INVESTIGATION OF SECONDARY METABOLITES PRESENT IN METHANOLIC AND ALKALOIDAL EXTRACTS OF HUNTERIA UMBELLATA LEAVES USING COMPUTATIONAL MOLECULAR NETWORKING TOOL

Rukayat A. Adedeji[1*]., Musibau Opemipo[1]., Oluwabukunmi Babalola[1]., Solomon O. Julius[2]., Stephenie C. Alaribe[1*].

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Nigeria
Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria

Abstract

Background: Plants have long served as a vital source of therapeutic agents in both traditional and orthodox medicine. However, with the shift in drug discovery towards synthetic laboratory compounds, there has also been a decline in the exploration of natural sources for drug development. Research attention needs to be diverted toward natural drug discovery and more importantly, towards the development of new and improved methods of isolating, analyzing, identifying, and characterizing chemical moieties obtained from plants.
Objectives: To investigate the secondary metabolites present in the methanolic and alkaloidal extracts of *Hunteria umbellata*, a medicinally significant plant, and explore potential links between the identified compounds and the plant's pharmacological uses.
Methods: Metabolomic technique was employed, utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS) to separate the components within the extracts. The isolated compounds were then identified based on their mass-to-charge (m/z) ratios. The resulting chromatograms and spectra were analyzed using a computational molecular networking tool to match the m/z values to known compounds in mass spectrometry libraries.
Result: Eighteen compounds were successfully isolated from the methanolic and alkaloidal extracts, including Yohimbine; (-)-Epicatechin; Picrinine; Tubotaiwine; Quercetin-3-O-robinobioside; and Pheophorbide A. Many of these compounds have been previously studied for their clinical relevance, such as antidiabetic, antioxidant, and anti-inflammatory activities, supporting the medicinal uses traditionally attributed to Hunteria umbellata. Notably, the identification of Pheophorbide A, a compound with potential photodynamic therapy applications, opens new avenues for further investigation.
Conclusion: These findings provide significant insight into the bioactive components of Hunteria umbellata, supporting its traditional medicinal uses. The identification of clinically relevant compounds not only validates traditional practices but also highlights the plant's potential for contributing to modern drug discovery efforts.

KEYWORDS: Medicinal Plant, *Hunteria umbellata*, Computational Molecular Networking, LC-MS/MS, Omics

Introduction

Plants are an important source of medicine and play a key role in world health (Sandberg et al., 2001). Medicinal plant use is ever on the rise as they have been known to be an important source of everyday medicine and groundbreaking discoveries in the treatment of cancer, Alzheimer's, sickle-cell anemia, and certain autoimmune diseases.
Plants remain an important and continuing source of novel chemotherapies even though the majority of modern drug discoveries are a result of synthetic compounds and monoclonal antibodies development (Brower, 2008).

Hunteria umbellata K. Schum, a tropical rainforest tree found in western and central Africa, is a member of the Apocynaceae family (Oboh et al., 2019; Igbe et al., 2009a; Adeneye and Adeyemi, 2009). The Apocynaceae family is one of the most extensively studied plant families and many genera including Hunteria have been studied for their chemical composition, pharmacological activity and economic importance.

Members of the Hunteria genus are quite popular amongst traditional practitioners and a good number of their use in herbal medicine preparation have been justified in scientific studies. For instance, a study on the anti-inflammatory activity of *Hunteria zeylanica* found that crude alkaloid extracts of the plant's stem bark produced significant inhibition of acute inflammation (Igbe et al., 2009b).

The major challenge with incorporating herbal products into the national healthcare plan in African countries is the lack of unbiased concrete scientific evidence to ascertain the identity, purity, and quality of these products. The majority of the current analytical techniques available for identifying components of herbal products are not sensitive to the complex nature of these products. Thus, the inadequacy in properly accounting for all the chemical constituents present in a plant preparation is one of the major reasons why people do not trust its efficacy or safety. In order to fully elucidate the chemical complex of a natural plant/product, it is important to use an analytical technique that goes beyond the traditional methods of revealing the structural components. The various omics technologies have revolutionized how traditional medicine-based drugs are discovered and processed to produce efficacious and high-quality drugs. Metabolomics tools like liquid chromatography-tandem mass spectrometry (LC-MS/MS) use mass data of components to exhaustively elucidate these components beyond what simple nuclear magnetic resonance (NMR) would do. LC-MS/MS data will account for the total ions present and thus would identify any compound or fragments attached to a major compound.

Global Natural Products Social Molecular Networking (GNPS; http://gnps.ucsd.edu) is an open-access knowledge base for the community-wide organization and sharing of raw, processed, or identified tandem mass (MS/MS) spectrometry data. This platform is used in this

study as a computational molecular networking tool for analyzing the spectra from LC-MS/MS analysis

GNPS is a data-driven platform for the storage, analysis, and dissemination of MS/MS spectra knowledge (Wang et al., 2016). It enables the community to share raw spectra, continuously annotate deposited data, and collaboratively curate reference spectra (referred to as spectral libraries) and experimental data (organized as datasets) (Wang et al., 2016). GNPS provides the ability to analyze a dataset and compare it to all publicly available data.

Molecular networks are visual representations of the chemical space present in MS experiments. GNPS is employed for molecular networking, a spectral correlation and visualization approach that detects sets of spectra from related molecules (so-called spectral networks), even when the spectra themselves are not matched to any known compounds. Spectral alignment detects similar spectra from structurally related molecules, assuming these molecules fragment in similar ways, as reflected in their MS/MS patterns, analogous to the detection of related protein or nucleotide sequences by sequence alignment (Wang et al., 2016).

This tool enables a cycle of annotation in which users curate data, enable product identification, and a knowledge base of reference spectral libraries and public data sets are created.

This study aims to isolate, purify, and identify the secondary metabolites present in the crude extract of *Hunteria umbellata* K.Schum. This is followed by the characterization of these secondary metabolites using a computational molecular tool with an MS library data bank.

Materials And Method

Materials

Fresh leaves of *Hunteria umbellata* were collected for this study. The chemicals used for extraction included 98% methanol, 100% chloroform, 2M sulfuric acid, and ammonia. Experimental procedures involved the use of a rotary evaporator, LC-MS/MS setup, lab mill laboratory grinder, analytical balance, and a hot air oven. Additional materials included glass funnels, beakers, foil paper, stirring rods, Whatman No. 1 filter paper (32.0 cm, CAT No: 1001320, Whatman England), spatulas, and ziploc bags.

Collection And Identification

Fresh samples of the leaves and stem bark of *Hunteria umbellata* were collected and identified by a taxonomist in Ibadan, Oyo State, Nigeria. The sample was transported to Lagos, identified and authenticated by Dr Nodza George at the herbarium in the University of Lagos, Akoka. Voucher number LUH-8748 was obtained.

Preparation Of Plant Material For Extraction

The leaves were rinsed thoroughly using distilled water to remove sand, dust, and any adhering particles from the leaf surface after which the leaves were flapped to drain most of the water and then spread out on a flat clean surface for the remaining water droplet to evaporate. The leaves were then plucked from the stem and spread out to air dry for 8 days during which the leaves were regularly turned over for optimum drying.
The dried leaves were placed in the hot air oven for 30 minutes before grinding to make the leaves material crunchy enough for the milling process. Afterwards, the crispy leaves were crushed by hand to increase the surface area of the materials which makes it easier to feed the materials into the grinding machine and also makes the grinding process more efficient.
Lab mill laboratory grinder was used to grind the leaves into powder after which the powdered plant leaves were stored in a polythene bag and labeled appropriately in preparation for subsequent steps. A small quantity of the powder Hunteria leaves was kept in a Ziploc bag, labeled, and put away for reference and documentation purposes.

Extraction Of Phytochemicals

Using Manske's method (Manske and Holmes, 2014), the leaves and stem bark of *Hunteria umbellata* were extracted for their phytochemical constituent and specifically their alkaloidal content.

Methanol Extraction

A 500g bag of ground HU leaves was emptied and transferred into a glass jar and a sufficient volume of methanol was added to soak the plant in. The sample was macerated for 72 hours after which the leaves were filtered using a Whatman filter to obtain the first methanol filtrate. The filtrate obtained from leaves was concentrated using a rotary evaporator. The concentrate obtained was kept in a beaker and covered using foil paper. About 5g of the pure methanol extract was kept for reference purposes. The recovered methanol from each concentration was used to resoak the leaves again and more methanol was added to top off the plant material for the second maceration. The methanol filtrate was subsequently obtained after 24 hours, concentrated, and resoaked till the plant content was exhaustively extracted. All the methanol extract obtained from each maceration was pooled and one-quarter of this extract was transferred into a sample bottle to be sent for analysis while the remaining three-quarter proceeded to the alkaloid extraction step.

Alkaloid Extraction

The methanol extract obtained in the previous step was dissolved in water and repeatedly titrated with 2M sulphuric acid to acidify the solution to pH 2 (tested using a pH meter). Steam

distillation was done next on the acidified solution to remove all traces of the methanol then the acidified Hunteria solution was kept in the refrigerator for 5 days after which it was taken out and filtered. The filtrate was agitated with 100% chloroform to separate the alkaloidal salts which are freely soluble in water but not in organic solvents like chloroform. An aqueous and organic phase layer was formed from the previous step. The aqueous phase containing the alkaloidal salt was separated and carefully basified with ammonia to liberate free alkaloids, hence, an aqueous and organic phase was formed. The organic phase containing the free alkaloid was separated using a separating funnel and evaporated to dryness using a rotary evaporator to obtain the crude alkaloids. The crude alkaloid of Hunteria was pooled into a screw-capped sample bottle to be shipped for analysis.

LC/MS Analysis

Samples were dissolved in methanol and analyzed using an Agilent LC-MS system composed of an Agilent 1260 Infinity HPLC coupled to an Agilent 6530 ESI-Q-TOF-MS operating in positive mode. A Sunfire analytical C18 column (150 × 2.1 mm; i.d. 3.5 µm, Waters) was used, with a flow rate of 250 µL/min and a linear gradient from 5% B (A: H2O + 0.1% formic acid, B: MeOH) to 100% B over 30 min. ESI conditions were set with the capillary temperature at 320 °C, source voltage at 3.5 kV, and a sheath gas flow rate of 10 L/min. The divert valve was set to waste for the first 3 min. There were four scan events: positive MS, a window from m/z 100−1200, and then three data-dependent MS/MS scans of the first, second, and third most intense ions from the first scan event. MS/MS settings were: three fixed collision energies (30, 50, and 70 eV), a default charge of 1, a minimum intensity of 5000 counts, and an isolation width of m/z 2. Purine C5H4N4 [M + H]+ ion (m/z 121.050873) and hexakis(1H,1H,3H-tetrafluoropropoxy)- phosphazene C18H18F24N3O6P3 [M + H]+ ion (m/z 922.009) were used as internal lock masses. Full scans were acquired at a resolution of 11 000 (at m/z 922). A permanent MS/MS exclusion list criterion was set to prevent oversampling of the internal calibrant (Fox Ramos et al., 2019).

Computational Molecular Networking

The Global Natural Product Social Molecular Networking (GNPS) website was used as the computational molecular networking tool.

The mass spectrometry file data was converted into an mzXML which is the accepted format by the tool. GNPS webpage was accessed on a computer system and account details were entered. From the main GNPS page, the "create molecular network" tab was clicked on which led to the workflow input page to start networking.

The mzXML file was uploaded using the provided tabs, and the following parameters were entered: for Molecular Networking, a precursor ion mass tolerance of 0.02 Da, fragment ion

mass tolerance of 0.02 Da, minimum pairs cosine of 0.6, minimum cluster size of 1, score threshold of 0.6, and no filtering of peaks in a 50 Da window; for Spectral Library Search, a precursor ion mass tolerance of 0.02 Da, fragment ion mass tolerance of 0.02 Da, minimum matched peaks of 6, score threshold of 0.6, top hits per spectrum set to 3, and no filtering of peaks in a 50 Da window.

The workflow was then submitted and the status page displayed, which showed the status of the molecular networking job. On completion, DONE was displayed on the job status page.

The result was downloaded from the "View Unique Library Compounds" window. The software Cytoscape was used to view the clusters

Results

The data from the obtained chromatogram and exported CSV file of isolated compounds was extracted to characterize the active constituent of *Hunteria umbellata* leaves. The identified compounds were then characterized and linked to known biological activity. The details of the identified compounds, their structure, and potential activities are described later in this session.

Percentage Yield Of Extract

The percentage yield of the crude methanol and alkaloid extract can be calculated using the formula shown below.

$$\%\text{Yield} = \frac{\text{Weight of Leave Extract (g)}}{\text{Total Weight of Leave Material (g)}} \times 100$$

Hence, the percentage yield of the crude methanol extract is calculated

$$\%\text{Yield} = \frac{19.77\text{g}}{500\text{g}} \times 100$$

$$= 3.95\%$$

The percentage yield of the alkaloid extract is calculated

$$\%\text{Yield} = \frac{4.37\text{g}}{500\text{g}} \times 100$$

$$= 0.87\%$$

Presentation And Analysis Of Isolated Compounds From The Methanolic Extract Of HU

Table 1: Table of Isolated Compounds from the Methanolic Extract of HU

Compound Name	Precursor MZ	Exact Mass	Spec MZ	Class	Subclass	NP classifier superclass
(-)-Epicatechin • (2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	291.087	0	291.086	Flavonoids	Flavans	Flavonoids
2-[[5-(4-hydroxy-3,5-dimethoxyphenyl)-6,7-bis(hydroxymethyl)-1,3-dimethoxy-5,6,7,8-tetrahydronaphthalen-2-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol	600.267	582.234	600.264	N/A	N/A	Lignans
Quercetin-3-O-robinobioside • 3-[[6-O-(6-Deoxy-alpha-L-mannopyranosyl)-beta-D-galactopyranosyl]oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one	611.159	610.153	611.16	Flavonoids	Flavonoid glycosides	Flavonoids
Procyanidin B2 • (-)-Epicatechin (4β-8)-(-)-epicatechin = (2R,2'R,3R,3'R,4R)-2,2'-Bis(3,4-dihydroxyphenyl)-3,3',4,4'-tetrahydro-2H,2'H-4,8'-bichromen-3,3',5,5',7,7'-hexol	579.15	0	579.15	N/A	N/A	N/A
Tubotaiwine •	325.191	324.184	325.191	N/A	N/A	Tryptophan

methyl(1S,11S,17R,18S)-18-ethyl-8,14-diazapentacyclo[9.5.2.01,9.02,7.014,17]octadeca-2,4,6,9-tetraene-10-carboxylate						alkaloids
10-hydroxygeissoschizol • (3E)-3-ethylidene-2-(2-hydroxyethyl)-2,4,6,7,12,12b-hexahydro-1H-indolo[2,3-a]quinolizin-9-ol	313.191	312.184	313.191	Indoles and derivatives	Pyrido indoles	Tryptophan alkaloids
(2R,3R,4S,5S,6R)-2-[[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2H-chromen-3-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol	453.139	452.132	453.139	Flavonoids	Flavonoid glycosides	Flavonoids
Triphenylphosphine oxide • Diphenylphosphorylbenzene	279.093	0	279.093	Benzene and substituted derivatives	Phenylphosphines and derivatives	N/A
Yohimbine • Methyl (1S,15R,18S,19R,20S)-18-hydroxy-1,3,11,12,14,15,16,17,18,19,20,21-dodecahydroyohimban-19-carboxylate	355.202	0	355.201	N/A	N/A	Tryptophan alkaloids
C-fluorocurarine • (19E)-4-Methyl-17-oxo-2,16-didehydrocur-19-en-4-ium	307.181	307.181	307.18	N/A	N/A	N/A
N4-methylantirhine	311.212	311.212	311.212	Indoles and derivatives	Pyrido indoles	N/A
(3beta,5xi,9xi,13alpha,17alpha,18xi)-3-Hydroxy-13,28-epoxyurs-11-en-28-one	455.352	454.345	455.352	Prenol lipids	Tri terpenoids	Triterpenoids
3-Hydroxy-4-oxo-gamm	567.42	0	567.419	Prenol lipids	Tetraterp	Carotenoids

a-carotene (5'Z)-3-Hydroxy-beta,psi-caroten-4-one					enoids	(C40)
2,6-Di-tert-butyl-4-hydroxymethylphenol	219.175	0	219.174	N/A	N/A	N/A
Triphenyl phosphate	327.078	0	327.078	Organic phosphoric acids and derivatives	Phosphate esters	N/A
Pheophorbide A • (3S,4S)-9-Ethenyl-14-ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-3-phorbinepropanoic acid	593.269	592.268	593.275	Tetrapyrroles and derivatives	Chlorins	Tryptophan alkaloids
Burnamine • (16R)-2α,5α-Epoxy-1,2-dihydro-17-hydroxyakuammilan-16-carboxylic	369.181	368.174	369.181	N/A	N/A	Tryptophan alkaloids

Keys: NP = Natural Product, MZ = mass/charge, Spec = Spectral, HU = *Hunteria umbellata,*
• is used as a separator for the common and IUPAC names of compounds

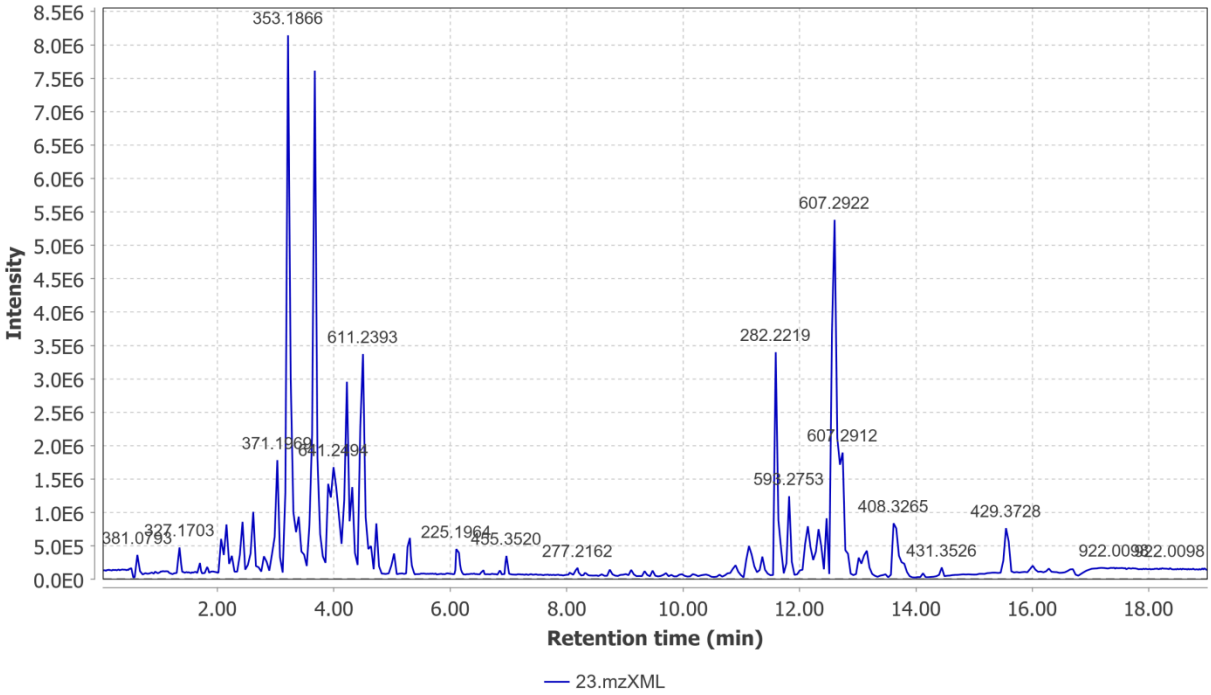

Fig 1: A Chromatogram showing the spectrum peaks of the isolated compounds in the methanolic extract of HU

Structures of some Isolated Compounds Present in the Methanolic Extract

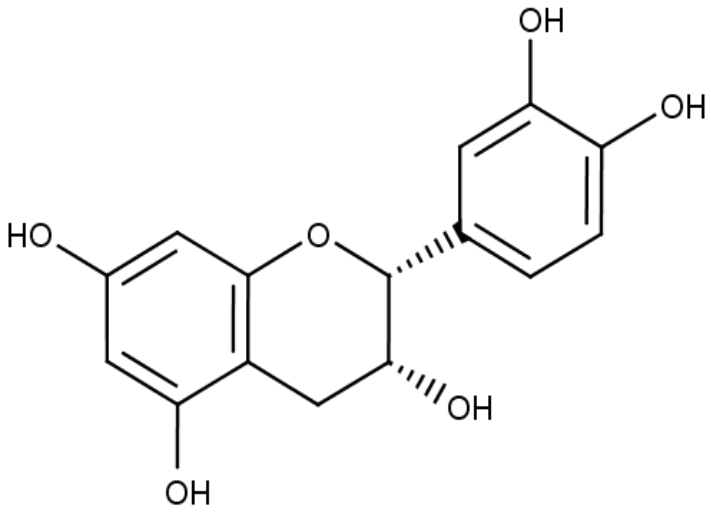

Fig 2: Structure of (-)-Epicatechin

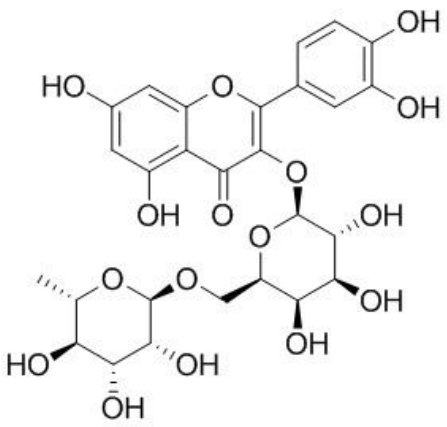

Fig 3: Quercetin-3-O-robinobioside

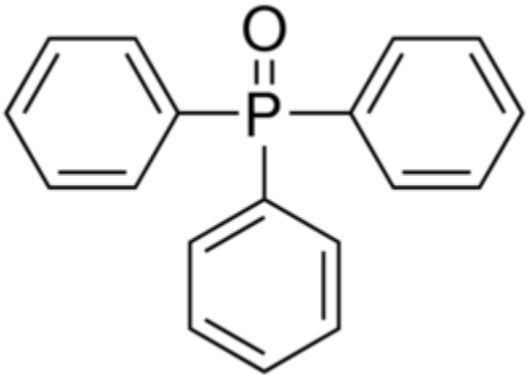

Fig 4: Triphenylphosphine oxide

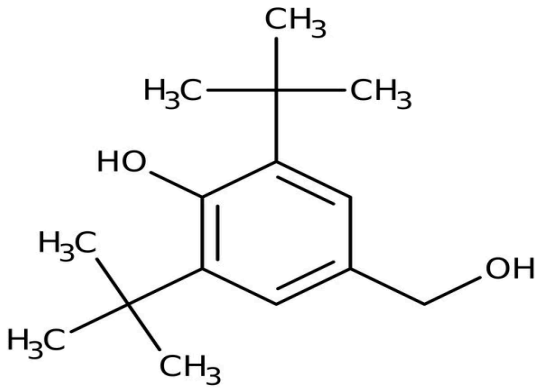

Fig 5: 2,6-Di-tert-butyl-4-hydroxymethylphenol

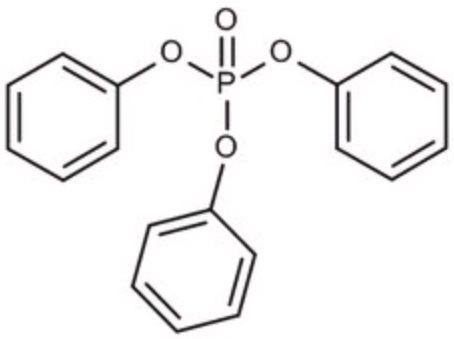

Fig 6: Triphenyl phosphate

Presentation And Analysis Of Isolated Compounds From The Alkaloidal Extract Of Hu

Table 2: Table of Isolated Compounds from the Alkaloidal Extract of HU

Compound Name	Precursor MZ	Exact Mass	Spec MZ	Class	Subclass	NP classifier superclass
Picrinine • Methyl 14-ethylidene-18-oxa-2,12-diazahexacyclo[9.6.1.19,15.01,9.03,8.012,17]nonadeca-3,5,7-triene-19-carboxylate	339.17	338.163	339.17	Indoles and derivatives	Pyridoindoles	Tryptophan alkaloids
(3beta,5xi,9xi,13alpha,17alpha,18xi)-3-Hydroxy-13,28-epoxyurs-11-en-28-one	455.352	454.345	455.352	Prenol lipids	Triterpenoids	Triterpenoids
Pheophorbide A • (3S,4S)-9-Ethenyl-14-ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-3-phorbinepropanoic acid	593.269	592.268	593.275	Tetrapyrroles and derivatives	Chlorins	Tryptophan alkaloids

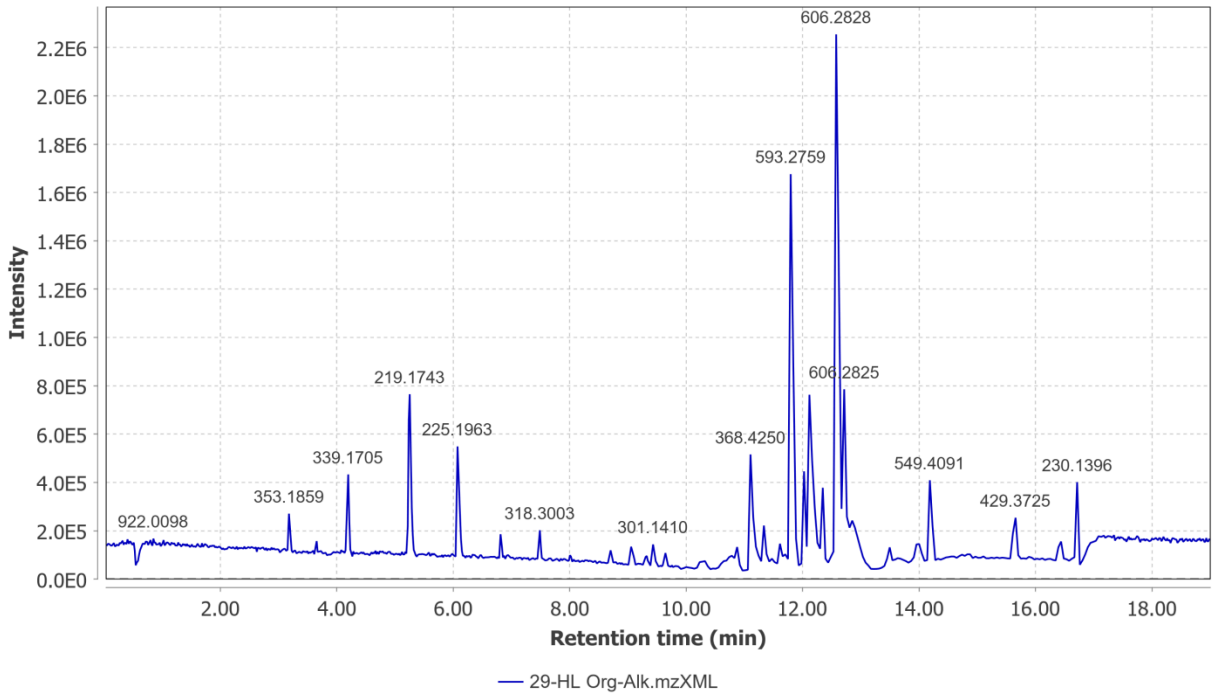

Fig 7: A Chromatogram showing the spectrum peaks of the isolated compounds in the alkaloidal extract of HU

Structures of the Isolated Compounds Present in the Alkaloidal Extract

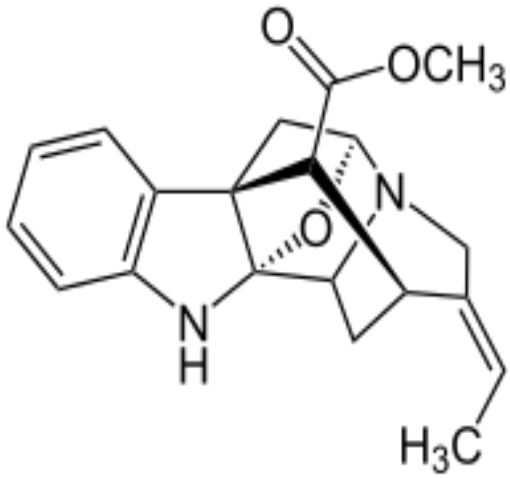

Fig 8: Picrinine

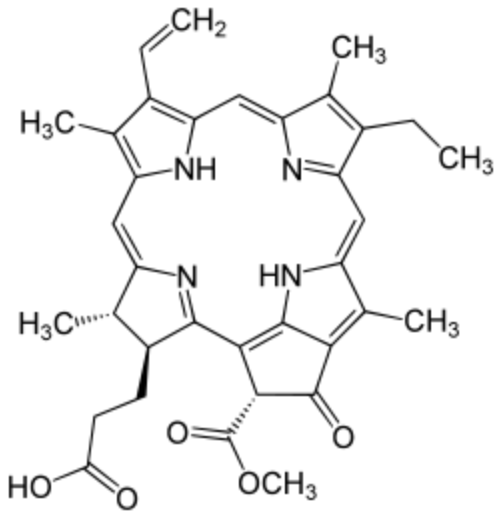

Fig 9: Pheophorbide A

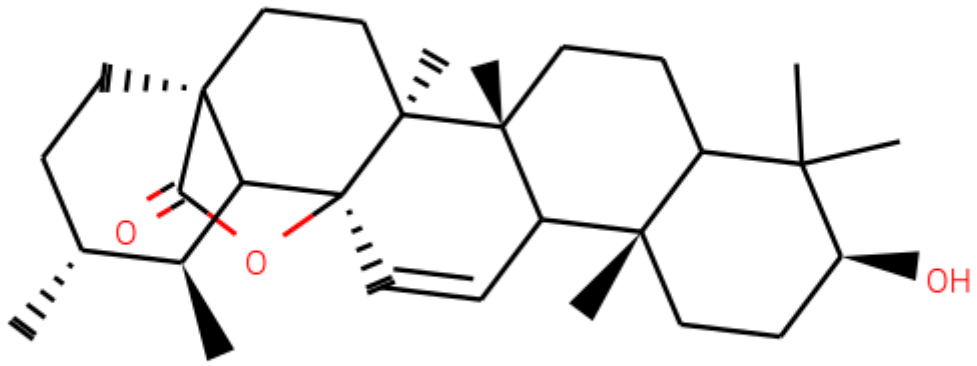

Fig 10: (3beta,5xi,9xi,13alpha,17alpha,18xi)-3-Hydroxy-13,28-epoxyurs-11-en-28-one

Discussion

This study was carried out to investigate the phytochemical content of *Hunteria umbellata* K.Schum using a computational molecular networking tool. The result of the LC-MS/MS investigation showed that the leaf of *Hunteria umbellata* contains several secondary metabolites many of which belong to the alkaloid, flavonoid, lipid, and carotenoid groups of phytochemicals which are well known for their medicinal importance.

(-)-Epicatechin; Quercetin-3-O-robinobioside;
(2R,3R,4S,5S,6R)-2-[[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2H-chromen-3-yl]oxy]-6-(3beta,5xi,9xi,13alpha,17alpha,18xi)-3-Hydroxy-13,28-epoxyurs-11-en-28-one (hydroxymethyl)oxane-3,4,5-triol are the flavonoids isolated from the methanolic extract of HU. The antioxidative effect of catechol-type flavonoids have been extensively studied and in one such study, (-)-Epicatechin was examined and found to inhibit the accumulation of phosphatidylcholine-hydroperoxides initiated by 2,2′-azobis(2-amidinopropane)hydrochloride (AAPH) in a concentration-dependent manner (Terao et al., 1994). Their antioxidant effect was also compared with a well-known commercial antioxidant, α-tocopherol, and found to have a longer-lasting inhibitory effect. This finding supports the use of the plant as an immune booster in local medical practices in African communities. Also, because of the link between the production of reactive oxygen species and inflammation, the antioxidative action of (-)-Epicatechin may also validate the use of HU as an analgesic and anti-inflammatory agent (Adeneye et al., 2011).

Another interesting finding is the insulin-like activities of (-)-Epicatechin in an in vitro study which observed that (-)-Epicatechin stimulates oxygen uptake in fat cells, inhibits lipolysis, and increases glycogen content while increasing glucose uptake (Ahmad et al., 1989). Also, the competitive binding experiment showed that (-)-Epicatechin does not share a binding site with insulin, thus, has insulin-like and insulinogenic properties and can be used in formulating novel antidiabetic drugs (Ahmad et al., 1989; Mechchate et al., 2021). These findings corroborate the traditional use of H. umbellata in diabetes management.

These flavonoids can also be useful in the management of clinical conditions associated with oxidative stress because of their antioxidant action. Such conditions as Cancer, Parkinson's, aging, Dementia, Alzheimer’s disease, and Inflammatory disorders could be managed with drugs containing these compounds as an active ingredient.

Tubotaiwine; 10-hydroxygeissoschizol; Yohimbine; Pheophorbide A; Burnamine; Picrinine; (3beta,5xi,9xi,13alpha,17alpha,18xi)-3-Hydroxy-13,28-epoxyurs-11-en-28-one are the isolated alkaloids from methanolic and alkaloidal extract of HU leaves.

Tubotaiwine, a tryptophan alkaloid has been studied and found to possess antimicrobial activity among other activities which may explain why the plant is traditionally used in the treatment of infection (Van Beek et al., 1984). Another biological study found tubotaiwine to be an opioid receptor ligand which might suggest some psychoactive or analgesic activity corroborating its local use for pain management (Stafford et al., 2009).

HU has been found to be effective in the treatment of male sexual dysfunction in traditional medicine. A study that looked at the aphrodisiac impact of HU seed extract in male rats recently backed up this notion. The study found that taking HU seed extract raises nitric oxide and antioxidant levels, as well as sexual behavior measures including the mounting number and intromission number (Oboh et al., 2019). On the other hand, administration of HU caused a significant decrease in arginase activity, mounting latency, and intromission latency. This activity may be attributed to the tryptophan alkaloid, Yohimbine isolated from the methanolic extract of HU. Yohimbine is an alpha-2 adrenergic antagonist that has been well-studied and found to be efficacious in the treatment of erectile dysfunction (Tam et al., 2001). This compound is commercially available in the US as Yohimbine hydrochloride (the standardized form) as a prescription drug.

Pheophorbide A is another alkaloid isolated during this study with a strong potential in cancer therapy. It is effective as a photosensitizer that is useful in photodynamic therapy for cancer treatment (Chen et al., 2009).
Burnamine has been investigated for hypoglycemic activity but no significant activity has been recorded to validate the use of the HU plant for diabetes treatment unlike Akuammicine, a structurally similar monoterpenoid indole alkaloid (Erharuyi et al., 2014). Since Akuammicine has demonstrated concentration-dependent glucose uptake, Burnamine might also be modifiable to produce this activity upon further studies, especially structural activity relationship study.
Picrinine is another monoterpenoid indole alkaloid that has been researched and documented to possess anti-inflammatory activity and, hence, useful as an antitussive, expectorant, and in asthma attack relief (Shang et al., 2010).
Procyanidine B2 is a B-type proanthocyanidin that was also isolated. A laboratory study suggests Procyanidin B2 plays an important role in hair cycle progression and might be useful in promoting hair growth (Kamimura and Takahashi, 2002).
There has been the suggestion that C-fluorocurarine possesses a hypotensive effect and might be useful in treating hypertension but these claims are yet to be validated by strong scientific evidence. 2,6-Di-tert-butyl-4-hydroxymethylphenol is another phenolic compound isolated from HU leaves and reported to possess antioxidant action but will require more research to confirm that and its other activities.
The other compounds isolated are relatively novel moieties, with limited information currently available. Further studies are required to determine their medicinal relevance or potential cytotoxicity.

Conclusion

The findings of this study can be used to conclude that the methanolic and alkaloidal extract of *Hunteria umbellata* leaves contain a wide variety of medicinally significant compounds which aligns with both the local medicinal uses of the plant and existing pharmacological literature on the plant extract. The presence of these bioactive constituents underscores the therapeutic value of *Hunteria umbellata* prompting the need for further exploration in drug development and natural product research.

References

1. Adeneye A. and Adeyemi, O., 2009. Hypoglycemic effects of the aqueous seed extract of Hunteria umbellata in normoglycemic and glucose and nicotine induced hyperglycemic rats. International Journal of Natural Products Research, vol. 2, no. 1, pp. 9–18.
2. Adeneye, A.A., Sofidiya, M.O., Adenekan, O.S., 2011. Anti-inflammatory and antioxidant activities of Hunteria umbellata seed fractions. Pharmacologia 2(6), p.165-171.
3. Ahmad, F., Khalid, P., Khan, M. M., Rastogi, A. K., & Kidwai, J. R., 1989. Insulin like activity in (−) epicatechin. *Acta diabetologia latina*, *26*(4), pp. 291-300.
4. Chen, K., Preuss, A., Hackbarth, S., Wacker, M., Langer, K., & Röder, B., 2009. Novel photosensitizer-protein nanoparticles for photodynamic therapy: photophysical characterization and in vitro investigations. Journal of photochemistry and photobiology. B, Biology, 96(1), 66–74.
5. Erharuyi, O., Falodun, A., & Langer, P., 2014. Medicinal uses, phytochemistry and pharmacology of Picralima nitida (Apocynaceae) in tropical diseases: A review. Asian Pacific journal of tropical medicine, 7(1), 1-8.
6. Fox Ramos, A.E., Pavesi, C., Litaudon, M., Dumontet, V., Poupon, E., Champy, P., Genta-Jouve, G. and Beniddir, M.A., 2019. CANPA: computer-assisted natural products anticipation. Analytical chemistry, 91(17), pp.11247-11252.
7. Igbe, I., Omogbai, E.K. and Ozolua, R.I., 2009. Hypoglycemic activity of aqueous seed extract of Hunteria umbellata in normal and streptozotocin-induced diabetic rats. Pharmaceutical Biology, 47(10), pp.1011-1016.
8. Igbe, I., Ozolua, R. I., Okpo, S. O., & Obasuyi, O., 2009b. Antipyretic and analgesic effects of the aqueous extract of the fruit pulp of Hunteria umbellata K Schum (Apocynaceae). Tropical Journal of Pharmaceutical Research, 8(4).
9. Kamimura, A., & Takahashi, T., 2002. Procyanidin B-2, extracted from apples, promotes hair growth: a laboratory study. British Journal of Dermatology, 146(1), pp. 41-51.
10. Manske, R. H. F., & Holmes, H. L. (Eds.)., 2014. The alkaloids: chemistry and physiology. Elsevier.
11. Mechchate, H., Es-Safi, I., Haddad, H., Bekkari, H., Grafov, A., & Bousta, D., 2021. Combination of Catechin, Epicatechin, and Rutin: optimization of a novel complete antidiabetic formulation using a mixture design approach. The Journal of Nutritional Biochemistry, 88, 108520.
12. Oboh, G., Adebayo, A. A., Ademosun, A. O., & Abegunde, O. A., 2019. Aphrodisiac

effect of Hunteria umbellata seed extract: Modulation of nitric oxide level and arginase activity in vivo. Pathophysiology : the official journal of the International Society for Pathophysiology, 26(1), pp. 39–47.

13. Sandberg F., Corrigan D., 2001. Natural remedies: Their origins and uses. Abingdon: Taylor and Francis. 2001.
14. Shang, J. H., Cai, X. H., Feng, T., Zhao, Y. L., Wang, J. K., Zhang, L. Y., ... & Luo, X. D., 2010. Pharmacological evaluation of Alstonia scholaris: Anti-inflammatory and analgesic effects. Journal of ethnopharmacology, 129(2), pp. 174-181.
15. Stafford, G. I., Jäger, A. K., & Van Staden, J., 2009. African psychoactive plants. African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality, 323, 346.
16. Tam, S. W., Worcel, M., & Wyllie, M., 2001. Yohimbine: a clinical review. Pharmacology & therapeutics, 91(3), pp. 215-243.
17. Terao, J., Piskula, M., & Yao, Q., 1994. Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Archives of Biochemistry and Biophysics, 308(1), pp. 278-284.
18. Van Beek, T. A., Kuijlaars, F. L. C., Thomassen, P. H. A. M., Verpoorte, R., & Svendsen, A. B., 1984. Antimicrobially active alkaloids from Tabernaemontana pachysiphon. *Phytochemistry*, *23*(8), pp.1771-1778.
19. Vicki Brower, Back to Nature: Extinction of Medicinal Plants Threatens Drug Discovery, JNCI: Journal of the National Cancer Institute, Volume 100, Issue 12, 18 June 2008, Pages 838–839.
20. Wang, M., Carver, J.J., Phelan, V.V., Sanchez, L.M., Garg, N., Peng, Y., Nguyen, D.D., Watrous, J., Kapono, C.A., Luzzatto-Knaan, T. and Porto, C., 2016. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. Nature biotechnology, 34(8), pp.828-837.