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CHARACTERIZATION OF LIPIDS FROM THE LEAVES OF *Symphonia globulifera* Linn.

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ABSTRACT: Lipids from the leaves of *S. globulifera* was extracted and characterized. The total lipid was 4.86%. The lipid content of *S. globulifera* leaf contained 80% neural lipids, 15% glycolipid, and 5% phospholipids. TLC revealed six lipid classes: diglyceride, cholesterol, esters, triglyceride, free fatty acid, sterol which is classified according to their RF ratios. The mass spectrum revealed that the methyl group appeared at $\delta 80.83$ and were confirmed by signals between $\delta 174.2$ indicating carboxylic group. This compound was characterized as ergosterol carboxylic acid. The phytochemical analysis revealed moderate amount of saponins, flavonoids, tannins, glycosides and trace amount of alkaloids. The proximate analysis of *Symphonia globulifera* leaf revealed the presence of 47.71% moisture, 6.25% ash, 2.10% fibre, 14.88% crude protein, 3.98% crude lipid and 74.8% carbohydrate.

INTRODUCTION

Symphonia globulifera commonly called Boarwood is the family Gultiferae which contains about 40 genera and 1000 species. However, only a species grow in Nigeria (Mabberly, 1987). They are trees, shrubs, lianes and herbs with yellow or bright coloured resinous juice. A number of useful timbers, drugs, dyes, gums, pigments and resin are derived from members of the family (Trease and Evans, 1989).

Most of tropical Africa is already beset with food crisis which threatens nutritional potential (Okigbo, 1993) especially on their lipid constituents. It has been estimated that only about 1% of 250,000 known species of higher plant is utilized from human needs and there is much need for the discovery of new products from lipids with special consideration on colour, flavour and toxicity (Harborne, 1973). A few workers have attempted to characterize the leaf lipids: *Zea mays leaves* (Douglas and Paleg, 1981). Spinach leaves, wheat leaf oosomes (Murphy and Parker, 1984) and soybean leave (Wilson and Burton, 1997). Little or no literature exist on the characterization of leaves from *Symphonia globulifera* (Trease and Evans, 1989). This study is therefore aimed at characterization of *Symphonia globulifera leaf* in order to ascertain the suitability of these lipids and other constituents for edible and industrial purposes.

MATERIALS AND METHODS

The plant *Symphonia globulifera* linn. leaves were obtained from the forest located at Itak Ikot Akap in Ikono Local Government Area of Akwa Ibom State. The plant was identified and authenticated by taxonomist in the Department of Botany and Ecological Studies, University of Uyo. The leaves of *S. globulifera* were ground using mortar and pestle and thereafter dried to obtain coarse powder.

The oil content was extracted by grinding 50g each of the sample with 100ml of propanol in a sterile mortar and pestle and homogenized using 100ml of chloroform- methanol (2:1^{v/v}) and then purified (Folch *et al.*, 1957). Butylated hydroxytoluene (0.005%) was added as antioxidant to protect polysaturated fatty acids. The mixture was filtered and the filtrates were evaporated to dryness in a rotatory evaporator at 50^oC. The extract was purified in accordance with the procedure of (Folch *et al.*, 1957). The organic layer of the lipid extract, which excluded all moisture, retained in the aqueous layer; was used for lipid analysis.

Lipid Analysis

Authentic lipid standards and the controls were obtained from sigma chemicals (ST. Louis, MD). Solvents used were of analytical grade and were re-distilled and kept cold before use. Teflon lined screw cap vials were used in all instances. All extractions were carried out at 0^oC and the extract kept in solution at all times. Total lipids were determined gravimetrically in aliquots of the purified extract. The total lipids (TL) were fractionated into neutral lipids (NL), glycolipids (GL) and phospholipids on silicic acid column (Rouser *et al.*, 1967) using chloroform, acetone and methanol respectively. The pure compound coded T was subjected to spectral analysis using mass spectrum 13^c and nuclear magnetic resonance proton and preliminary phytochemical test were carried out for alkaloids, steroids and saponins according to Sofowora (1982) while terpenes, flavonoids and tannins were done according to Trease and Evans (1989). The proximate composition of *Symphonia globulifera* leaves were used to determine the nutritional values such as the moisture content, dry matter content, crude protein, crude lipid, crude fibre, ash content and carbohydrate content. These parameters were analysed according to method of the Association of Official Analytical Chemist (AOAC, 1975).

RESULTS

The total lipids content of dry leaves of *Symphonia globulifera* was 4.8%. This was further classified into 80% neutral lipids, 15% glycolipids and 5% phospholipids (Table 1). The colour of the lipid was yellow and oily at room temperature. The thin layer chromatography revealed four categories of lipids which were further classified into diglycerides, cholesterol, esters, triglyceride, free fatty acid and sterol according to their R_f ratios. The spectral analysis of fatty acid from *Symphonia globulifera* leaves showed an (M+H)⁺ peak at M/Z, indicating the exact mass of the compound. The HNMR revealed five methyl groups appearing as multipet at δ80.83, while methylene groups appeared between δ1.61 and δ4.57 as multipet and quartet. Methane group showed up as triplet at δ5.34 (Fig. 1 – 2).

Phytochemical properties of *S. globulifera* leaves showed the presence of moderate amount of saponins, flavonoids, tannins, anthraquinons, glucosides, cardiac glycosides, cyanogenetic glycosides while trace amount of alkaloids and phlobatannis were also revealed in this leaf (Table 2). The proximate analysis revealed the presence of moisture 47.71%, dry matter content 52.29%, crude protein 14.88%, crude lipid 3.29%, crude fibre 2.10%, ash content 2.26% and carbohydrate 72.84% (Table 3).

Table 1: Lipid composition of *Symphonia globulifera* leaves

Lipid fraction	Result (g)	Percentage (%)
Total Lipid	12.30g	45.8%
Neutral Lipid	3.888g	80%
Glycolipids	0.729g	15%
Phospholipids	0.243g	5%

Table 2: Phytochemical properties of ethanolic extract of *Symphonia globulifera* leaves

Test	Inferences
Sapanins	++
Flavonoids	++
Tannins	++
Anthraquinones	++
Alkaloids	+
Glycosides (reducing sugar)	++
Cardiac glycoside	++
Cyanogenetic glycoside	++
Phlobatannins	+

Table 3: Proximate properties of the leaves of *Symphonia globulifera*

Test parameters	Results (%)
Moisture content	47.71%
Dry matter content	52.29%
Crude Protein	14.88%
Crude Lipid	3.92%
Crude fibre	2.10%
Ash Content	6.26%
Carbohydrate	72.84%

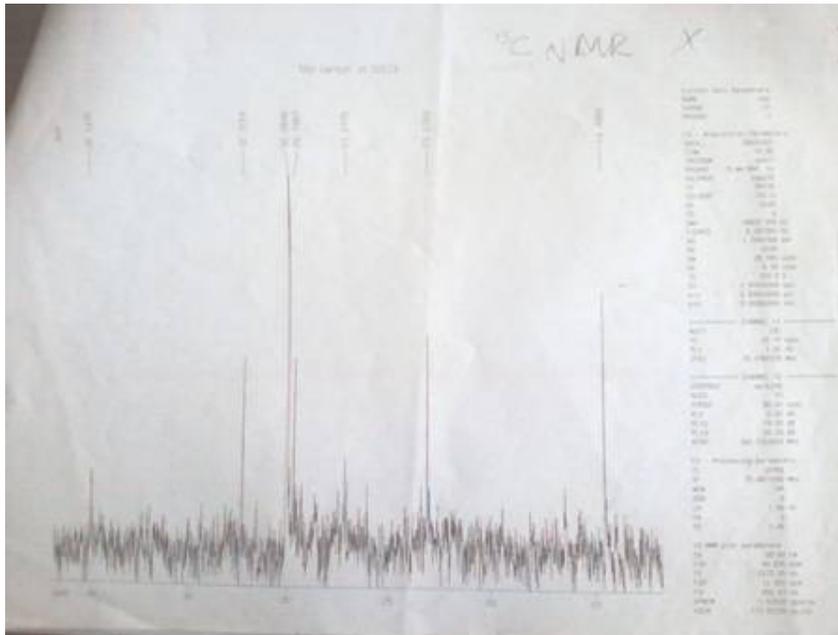


Figure 1: ^{13}C NMR spectra of *Symphonia globulifera*

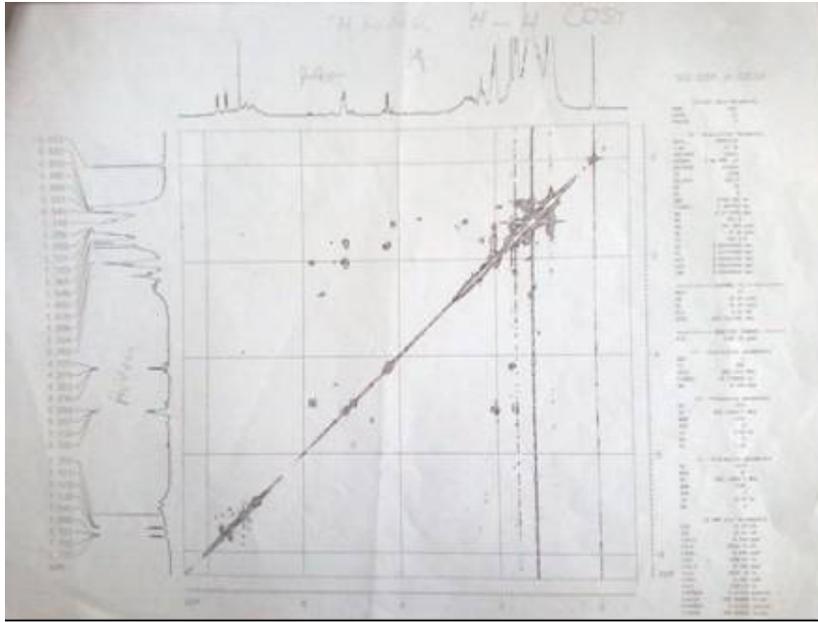


Figure 2: HNMR spectra of *Symphonia globulifera*

DISCUSSION

The total lipid content of *S. globulifera* was 4.86%. Of this quantity, the neutral lipids represented 80% while the glycolipids and phospholipids were 15% and 5% respectively. Esenowo *et al.*, (2005) reported similar findings when he observed that the total lipid of *Dacryodes edulis* seed was 12.5%. The neutral lipids, glycolipids and phospholipids of *D. edulis* were 10%, 0.85% and 1.65% respectively. The thin layer chromatographic fraction of lipids revealed that the neutral lipids were the major components while the phospholipids were the minor component. Similar results have earlier been reported by Joshi and Doctor, (1975) who were able to separate crude cotton seed oil into nine classes using Thin Layer Chromatography.

The lipid classes obtained from *S. globulifera* leaf were oleic acids, palmitic acids, cholesterol. This result is in line with the report by Eke, (1980) who revealed that fatty acid profiles examples Oleic acid, palmitic acid, lauric acid and myristics have a good potential value as edible oil and for production of soaps, cosmetics and margarine. It is suggested that the lipid of *S. globulifera* can be used as edible oil and for industrial purposes.

During the mass spectrum examination of *S. globulifera* leaf lipids, the presence of ¹³CNMR confirmed many of the features in HNMR spectrum. For instance the methyl groups that appeared at δ 80.83 were confirmed by signals between δ 14.8 to δ 20.1 in ¹³CNMR. The signal at δ 174.2 indicated a carboxylic group. This compound is therefore characterized as ergosterol carboxylic acid.

The phytochemical analysis revealed that the leaves of *S. globulifera* contains moderate amount of saponnins, flavonoids, tannins, anthraquinons, glycosides, cardiac glycosides, cyanogenetic glycosides. The presence of saponnins confirms that this plant could be used for ritual bath, concoctions, medicated soap and detergent. The presence of cardiac glycosides could render it effective in the management of hypertension while the tannins could be applied to wounds sores and skin diseases (Trease and Evans, 1989).

The crude fibre of *S. globulifera* was 2.10%. Ifon and Bassey (1979) showed that the fibre content of leafy vegetables common to Nigerians range between 8- 15% therefore the leaves of *S. globulifera* can be used for weaning infants (Bassey *et al.*, 1979). Aside from moisture content of this leaf which is 12.48%, carbohydrate was one of the highest compositions in this leaf. This compares well with other leafy vegetable like *Diplazium sammantii* as reported by (Bassey *et al.*, 2001) which has 68,6% and 65.3% in portulova. This leaf has a high content of carbohydrate and can be used for energy food.

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