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Standardization of *Jatropha curcas* .L

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Jatropha curcas is a tree and shrub plant found in northern Nigeria, traditionally used by the population of this country for the treatment of various ailments. Despite its medicinal value and popularity in northern Nigeria, no scientific data for standardization of the starting material to further proved to support its pharmacognostic value. The extreme morphological variability of the species leads to its misidentification. Therefore, the present study aimed at studying its morphological, anatomical, and chemical features, to provide baseline information needed on herbal product formulation from the plant. An established method of fast green and safranin method was used for morpho-anatomical analysis and GC/MS was used to analyse the ethanolic extract from the leaf part. The leaf is leptophyllous with leaf length 10-18 and width 8-14 cm, respectively. Open vascular system; lose U shape with ¼ of it circled at the abaxial surface and ¾ open at the adaxial surface. Eight compounds were profiled with decane compound with the highest abundance. The pharmacognostic features documented from the present study will serve as a key for the taxonomic identification of Jatropha curcas in Nigeria.

Keywords: Morphology, Leaf.

INTRODUCTION

Euphorbiaceae family approximately contain 290 genera with more than identified 750 species all over the world (Cavalcante, da Conceição Santos, & da Silva Almeida, 2020; Gerometta, Grondin, Smadja, Frederich, & Gauvin-Bialecki, 2020). They are comprised of trees, shrubs, and herbs. Members of this family are previously reported and xerophytes species(Cavalcante et al. 2020). Their species are known producers of latex. Member Jatropha is of significant biological importance to the human (Holl, Gush, Hallowes, & Versfeld, 2007). They served as alternative medicine, food and cultivated as ornamental. The species are predominantly found in the tropical and subtropical regions of the world(Holl et al. 2007). Latex extracted from members of this family are utilised to healed wounds and mixed with salts for tooth improvements. Genus jatropha originated from America but now can be found all over the world mostly in tropical and subtropical regions of the world (Mahmoud, Labaran, & Yunusa, 2020).

The species was likely to be dispersed through the aid of shipping. *J. curcas* is the most abundant species from the genus as a result of its ability to endure drought and easily to propagate through the stem (Kong, Biddle, Foale, & Adkins, 2020). The genus belongs to the tribe *Joannesieae* of *Crotonoideae* with approximately 170 species. Is a small, large shrub or perennial tree with a height of 7 to 10 m. the life expectancy of the plant is up to 50 years (Singh, 2016).

Traditionally, in Nigeria, the plants are known to be used for the treatment of hypotension, diabetes, fever, ulcer, and many more diseases. In developing countries, herbal drug plays an important role in the management of health (Lawal et al. 2010). It has been established all plant parts as a potential source of medication. Despite its importance, the major obstacle hindering the progression in herbal drug

development is the lack of documentation and standard quality control of the medicinal plants (Dike, Obembe, & Adebiyi, 2012). Looking at its importance, there is an immediate need for standardization of each of the plant materials utilised in the traditional medicinal system. Standardization of plant material is achieved through morpho-anatomical studies and chemical profiling (Fatihah, Nashriyah, Zaimah, Khairil, & Ali, 2014). According to WHO (1988), the macro and microscopic analysis of plant material with medicinal value is of paramount importance toward establishing the fingerprinting for identification and purity of its contents before further investigation (Olsen, 1988). However, microscopic analysis of medicinal plants is a complex task due to the heterogeneous composition of the plant content (Abdulrahman, Ali, Fatihah, Khandaker, & Mat, 2018b). Proper identification and standardisation of quality assurance of the plant material will significantly contribute to ensuring quality herbal medicine leading to efficacy and safety of end users.

MATERIALS AND METHODS

Plant Materials

The plant material was collected and submitted to the department of botany for identification and deposition in the herbarium. The following voucher number assigned ABU08961 and ABU0772 respectively. Morphological study of the plant leaf was subjected to the following observations: leaf length, leaf width, leaf attachments, leaf organization, leaf colour, leaf odour, lamina shape, lamina symmetry, the position of the petiole, petiole length, attachments, presence of tertiary vein, vein type, vein spacing, (Abdulrahman, Ali, Fatihah, Khandaker, & Mat, 2018a).

Anatomical studies were carried out as described in (Table 1 and 2). The abaxial and adaxial leaf surfaces were also polished with nail polish and masked with sellotape and directly transfer to glass slides to observe stomata.

(Gas chromatography Mass spectrometry) Conditions

Agilent GC/MS was used coupled with the mass spectrometer. The compound was separated on a column: HP-5MS 30 m x 0.25 mm, 0.25 mm film thickness with a programmed temperature initially at 59 °C for 9 min, then to 230 °C for 1 min at 3°C/min with 1 min hold. The injector was at 245 °C and the flow rates of the carrier helium gas 1 mL/min. Volt 70 e V was used for the MS with the ion source and analyser temperature at 260 °C (Wesołowska, Jadczak, Kulpa, & Przewodowski, 2019).

Table 1: Decolourization and Staining Process (Abdulrahman et al., 2018a)

S/N	Solutions	Time	Description
1	Sodium hydrochloride	30 minutes	Time varies and needs to wait until it decolorizes sometimes up to one hour depend on the plant part
2	Distilled water	5 minutes	To remove excess bleach from the plant part
3	Fast green	30 minutes	To stain the vascular bundle sometimes it varies depending on the plant part
4	Distilled water	1-2 minutes	To remove excess color
5	Safranin	1-5 minutes	To stain other cells

Note. S/N = serial number

Table 2 : Dehydration Process

S/N	Solutions	Time	Description
1	50 % Ethanol	2 minutes	Petri dish was covered
2	70 % Ethanol	2 minutes	Concentrated hydrochloric acid, a drop was added to the solution and shake, gently to remove excess colors
3	95% Ethanol	2 minutes	Petri dish was covered
4	100% Ethanol	2 minutes	Petri dish was covered

Note. S/N = serial number

RESULTS

Leaf length 10-18 and width 8-14 cm, respectively. The leaf is leptophyllous, lamina apex; Acute, lamina symmetry; base asymmetrical Petiole 5 – 16 cm. The leaf color is green. The leaf is broad with alternate attachments. One leaf at each node. Base swollen petiole, the leaf is oblong - widest part at the middle 2/3 of the leaf space. The base angle of the leaf is obtuse. The leaf margin is serrate. The marginal indentation is unlobed (Fig. 1). The vines are actinodromous: a situation whereby four or more primary veins diverging radially from the single point of the petiole. The adaxial surface of the epidermal is smoot with square shapes, small oil secretion glands was also observed at the adaxial surface

of the leaf while the abaxial surface of the leaf were found to be a protruding surface with no il secretion gland (Fig 2). At both the adaxial and abaxial surface of the leaf, no hypodermal layer was observed. The stomata were found merged to the epidermis. Anisocytic stomata were seen. The stomata was found to be confined to the abaxial surface; hypostomatic stomata. The leaf lamina transverse section palisade layer was absent; spongy mesophyll layer was seen occupying 2/3 of the leaf lamina at the abaxial part (Fig. 2). The transverse section of the midrib revealed collenchyma cells confined to the abaxial surface with the extracellular surface. Sclerenchyma cells ensheathing the vascular tissue at the adaxial and abaxial surface. Immediately after the Sclerenchyma cells, layers of parenchyma cells filled the adaxial section of the midrib. It is an open vascular system; the vascular tissue is lose you shape with ¼ of it circled at the abaxial surface and ¾ open at the adaxial surface (Fig. 2). No trichome was seen at the midrib section. The G C/MS analysis of the *ethanolic* extract of the leave revealed 8 compounds quantitatively as shown in Figure 2 and Table 3.

Figure 1: Morphological structure of *Jatropha curcas* .L

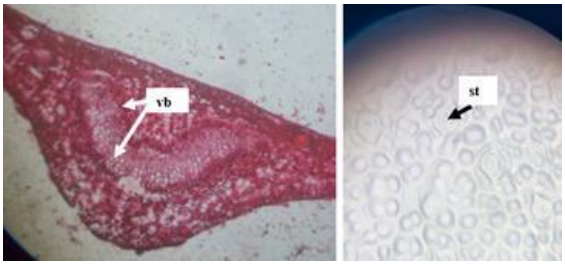

Figure 2 :Midrib and stomata of *Jatropha curcas* .L: vb; vascular bundle and st; stomata

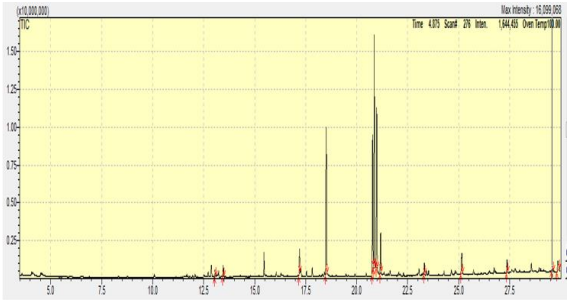

Figure 3: Chromatogram of ethanolic leaf extract

Table 3 Compound identified from GC/MS

S/N	RT	Compound		Area
1	4.0750	Decane	$C_{10}H_{22}$	2.1
2	8.3135	Undecane	$C_{11}H_{24}$	1.2
3	11.6740	Tetradecane	$C_{14}H_{30}$	0.5
4	18.5095	*Hexadecanoic acid*	$C_{16}H_{32}O_2$	2.1
5	20.8635	9-Octa acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	4.8
6	20.9855	(*Z*)-Octadec -9-en-1-ol	$C_{18}H_{36}O$	7.8
7	21.1730	Octadecenoic	$C_{18}H_{34}O_2$	18.7
8	25.1605	Dimethyl phthalate	$C_{10}H_{10}O_4$	0.9

DISCUSSION

Coming up with a standard procedure is the key point in the correct identification and quality control of herbal medicine (Chan, 2005; van der Kooy, Maltese, Choi, Kim, & Verpoorte, 2009). Identification of the starting material is the prime stage in herbal drug formulations (Sahoo & Manchikanti, 2013; Thakur, Ghodasra, Patel, & Dabhi, 2011). *Jatropha curcas* has long been in utilization for the treatment of various ailments and health improvements in Nigeria (Makkar & Becker, 2009). Therefore, it is necessary to layout standard procedures for the standardization of its starting materials for usage in herbal formulation. Morphology and anatomic investigation are

simple, reliable, and the cheapest form of standardization of the starting material (NirmlaDevi et al. 2011). World health organisation has made it paramount; morphological and anatomical description of utilised traditional medicinal plants to establish its purity and identity before any phytochemical analysis (Niranjan Babu, 2010). The flowers and fruits are found only once a year, variety in the anatomical characters of the leaf has been used as a yardstick for taxonomic delineation of various medicinal plants (Mahmoud et al. 2020). *Jatropha curcas* has a compound leaf morphology; leptophyllous, lamina apex; Acute, lamina symmetry; base asymmetrical. The above characters were previously reported in *Jatropha* anatomical comparative analysis of infrageneric relationships and petiole examination (Pompelli et al. 2010). Similarly, morphological features where utilised in the identification and discrimination (Fatihah et al., 2014). The anatomical leaf surface of *Jatropha curcas* showed the epidermal surface is covered with a smooth and thin cuticle. Several studies reported the usage of the leaf epidermal surface for taxonomic identification of medicinal plants (Shah et al. 2018). The leaf lamina transverse section of the leaf showed an abundance of spongy mesophyll layer. The following features are a good diagnostic character for the identification of *Jatropha curcas*. Merged stomata to the epidermis were recorded. As reported by Hussin et al. (1992), the anatomy of stomata is one of the most important taxonomic characters for plant identification; types of the stomata, location and occurrence are good characters for plant delineation. An open vascular system: the vascular tissue is lose you shape with ¼ of it circled at the abaxial surface and ¾ open at the adaxial surface. Vascular tissue arrangement has been previously reported to be sufficient enough to discriminate or delimited plant species into various groups (Hussin, Cutler, & Moore, 1992). Much scientific research delimited plant species with the arrangement of the vascular tissue at the midrib (Aung, Yaakob, Abdullah, Rayung, & Li, 2015; Hussin et al. 1992; Kantachot, Chantaranothai, & Thammathaworn, 2007). Knowledge of the secondary metabolite will similarly complement the results of the microscopic and macroscopic analysis. The GC/MS analysis of the leaf extract revealed eight secondary metabolites.

CONCLUSION

Findings from the present study established principle parameters or characters for the identification to avoid adulteration of *Jatropha curcas* in the formulation of the herbal product from its material.

CONFLICT OF INTEREST

The author declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

The author designed the experiments, performed data analysis, and wrote the manuscript.

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