<td>present</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>Formation of yellow color precipitate indicates the presence of flavonoids.</td>
<td>present</td>
</tr>
<tr>
<td></td>
<td>Alkaline Reagent test</td>
<td>Formation of intense yellow colour, which becomes colourless on addition of dilute acid.</td>
<td>Present</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Borntrager's</td>
<td>Formation of rose-pink color in the ammoniacal layer</td>
<td>Present</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Keller-Kili test</td>
<td>Formation of a purple, reddish brown, brown ring at the interface and green colour in the acetic layer.</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>Persistence of the foam formed for about ten min.</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Froth Test</td>
<td>Formation of more than 1cm layer of foam which stood for more than 5 min.</td>
<td>Present</td>
</tr>
<tr>
<td>Steroids/Terpenes</td>
<td>Salkowski's Test</td>
<td>The presence of a golden yellow colouration.</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Liebermann-burchard test</td>
<td>The appearance of brown ring at the interphase.</td>
<td>Present</td>
</tr>
<tr>
<td>Tannins</td>
<td>Lead acetate test</td>
<td>The presence of white precipitate.</td>
<td>present</td>
</tr>
<tr>
<td>Phenols</td>
<td>Ferric chloride test</td>
<td>Formation of bluish black colour.</td>
<td>Present</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Borntrager test</td>
<td>Formation of a rose-pink colouration in the aqueous layer prior to the addition of 10% ammonia solution.</td>
<td>present</td>
</tr>
</tbody>
</table>

Figure 1: DPPH radical scavenging activity of various fractions of Pax herbal bitters.

Figure 2: Total phenolic content (TPC).

Figure 3: Total Flavonoid Content (TFC).

Figure 4: GC-MS peaks of n-hexane fraction.
Table 3: Names of chemicals, molecular formula and peak area of compounds from n-Hexane fraction.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time (RT) min</th>
<th>Name of compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Area of compound</th>
<th>% peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.570</td>
<td>(9-Oxabicyclo[3.3.1]non-6-en-3-yl) Methanol Cyclohexanol</td>
<td>C₈H₁₂O</td>
<td>124.18</td>
<td>159246</td>
<td>0.327</td>
</tr>
<tr>
<td>2</td>
<td>5.948</td>
<td>Isoborneol</td>
<td>C₁₀H₁₈O</td>
<td>154.25</td>
<td>295990</td>
<td>4.540</td>
</tr>
<tr>
<td>3</td>
<td>6.200</td>
<td>Terpinen-4-ol</td>
<td>C₁₅H₂₄O</td>
<td>206.32</td>
<td>2213568</td>
<td>1.576</td>
</tr>
<tr>
<td>5</td>
<td>11.79</td>
<td>2,4-Di-tert-butylphenol</td>
<td></td>
<td></td>
<td>2245823</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>12.334</td>
<td>Cyclohexanemethanol</td>
<td>C₁₅H₂₄O</td>
<td>114.19</td>
<td>2892249</td>
<td>5.932</td>
</tr>
<tr>
<td>7</td>
<td>12.419</td>
<td>(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol</td>
<td>C₁₅H₂₅O</td>
<td>204.34</td>
<td>2315301</td>
<td>4.748</td>
</tr>
<tr>
<td>8</td>
<td>12.797</td>
<td>(1αR,4S,7αS,7βR)-1,1,7-Tetramethyl-1α,2,3,4,6,7α,7β-octahydro-1H Cycloprop[e]azulen-7-ol</td>
<td>C₁₅H₂₅O</td>
<td>220.00</td>
<td>1368379</td>
<td>2.086</td>
</tr>
<tr>
<td>9</td>
<td>13.318</td>
<td>(1αR,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene</td>
<td>C₁₅H₂₅O</td>
<td>204.35</td>
<td>4548381</td>
<td>9.328</td>
</tr>
<tr>
<td>10</td>
<td>13.569</td>
<td>Isopathulenol</td>
<td>C₁₅H₂₅O</td>
<td>220.35</td>
<td>9771826</td>
<td>20.041</td>
</tr>
<tr>
<td>11</td>
<td>13.804</td>
<td>(1S,4αR,7R)-1-4α-Dimethyl-7-(prop-1-en-2-yl)-1,2,3,4,4α,5,6,7-octahydreronaphthalene</td>
<td>C₁₅H₂₅O</td>
<td>204.35</td>
<td>1042707</td>
<td>2.138</td>
</tr>
<tr>
<td>12</td>
<td>13.896</td>
<td>Alloaromadendrene</td>
<td>C₁₅H₂₅O</td>
<td>204.35</td>
<td>690036</td>
<td>1.415</td>
</tr>
<tr>
<td>13</td>
<td>14.411</td>
<td>Ledene alcohol</td>
<td>C₁₅H₂₂O</td>
<td>220.35</td>
<td>2267111</td>
<td>4.650</td>
</tr>
<tr>
<td>14</td>
<td>15.921</td>
<td>p-Heptylacetophenone</td>
<td>C₁₅H₂₂O</td>
<td>218.34</td>
<td>4600378</td>
<td>9.345</td>
</tr>
<tr>
<td>15</td>
<td>18.027</td>
<td>2,4-Decadienamide, N-isobutyl -, (E,E)-</td>
<td>C₁₅H₂₅NO</td>
<td>223.35</td>
<td>6190676</td>
<td>12.696</td>
</tr>
<tr>
<td>16</td>
<td>22.398</td>
<td>Shogaol</td>
<td>C₁₇H₂₄O₃</td>
<td>276.38</td>
<td>7388812</td>
<td>15.154</td>
</tr>
</tbody>
</table>

Table 4: Correlation of identified compounds with reported biological activity.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Name of the compound</th>
<th>Biological activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(9-Oxabicyclo[3.3.1]non-6-en-3-yl) Methanol Cyclohexanol</td>
<td>No yet reported</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Isoborneol</td>
<td>Antioxidant, neuroprotective</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Terpinen-4-ol</td>
<td>Anti-inflammatory, antifungal, antibacterial</td>
<td>16, 17</td>
</tr>
<tr>
<td>5</td>
<td>2,4-Di-tert-butylphenol</td>
<td>Antioxidant, antifungal</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cyclohexanemethanol</td>
<td>Anti-inflammatory, antiviral</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol</td>
<td>Anti-inflammatory, neuroprotective, antidepressant, anti-alcoholism</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1H-Cycloprop[e]azulen-7-ol</td>
<td>Immunoinhibitory</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene</td>
<td>Anti-inflammatory, neuroprotective, antidepressant, anti-alcoholism</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Isopathulenol</td>
<td>Immunoinhibitory</td>
<td>18</td>
</tr>
<tr>
<td>11</td>
<td>Naphthalene, 1,2,3,4,4α,5,6,8α-octahydro-4α,8-dimethyl-2-(1-methylethenyl)-, [2R(2α,4α,α,α,8α,βa.)]-</td>
<td>Antifungal</td>
<td>19</td>
</tr>
<tr>
<td>12</td>
<td>Alloaromadendrene</td>
<td>Antioxidant, anti-aging, antimicrobial agent</td>
<td>15, 12</td>
</tr>
<tr>
<td>13</td>
<td>Ledene alcohol</td>
<td>Antioxidant, antifungal</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>p-Heptylacetophenone</td>
<td>Anti allergic</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2,4-Decadienamide, N-isobutyl -, (E,E)-</td>
<td>Antioxidant and protection agent</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Shogaol</td>
<td>Anticough, induce apoptosis, memory enhancer</td>
<td>21, 22</td>
</tr>
</tbody>
</table>
Figure 5: Mass spectra of some of the identified compounds from Paxherbal Bitter.

Figure 6: FTIR of Crude extract.

Figure 7: FTIR peaks of methanolic fraction.

Figure 8: FTIR peaks of n-Hexane fraction.

Figure 9: FTIR from DCM Residue.

Figure 10: FTIR peaks of DCM fraction.

Table 5: FTIR peak values and functional groups of crude extract of Pax herbal bitters.

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3399.00</td>
<td>Alcohols, phenols, carboxylic acid</td>
</tr>
<tr>
<td>2937.14</td>
<td>Alkane, aldehyde</td>
</tr>
<tr>
<td>2371.42</td>
<td>Unknown</td>
</tr>
<tr>
<td>1617.33</td>
<td>Amine, amide, alkene</td>
</tr>
<tr>
<td>1446.76</td>
<td>Alkane, Nitro</td>
</tr>
<tr>
<td>1364.45</td>
<td>Nitro, alkane, fluoride</td>
</tr>
<tr>
<td>1269.91</td>
<td>Esters, ethers, anhydride, alcohol, carboxylic acids</td>
</tr>
<tr>
<td>1066.29</td>
<td>Esters, ethers, cyanide</td>
</tr>
<tr>
<td>592.00</td>
<td>Alkyl halide</td>
</tr>
<tr>
<td>469.66</td>
<td>Alkyl halide</td>
</tr>
</tbody>
</table>
In this research, we profiled the metabolites in Pax herbal bitters using three different spectroscopic methods. Samples used for analysis were purchased from pharmacies in Ibadan, South western Nigeria.

Qualitative phytochemical screening of crude Pax herbal bitters indicated the presence of flavonoids, saponins, alkaloids and steroids while phenols, tannins, anthraquinone, glycosides and terpenoids were moderately present. Alkaloids have pharmacological applications as anesthetics and CNS stimulants, antimicrobial, anticancer, memory enhancing, and many others. Antioxidants and free radical scavengers have been attributed to flavonoids. Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals to combat cancer, as an anti-inflammatory agent and to prevent heart ailments to an appreciable degree. Terpenoids and steroids are a large and diverse class of naturally occurring chemicals found in all classes of living organisms. Glycosides are...

### Table 6: FTIR peak values and functional groups of methanolic extract of Pax herbal bitters.

<table>
<thead>
<tr>
<th>Peak values</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3407.00</td>
<td>Amine, amide, carboxylic acid</td>
</tr>
<tr>
<td>2937.14</td>
<td>Alkane stretch, aldehyde</td>
</tr>
<tr>
<td>2337.14</td>
<td>Unknown</td>
</tr>
<tr>
<td>1629.00</td>
<td>Alkene (C=O), amide (C=O)</td>
</tr>
<tr>
<td>1446.15</td>
<td>Alkanes (CH₃)</td>
</tr>
<tr>
<td>1393.00</td>
<td>Alkanes (CH₂)</td>
</tr>
<tr>
<td>1273.31</td>
<td>Amine</td>
</tr>
<tr>
<td>1021.53</td>
<td>Amine</td>
</tr>
<tr>
<td>694.61</td>
<td>C-X, Aromatic alkane out of plane</td>
</tr>
<tr>
<td>570.59</td>
<td>Alkyl halide (C-X (X= Br, I))</td>
</tr>
</tbody>
</table>

### Table 7: FTIR peak values and functional groups of DCM extract of Pax herbal bitters.

<table>
<thead>
<tr>
<th>Peaks values</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3423.00</td>
<td>Amine, amide, alcohol including phenol</td>
</tr>
<tr>
<td>2930.92</td>
<td>Alkane stretch</td>
</tr>
<tr>
<td>1705.91</td>
<td>Ketone, C=O (COOH), carboxylic acid</td>
</tr>
<tr>
<td>1610.67</td>
<td>Non-acid carbonyl</td>
</tr>
<tr>
<td>1511.91</td>
<td>Nitro</td>
</tr>
<tr>
<td>1454.49</td>
<td>Aromatic alkane, Nitro (R-NO₂), N=O</td>
</tr>
<tr>
<td>1370.20</td>
<td>Nitro, Alkane</td>
</tr>
<tr>
<td>1262.16</td>
<td>C-X (X=F), S=O</td>
</tr>
<tr>
<td>1162.17</td>
<td>Alcohol, ethers, esters, anhydride</td>
</tr>
<tr>
<td>1122.27</td>
<td>Amines, alcohol, esters, anhydride</td>
</tr>
<tr>
<td>1035.39</td>
<td>Alcohol</td>
</tr>
<tr>
<td>625.63</td>
<td>Alkyl halide</td>
</tr>
<tr>
<td>566.50</td>
<td>Alkyl halide</td>
</tr>
</tbody>
</table>

### Table 8: FTIR peak values and functional groups of DCM residue of Pax herbal bitters.

<table>
<thead>
<tr>
<th>Peak values</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3396.21</td>
<td>Alcohols, phenols, carboxylic acids, primary and secondary amine</td>
</tr>
<tr>
<td>2931.14</td>
<td>Alkane, aldehyde</td>
</tr>
<tr>
<td>1621.53</td>
<td>Alkene, amide</td>
</tr>
<tr>
<td>1444.30</td>
<td>Alkane, Nitro</td>
</tr>
<tr>
<td>1363.60</td>
<td>Nitro, sulfones, sulfonyl chloride</td>
</tr>
<tr>
<td>1270.46</td>
<td>Esters, ethers, anhydride, alcohol</td>
</tr>
<tr>
<td>1076.51</td>
<td>Alcohol, amines, esters, ethers</td>
</tr>
<tr>
<td>601.63</td>
<td>Alkyl halide</td>
</tr>
<tr>
<td>404.14</td>
<td>Alkyl halide</td>
</tr>
</tbody>
</table>
Elufioye and Mada.: Chemical analysis and antioxidant activity of Pax herbal bitters

Table 9: FTIR peak values and functional groups of n-Hexane fraction of pax herbal bitters.

<table>
<thead>
<tr>
<th>Peak values</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3375.00</td>
<td>Alcohol, phenol, carboxylic acid</td>
</tr>
<tr>
<td>2933.00</td>
<td>Alkane, aldehyde</td>
</tr>
<tr>
<td>2360.00</td>
<td>Unknown</td>
</tr>
<tr>
<td>2045.71</td>
<td>Alkyne, nitriles</td>
</tr>
<tr>
<td>1711.75</td>
<td>Ketone, carboxylic acid</td>
</tr>
<tr>
<td>1504.14</td>
<td>Nitro, aromatic</td>
</tr>
<tr>
<td>1454.93</td>
<td>Alkane, Nitro</td>
</tr>
<tr>
<td>1373.05</td>
<td>Nitro, alkane, fluoride</td>
</tr>
<tr>
<td>1246.60</td>
<td>Esters, ether, alcohol, anhydride</td>
</tr>
<tr>
<td>1156.47</td>
<td>Cyanide, esters, ether</td>
</tr>
<tr>
<td>1036.76</td>
<td>Esters, ether</td>
</tr>
<tr>
<td>939.69</td>
<td>Alkyl halide</td>
</tr>
<tr>
<td>606.60</td>
<td>Alkyl halide</td>
</tr>
</tbody>
</table>

FTIR spectroscopy is a useful method for obtaining information on the chemical nature of natural product mixture. It detects the vibrational frequencies and intensities of individual functional groups of the components in the mixture with high sensitivity and time resolution and permit quantification of specific classes of dissolved organic matters including aromatic and aliphatic organic compound containing oxygen, nitrogen, and sulfur functional groups. The use of FTIR spectral fingerprinting for herbal preparation tends to focus on the identification and assessment of the stability of the functional groups in chemical constituents. The results of the FTIR spectrum of n-Hexane extract of Pax Herbal Bitters is shown in Tables 5-9. About fourteen areas were identified in the mid infrared (MID) domain and the fingerprint region. The FTIR confirmed the presence of alcohols, phenols, alkanes, alkenes, alkyl halide, aldehydes, esters, carboxylic acids, aromatics, nitro compounds and amines in all the extracts. FTIR spectroscopy has been proven to be reliable and sensitive method for detection of biomolecular composition and can assist the manufacturer in controlling and ensuring the consistency and quality standard of products. UV absorbance has been shown to be useful in estimating dissolved aromatic carbon content for instance the phenolic hydroxyl groups in a sample. The qualitative UV spectrum profile of crude, n-Hexane and DCM extracts of PaxHerbal Bitters in Figures 11 to 13 was selected from a wavelength 190 to 900 nm of both UV and VIS region. The various peaks as seen above also contributed to structural elucidation of compounds which may be present in the polyherbal mixture.

CONCLUSION

The herbal bitters studied revealed the presence of several chemicals with reported biological activity which may be contributing to the pharmacological claims of the product.

ACKNOWLEDGEMENT

We would like to acknowledge the National Institute of Technology for providing the library data that enabled the identification of the chemical constituents in the samples.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS

DCM: Dichloromethane; DPPH: 2, 2-diphenyl-1-picrylhydrazyl hydrate; TPC: Total phenolic content; TFC: Total flavonoids content; GC–MS: Gas Chromatography–Mass Spectroscopy; FTIR: Fourier-transform infrared spectroscopy; UV: Ultra-violent.

REFERENCES

12. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging drugs used in the treatment of heart diseases and are found as secondary metabolites in several medicinal plants. Qualitative analysis of the samples revealed highest phenolic in the methanol (20 mg/g) followed by DCM extract (5.39 mg/g) while flavonoids were found to be in almost equal amount DCM and methanolic extract (2.548 mg/g). This result supports previous report that bitters contain flavonoids, phenols and polyphenols which are believed to be responsible for antioxidants activity. Flavonoids are polyphenols found mostly in fruits, vegetables and certain beverages that have diverse beneficial effects. DPPH radical scavenging antioxidant activity determined was also found to be higher in the DCM extract compare to methanolic and the crude extracts thus corroborating the antioxidant potentials of flavonoids in bitters.

A combination of GC-MS, FTIR and UV spectroscopic methods was used to characterize the various fractions of the bitters. Over the past years, many highly accurate and sensitive methods for the analysis of complex mixtures of compounds have been developed. However, GC-MS covers relatively larger classes of compounds.

GC-MS analysis of n-Hexane fraction revealed sixteen compounds thirteen of which has been reported with biological activities while three have not been linked to any biological activity as shown in Tables 3 and 4. The GC-MS spectra of compounds identified from n-Hexane fraction showing the retention time and peak area of the various compounds are shown in Figure 4. A typical mass spectrum of some of the chemicals obtained after matching with data in the NIST library is also shown in Figure 5 and some of their structures are as shown in Figure 14. Terpinen-4-ol has been reported to be the most active ingredient obtained after matching with data in the NIST library is also shown in Figure 4. A typical mass spectrum of some of the chemicals showing the retention time and peak area of the various compounds are shown in Figure 5. It is a strong antitussive and it has been reported to reduce blood pressure and gastric contraction. It has also been linked also to memory and cognitive enhancing properties.

FTIR spectroscopy is a useful method for obtaining information on the chemical nature of natural product mixture. It detects the vibrational frequencies and intensities of individual functional groups of the components in the mixture with high sensitivity and time resolution and permit quantification of specific classes of dissolved organic matters including aromatic and aliphatic organic compound containing oxygen, nitrogen, and sulfur functional groups.

Elufioye and Mada.: Chemical analysis and antioxidant activity of Pax herbal bitters

GRAPHICAL ABSTRACT

SUMMARY

• Pax herbal bitters contain several chemical compounds with previously proven biological activities that may account for the claimed pharmacological effects.

ABOUT AUTHORS

Dr. Taiwo O. Elufioye: is a senior lecturer in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria.

Cite this article: Elufioye TO, O. GC-MS, FTIR, UV Analysis and in vitro Antioxidant Activity of a Nigeria Poly Herbal Mixture: Pax Herbal Bitters. Free Radicals and Antioxidants. 2018;8(2):74-81.