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

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Abstract: The powdered dried root of *Hippocreata 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 $ROSW_0$ 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: *Hippocrarea 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) *Euoymus*(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, Tanganyika (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 almost 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 *Hippocreata 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 Kieseigel 60 with pore size range of 0.063 – 0.200 mm, respectively. The pre-coated silica gel($60F_{254}$) plates for thin-layer chromatography were manufactured by Merck. IR spectra were obtained using FTIR Genesis and GCMS machine was the Xcalibur.

Extraction of 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^oC) and was coded $ROSW_0$. Fraction $ROSW_0$ 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 $x10^{-2}$, 3.67 $x10^{-3}$, 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; Poornima and Ravishankar,

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; Ryam 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, isoquinoline alkaloids (particularly berberine) (inhibitorshtmhttps://sites.google.com/).

Table: 1 Qualitative and Quantitative Phytochemical Analysis of the Root of *Hippocratea welwitchii*

Metabolites	Presence	Quantity (µg/g)
Tannins	-	-
Phlobatannins	-	-
Chlorogenic acid	-	-
Anthraquinones	-	-
Saponins	+	1.666×10^{-2}
Alkaloids	+	3.67×10^{-3}
Phenols	+	2.64×10^{-2}
Balsams	-	-
Anthracenes	-	-
Flavonoids	-	-
Resins	-	-
Sterols	-	-
Glycosides	+	2.01×10^{-2}
Terpenoids	+	3.08×10^{-3}

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

The IR spectrum of $ROSW_0$ (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 $ROSW_0$ was not pure but contained among many compounds, two identifiable components, $ROSW_{0\text{-}1}$(RT=11.15 mins) and $ROSW_{0\text{-}2}$(RT=12.37 mins) with molecular ion peaks at 468 and 424, respectively. The MS of compound $ROSW_{0\text{-}1}$(Fig. 3) showed significant fragment ions at m/z 468[M^+], 425, 250, 218, 207, 204/205(Scheme 1) while that of $ROSW_{0\text{-}2}$ (Fig. 4) gave significant fragment ions at m/z 424[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).

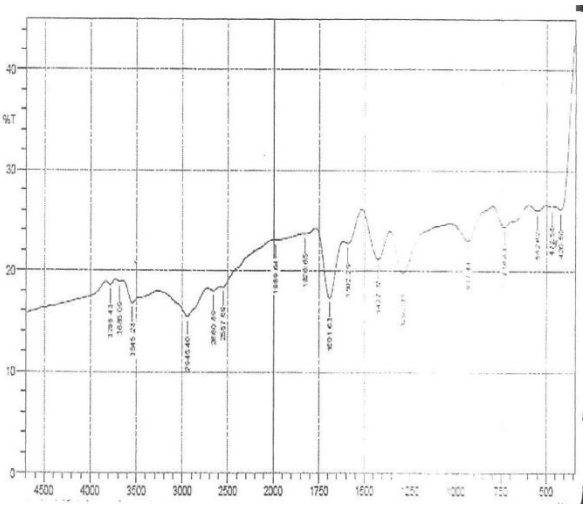


Fig. 1: IR spectrum of fraction $ROSW_0$

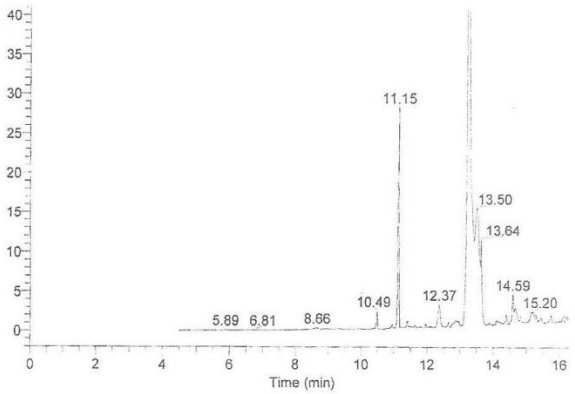


Fig. 2: Gas chromatogram of fraction $ROSW_0$

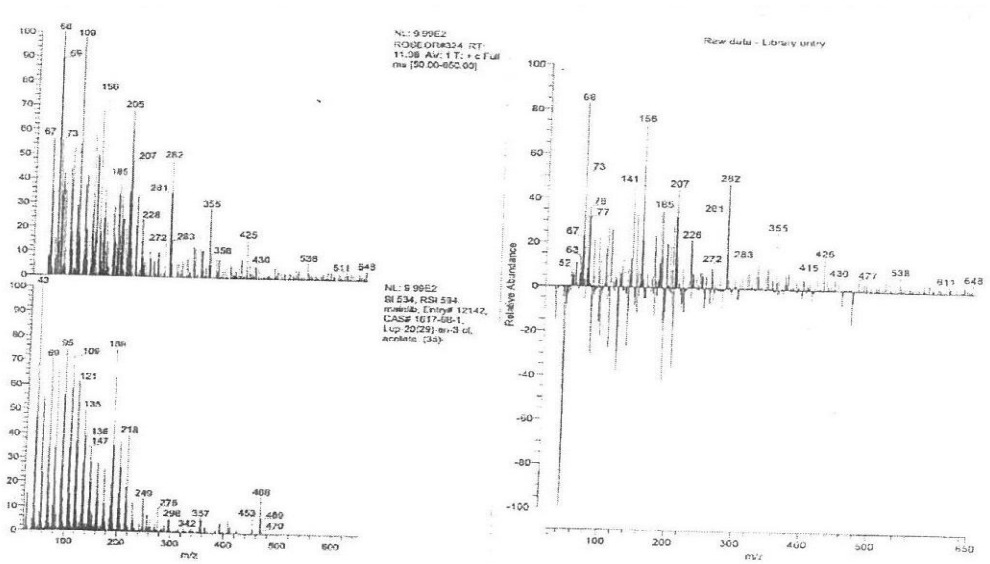


Fig. 3: Mass spectrum of component $ROSW_{0\text{-}1}$

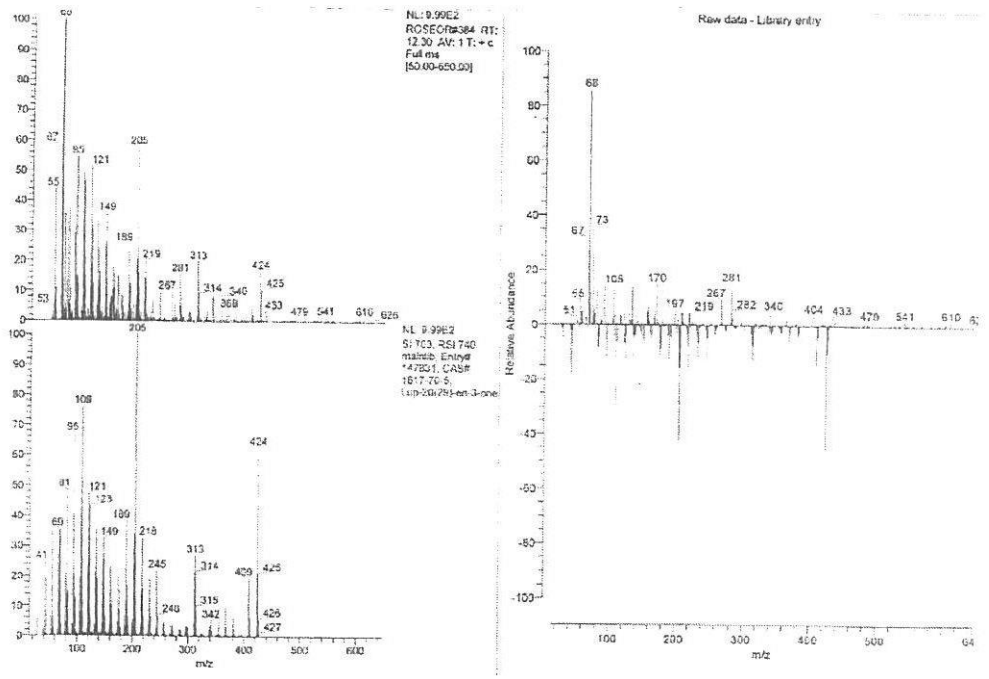
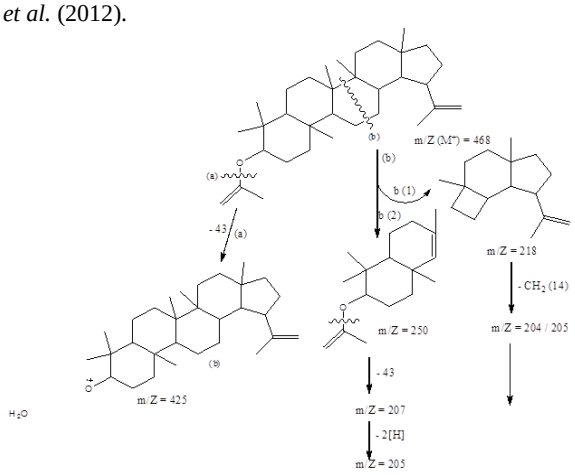
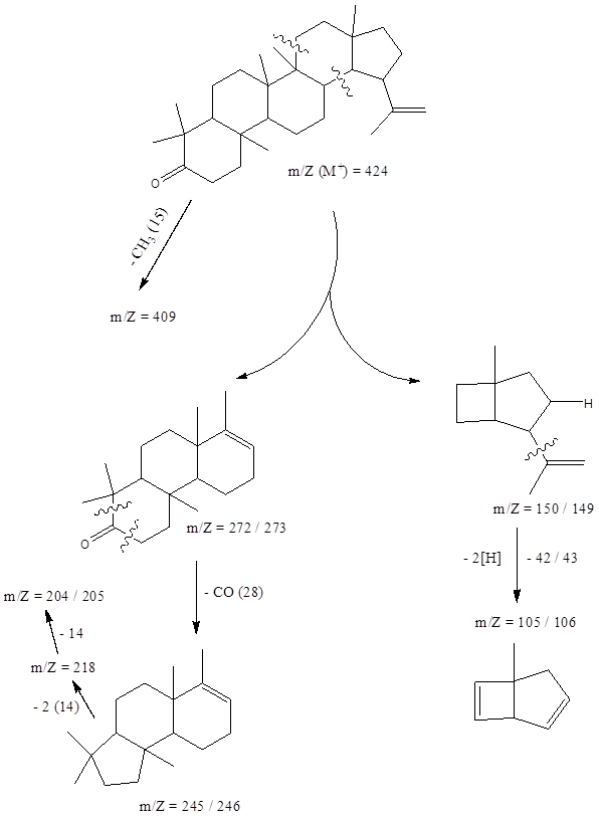


Fig. 4: Mass spectrum of component ROSW$_{0-2}$

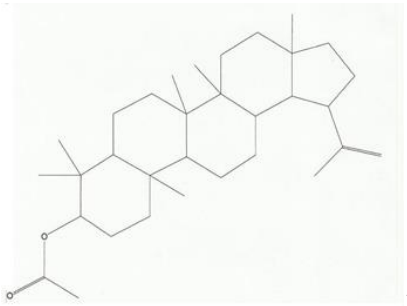
The two compounds apart from their molecular ions display the characteristic fragment ion at m/z 218. Based on their MS analyses and by direct comparison with computer MS library data the structures of ROSW$_{0-1}$ and ROSW$_{0-2}$ were assigned structures **1** and **2,** corresponding to lup-20(29)-en-3-ol, 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 opiod pathway and this may be responsible for the anti-epilepsy property of the plant earlier reported by Okoh- Esene *et al.* (2012).



Scheme 1: MS Fragmentation pattern for component ROSW$_{0-1}$



Scheme 2: MS fragmentation pattern for component ROSW$_{0-2}$



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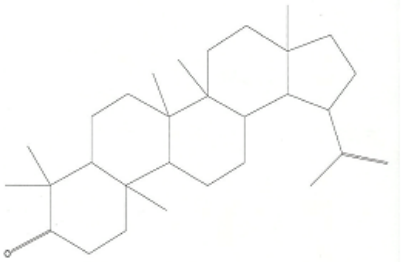

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Conclusion

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.

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