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Pharmacognostic study of *Ocimum gratissimum* Linn.: Pharmafood plant.

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Abstract

Objective: To study the preliminary characteristics and pharmacognostic of *Ocimum gratissimum* Linn., medicinal plant, used as food in some parts of West Africa.

Methods: The pharmacognostic study involved performing macroscopic and microscopic examination of fresh and dry medicinal plant. The study of physicochemical parameters consisted of the determination of moisture content, total ash and insoluble sulfur in hydrochloric acid.

Results: pharmacognostic examination revealed a small plant with small stalked leaves. The fruits are in the form of small capsules. The anatomo-histological section, showed trichomes, palisade cells, spongy parenchyma, vascular unit. On the stem, primary tissues, a fundamental parenchyma is observed. Micrographic revealed oily cells, spiral and wood beams spiral beams.

Conclusion: The pharmacognostic analysis and physico-chemical characteristics can help in the efficient use of this plant.

Keywords: Pharmacognosy, Pharmafood, *Ocimum gratissimum* Linn.

1. Introduction

Medicinal plants are of great importance to human health. That is why the World Health Organization estimates that 80% of the populations in developing countries rely on traditional medicine for their primary health care [1]. Therefore, medicinal plants have played an important role in the socio-cultural and therapeutic need of people. Some of these plants are used as spices and food [2,3], but also serve as natural sources for research and development of new drugs [4]. In Africa and other parts of the world, many ethnobotanical surveys showed that *Ocimum gratissimum* Linn., a well-known plant is widely used in the treatment of various infections.

In India, the plant is used for curing stomach ache, diarrhea and skin infections, but also in religious ceremonies and rituals, the leaves are used as laxative and consumed as tea. The use of essential oils is remarkable in medicine, including headaches, flu, fever, sore throat, ears or eyes. The seeds have laxative properties and are prescribed against gonorrhoea [5].

In West Africa, particularly in republic of Benin *O. gratissimum* Linn. is used both for culinary and therapeutic purposes for the treatment of intestinal, skin and eye infections [6].

In Nigeria, *O. gratissimum* Linn. is known for its various uses in traditional medicine [7]. Indeed, the drug is reported for its action against gastrointestinal infections (diarrhoea, dysentery), infections of the skin (dermatitis, eczema, scabies), infections of the upper respiratory tract, associated with cough, asthma and bronchitis, wounds and sores, insect bites, nosebleeds, stroke, anemia [8,7].

In Côte d'Ivoire, the plant has a versatile action; it is recommended in the treatment of many diseases. Indeed, the leaves are recommended to treat sinusitis, influenza, sore throat, coryza, colds and help to fight against dizziness. Leafy stem is used in the treatment of hypertension, diabetes, and varicella [9].

Despite scientific and medical values proven by several studies on this medicinal plants *Ocimum gratissimum* Linn., analyzes of pharmacognosy and study of physicochemical parameters like moisture content, total ash and acid insoluble in hydrochloric are very few. Therefore, this study reported a macroscopic, anatomical and micrographic examination of plant, leaf, stem, fresh and dry crude drug (leaf powder), as well as a study of physicochemical parameters on moisture content, total ash value, sulfuric and hydrochloric acid insoluble ash value.

2. Materials and methods

2.1 Plant material

The plant material consists of leaves and stems with leaves of *Ocimum gratissimum* Linn. (Lamiaceae) [O g]. The choice of drugs was guided by the literature [10, 11, 12], its availability and accessibility. The leafy stems of *O. gratissimum* Linn. were obtained from Abengourou, in eastern part of Côte d'Ivoire, in February 2012. The plant has been formally identified by the Laboratory of Botany and a herbarium was formed and deposited at the National Centre of Floristic (CNF) University Felix Houphouët Boigny having the n° 2; Fofié Yvette.

2.2. Methods

Plants were cut, cleaned and then dried at the laboratory temperature (24-26 °C). The dry plant material is coarsely pulverized using a grinder type Retsch® SM 2000. The dry plant material was used for the pharmacognostic studies, and the physicochemical examination. The fresh organs were preserved for the macroscopic, anatomic and histological study.

2.3. Macroscopic Studies

The macroscopic study is a morphological study. This study allowed us to make a description of the plant and determine the shape, texture and color of the drug.

2.4. Organoleptic characteristics study

The study of the organoleptic characteristics covered the powdered drug and concerned the taste, appearance, color and odor of the drug. The smell test is carried out with 1 mg of powdered drug taken between the thumb and forefinger. The odoriferous constituents released slowly and were tested repeatedly. The intensity of the odor was first tested by the following parameters: "No, Low, Sharp, and Strong" Then was determined odor type: "Aromatic, fruity".

For taste, 5 grams of drugs are placed and kept in the mouth without swallowing, for 10 to 30 seconds. After spit sample, the mouth is rinsed and then enjoyed the taste: «Piquant, Fade, sour, bitter, sweet, salty, warm. This important study allows for the identification of drugs and standardization. The appearance and color required observation.

2.5. Microscopic studies

The anatomo-histological studies have respectively identified tissues and locate sites of secondary metabolites. These studies were made on sections of leaves and stem, and the powder of *Ocimum gratissimum* Linn.

2.5.1. Anatomo-histological

It consisted essentially of an optical microscopic observation. Thin cross sections were cut using a microtome in the vegetative organs of fragments. Then, the sections were treated according to the technique recommended by Gabe [13]. The colorant used was Carmine-green miranda. The best sections were mounted and stored between slide and cover slip in glycerin medium for observations and images taken with a digital camera incorporated in the microscope.

2.5.2. Micrographic study

A small amount of fine powder was mixed with a few drops of 5% KOH on an object slide and covered with a cover slip. The observation was conducted under an optical microscope with 10x objective. The characteristic features of the powder drug were recorded and photographed.

2.6. Physicochemical study

The study of physicochemical parameters involved determining the moisture content and ash content; it was conducted using the following protocol.

2.6.1. Determination of moisture Content

The determination of the water content was performed by the gravimetric method according to the protocol created by Linden and Lorient [14] and Mukherjee [15]: 5 tests were performed taken 5 g of powder introduced into 5 calibrated crucibles. The 5 samples were dried in an oven at 105 °C for 24 hours. The crucibles were cooled in a desiccator and weighed. The masses obtained were used to calculate the mass loss and calculate the water content of the powder as a percentage.

2.6.2. Determination of ash content

2.6.2.1. Total ash: The dried powder used for the determination of the water content was reduced to ash in a furnace at 600 °C for 6 hours. After cooling in a desiccator, the ash were weighed. The masses obtained were used to calculate the masses of ash and calculate the total ash content, expressed as a percentage.

2.6.2.2. Determination of acid insoluble ash value in 10% hydrochloric acid: Total ash obtained was used with 20 ml of 10% hydrochloric acid. The whole mixture was boiled in a water bath for 15 minutes. The solution obtained was filtered through Whatman paper. The residue was collected in a crucible and calcined in an oven at 600 °C for 6 hours. The crucible was cooled in a desiccator. The mass of insoluble ash in hydrochloric acid was expressed as a percentage.

2.6.2.3. 50% Sulfuric ashes: Five tests were performed taken 5 g of powder, introduced into crucibles. It was added to the contents of each crucible 5 ml of 50% H₂SO₄. The solution was placed in the oven at the temperature of 600 °C for 6 hours. After calcination and cooling in a desiccator, the ash were weighed and the mass of sulfuric ash was expressed as a percentage.

3. Results

3.1. Macroscopic study

This study allowed us to identify the plant material, and is the first step in the characterization of the drugs.

Ocimum gratissimum Linn. [16]

Morphological characteristics

Shrub up to 2 m, the stem of *Ocimum gratissimum* Linn. is much branched. Herb leaves petiolate, opposite, ovate to oblong in shape, are toothed on the edges. The inflorescences are racemes and form a set paniculé terminal. Flowers, small, irregular, whitish and grouped in spikes (Figure 1). The fruits are in the form of small capsules open at the apical end (Figure 2).

Phytogeography

Ocimum gratissimum Linn. is a pantropical species found in tropical America, tropical Africa, India and Southeast Asia due to its ease of adaptation. It is one of the most invasive plants of South Pacific New Caledonia.

Ecology

Ocimum gratissimum Linn. species is widely naturalized in many areas. Major invasive plant, it is reported as a weed for pastures and prefers moist, fertile areas, but tolerates drought after flowering. It is also found in degraded forests, dry forests and in

secondary thickets. This herb is commonly found along roadsides.

Specimens studied

Ahouabo, in May 1972, Adjanohoun and Ake-Assi n° 225. Akoupé, July 1972 Adjanohoun and Ake-Assi n° 304. Abengourou, in February 2012, Fofié Yvette n° 2.



Fig 1: Feuilles et inflorescences de *Ocimum gratissimum* Linn.

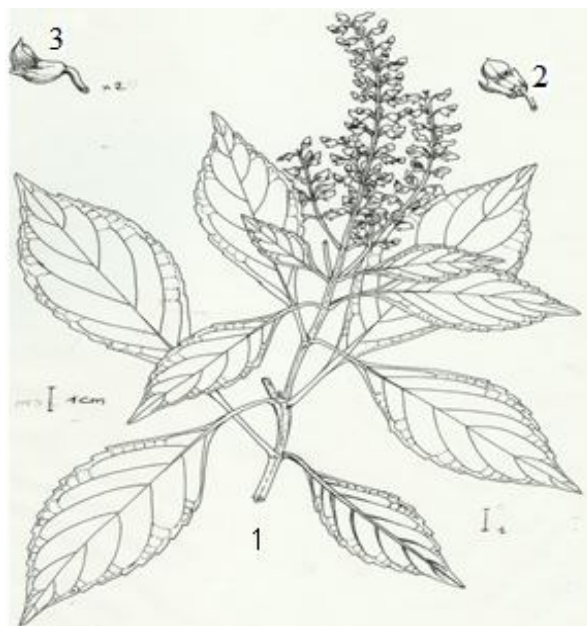


Fig 2: Différentes parties de *Ocimum gratissimum* Linn.

1 : port de la plante, 2 : gaine foliaire, 3: fruit Source: FOFIÉ YVETTE

3.2. Organoleptic study

All organoleptic characteristics found in this study are shown in Table 1.

Drugs Characters	<i>Ocimum gratissimum</i> Linn. (leaf)
Taste	Characteristic
Odor	Aromatic
Color	Green
Aspect	Powdered
Texture	Coarse

3.3. Microscopic studies

Microscopic study of the cross section of the leaf and stem, plays an important role in the diagnostic, identification and differentiation of the studied drug.

3.3.1. Anatomo-histological

The cross section of the lamina (Plate 1) has two zones: a midrib slightly curved at the top and strongly convex in the lower face and the thinnest blade face. In the middle of the blade, the cylindrical midrib, shows two epidermis (upper and lower) and mesophyll which includes various tissues (fundamental and medullary parenchyma, primary wood and primary phloem, collenchyma). The epidermis is highly cutinized and also rows of palisade cells. Detectors of bristles are present on the upper side of blade. The spongy parenchyma consists of rounded cells, some of which are isolated sclerotized. The collenchyma without meatus consisting of cells, uniformly thick and is placed under the epidermis. The basic parenchyma is most abundant on the lower side. The vascular system forms a beam disposed in closed bow with a sheath of primary phloem and primary wood. Medullary parenchyma is very small.

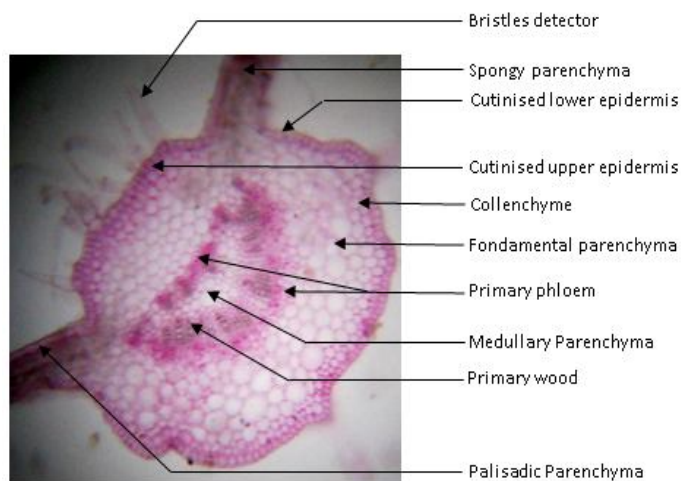


Plate 1: Cross section of a portion of *Ocimum gratissimum* Linn. Laboratory of Applied Biochemistry (University of Ouagadougou), July 2012 Focus: Gx40; Coloration: Carmino-green; Liquid medium: glycerin water

The cross section of the hexagonal stem (Plate 2) has two parts: the bark and the central cylinder. The bark is thin, has three primary tissues (epidermis, cortical parenchyma and collenchyma). The epidermis consists of a single base of small contiguous rectangular cells, the wall is thin and cellulose. The cortical parenchyma consists of several layers of polygonal cells with thin walls.

The central cylinder is more developed than the bark. A fundamental parenchyma is observed in which differs from primary tissues (wood, phloem parenchyma spinal cord) and two secondary tissues (wood and secondary phloem). The primary wood is centrifuged and the secondary phloem and secondary wood are arranged in radial alignment. The marrow parenchyma includes meatus, and is formed of large polygonal cells cellulose walls.

Table I: Organoleptic character of *Ocimum gratissimum* Linn. leaves.

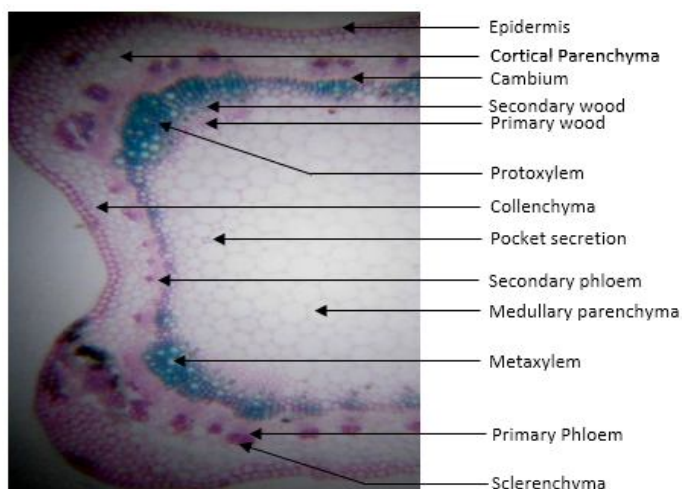


Plate 2: Cross section of a portion of stem of *Ocimum gratissimum* Linn.; Laboratory of Applied Biochemistry (University of Ouagadougou), July 2012 Focus: Gx40; Coloration: Carmino-green; Liquide medium: glycerine water

3.3.2. Micrographic study

Micrographic analysis of the powder has allowed us to detect the

characteristic elements of the drug and also the contaminants and to confirm the purity of the drug. Thus we note the presence:

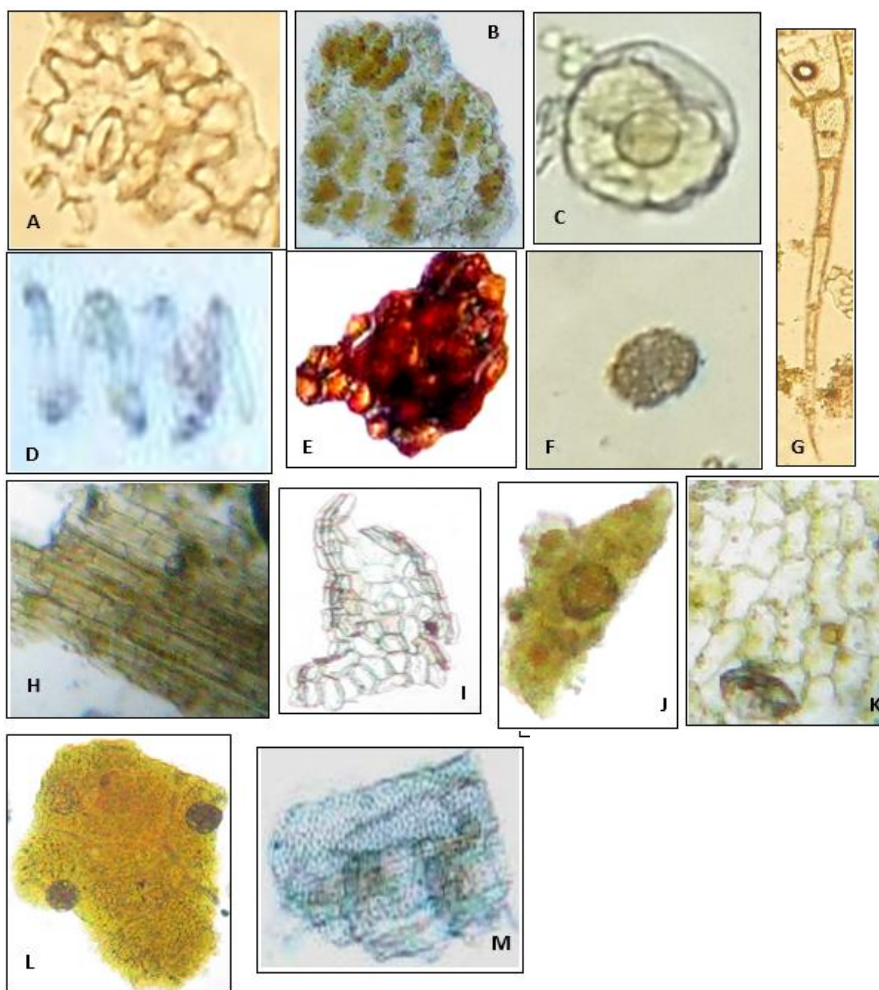


Fig 3: Characteristic elements of the *Ocimum gratissimum* Linn powder.

A: Stomata on epidermis fragments; **B, C and J:** Oily cells; **D:** Spiral beam; **E:** Fragments of sclerenchymatous fibers; **F and L:** Cystoliths; **G:** Bristle; detectors (few); **H:** Tissue fragments (many); **I:** Upper epidermal cells; **K:** Fragments of epidermis (many); **M:** Spiral wood beam

3.3.3. Physicochemical study

This study is very important in that it helps identify poor handling practices and assess the quality of the proposed drug under study. Various parameters were defined and recorded in Table 2.

Table 2: Physicochemical parameters of *Ocimum gratissimum* Linn.

Parameters Drug	Humidity %	Total ash %	Sulfuric ash %	Insoluble ash in hydrochloric acid %
<i>Ocimum Gratissimum</i> Linn.	8,97±0,01	8,45±0,02	10,27±0,01	0,96±0,01

4. Discussion

Our study has focused on examining pharmacognosic study of *Ocimum gratissimum* Linn. (Lamiaceae) leaves. The scientific books have allowed us to review a large number of bibliographic data on this drug, including the systematic and various domestic uses in traditional medicine. Normalization of the macroscopic and microscopic characteristics of the *O. gratissimum* Linn. drug remains essential in other to identify and avoid falsification. Thus comparing the cross section of the leaf and stem anatomy showed structural similarities.

Both sections have a spinal cord parenchyma, a phloem, xylem and collenchyma. It is observed in a thin sheet cuticle on the upper epidermis and the lower epidermis. Also palisade tissue above the spongy parenchyma. In the stem, secreting pockets are visible on the surface of the medullary parenchyma, as well as supporting cells sclerenchyma primary tissue (primary phloem). The distinct cortical parenchyma can be seen towards the periphery of the cut.

Organoleptic characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs [17]. Thus leaves green in color, emit a very fragrant and aromatic minty odor when bruised. The powdery appearance of the sprayed leaves, has a coarse texture.

The micrograph performed on the powder has highlighted a number of characteristic elements namely: the epidermal cells, the type of stomates, the spiral beams, the cystoliths, the trichomes, spiral wooden beam, oily cells, are diagnostic substances for drugs of plant origin. These diagnostic elements are consistent with botanical standards and WHO guidelines [18,19].

The study of physicochemical parameters such as moisture content and ash values are useful to determine the physiological and non-physiological state of ash, of detecting the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of 8.97±0.01, which is below 10%. This result comply to the standards established by the International Pharmacopoeia, because this water content rate, prevent oxidation reactions, fermentation and give less chance to microbial growth and contamination in drugs [20]. Therefore, for proper storage of drugs made with the leaves of *O. gratissimum* Linn., it would be desirable to use those whose water content is less than or equal to 10%.

The determination of total ash gave us a rate of 8.45±0.02. This value indicate the level of minerals in drugs [21]. Sulfuric ash for their rate of 10.27±0.01. They result from the conversion of organic salts to sulphates [21]. This value substantially equal to the average of 10,80% found during the determination of sulfuric ash in the different samples of *Sclerocarya birrea* (A. Rich) Hoscht [22]. Insoluble ash in hydrochloric acid gave a rate of 0.96±0.01. Indeed, the ash insoluble in hydrochloric acid tell us about the contamination of the drug by siliceous elements [21]. This result is in agreement with those of [22] and Srikanth *et al.* [23], who found rate of 0.97% and 0.5% respectively.

5. Conclusion

This study allowed us to detect the presence of various pharmacognosic parameters in *Ocimum gratissimum* Linn drug, according to botanical standard and WHO guidelines. The results of the assay of water content are satisfactory as the level presented in the tested drug allows good storage and prevents the oxidation, fermentation and microbial growth.

The dosage of the ash content did not permit distinction of overload in the drug presented in the form of powder.

In light of these results, pharmacognosic analysis and physicochemical characteristics can help in the efficient utilization of this plant, as part of a policy of standardization, identification and research of *O. gratissimum* Linn. drugs.

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