

Identification of Phytochemicals Present in the Leaves of *Pterocarpus Mildbraedii* Harms by GC/MS Analysis

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Abstract: Phytochemicals are non nutritive bioactive chemical compounds that occur naturally in plants, which are considered to have beneficial effect on human health and have disease preventive properties. The phytochemical compositions of the leaves of *Pterocarpus mildbraedii* Harms, used widely in soup preparation in Nigeria were studied. The ethanol extract of the leaves was subjected to GC-MS analysis. Seven (7) Phytoconstituents were identified from the leaves with fagasterol or lupeol constituting the bulk of the oil (42.04%), followed by oleic acid (20.38%), and palmitic acid (11.46%). Other constituents are: 1,2,3,4 Butanetetrol or Erythritol (5.73%), N,N Dimethyl-2-propyn-1- amine (4.46%), 1,2-Benzenediol (7.64%), 4-Hydroxypiperidine (8.28%), and n-Hexadecanoic acid (11.46%). The presence of these compounds in the plant proved that the plant can be used as a potential food and drug.

Keywords: oha, hypocholesterolemic, chemoprotective.

I. Introduction

Nigeria is blessed with many medicinal plants whose roots, barks, seeds and leaves are used for the treatment of different diseases. The leaves of *Pterocarpus mildbraedii* Harms locally known as “Oha” in the Eastern Nigeria is one of the vegetables consumed widely in Nigeria. Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw, or cooked (Fayemi, 1999, Dhellot *et al.*, 2006, Hassan *et al.*, 2007). Vegetables are valuable in maintaining alkaline reserve of the body. They are valued mainly for their high carbohydrate, vitamin and mineral contents (Awol, 2014). Vegetables are rich sources of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorus (Fasuyi, 2006). In addition, they contain phytochemicals or anti nutrients which reduce their bioavailability (Akindahunsi and Salawu, 2005). According to Aletor and Adeogun 1995, some anti nutritional compounds exhibit protective effects, thus making them to serve a dual purpose of reducing some essential nutrients and protecting the body against a number of biochemical, physiological and metabolic disorders. Vegetables may be edible roots, stems, leaves, fruits or seed. Each group contributes to diet in its own way (Robinson, 1990 ; Chinma and Igyor (2007).). However, there are some inexpensive leafy vegetables whose nutritive and anti-nutritive potentials are yet to be adequately studied and utilized. Among these leafy vegetables are the leaves of *Pterocarpus mildbraedii* Harms. The leaves which are used for soup preparation give good taste and palatability. *P. mildbraedii* Harms is found in Sierra Leone, Liberia, Ghana, Nigeria, Cameroon, Equatorial Guinea and Tanzania. *Pterocarpus mildbraedii* Harms is a green leafy vegetable which grows more like a big tree; it reaches a height of 2m (6.6ft) and its stem has a diameter of 20m (0.79 inch). *P. mildbraedii* Harms has a smooth, gray or pale brown bark, exuding red gum when cut. Two species are recognized locally (*Pterocarpus mildbraedii* Harms (Oha) and *Pterocarpus santalinoides* (uturukpa). *P. mildbraedii* is a good source of beneficial chemicals. It has antioxidant, hypocholesterolemic, chemoprotective and anti-bacterial properties (Ujowundu *et al.*, 2010).

The nutritional and antinutritional constituent of the leaves of *Pterocarpus mildbraedii* Harms have been reported by Ujowundu *et al.*, 2010 and Onyeka and Nwambakwe, 2007. Ujowundu *et al.*, 2013 reported that the leaves of *Pterocarpus mildbraedii* Harms among six other green leafy vegetables studied are the most enriched. The leaves are good sources of alkaloids, flavonoids and tannins. This indicates that the leaves of *Pterocarpus mildbraedii* Harms can serve as antispasmodic, analgesic, diuretic, anti-inflammatory and antioxidant agents (Uchegbu and Okwu, 2012; Uchegbu *et al.* 2013). The leaves of *Pterocarpus mildbraedii* Harms were also reported to have adequate minerals and high amount of vitamins A, B₁, B₂, B₅, B₆ and C. (Ujowundu *et al.* 2010). The decoction of this very plant is used in treatment of fever which is accompanied by indigestion or diarrhea. Decoction was given in case of malaria.

In spite of the nutritional quality of the leaves of *Pterocarpus mildbraedii* Harms, the phytoconstituents have not been fully documented. This present research will examine the phytochemical constituents of these leaves using GC-MS to ascertain their usefulness as healthy food and potent drug.

II. Materials And Methods

2.1 Plant materials: sample collection / preparation

Fresh leaves of *P. mildbraedii* Harms were bought from Ekeonuwa market in Owerri, Imo state Nigeria. The leaves were washed, allowed to drain, then pounded with mortar and pestle. The pounded leaves were soaked in ethanol for 48 hours, 1ml of the extract was subjected to GC/MS analysis.

2.2 Experimental procedure of gas chromatography – mass spectrometry (GC-MS).

The GC analysis were carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV- 101) coated with polymethyl silicon (0.25mm x 50m) and the conditions were as follows: Temperature programming from 80- 200°C held at 80°C for 1 minute, rate 5°C/min and at 200°C for 20 min. FID temperature 300°C, injection temperature of 250°C and carrier gas nitrogen at a flow of 1ml /min, split ratio 1:75. GC- MS analysis was conducted using GCMS- QP 2010 PLUS SHIMADZU JAPAN with injector temperature of 230°C and carrier gas pressure of 100 kpa. The column length was 30m with a diameter of 0.25mm and the flow rate of 50ml/min. the elutes were automatically passed into a mass spectrometer with a detector voltage set at 1.5kv and sampling rate of 0.2 sec. The mass spectrometer was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents like ethanol, chloroform, diethyl ether, hexane were all analytical grade and were procured from MERCK, GERMANY.

Component Identification :

Oil components were identified by matching the peaks with Computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks with those from literature (Okwu and Ighodaro, 2010; Uchegebu *et al*, 2014).

III. Results and Discussion

The ethanol extract of the leaves of *P. mildbraedii* Harms on GC-MS analysis revealed rich phytochemicals. The molecular formula, the molecular weight, the retention time and the percentage constituents of the compounds are shown in Table 1.

The first compound is a polyhydroxyl compound, 1,2,3,4-Butanetetrol or Erythritol with molecular formula, $C_4H_{10}O_4$ (m/z 122). It constitutes 5.73% of the oil. The second compound is an amine, N,N-Dimethyl-2-propyn-1-amine. The formula is C_5H_9N , with molecular mass of 83, it constitutes 4.46% of the oil. The third compound is identified as 1,2 - Benzenediol or 0-Dihydroxybenzene with molecular formula $C_6H_6O_2$ (m/z 110). The base peak occurred at m/z 110. Its percentage composition is 7.64%.

The fourth compound is 4-Hydroxypiperidine. Its molecular formula is $C_5H_{11}NO$ with the molecular mass of 101. It comprises 8.28% of the oil. The base peak occurred at m/z 82. The fifth compound is n-Hexadecanoic acid or palmitic acid with molecular formula, $C_{16}H_{32}O_2$ m/z 256. It comprises 11.46% of the oil. The sixth compound is identified as 9-octadecenoic acid or oleic acid. The molecular formula is $C_{18}H_{34}O_2$ with molecular mass 282. It constitutes 20.38% of the oil. The seventh compound is a steroid known as lupeol or fagasterol with molecular formula $C_{30}H_{50}O$, m/z 426. It constitutes the highest percentage, 42.04% of the oil.

The leaves of *P. mildbraedii* Harms contain 1,2 - Benzenediol. Benzenediol has been reported to be present in vegetables, fruits, coffee, wine and tea. It has been found to be the remedy for cardiovascular diseases, including stroke, atherosclerosis and some infections (Oke, 1968). The benzenediol was also found to be the remedy in the treatment of cancer, renal diseases, diabetes, arthritis and toxic to microorganisms (Awa *et al*, 2012).

P. mildbraedii leaves were found to contain lupeol or fagasterol. Lupeol is a pharmacologically active triterpenoid. It has several potential medicinal properties. Lupeol has a complex pharmacology, displaying antiprotozoal, antimicrobial, antiinflammatory, antitumor and chemopreventive properties (Gallo, and Sarachine, 2009). It is an effective inhibitor in laboratory models of prostate and skin cancers (Prasad *et al*, (2008); Nigam *et al*, (2007); Saleem *et al*, (2004).

The leaves of *P. mildbraedii* were also found to contain oleic acid. Oleic acid is used as emollients, small amount of oleic acid is used as an excipient in pharmacy, and consumption of oleate in olive oil has been associated with a decreased risk of breast cancer and reduction of blood pressure (Teras *et al*, 2008).

n-Hexadecanoic acid was also found to be present in *P. mildbraedii*., in India, medicated oils rich in n-Hexadecanoic acid are used in the treatment of rheumatism and inflammation (Aparna *et al* 2012 and Smolinske and Susan, 1992).

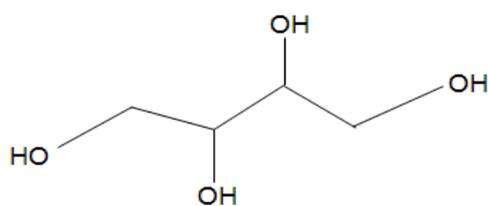
The leaves of *P. mildbraedii* were found to contain a phenolic compound 1,2,3,4-Butanetetrol. Plants that have significant medicinal values have been found to be rich in phenolics. Phenolic compounds are known to have antioxidant properties (Uchegbu *et al*, 2013). Antioxidants are considered as any species that reduce the oxidizing species i.e. free radicals that would damage the substrate (Uchegbu *et al*, 2013; Benzie and Stain 1999). The presence of this phenolic compound in the leaves of *P. mildbraedii* shows that the extracts from the leaves could be used as an antioxidant and thus may be used in the treatment of diseases such as cancer, liver and heart diseases.

IV. Conclusion

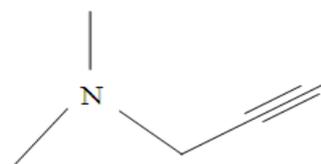
Results of the investigation have revealed that the leaves of *P.mildbraedii* are not only palatable in soup but also very medicinal. All the phytochemicals present in the leaves proved that the plant could serve as anti-inflammatory, anti-oxidant, anti-tumor and anti microbial agent. The leaves of *Pterocarpus mildbraedii* Harms could be used in the treatment of diseases such as cancer, liver, kidney, heart diseases, arthritis, cardiovascular disease and diabetes. The nutritional and health benefits of the leaves of *P.mildbraedii* Harms have proved the plant to be a potential source of useful drugs and quality food. It is believed that the results of this study will help to stimulate consumption or utilization of this leafy vegetable as it is a good source of micronutrients needed for healthy growth. The phytopharmacological activities reported in the work proved that the medicinal value of *P.mildbraedii* is unimaginable. The presence of bioactive compounds and pharmacological activities proved the potency of the plant in the development of novel drugs in future.

Table 2: GC-MS analysis of ethanol extracts of the leaves of *P. mildbraedii*

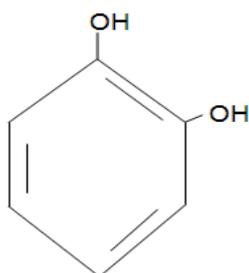
Peak	Compound	Molecular Formula	Molecular Weight	Retention Time	% Content	Fragment peak M/z
1	1,2,3,4-Butane tertrol or Erythritol	C ₄ H ₁₀ O ₄	122	5.417	5.73	27(20%),31(50%),44(98%),61(100%), 91(30%).
2	N,N Dimethyl-2-propyn-1-amine	C ₅ H ₉ N	83	6.425	4.46	39(30%), 42(100%), 58(20%), 82(90%).
3	1,2-Benzenediol	C ₆ H ₆ O ₂	110	9.008	7.64	53(15%), 64(40%), 81(20%), 110(100%)
4	4-Hydroxypiperidine	C ₅ H ₁₁ NO	101	12.675	8.28	27(20%), 28(40%), 42(60%), 56(100%), 82(40%), 101(60%).
5	n-Hexadecanoic acid or palmitic acid	C ₁₆ H ₃₂ O ₂	256	18.508	11.46	27(20%), 41(75%), 43(100%), 60(80%), 73(90%), 129(30%), 256(20%).
6	9-octadecenoic acid or oleic acid	C ₁₈ H ₃₄ O ₂	282	21.208	20.38	27(25%), 41(100%), 55(80%), 69(40%), 83(30%), 97(25%).
7	Lupeol or fagarsterol	C ₃₀ H ₅₀ O	426	27.817	42.04	41(65%), 43(100%), 68(99%), 81(80%), 95(75%), 109(75%), 121(60%), 135(60%), 207(60%), 426(20%).



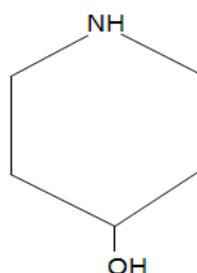
(1) 1,2,3,4-Butanetetrol



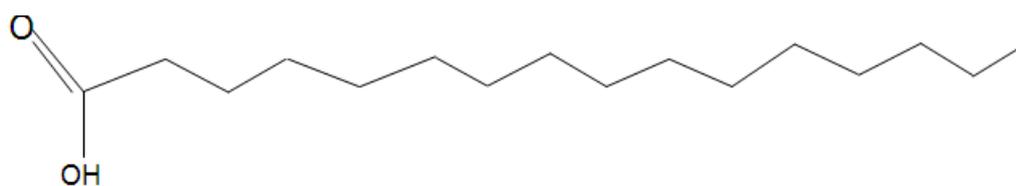
(2) N, N-Dimethyl-2-propyn-1-amine.



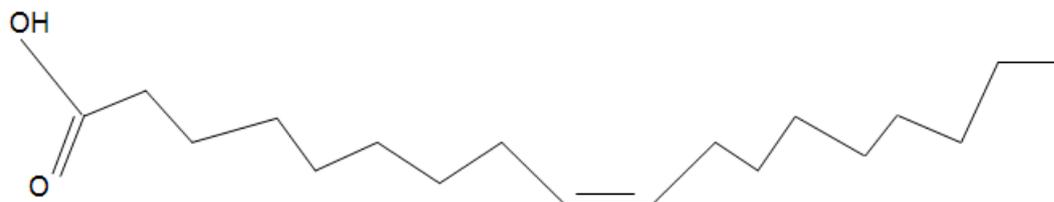
(3) 1,2-Benzenediol.



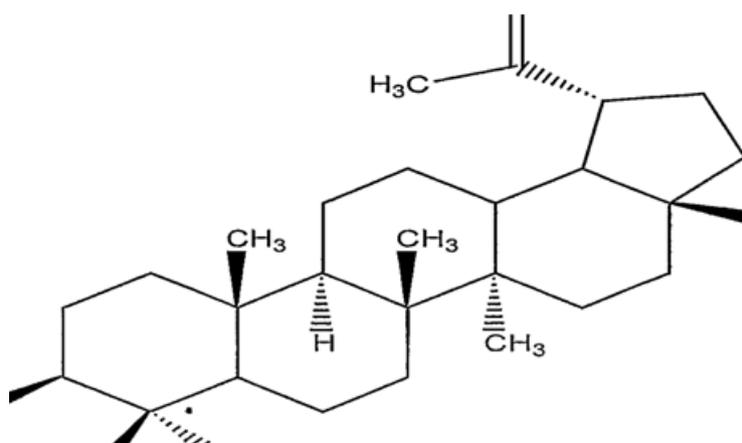
(4) 4-Hydroxypiperidine.



(5) n-Hexadecanoic acid



(6) 9-Octadecenoic acid (oleo acid)



(7) Lupeol or Fagasterol.

Fig 1: Structures of compounds from *Pterocarpus mildbraedii* leaves.

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