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Comparison of chemical compositions of essential oils from the fresh and dried leaves of *Tapinanthus bangwensis* (Engl. and K. Krause) Danser [Loranthaceae]

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Abstract
The essential oil of *T. bangwensis* (Loranthaceae) leaves was obtained by hydrodistillation and the essential oils chemical constituents were analysed by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). Volatile oils were obtained differently from fresh and dried leaves of *T. bangwensis* in yields of 0.76% and 0.67% (v/w) respectively. A total of ten and nine compounds representing 99.9% and 99.8% of the total oil contents were identified respectively from the fresh and dried samples. Major constituents in the fresh leaves are pentacosane (18.4%) heptacosane (12.5%), hexacosane (12.5%), heneicosane (12.4%), tetracosane (10.5%), and octacosane (10.4%) while the dried leaves consist mainly of (*Z*)-9-octadecenamide (27.9%), *n*-hexadecanoic acid (13.8%), pentadecane (12.5%), hexadecane (11.2%) and tetradecanamide (11.2%). It was found that *n*-hexadecanoic acid was present in both samples though in different percentage. The oil constituents of fresh sample were found to be majorly hydrocarbons while amide was found to be the major constituent of the dried sample.

Keywords: *Tapinanthus bangwensis,* Loranthaceae, Volatile oils, Gas Chromatography-Mass Spectroscopy [GC-MS], Hydrocarbons

Introduction
Tapinanthus bangwensis plant belongs to the family of Loranthaceae (Mistletoes). Mistletoes are plant parasites with the intention of obtaining food and water on other plants. They are highly specialized angiosperms which are well known as broad host range hemiparasites of a variety of different gymnosperms and angiosperms [1]. The host plants include cocoa tree, kolanut tree, rubber tree and orange tree [2, 3]. They cause important damages to their hosts with great economic loss [4, 5]. Mistletoes comprise about 900 species in 65 genera, which are mainly found in Africa, Asia, Australia and South America [6]. Mistletoes have been reported to be used in religious rites and in medicinal use which includes anti-diabetic, antioxidant and antihypertensive [7-9]. Mistletoe leaves is a good source of tannin, vitamin B1, vitamin C and calcium [10]. The leaves extracts of *Tapinanthus globiferus* is an antitrypanosomal [11]. The aqueous extract of mistletoe is used to normalize blood sugar and cholesterol levels in rat according to Iheanacho *et al*., 2008 [12]. The reduction in the blood sugar level noticed by mistletoe is as a result of the presence of lectins which are a protein that bind sugars as reported by Hostanska *et al*., 1995 [13]. *Tapinanthus bangwensis* is a parasitic woody shrub which grows on different trees like kola nut, orange, cocoa. It is called 'African Mistletoe', native to Senegal, West Cameroun, Congo basin but are also found in most parts of the world especially West Africa, they are indigenous to Nigeria. It is the commonest of the West Africa mistletoes [14]. They are applied in ethnomedicine for treating diverse ailments including leprosy. It serves as purgatives, anti-rheumatism and anthelmintic [14]. The methanolic extracts of *Tapinanthus bangwensis* had been reported to have anti-inflammatory [15] and antibacterial activities [16]. Phenolic compounds such as gallic acids derivatives have been isolated from *T. bangwensis* and are recognised to show anti-inflammatory, antimicrobial and anticancer activities [17]. Also, *T. bangwensis* leaves is rich in iron, calcium, manganese and with trace of magnesium, sodium and potassium [18]. *T. bangwensis* is used in Nigeria especially to treat hypertension and diabetes [19]. This study was carried out to investigate the essential oil constituents of the fresh and dried leaves of the *T. bangwensis* species grown in Nigeria; a comparative study.

Material and Methods

Plant Materials: The fresh leaves of *Tapinanthus bangwensis* growing on *cola acuminata* Schott and Endl. were harvested at Ago-Iwoye, Ogun state, in May 2009. The plant sample was authenticated by Prof. A. A Ayodele of the University Herbarium, Department of Botany University of Ibadan, Nigeria where a voucher specimen was deposited with herbarium number UIH 22399

Isolation of Essential Oils: The oils were obtained by hydrodistillation of fresh leaves (500 g) and air dried leaves (400 g) using a Clevenger-type apparatus for 3 hour in each case. The oils were dried over anhydrous sodium sulphate (Na_2SO_4) and stored in vials at a low temperature until analysis.

Gas Chromatography (GC) Analysis: The volatile oil samples were subjected to GC analysis on a Shimadzu model QP2010 PLUS chromatography fitted with a flame ionization detector (FID) and DB-5 (30 x 0.25 mm, 0.25 µm film thickness). The column inlet pressure was 100.2 kPa. Helium was used as carrier gas with a flow of 1mL/min. The GC oven temperature was programmed at 60 ºC (hold for zero min.), heated to 140ºC at 5 ºCmin^{-1}, with a final hold time of 10 min. at 280 ºC. Injector and detector temperature were fixed at 200 ºC and 250 ºC respectively.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis: The gas chromatography-mass spectrometry (GC–MS) analysis was performed on a Shimadzu model QP2010 gas chromatography with split injector interfaced to a 5973 mass selective detector operated at 70 eV with a mass range of m/z 40–420. The oven temperature was programmed from 60-280 °C (hold for 5 minutes) at a rate of 3 °C/min. The same operations and temperature programmings were used as for GC. FID chromatogram was used to calculate the relative percentage amounts of the separated compounds.

Identification of Components: Identification of each individual constituent of the essential oil was achieved based on their retention indices (determined with a reference to a homologous series of normal alkanes) and by comparison of their mass spectral fragmentation patterns (NIST database/ Chemstation data system) with data previously reported in the literature [20, 21].

Results and Discussion

The yield of essential oils obtained from the fresh (500g) and dried (400g) leaves of *T. bangwensis* were 0.76 % v/w and 0.67 % v/w respectively. The essential oils from the samples were colour less with a herbal smell. A total of 19 compounds were identified from both samples representing 55% of the oil from the fresh sample and 45% of the oil from the dried sample**.** The GC and GC-MS analyses of the fresh leaves of *T. bangwensis* revealed a total of ten compounds representing 99.9 % of the essential oil, while nine compounds representing 99.8 % were identified in dried leaves essential oil. The constituents of both the fresh and dried leaves volatile oils of *T. bangwensis* are presented in Table 1. The major constituents in the fresh leaves oil of *T. bangwensis* were pentacosane (18.4%), heptacosane (12.5 %), hexacosane (12.5%), heneicosane (12.4%) tetracosane (10.5%) and octacosane (10.4%) while dried leaves oil consist mainly of (*Z*)-9-octadecenamide (27.9%), n-hexadecanoic acid (13.8%), pentadecane (12.5%), hexadecane (11.2 %) and tetradecanamide (11.2 %). Minor constituents in the fresh leaves oil were nonacosane (9.4 %), pentacosane (8.2 %), n-hexadecanoic acid (7.1 %), hexadecanol (5.0 %) and undecane (1.7 %) while minor constituents in the dried leaves oil were 9-hexadecenoic acid (9.9 %), 3,7-dimethyl nonane (6.1 %), (2,2^1 – oxy-bis-propane) di-isopropyl ether (3.6 %) and 3,7-dimethyl nonane (3.6 %). n-hexadecanoic acid was detected in both oils. Its percentage is 7.1 % and 13.8 % for fresh and dried leaves respectively. Tetracosane, pentacosane, hexacosane, heptacosane, pentadecane and hexadecane found in this plant had been reported to be present in *Viscum album* and *Taxillus Kaempferi* [22]. However, octacosane and nonacosane are added essential oil constituents of mistletoe.

The Table 2 showed that the essential oils of the fresh leaves are characterised with a high percentage of hydrocarbon, fatty acid, and an alcohol while dried leaves are characterised with amide, ether and hydrocarbons. From the result, it showed that there is a very significant difference in the constituents of essential oils obtained from the fresh and dried leaves of the plant. This could have been as a result of drying condition(s) and climatic factors. The presented results showed clearly that essential oils of both the fresh and dried leaves could be a good anti-inflammatory since both samples contained n-hexadecanoic acid that had been reported to be anti-inflammatory [23].

Table 1: Constituents of Essential Oil from Fresh and Dried Leaves of *T. bangwensis.*

Compound	Retention Index	% Composition Fresh	% Composition Dry
(2,2^1-oxy-bis-propane) di-isopropyl ether	565		3.6
3,7-dimethyl nonane	986		3.6
Undecane	1100	1.7	-
2,3,8-trimethyl decane	1121	-	6.1
Pentadecane	1500	-	12.5
Hexadecane	1600	-	11.2
Tetradecanamide	1822	-	11.2
Hexadecanol	1854	5.0	-
n-hexadecanoic acid	1968	7.1	13.8
9-hexadecenoic acid	1976	-	9.9
Heneicosane	2100	12.4	-
(z)- 9- octadecenamide	2228	-	27.9
Tetracosane	2400	10.5	-
Pentacosane	2500	18.4	
Hexacosane	2600	12.5	-
Heptacosane	2700	12.5	-
Octacosane	2800	10.4	-
Nonacosane	2900	9.4	-
Total		99.9%	99.8%

- Not detected

Table 2: Comparison between the class of organic compounds in Fresh and Dried leaves essential oil of *T. bangwensis.*

Class of Organic Compound	Amount of essential oils (%)	
	Fresh Leaves	**Dried Leaves**
Hydrocarbon	87.8	33.4
Alcohols	5.0	-
Fatty acid	7.0	13.8
Amides	-	39.1
Ester	-	3.6
Carboxylic acid	-	9.9

Conclusion

The chemical constituents of the essential oil from both fresh and dried leaves of *T. bangwensis* showed that it could be a good source of hydrocarbons since its constituents were majorly hydrocarbon. Further studies could be done on its biological activities such as antimicrobial, anti-inflammatory, toxicity and to compare volatile oil constituents of *T. bangwensis* on different host trees. This study is reporting the chemical composition of essential oils of fresh and dried leaves of *T. bangwensis* in South-West of Nigeria for the first time to the best of our knowledge.

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