



SHORT COMMUNICATION

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF THE ESSENTIAL OIL OF *Euphorbia heterophylla* LEAVES (Euphorbiaceae) INDIGENOUS TO NIGERIA.

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Abstract

Essential oil was extracted from the fresh leaves of *Euphorbia heterophylla* indigenous to Nigeria using hydro distillation method. The essential oil extracted was analyzed using Gas chromatography mass spectroscopy technique (GC-MS). Antibacterial activities of the essential oil were investigated using Agar well diffusion method. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aureginosa*, *Klebsillae pseumoniae* were used. Stigmaterol, Beta amyron, acetamide and squalene were present in the essential oil, with stigmaterol being the predominant constituent with a percentage composition of (23.99 %). The Essential oil fraction was more active against *Staphylococcus aureus* and *Pseudomonas aureginosa* at a concentration of 5 to 40 mg/ml.

Keywords: Antibacterial, essential oil, *Euphorbia heterophylla*, Stigmaterol

Introduction

Essential oils (E.O) are volatile oils that give plants their characteristics odors and are used especially in perfumes and flavorings, and for aromatherapy. They are soluble in alcohol, ether but insoluble in water. These volatile oils are generally liquid and colorless at room temperature. They have a characteristics odor, are usually liquid at room temperature and have a density less than unity with exception of few cases (e.g. cinnamon and vetiver). These volatile oils, contained in herbs are responsible for different scents of fruits. E.O also possesses antifungal or insecticide and deterrent activities. These E. Os are complex mixtures that may contain 300 different compounds. These components belong mainly to the vast

majority of the terpene family (Wiisal *et al.*, 2016).

Euphorbia heterophylla L. is widely used in African medicines and in tropical countries. In Africa, a decoction or infusion of the stems and fresh/ dried leaves is taken as a purgative and laxative to treat stomach ache and constipation and to expel intestinal worms (kokwaro, 1993). In Nigeria, the latex and preparations of the leaves and root are applied to treat skin tumors. In peninsular Malaysia, a leaf extract is taken to treat body pain. In Benin, the leaves are eaten as a vegetable, despite their laxative action (Falodun, 2004). 1,8 cineole (32.03%), camphor (16.54%), Beta elemene, limonene and alpha pinene were present in *Euphorbia heterophylla* grown and collected in Egypt (Elsahamy *et al.*,2019). The essential oil of

Euphorbia heterophylla grown in Nigeria is poorly studied. This study investigates the chemical constituents of essential oil fraction of *Euphorbia heterophylla* grown in Nigeria using Gas chromatography Mass spectroscopy technique and the antibacterial activities of this essential oil.

Methods

Collection of Plant & Extraction of Essential Oil

Fresh leaves of *Euphorbia heterophylla* were collected from Ijebu-Ode, Ogun state Nigeria and authenticated at the forestry research institute of Nigeria (FRIN) in Ibadan, Oyo state with tag no FHI No 112442. The leaves were chopped and subjected to hydro distillation extraction for 4 hrs using Clevenger type apparatus. The obtained oil was preserved in a sample bottle and stored under refrigeration.

Gas Chromatography and Gas Chromatography Mass Spectroscopy (GC and GC/MS) Analysis

Oil sample analysis were performed on Agilent Gas Chromatograph 7890 required with a Flame Ionization Detector and a non-polar HP-5MS capillary column made of 5% Phenylmethylsilicone, 95% dimethylpolysiloxane (30 m x 0.25 mm i.d., 0.25 µm film thickness). Chromatographic conditions were as follows: Helium as carrier gas at a flow rate of 1mL/min; injection volume was 0.1µL; column temperature was 60 °C for 10 min, and programmed at the rate of 3°C/min to 200°C, and finally held isothermally for 10 min. The detector and injection temperatures were 250 and 220 °C, respectively. Samples were injected by splitting, and the split ratio was 1:20. Analysis was performed on GC/MS (Agilent Model 7890 MSD) equipped with a HP 5MS capillary column (30 m x 0.25mm i.d., 0.25 µm coating) and temperature programming was done as described above. The carrier gas

used was Helium at a flow rate of 1.0 mL/min and the split mode had a ratio 1:20. The injection port was set at 220°C.

Identification of Components

Identification of components was done by comparing their relative retention times with the retention times of authentic standards. Further identification was performed by comparison of their mass spectra with Wiley Library data and commercially available data (NIST, 2011). Their retention indices were also compared with literature values (Adams, 2009).

Antibacterial screening (Dilution method agar well diffusion techniques)

Agar well diffusion techniques was used. 20 mL of agar plates were seeded with 1ml of an overnight both culture of each bacteria in sterile petri dish. The seeded plates were allowed to set after a uniform distribution of the bacteria. A standardized sterile cork was used to cut uniform wells in the surface of the agar. These wells were filled with different concentrations of extracts with a sterile syringe. Plates were allowed to stand or observed for 1 h at room temperature. All plants were incubated and observed for zones of inhibition.

Result and Discussion

The essential oil fraction of *Euphorbia heterophylla* contains stigmasterol (23.99%), beta amyrene (17.47%), squalene (5.32%) and acetamide (0.57%) with stigmasterol with the highest percentage of 23.99% as shown in table 1. This is the first time these compounds are reported in the essential oil fraction of *Euphorbia heterophylla* grown in Nigeria.

1, 8 cineole (32.03%), camphor (16.54%), Beta element, limonene and alpha pinene found in *Euphorbia heterophylla* grown and collected in Egypt (Elsahamy *et al.*, 2019). Essential oils have a very high variability of their composition, both in qualitative and quantitative terms.

Table 1: Chemical composition of essential oil from *Euphorbia heterophylla*

S/No	Retention Time (Mins)	Molecular Mass (G/Mol)	Compounds	Percentage Composition (%)
1	20.844	412.7	Stigmasterol	23.99
2	23.154	424.7	Beta amyrone	17.47
3	23.740	410.7	Squalene	5.32
4	19.063	59.1	Acetamide	0.57

Various factors are responsible for this variability and can be grouped into two components which are intrinsic factors related to the plant, and interaction with the environment (soil type) and climate and the maturity of the plant (Hussain, 2008). Also, extrinsic factors related to the extraction method and the environment. Other

parameters are; Seasonal variations, plant origin, degree of maturity of the plant, geographic origin (Marotti *et al.*, 1994). These factors may be responsible for the variation in the constituents present in *Euphorbia heterophylla* grown and collected from different countries.

Table 2: Antibacterial activities of essential oil from *Euphorbia heterophylla*

Concentration (mg /mL)	Inhibition zones			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas auregonosa</i>	<i>Klebsillae pneumoniae</i>
40	+	+	+	+
20	+	+	+	+
10	+	-	+	-
5	-	-	-	-

The essential oil fraction showed activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas auregonosa* and *klebsillae pneumoniae* at concentration of 20 mg/mL to 40 mg/mL as shown in Table 2. At concentration of 10 mg/mL, it showed

activity against *Staphylococcus aureus* and *Pseudomonas auregonosa* only. This indicated that the essential oil fraction from *E. heterophylla* collected from Nigeria is effective against all the bacteria used in this study at a concentration of 40 mg/mL

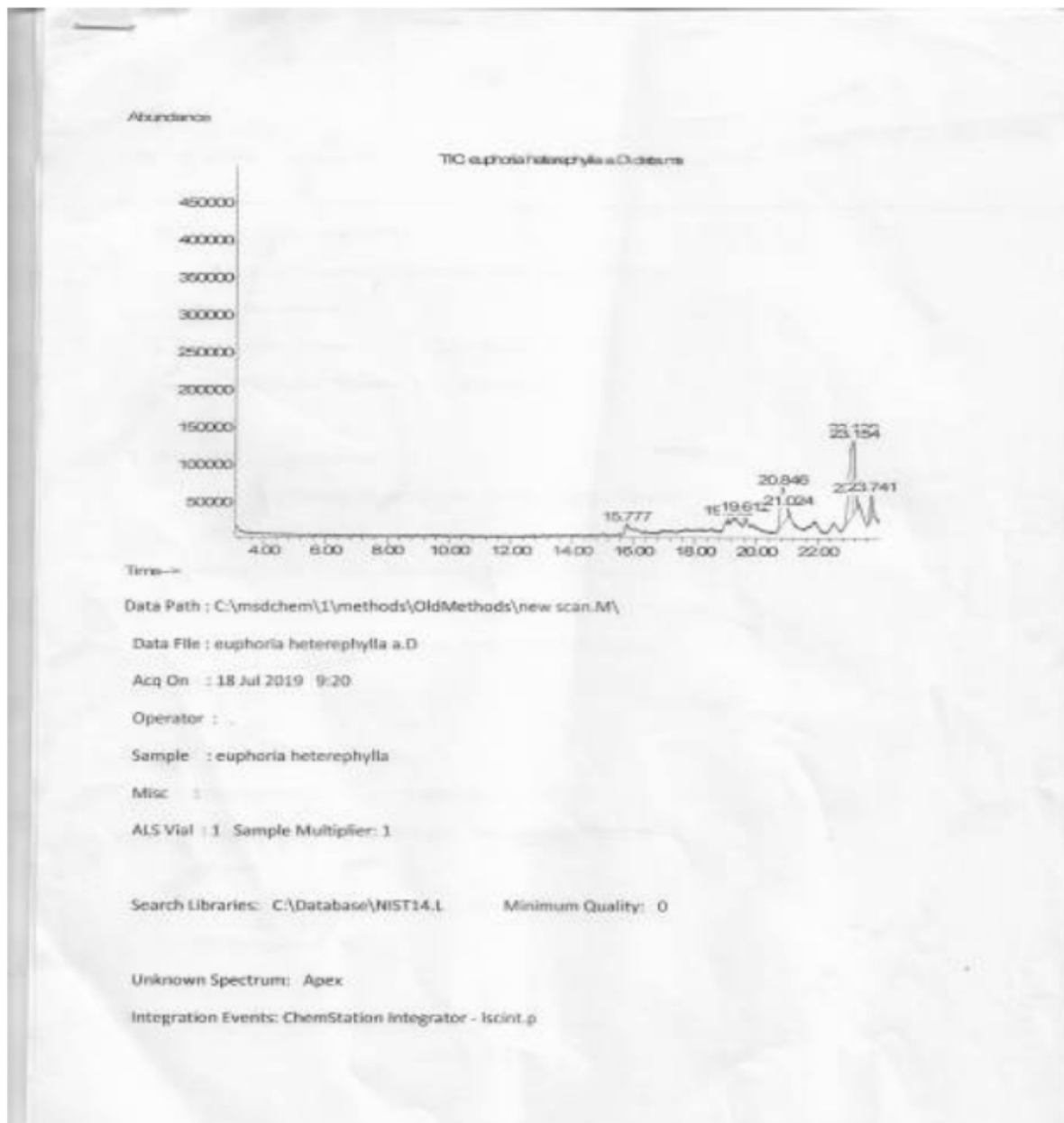


Figure 1: Chromatography Result

Conclusion

The traditional usage of this plant in the treatment of various ailments (such as a purgative and laxative to treat stomach ache, constipation and to expel intestinal worms) may be due to the chemical

composition of the essential oil fraction which showed antibacterial activity. Further work should be done to screen the essential oil fraction for antioxidant activity.

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