TWO TRITERPENOIDS ISOLATED FROM THE ROOT OF Hippocratea welwitschii (CELASTRACEAE)-Oliv

S. K. Okwute1*, R. U. Okoh-Esene2 and J. I. Okogun3

1Chemistry Department, University of Abuja, Federal Capital Territory, PMB 117, Abuja, Nigeria
2Sheda Science & Technology Complex (SHESTCO), PMB186, Garki Abuja FCT, Nigeria
3Pax- Herbal Clinic & Research Laboratory, Benedictine Monastery, PO Box 150, Ewu, Edo, State, Nigeria

*Corresponding author: profokwute@yahoo.com

Received: February 10, 2018 Accepted: July 25, 2018

Abstract: The powdered dried root of Hippocratea welwitschii, a plant known traditionally for its strong anti-epileptic activity was extracted with 95% ethanol to obtain the crude extract. The crude root extract was investigated for its chemical constitution. Phytochemical analysis of the crude extract showed that it contained glycosides, saponins, triterpenes, phenols and alkaloids. It was then fractionated into hexane, ethyl acetate, aqueous methanol and an insoluble yellow substance. The ethyl acetate fraction was subjected to chromatographic separation to give a sub-fraction coded ROSW1 which on IR spectral and GC-MS analyses led to the identification of two triterpenoids, lup-20(29)-en-3-ol, acetate and lup-20(29)-en-3-one. The phytochemicals, including the isolated triterpenoids may be responsible for the anti-epileptic activity of the plant in traditional medicinal practice.

Keywords: Hippocratea welwitschii root, phytochemical screening, two triterpenoids

Introduction
The genus Hippocratea belongs to the family of plants known as Celastraceae family (Wikipedia, 2010). The great majority of the genera are tropical with only celastrus (the staff vine) Euonymus (the spindles) and maytenus widespread in the temperate climates. Most of the representatives of this family are shrubs and some such as Hippocratea are climbers by their branchlets, twisting round their supports. Hippocratea welwitschii, is a shrub found in many parts of Africa, including Guinea, western Cameroon, South-South Nigeria, Angola, Uganda, Tanganikya (Tanzania). It is a constituent of some traditional medicinal remedies (Burkill, 1985).

In Nigeria the root is used to effectively manage severe cases of epilepsy. Reports of chemical studies on the plant are non-existent. This study therefore sought to evaluate the chemical constituents of the root of this plant with the aim of accounting for the traditional medicinal uses, particularly its use as an anti-epileptic remedy. We now wish to report the results of phytochemical screening of the root extract of Hippocratea welwitschii and the identification of two triterpenoids from the ethyl acetate-soluble fraction. To the best of our knowledge this is the first report on the chemical constituents of the plant.

Materials and Methods
The roots of this plant were dug up from an old farm in southern Nigeria, cleaned up to remove the sand particles and dried indoors in an airy corridor. The plant was identified and authenticated by a taxonomist, Mr Ozioko, of Bio-resource Development and Conservation Programme, number 114 Aku Road, Nsukka, Enugu state, Nigeria and was assigned the voucher number, BDCP 213. The dried roots were then broken into smaller bits and blended into powder with a blender- Waring commercial blender 8011E model 38BL 41 extracted and used for both chemical and biological analysis using standard methods.

All the solvents used were of analytical grade from British Drug House (BDH).

Column chromatographic methods (flash and gravitational) were employed using reverse phase silica gel (RP-18) and Kieselgel 60 with pore size range of 0.063 – 0.200 mm, respectively. The pre-coated silica gel(60F254) plates for thin-layer chromatography were manufactured by Merck. IR spectra were obtained using FTIR Genesis and GCMS machine was the Xcalibur.

Extracted from the root powder of Hippocratea welwitschii

Powdered root sample (1300 g) of Hippocratea welwitschii was extracted with 95% ethanol for forty-eight (48) hours using a soxhlet extractor. The extract was then filtered, evaporated in- vacuo using a rotary evaporator to give 127.0 g of crude extract. The extract was dissolved in water to leave an insoluble deposit which when dried was a brownish powdery substance (57.92 g). The aqueous solution was filtered and 10% concentrated sulphuric acid added, refluxed for three hours and then allowed to cool down. The excess acid was then neutralized with 0.01M sodium hydroxide and fractionated with aqueous methanol and hexane (300 ml of 10% aqueous methanol +300 ml x 2 of hexane). The hexane layer was washed with 200 ml of water, dried and evaporated to dryness to give a greenish yellow residue (11.55 g). The aqueous methanol layer was again extracted with ethyl acetate, following the same procedure as with hexane to give a straw coloured material (17.56 g). Evaporation of the aqueous methanol fraction gave a chocolate substance (38.23 g).

Qualitative and quantitative phytochemical analysis of the root extract of Hippocratea welwitschii

Phytochemical analysis of the root was carried out using standard methods of analysis (Trease and Evans, 1989; Sofowora, 1982, 1993). The quantities of the phytochemicals present were determined using the methods of Harborne (1973) and Obadoni and Ochuko (2001). The results are shown in Table 1.

Chromatographic purification of the ethyl acetate fraction

The ethyl acetate fraction (5.0 g) was eluted with mixtures of hexane and ethyl acetate on a column of silica gel 60, followed by preparative TLC to give a milky white powder (7 mg. m.p. 139.2-142.3°C) and was coded ROSW2. Fraction ROSW2 was then subjected to FTIR spectral and GCMS analyses to determine its degree of purity as well as its possible constituents.

Results and Discussion

The results of the phytochemical analysis in Table 1 above showed that the root of Hippocratea welwitschii contains saponins, alkaloids, phenols and glycosides in varying amounts 1.66 x 10^-2, 3.67 x 10^-2, 2.64 x 10^-2 and 2.01 x 10^-2 µg/g, respectively. Plant saponins generally help humans to fight fungal infections, combat microbes and viruses and knock out some tumor cells, particularly lung and blood cancers (Barakat et al., 1993; Poormima and Ravishankar, 2004).
Phytochemical Screening of Hippocratea welwitschii Extract

2009). They also bind blood cholesterol, thereby reducing heart problems but the most exciting and outstanding prospect for saponins are how they inhibit and kill cancer cells (Poornima and Ravishankar, 2009). It has also been reported that they do so without destroying normal cells in the process, as is the mode of some cancer fighting drugs (Poornima and Ravishankar, 2009; Ryan and Shattuck, 1994). Trace quantities of phenolic compounds help prevent the death of plants since phenolic compounds from plant extracts act as antimicrobial agents (Ofokansi et al., 2005). Anticonvulsant properties of many plants, used for treatment of epilepsy in traditional medicines around the world, have been attributed to phytochemicals found in them, e.g., flavonoids, saponins, isoquino line alkaloids (particularly berberine) (inhibitortm).Table: 1 Qualitative and Quantitative Phytochemical Analysis of the Root of Hippocratea welwitschii

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Presence</th>
<th>Quantity (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
<td>1.66 x 10^-2</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>1.66 x 10^-2</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>3.67 x 10^-3</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>2.64 x 10^-2</td>
</tr>
<tr>
<td>Balsams</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthracenes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>2.01 x 10^-2</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>3.08 x 10^-3</td>
</tr>
</tbody>
</table>

+ = Present; – = Absent; H.w = Hippocratea welwitschii

The IR spectrum of ROSW0 (Fig. 1) showed absorptions at 3686.69 - 3545.28 due to hydrogen-bonded OH group. It also gave an absorption band at 1691.63 ascribable to cyclic ketonic carbonyl moiety.

The gas chromatogram (Fig. 2) showed that ROSW0 was not pure but contained among many compounds, two identifiable components, ROSW0-a (RT=11.15 mins) and ROSW0-b (RT=12.37 mins) with molecular ion peaks at 468 and 424, respectively. The MS of compound ROSW0-a (Fig. 3) showed significant fragment ions at m/z 468[M+], 409, 272/273, 245, 218, 150/149, 105/106(Scheme 2), respectively, typical of lupane-type triterpenoids such as betulinic acid, lupeol and lup-20(29)-en-3-one (Herzt et al., 1972; Igoli and Gray, 2008; Dantarayana et al., 1982; Gabriel and Okwute, 2009; Okwute and Isyaka, 2014).

![Fig. 1: IR spectrum of fraction ROSW0](image1)

![Fig. 2: Gas chromatogram of fraction ROSW0](image2)

![Fig. 3: Mass spectrum of component ROSW0-a](image3)
The two compounds apart from their molecular ions display the characteristic fragment ion at m/z 218. Based on their MS analyses and by direct comparison with computer MS library data the structures of ROSW0-1 and ROSW0-2 were assigned structures 1 and 2, corresponding to lup-20(29)-en-3-ol, acetate and lup-20(29)-en-3-one, respectively. Lup-20(29)-en-3-one has been previously reported from the stem bark of *Pterocarpus erineceus* (Gabriel and Okwute, 2009) and from the bark of *Zanthoxylum budrunga* (Anwarul et al., 2001) and is known to demonstrate antibacterial and anti-fungal properties (Anwarul et al., 2001). Lup-20(29)-en-3-ol, acetate has been reported from *Maytenus acanthophylla* Reissek (Celastraceae) (Menezes et al., 2011) and is a multi-target drug (Dong et al., 2013) targeting key molecular pathways such as those involving NF-kappaB, among others. It has been a known anti-tumor, anti-inflammatory drug acting through the opioid pathway and this may be responsible for the anti-epilepsy property of the plant earlier reported by Okoh-Esene *et al.* (2012).
Conclusion
From the results of this study, one could attribute the antimicrobial and the previously reported anti-epilepsy activities of the crude 95% ethanol extract to the presence of phenols, alkaloids, saponins and terpenoids in the plant. Saponins and lupane-type triterpenoids are known to be sedative and have been used to manage cognitive diseases such as epilepsy. This work has identified two important triterpenoids, lup-20(29)-en-3-one and lup-20(29)-en-3-ol, acetate which are known to be anti-infective agents. Their presence therefore lends support to the use of the plant in the traditional management of infections and epilepsy.

References

Inhibitors: https://sites.google.com/site/epilepsynotes/home/nutrition-notes/phytochemicals
Menezes et al. 2011. The triterpenes 3β-lup-20(29)-en-3-ol and 3β-lup20(29)-en-3-yl acetate and the carbohydrates 1,2,3,4,5,6-hex-O-acetyl dulcitol as photosynthesis light reaction inhibitors. Molecules, 16(12): 9939-9956. Doi:3390/molecules16129939.
Sofowora EA 1982. Medicinal Plants and Traditional Medicines in Africa; John Wiley and Sons Ltd., Nigeria, pp. 64-79.