**STUDY OF THE FATTY ACID COMPOSITION OF *DIOSPYROS MESPILIFORMIS* PLANT LEAVES**

**Abstract**

The aim of this study was to determine the fatty acid composition of the plant's leaves. *Diospyros mespiliformis* is a plant used in traditional medicine for various therapeutic and cosmetic purposes. The plant's leaves, infused, decocted or applied as a plaster, are used to treat rheumatism, wounds and coughs. They are also used as a dressing to prevent infection. [1-4]

Fatty acids are known for their anti-inflammatory and antimicrobial properties, and for their ability to combat cardiovascular disease, arrhythmia, thrombosis, atherosclerosis, autoimmune disorders (lupus, nephropathy), Crohn's disease, Alzheimer's disease, breast cancer, colon cancer, prostate cancer, polyarthritis, rheumatism, asthma, multiple sclerosis, depression, diabetes and more. ...[5-15]

For all these reasons, the study of fatty acids in the plant's leaves was carried out in order to elucidate their molecular structures and to see their possible implications in the biological properties attributed to the leaves, but also the advantages that the fatty acid composition may offer for other purposes.

A total of 8 fatty acids were detected. The results revealed respective fatty acid contents in descending order of palmitic acid (33.46%), α-linolenic acid (19.03l%), stearic acid (13.64%), vaccenic acid (9.957%), linoleic acid (8.529%), palmitoleic acid (6.554%), myristic acid (5.784%), arachidic acid (2.777%).

**Key words:** Fatty acids, leaves, *Diospyros mespiliformis*

**Introduction**

Fatty acids are constituents of plant membranes, and may also have an intracellular role and activity. They are the elementary constituents of lipids, composed solely of carbon, hydrogen and oxygen atoms. They may be present as esters on a glycerol backbone, or free.

They play several roles in plants. They can act as signals in plant-pathogen interactions, act as antimicrobials and contribute to plant resistance to other types of stress. Fatty acids also play an important role in humans. Fatty acids are the second most important source of energy after carbohydrates. In addition to their physiological and energetic role, they also have numerous biological activities, notably their ability to prevent diseases such as cardiovascular disease, dyslipoproteinemia, arrhythmias, thrombosis and atherosclerosis, autoimmune disorders (lupus, nephropathy), Crohn's disease, Alzheimer's disease, Parkinson's disease, breast cancer, colon cancer, prostate cancer, polyarthritis, rheumatic diseases, asthma, psoriasis, multiple sclerosis, depression and diabetes. [5-15]

It is therefore important to be able to qualify and quantify the fatty acids present in the leaves of the *diopyros mespiliformis* plant, to see if they can be linked to the biological activities assigned to the leaves in their various uses. This qualification and quantification should also make it possible to explore other perspectives with regard to the biological activities associated with the compounds found but not yet exploited. Given the popularity of these compounds, which are attributed with numerous beneficial physiological and biological effects, it seemed worthwhile to carry out a study on the fatty acids contained in the plant's leaves.

* + - 1. **Botanical description of the plant**

*Diospyros Mespiliformis* Hochst Ex A. DC, a member of the Diospyros genus in the Ebenaceae family, is a 10 to 15 m-high tree with a robust, cylindrical trunk and brittle, charcoal-like black bark. It is a species characteristic of savannah woodlands (dry forests) and sometimes wet forests.

It is characteristic of heavy, well-drained soils. [6-13] The species is found almost everywhere on the surface of the globe. It is found in the flora of several countries, in sub-Saharan Africa and the Gulf of Guinea, southern Africa, central Africa and northern Africa in Egypt. [9-30] Its presence has been reported in the Near East (Yemen, Israel and Saudi Arabia), in North and South America, and in Madagascar. [6, 10, 12, 18, 19, 31, 22] The plant's leaves are used as an astringent, febrifuge, haemostatic, laxative, stimulant and vermifuge. Infusions are used to treat fever, pneumonia, syphilis, leprosy and yaws. The leaves are also used to treat headaches, arthritis and skin infections. [33]

* + - 1. **Materials and methods**

**2.1. Harvesting and preservation of plant leaves**

The raw material used in our study was harvested in Ndiemane, a village located on the Senegalese coast between Mbour and Joal-Fadiouth. After harvesting, the leaves were washed with water and then dried under cover at room temperature in our laboratory. After drying, the samples were ground.

**2.2. Extraction of plant leaves**

Vegetable oil is extracted continuously from leaf powder using a Soxhlet extractor. To achieve this, 20 g of leaf powder were weighed into a cellulose cartridge (Schleicher & Schuele), which was then placed in the extractor. The extractor was connected to a cooler and a flask. A volume of 250 mL of n-hexane (HPLC-S, Biosolve) was used to extract the oil. Part of this volume was poured into the extractor to allow the plant material to macerate overnight before starting the extraction. The rest was poured into the flask with a few boiling stones (pumice, VWR). The flask was then brought to the boil using a flask heater. The solvent vapors condense in the condenser and flow into the extractor, where they extract the lipids from the sample. The solvent in contact with the sample is continuously renewed. The device is heated for five hours after the first reflux of solvent has been observed.

Hexane was then evaporated from the recovered extract to obtain the extracted oil only. This was done using a rotary evaporator (Heidolph LABOROTA water bath, Labobase/Laborota 4000 Heidolph G4 high type pump) at a temperature of 60°C and a pressure starting from 500 mbar and gradually decreasing to 100 mbar to remove the last traces of solvent.

Mass yield is the ratio between the mass of plant oil extracted and the mass of plant material extracted. It is expressed as a percentage (g/100 g) of fresh matter according to the following formula:

Extraction rate = $\frac{Mass of oil extracted}{Mass of vegetable matter}$ × 100 (1)

The extraction operation was repeated three times and the average of these three repetitions was calculated.

**2.3. Fatty acid analysis**

Total fatty acids were determined after esterification with BF₃ in methanol

**2.3.1. Principle**

It involves esterifying fatty acids (free or esterified) to lead to free fatty acid methyl esters (FAME, Free Acid Methyl Ester) and then identifying the latter by GC.

**2.3.2. Reagents and solvents**

The reagents used consisted of NaCl, H₂SO₄, BF₃ 14% solution in MeOH (Sigma-Aldrich) and Supelco FAME. The solvents used during the study were hexane and methanol.

**2.3.3. Preparation**

To carry out the study, several solutions were prepared in advance, including 10% aqueous sulfuric acid, BF3 methanolic solution and saturated aqueous NaCl solution.

**2.3.4. Material**

The equipment used consisted of a Bain thermostatized at 70°C and a 10 mL sovirel tube with a Teflon-lined stopper.

**2.3.5. Procedure**

The fatty acid profile was determined by gas chromatography. For this purpose, the fatty acids had to be converted into methyl esters to make them more volatile. A mass of around 10 mg of fat was placed in the sovirel tube, into which 0.2 mL of hexane (Biosolve) and 0.5 mL of BF3 solution were then added. The whole was incubated at 70°C for 90 minutes. Once the reaction medium had cooled, 0.5 mL saturated NaCl solution and 0.2 mL 10% H2SO4 were added. The mixture was homogenized by vortexing. Finally, 8 mL hexane (Biosolve) was added to dilute the sample. This solution was injected into a GC-FID (Gas Chromatography - Flame Ionization Detector). The apparatus used in the laboratory is an HP 6890 with a VF Wax ms 0.25 µm x 250 mm x 30 m polar capillary column. A volume of 0.5 µL was injected in ON-COLUMN mode. The temperature program was as follows: injection at 55°C, rise to 150°C at a rate of 30°C/min, then directly to 250°C at a rate of 5°C/min, remaining at this temperature for 15 minutes. Helium was used as carrier gas at a flow rate of 1.7 mL/min.

The peaks of the chromatogram obtained were identified using the retention times obtained for a control containing many fatty acids. The analysis was repeated three times on the extract. The fatty acid profile is expressed in relative terms, i.e. the composition of each fatty acid is expressed as a percentage of the total fatty acid content (TFA). For this purpose, the areas of the peaks corresponding to the fatty acids have been summed, and the relative concentration of a fatty acid is equal to the ratio of its area to that of the sum of all the areas, as shown by the following formula:

(AG %) = $\frac{AG area}{Total area AG}$ × 100 (2)

1. **Results and discussion**

**3.1. Results**

After drying the hexane extract, we obtained a mass of 8.351g, giving an extraction rate of 41.75%.

The results of the GC analysis were as follows:



**Figure 1:** Chromatogram from GC-FID analysis of the injected solution

GC-FID analysis revealed a total of eight fatty acids with distinct percentages of presence as shown in the table below.

**Table 1:** fatty acid composition of plant leaves

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Acid** | **RT (min)** | **Area** | **%** | **Nomenclature** |
| Myristic | 10.384 | 52.596 | 5.784 | C14 |
| Palmitic | 13.395 | 304.209 | 33.456 | C16 |
| Palmitoleic | 13.634 | 59.599 | 6.554 | C16:1w7 |
| Stearic | 16.541 | 123.994 | 13.636 | C18 |
| Vaccenic | 16.837 | 90.545 | 9.957 | C18:1w7 |
| Linoleic | 17.543 | 77.555 | 8.529 | C18:2w6 |
| α-Linolenic | 18.541 | 175.518 | 19.303 | C18:3w3 |
| Arachidic | 19.667 | 25.257 | 2.777 | C20 |

The percentages of fatty acid present in the plant leaves, in descending order, were 33.46% for palmitic acid, 19.03% for α-linolenic acid, 13.64% for stearic acid, 9,957% for vaccenic acid, 8,529% for linoleic acid, 6,554% for palmitoleic acid, 5,784% for myristic acid and 2,777% for arachidic acid.

**Figure 2:** Circular diagram of fatty acids in plant leaves

**3.2. Discussions**

Chromatographic analysis yielded several pieces of information. Firstly, the extracted oil contains fatty acids with chains ranging from 14 to 20 carbon atoms. Secondly, the fatty acids found in decreasing order of abundance are palmitic acid, α-linolenic acid, stearic acid, vaccenic acid, linoleic acid, palmitoleic acid, myristic acid and arachidic acid respectively.



**Figure 3:** Structures of saturated fatty acids



**Figure 4:** Structures of unsaturated fatty acids

These results could be linked to a number of factors, including the molecular composition of the cutin in tree leaves, soil texture or edaphic parameters, other pest stresses, extraction method and geography. [33-37]

This disparity in the content of the various fatty acids found on leaves, and the biological activities associated with them, could explain some of the biological activities attributed to leaves, as well as their many traditional medical, cosmetic and nutritional uses.

* Myristic acid, found in the fatty acid composition of leaf extract, is known for its ability to increase cholesterol availability. It is also used as an additive for the active ingredients of certain drugs used in plaster or oil form to facilitate their penetration of the skin barrier. [37-39]
* Palmitic and oleic acids have anti-inflammatory, regenerative and moisturizing properties. [40-41]
* Stearic acid plays an important role in cosmetics, and is present in deodorant stick formulations in the form of sodium soap, giving them a low acidity and a certain consistency. [42]
* Palmitic and stearic acids are also used as skin barrier penetration additives for the active ingredients in certain drugs. They also promote melanogenesis. [43], [38]
* Trans-vaccenic acid can have negative effects on human health. However, the cis isomer from ruminants has no negative effect on human health and may even have a cholesterol-lowering effect. [44-45]
* Linoleic acid, when consumed in moderation, reduces platelet aggregation and may also play an antithrombotic, anti-inflammatory and ischemia-reducing role. It also helps repair fibrinogen cells and arrhythmia. It also leads to the production of vitamin D. It corrects the symptoms of a fatty acid-deficient diet on the brain and retina in children. [46-48]
* α-Linolenic acid is ideal for medicinal and nutritional applications, as it has antimicrobial, antidiabetic and cardioprotective properties. [49] It regulates a number of bodily functions, including blood pressure, blood viscosity, immune responses and anti-inflammatories. [50]
* Linoleic and α-linolenic acids are essential fatty acids and also serve as additives to certain drugs, enabling them to penetrate the skin barrier. They have the ability to inhibit the production of melanogenesis. [51], [38]
* Arachidic acid is involved in inflammation and wound healing. [37]

In view of the biological activities attributed to the various acids on the leaves, the presence of linoleic acids, known for their antibacterial properties, could be part of the reason for its use to treat wounds. The presence of stearic, palmitic, linoleic and α-linolenic acids, known for their biological activities on the skin, muscles, vision and brain, could demonstrate the use of the plant's leaves in nutrition, cosmetics and wound healing.

**Conclusion**

The aim of this study was to elucidate the fatty acid composition of the plant's leaves, their possible implications in the biological properties attributed to the leaves, but also to see what advantages the fatty acid composition may offer for other purposes. Verification of the fatty acid composition of the plant's leaves revealed the presence of a total of eight fatty acids, four of them saturated and four unsaturated. Analysis of the biological data associated with the acids found also suggests the possible involvement of these acids in many of the medicinal properties or uses associated with the plant's leaves. It should be noted, however, that although they seem very interesting in terms of the benefits they could offer, further studies are needed to establish their likely degrees of involvement in the activities allocated to the leaves.

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