International Journal of Agriculture, Forestry and Fisheries
2019; 7(2): 12-17
http://www.openscienceonline.com/journal/ijaff
ISSN: 2381-4365 (Print); ISSN: 2381-4373 (Online)

Open Science

Pharmacognostic and Physicochemical Profile of the Leaves of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell

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To cite this article

Olamilekan Lanre Awotedu, Paul Oluwatimilehin Ogunbamowo. Pharmacognostic and Physicochemical Profile of the Leaves of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell. *International Journal of Agriculture, Forestry and Fisheries.* Vol. 7, No. 2, 2019, pp. 12-17.

Received: July 10, 2019; **Accepted**: August 28, 2019; **Published**: September 6, 2019

Abstract

Authentication and standardization of *Synsepalum dulcificum* using pharmacognostic and physicochemical profile is an avenue to check and create a correct taxonomic information that could help in the proper identification and its use as herbal remedy. The pharmacognostic profile evaluated includes the macroscopy, microscopic features, and chemo microscopic characters. Also the physicochemical analysis of the dried powdered sample was examined using standard chemical methods. The macroscopic features and microscopic evaluation of the leaf sample revealed that the pharmacognostic value are important in the proper authentication of the plant. Macroscopic features reveals that the leaf is light green, with faint odour and bitter taste. The leaves are simple, opposite, and entire having a slightly hard and smooth texture. The shape is small and cylindrical, with alternate arrangement and the apex is obtuse. The chemo-microscopic analyses also reveal that the leaf contains lignin, starch, calcium oxalate crystals and cellulose. The microscopic feature shows that the leaf epidermis at both lower and upper epidermis is rectangular to polygonal and slightly undulating. The anticlinal walls are very wavy and irregular at the lower epidermis, but regular at the upper epidermis. The cell density (425μm) are numerous at the upper epidermis. The stomata are paracytic and frequent at the abaxial epidermis, but absent at the adaxial epidermis. Meanwhile, trichomes are absent. The physicochemical analyses also show high values for loss on drying (11.49%), methanol extractive (16.64%), water extractive (19.42%), total ash (11.21%), water soluble ash (4.83%) and acid insoluble ash (1.02%) which falls within the WHO (World Health Organization) standards for crude drugs from medicinal plants. Information gathered from these studies can be used as good indicators in the identification and standardization of herbal plants usage as monograph in crude drugs.

Keywords

Authentication, Standardization, Taxonomy, Micromorphology, Pharmacopoeia

1. Introduction

Standardization of herbal medicines (drug) is a process of establishing or prescribing a set of peculiar identities or specific characteristics which are generally unique and of unshared qualities. However, amongst various techniques used in identification of plant drugs, pharmacognostic study is always the most reliable. Basic component are standardization and authentication of natural drugs [1]. Plant used in traditional medicines are authenticated through morphological, physico-chemical and phytochemical analyses [1]. *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell belongs to Sapotaceae family. It is a small tree (Shrub) and indigenous to tropical west Africa. It grows up to 15-20ft (6.1m) high in its natural habitat. Moreover, it does not grow above 10ft in cultivation [2]. It has a small leaf and can be 5-10cm long, 2-3.7cm wide and glabrous below and are clustered at the end of the branchlets. It is known as Agbayun in Yoruba. It has a small berry fruit that is

commonly called ‘‘miracle fruit” because it has this unique taste modifying ability to turn sour edible things to taste sweet. Although the fruit itself is not sweet, but it removes acid sourness from what one eats. The sugar content of the fruit is very low, and it contains ‘miraculin’ that is a glycoprotein [3]. The protein called miraculin that is present in the miracle fruit is responsible for the unusual taste by attaching itself to the sweet receptor cells present in the tongue, however, suppressing the sourness ability of the central nervous system [4]. The unusual effect stays for some minutes until it is being eliminated by saliva. The unusual taste modification of the miracle fruit assumes an alternative sweetener to cover the sourness of any edible products [5]. The miracle fruits are very small, up to 2-3cm long, they are usually red in colour when ripe. It contains an edible pulp surrounded by a thin epidermal layer which contains a single seed [6]. The present investigation on the leaves of *Synsepalum dulcificum* is established to access a taxonomic and medicinal standard that will help in proper identification of the plant. Furthermore, the study will enhance greatly the quality of herbal products and for preparation of its monograph for inclusion in the Pharmacopoeia.

2. Materials and Method

2.1. Collection and Preparation of Plant Sample

The shrub was collected from the central nursery of Forestry Research Institute of Nigeria. The fresh sample was identified and authenticated by a taxonomist of the taxonomy unit of the institute. The fresh leaf sample was used for the transverse and epidermal section of the microscopic analysis and also used for the macroscopic (organoleptic evaluation) while the powdered leaf sample was used for chemo-microscopic and phytochemical evaluation.

Figure 1. *Synsepalum dulcificum leaves.*

2.2. Pharmacognostic Study

2.2.1. Macroscopic Study

The macroscopic and organoleptic features of the leaves were evaluated. The shape, texture, apex, size, base, arrangement, margin, venation, colour, taste and odour of the leaves were observed. The quality control methods of the macroscopic features were carried out and photographs were taken at different magnifications using digital camera. All the macroscopic procedures are as described by [7].

2.2.2. Microscopic Study

Qualitative leaf examination: The surface of the leaf portions of *Synsepalum dulcificum* was prepared by peeling-up the upper (Adaxial) and lower (Abaxial) epidermis separately with sterilized forceps, and later soaked in a well-covered petri dishes containing nitric acid (HNO_3) for some hours depending on the plant. This is to soften and aid the perfect removal of the mesophyll layers. The disintegration of the mesophyll layers is usually indicated by bubbles. The upper and lower layers removed were placed in another petri dish containing distilled water, which is later put in a clean petri dish containing ethanol to harden the tissue cells. Upon removal, it was then stained with safranin o and later dipped again inside distilled water to reduce the concentration of the staining agent. It was then mounted on a slide, a drop of glycerol was added and a cover slip was used to cover it, followed by a sealant to protect the edges. Epidermal and other pharmacognostic features were observed from the surface of the leaf. Also, the transverse section of the leaf was prepared with a sledge micrometer. The cut leaf blade was put inside a container and was stained with safranin o for some minutes, it was later cleansed in distilled water and ethanol. It was stained and re-cleansed again. It was then placed in a container containing 1ml of ethanol/xylene until the epidermis is very clear. The epidermal layer was then mounted on a microscopic slide adding a drop of glycerol. The standards methods of Brain and Turner, [8]; WHO, [9]; Evans, [10] were adopted.

Quantitative leaf examination

The microscopic slide prepared for the epidermal section as described above was viewed using a set-up camera Amscope at × 10 objective (1 small stage micrometer division = 10µm; calibration factor = 2.7) was set for the determination of stomatal index, stomatal number, and palisade ratio. The length and width of stomata and trichomes were measured by calibrating the eyepiece micrometer using stage micrometer as described by Evans [10].

Stomata number

$$\mathrm{SN} = \frac{\text{Number of stomata on each epidermis}}{\text{Mean}}$$

Where SN- Stomata number

Stomata index

$$I = \frac{S}{E + S} \times 100$$

Where I – Stomata Index

S – No of Stomata per unit area

E – No of epidermal cells in the same unit area.

Palisade ratio

$$\mathrm{PR} = \frac{\text{Number of epidermal cells on each epidermis}}{10}$$

2.2.3. Chemo-microscopic Examination

Powdered leaves samples of *Synsepalum dulcificum* were

first cleared of obscured materials by soaking them in 70% chloral hydrate solution. Fragments of the leaves were transferred onto clean microscopic slide with dilute glycerol to prevent dehydration or hardening. Detecting reagents for metabolites such as starch, lignin, cellulose, calcium oxalate crystals, fixed oils and fat, calcium carbonates, mucilage and tannins were applied for their presence in cells inclusions or cell wall. These examinations were carried out according to the methods described by Brain and Turner, [8]; WHO, [9]; Evans, [10].

2.3. Physicochemical Evaluation

The physicochemical parameters evaluated in this study includes the following; the ash values (Total ash content, Water soluble Ash, Acid insoluble ash, Nitrated ash), loss on drying, and the extractive values (Methanol and Water). All these parameters were determined using WHO guidelines.

3. Result

3.1. Macroscopic Examination

Macroscopically, the leaf (Figure 1) of *Synsepalum dulcificum* has a light green colour, with a faint smell and a bitter taste. The leaf was observed to be small and cylindrical with an alternate arrangement. The leaves have a fractured surface and is non glandular. The leaf composition is simple, opposite and entire, venation is pinnate and the margin is smooth, with the apex of the leaf having an obtuse shape. The base is unequal, while, the texture is slightly hard and smooth. The surface is smooth and the petiole is very short (Figure 1). The summary of the macroscopic and organoleptic observations is given in Table 1.

Table 1. *Macroscopic and organoleptic characters of the leaves of Synsepalum dulcificum.*

Features	Descriptions
Leaf Shape	Small and Cylindrical
Arrangement	Alternate
Fractured Surface	Non glandular
Petiole	Short
Lamina	
Composition	Simple, Opposite and entire
Venation	Pinnate
Margin	Smooth
Apex	Obtuse
Base	Unequal
Texture	Slightly hard and smooth
Surface	Smooth
Organoleptic Properties	
Colour	Light green
Odour	Faint
Taste	Bitter

3.2. Microscopic Examination

The result of the microscopic evaluation of the leaf of *Synsepalum dulcificum* is expressed in Table 2. The observed shapes of the lower and upper epidermis showed that they are rectangular to polygonal and slightly undulating. The lower epidermis anticlinal walls are wavy and irregular, while it is regular for the upper epidermis. Cuticles are present at both epidermises. The values in parenthesis as obtained for mean cell length (31.08 and 20.60μm), cell width (25.88 and 13.46μm), cell density (79.0 and 425μm), mean stomata (60 and absent), stomata index (43.17% and absent) of the lower and upper epidermis respectively. Paracytic stomata are present on the lower epidermis, while they are not present on the upper epidermis. The trichomes are not present on both epidermises.

Table 2. *Epidermal characters and their qualitative and quantitative descriptions.*

Epidermal Features	Characters	
	Lower Epidermis (Abaxial)	Upper epidermis (Adaxial)
Cells		
Shape	Rectangular to Polygonal and slightly undulating	Rectangular to polygonal and slightly undulating
Anticlinal Walls	Very wavy, Irregular	Regular
Cuticle	Present	Present
Mean Length (μm)	31.08	20.60
Mean Width (μm)	25.88	13.46
Density (μm)	79.0	425
Stomata		
Type	Paracytic	Absent
Frequency	Numerous	Absent
Mean Stomata	60	Absent
Stomata Index (%)	43.17%	Absent
Trichomes		
Type	Absent	Absent

3.3. Chemo-microscopic Evaluation

Table 3 shows the result of chemo microscopic evaluation of the leaves of *Synsepalum dulcificum*, using the powdered samples. The result as expressed in Table 3 revealed the presence of starch, cellulose, Calcium oxalate crystal, lignin, while fats, calcium carbonate, and mucilage are absent. These compounds have shown various pharmacological actions and thus, may be responsible for the activities associated with the plant.

Table 3. *Result of Chemo microscopic evaluation of the leaves of Synsepalum dulcificum.*

S/N	Parameter	Observation	Result
1	Lignin	A red colouration observed	+
2	Starch	A Blue colouration	+
3	Fats	A Pink colouration	-
4	Calcium Oxalate Crystals	No effervescence	+
5	Calcium Carbonate	Effervescence	-
6	Mucilage	No Pink colouration	-
7	Cellulose	A blue Colouration observed	+

3.4. Physicochemical Analysis

The values reported for various physicochemical parameters evaluated for *Synsepalum dulcificum* leaves is expressed in Table 4. The result indicated shows that total ash value of the powdered sample was 11.21%, while water soluble ash was 4.83%. The acid insoluble ash was 1.02% and the nitrated ash was 15.76%. The loss of drying value of the powdered sample was 11.49%. The soluble extractive values for methanol was 16.64, while for water was 19.42. The maximum soluble extractive value was found in water.

Table 4. *Physicochemical analysis of Synsepalum dulcificum leaves.*

No	Parameters	% value (w/w) (whole plant)
	Ash Value	
	Total Ash	11.21
1	Water soluble Ash	4.83
	Acid insoluble Ash	1.02
	Nitrated Ash	15.76
2	Loss on drying	11.49
	Soluble extractive Values	
3	Methanol	16.64
	Water	19.42

4. Discussion

The botanical identity of a plant is basically done by establishing its pharmacognostic analysis. The standardization profile of herbal plants is necessary to assume a proper identification of the plant by checking and preventing them from adulteration [11]. Knowledge has it that plant drugs are naturally embedded with various chemical and biological constituents that enables them to function in the treatment of various disease conditions without a correct plant identity [12]. Macroscopic examination is a standardization technique that study the sensory profile of drugs, therefore, the pharmacognostic study of *Synsepalum dulcificum* can serve as diagnostic test especially its organoleptic characteristics [13]. The leaf has a light green colour, with a faint smell and a bitter taste (Figure 1). The leaf composition is simple, opposite and entire, the arrangement is alternate and the surface is non glandular. The observed leaf is small, cylindrical and alternate. The venation is pinnate and the margin is smooth, with the apex of the leaf having an obtuse shape. Surface is smooth and the petiole is very short. The base is unequal, while the texture is slightly hard and smooth. The simplest, correct, reliable and cheapest methods to establish an accurate and proper identification is through macroscopic evaluation [14]. The qualitative microscopic characters examined using a light microscope revealed the presence of cell descriptions like shape, stomata, and trichomes. The cell shape of *Synsepalum dulcificum* are rectangular to polygonal and slightly undulating both at the lower (Abaxial) and upper (Adaxial) epidermis. The anticlinal walls are very wavy and irregular at the abaxial epidermis and straight and regular at the upper epidermis (Figure 2A & B). Cuticles are present at both epidermises. The microscopic evaluation of the leaf showed that the leaf has paracytic stomata occurring at the lower epidermis alone thus indicating efficient gaseous exchange and transpiration at the abaxial epidermis. The frequency of the stomata at the lower epidermis shows its numerosity. The paracytic (parallel-celled) type is guarded by one or more subsidiary cells (Figure 2D). Trichomes are absent at both epidermises. The quantitative evaluation of the cells shows the mean cell length at lower epidermis (31.08µm) and upper epidermis (20.60µm). The mean cell width at lower epidermis (25.88µm) and upper epidermis (13.46µm), while the cell density for lower epidermis (79) and upper epidermis (425). The cell density on the adaxial are more numerous than that of the abaxial epidermis. The mean stomata number in the lower epidermis is 60 while the stomata index of the lower epidermis is 43.17%.

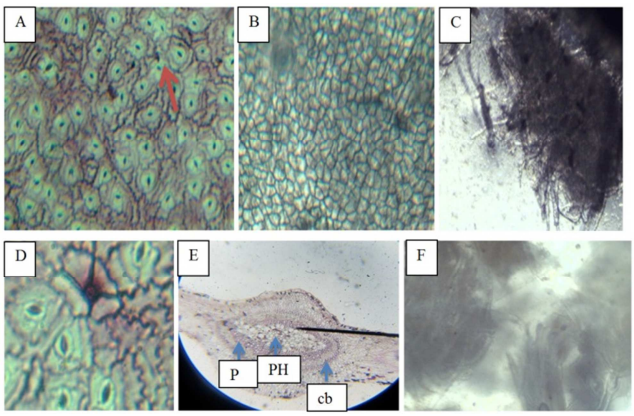

Figure 2. *Light Micrograph of the leaves of Synsepalum dulcificum (A) Leaf clearing showing sinuous epidermal cells and paracytic stomata type on the abaxial surface X10 magnification (B) Leaf clearing showing epidermal cells. (C) Powdered Leaf sample. (D) Leaf clearing showing paracytic stomata. X40 magnification. (E) Anatomical overview of the leaf blade showing parenchyma (pr), phloem (ph), and cambium (cb). (F) Powdered sample for microchemical test.*

The anatomical section of the leaf blade shows the presence of parenchyma, xylem and cambium (Figure 2E). Chemo-microscopic evaluation of the leaves of *Synsepalum*

dulcificum shows that calcium oxalate crystals were observed and they are believed to always have a physiological role administering excess calcium within plant cells, they also disperse light to the chloroplasts [15]. There is presence of starch grains (Figure 2C) and they appear in oval shape, plants store carbon in form of starch and they are converted into sugar when the plants need energy [16]. The powdered leaf sample investigated also showed the presence of lignin, starch, calcium oxalate crystals and cellulose. The botanical information provided in this study has set a standard for genuine and proper identification of *Synsepalum dulcificum* and distinguishes it from other co-generic species as an herbal drug that can be included in the pharmacopoeia of medicinal plants [17]. The result obtained in this study is contrary compared with that reported by Elufioye & Olaifa, [18] whose result tested positive for all the chemo-microscopical test in *Spigelia anthelmia* leaves. The evaluation of the physicochemical properties is important since they help in identifying adulterated and unstandardized crude plant drugs. Such parameters include ash value (Total ash, acid insoluble ash, water soluble ash, and nitrated ash), moisture content, and soluble extractive values (Methanol and Water). Moisture content is an imperative parameter that measures the efficiency of plant drying process which indicates the stability of the drug during storage time [19]. Micro-organism growth is slowed down because the phyto-constituents present in plant can be stored for a long period of time due to high drying time of the plant [20]. The loss on drying obtained in this study for *Synsepalum dulcificum* is 11.49%, which indicates an efficient and fast drying time. This is obviously higher than the value (10.8%) reported for *Diplazium esculentum* by Dash *et al.* [21], for *Achyranthes coynei*, (10.23%) by Gireesh *et al.* [22], and for *Limonium brasiliense* (14%) by Blainski *et al.* [23], which was higher than the value gotten in this study. The extractive soluble values gotten for a particular solvent gives an idea, insight and indicate the nature and amount of chemical constituents available in the crude drugs [24]. The amount of the soluble extractive values gotten in this study for methanol and water are (16.64 and 19.42) respectively. The maximum extractive values recorded for water is in accordance with values reported in similar studies; Kanakiya *et al.* [25] reported (20.12) for *Limonium stocksii*, while Gireesh *et al.* [22] reported (15.24) for *Achyranthes coynei*. The purity and quality of plants are determined by the ash values, because they indicate diverse impurities like silicate, oxalate, carbonate. Water soluble ash gives an estimate or exact idea of the inorganic compound present in the plant [26-27]. The acid insoluble ash is made up of silica and contaminants of earthy materials. Higher total ash values have been reported by different authors in various plants. Elufioye *et al.* [18] reported 14.67% for *Spigelia anthelmia;* Partha & Rahaman, [28] reported 15.85% for *Adenanthera pavonina*. Kanakiya *et al*. [29] reported (11.83) for *Limonium stocksii*. The total ash value (6.40) gotten from the findings of Ibrahim *et al.* [30] for *Crotolaria lachnosema* is very low compared to the value reported for this study.

5. Conclusion

The present study has shown the examined pharmacognostic profile which comprises of macroscopic characters, microscopic, chemo-microscopic and the physicochemical characters which are good indicators of how best plants can be properly identified and standardized. Macroscopic and organoleptic characters of the plant are quite distinct and revealed its physical features which establishes an integral part of the plant correct identification and standardization targeted options which could serve as yardstick for its crude drug usage. Microscopic features also reveal the absence of trichomes on both lower and upper epidermis which creates a distinct diagnostic characters in distinguishing this plant from other species. Also, the presence of chemo-microscopic compounds present suggest that they may be responsible for the pharmacological activities associated with the plant, like administering excess calcium within the plant and storing carbon in form of starch and converting them when the plants need energy. The physicochemical status from the study indicates the nature, amount and impurities of chemical constituents available in the crude drugs. The values obtained for moisture indicates efficiency and stability of the plants storage time if used as a drug. *Synsepalum dulcificum* is a plant which is known to have a taste modifying effect and the diverse taxonomic information provided in this study has set a standard for genuine and proper identification and standardization that distinguishes it from other co-generic species as an herbal drug.

Acknowledgements

Our unquantifiable appreciation goes to Mr Adeniyi of Forest Product and Utilization department (FPD & U) of Forestry Research Institute of Nigeria and Mr Joseph for their tremendous and valuable contributions towards the success of this work.

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