Anales de Biología 39: 55-62, 2017
DOI: http://dx.doi.org/10.6018/analesbio.39.06 ARTICLE

Taxonomic value of the leaf micro-morphology and quantitative phytochemistry of *Jatropha integerrima* Jacq. and *Jatropha podagrica* Hook. (Euphorbiaceae)-known horticultural plants in Nigeria

Opeyemi Saheed Kolawole[1], Mahboob Adekilekun Jimoh[2], Fred Yakubu[3] & Emmanuel Chukwudi Chukwuma[3]

1 Department of Biological Sciences, Faculty of Science, Federal University, Kashere, Gombe State.
2 Department of Biological Sciences, Osun State University, Osogbo, Osun State, Nigeria
3 Forestry Research Institute of Nigeria, Jericho Hills, Ibadan, Oyo State, Nigeria

Correspondence
OS. Kolawole
E-mail:
kolawoleopeyemisaheed@gmail.com
+234 8060909011
Received: 14 August 2016
Accepted: 28 February 2017
Published on-line: 14 March 2017

Resumen

Valor taxonómico de la micromorfología de la hoja y la fitoqímica cuantitativa de Jatropha integerrima *Jacq. y* Jatropha podagrica *Hook. (Euphorbiaceae)-conocidas plantas hortícolas en Nigeria*

Se examinó la micromorfología y la fitoquímica cuantitativa de *J. integerrima* y *J. podagrica para* para identificar caracteres útiles para la delimitación taxonómica, siguiendo protocolos estandarizados, descritos por autores anteriores. Ambas especies son hipostómicas (paracíticas). Los tricomas (multicelulares, no glandulares) estaban presentes sólo en la superficie adaxial de *J. integerrrima* y ausentes en otras superficies epidérmicas. Los análisis fitoquímos también señalaron pequeñas diferencias en la cantidad de compuestos bioactivos presentes en ambas especies. El contenido de fenol es el mayoritario en ambas especies: 37.65% en *J. integerrima* y 36.13% en *J. podagrica*. Las semejanzas y diferencias de los caracteres epidérmico-foliares y contenidos fitoquímicos pueden ser empleadas para delimitar taxonomicamente ambas especies.

Palabras clave: *Jatropha*, Microcaracteres, Fitoquímica, Taxonomía.

Abstract

The leaf micro-morphology and quantitative phytochemistry of *J. integerrima* and *J. podagrica* were examined to find useful characters for the delimitation of taxa, following standard protocols as described by previous authors. Both species are hypostomatic (paracytic). Trichome (multicellular, non-glandular) are only present on the adaxial surface of *J. integerrima* and absent in other epidermal surfaces. Phytochemical analysis also showed little differences in the amount of bioactive compounds present in both species. Phenol contents are the highest in the two species: 37.65% in *J. integerrima* and 36.13% in *J. podagrica*. The similarities and differences in the foliar epidermal characters and phytochemical content can be used to delimit the two studies species, as taxonomic characters.

Key words: *Jatropha*, Micro-characters, Phytochemistry, Taxonomy.

Introduction

The genus *Jatropha* is a member of the Euphorbiaceae, and comprises of approximately 175 species of succulent, caudiciform, herbaceous perennials and woody plants. It is characterized by simple to palmately arranged leaves; 3, 5 or 7 lobed or divided into a maximum of 11 segments (Abdulrahaman *et al.*, 2014). The genus is diverse, occurring in tropical and sub-tropical areas of the world. Extracts from different parts such as leaves, stem, bark and roots of *Jatropha* species have been used in ethno-medicines for a long time (Duke, 1985).

Jatropha integerrima Jacq.is an erect ornamental evergreen shrub, native to West Indies and grows commonly in Southern parts of India (Krishan & Paramathma 2009). It grows from 3 to 10 feet tall and to almost tree-like proportions with age in frost free climates. The leaves are 3 to 6 inch long by 2 inch wide, green and velvetly on upper surface and flecked with purple below, have sharp points on the lobes and are held on long leaf stems. *J. integerrima* flowers are 1 inch wide five-petaled deep red with yellow stamens are held in branched clusters on 4 inch long stalks at the branch tips. Various parts of *J. integerrima* are traditionally used as purgative, styptic, emetic, in treatment of warts, tumours, rheumatism, herpes, pruritis, toothaches, scabies, eczema and ringworm (Kirtikar *et al.* 2002). The leaves and branches of the plant have been shown to hold cholinesterase activity while latex of the plant has demonstrated anti-cancer activity (Gupta and Gupta 1997, Wele *et al.* 2007, Sharma and Singh 2010).

Jatropha podagrica Hook is a shrub native to tropical America. *J. podagrica* is an ornamental plant which is also employed to cure various infections in traditional medicine. *J. podagrica* is known for many biological activities such as anti-tumour, antimicrobial, molluscicidal and anti-insect (Kupchan *et al.* 1970, Bhushan *et al.* 2008). Different parts of *J. podagrica* are also used for antipyretic, diuretic, choleretic and purgative effects (Irvine 1961). Various medicinal and pesticidal properties, including antimicrobial, antitumour and insect antifeedant activities, have also been attributed to this plant (Aiyelaagbe *et al.* 1998, Aiyelaagbe *et al.* 2000, Bhaskarwar *et al.* 2008, Sanni 1988).

Previous phytochemical investigations of *J. podagrica* led to the isolation of japodic acid, erytrinasinate (Aiyelaagbe & Gloer 2008), n-hexacosane, β-amyrin, lupeolpalmitate, quercetin, apigenin, vitexin, isovitexin, rutin (Odebiyi 1985), podacycline A, podacycline B and 3-acetylaleuritolic acid (Ee *et al.* 2005).

Earlier studies on the genus were examined on the basis of morphological (Dehgan and Webster 1979, Dehgan 1980), wood anatomy (Oladipo & Illoh 2012a), numerical approach to the taxonomy using quantitative phytochemical parameters (Kolawole *et al.* 2014), leaf and seed electrophorensis (Oladipo & Illoh 2012b, Oladipo *et al.* 2008), leaf epidermal features (AbdulRahaman & Oladele 2010), quantitative phytochemical constituents (Kolawole *et al.* 2014), palynological and carpological studies (AbdulRahamnan *et al.* 2014) and recently morphometric study (Kolawole *et al.* 2016). Metcalfe & Chalk (1972) had pointed out that the historical development of botany has been such that physiological and anatomical investigations of plants have been unnecessarily separated from the studies of their systematic arrangement.

This paper aims to provide a comprehensive description and add to scanty information that is available in literatures on the foliar epidermal and phytochemical properties, with a view of finding additional diagnostic characters that may be used to distinguish the species.

Material and methods

Sample collection and identification

Fresh specimens of *J. intergerrima* and *J. podagrica* (Fig. 1) were collected from different locations in Nigeria and were identified at the Forest Herbarium, Ibadan (FHI). Voucher specimens were prepared according to the established protocol of Soladoye *et al.* (2011). Each specimen was assigned with a specific voucher number: *Jatropha intergerrima* Jacq. FHI109864 and *Jatropha podagrica* Hook. FHI109871. Voucher specimens were deposited at the Forest Herbarium, Ibadan (FHI) (Holmgren *et al.*, 1990).

Sample preparation

The dried leaves were ground into fine powder and transferred into airtight containers with proper labelling. They were then subjected to phytochemical screening which was carried out at the

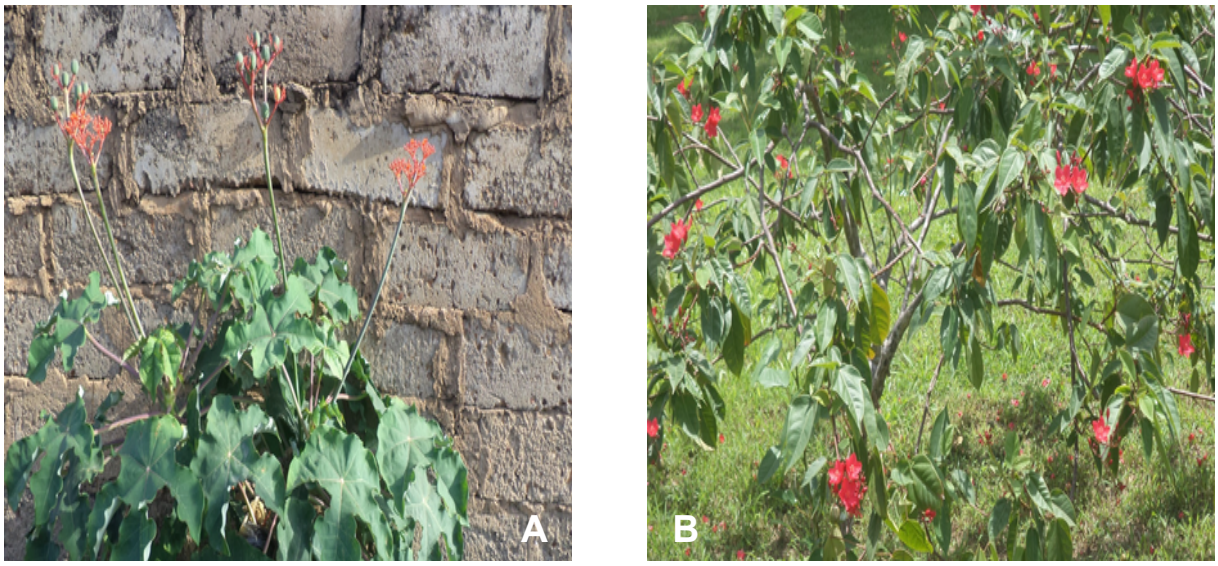

Figura 1. Especímenes vivos. **A:** *Jatropha podagrica* **B:** *Jatropha integerrima*.
Figure 1. Live specimens of the species studied. **A:** *Jatropha podagrica* **B:** *Jatropha integerrima*.

National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. This screening was done to determine the biologically active compounds present in the plant parts. Procedures were adapted from earlier works for plant analysis as described below and reported by Sofowora (1993) and Trease & Evans (2005). A detailed method of extraction, as well as purification techniques for active plant constituents described by Harborne (1998) and adopted by Soladoye & Chukwuma (2012) were also employed for the phytochemical extraction of the plant materials.

Foliar epidermal preparation

About 2-5cm^2 of the leaves was obtained from fresh specimens of *J. integerrima* and *J. podagrica* were and soaked in well covered glass petri-dishes containing concentrated trioxonitrate (v) acid for 2-3hr. Upon the disintegration of tissues and indication of bubbles, the specimens were carefully transferred unto clean petri-dish and rinsed with distilled water before the epidermises were separated using forceps. The mesophyll tissues of the leaves were also cleared with camel brush. They epidermises were thereafter stained with Safranin 0 for 5 minutes and again rinsed and continuous changes of distilled water until excess stain is washed off. The stained tissues were carefully mounted in 25% glycerol, unto clear microscopic glass slides and ringed with nail vanish. They were then studied under an Olympus light microscope at different magnifications. Observations were taken from 20 fields of view for each epidermal surface (abaxial and adaxial), of the *Jatropha* species studied (Khatijah & Zaharina 1998, Adedeji 2004, Chukwuma *et al.* 2014).

Phytochemical analysis

Determination of tannins

Tannin content was determined using the method outlined by Van-Burden & Robinson (1981). 0.5 g of the dried powdered leave sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and then shaken thoroughly for about 1 hour. The solution was filtered into a 50 ml volumetric flask and made up to the mark. 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Edeoga et. al., 2005).

Determination of flavonoids

To determine the flavonoid content in the leaves of the *Jatropha* species studied, the aluminium chloride colorimetric method was employed. 1 ml of each plant extract was mixed with 2 ml of methanol, 0.2 ml of 10 % aluminium trichloride ($AlCl_3$), 0.2 ml of 1M potassium acetate, and 5.6 ml of distilled water. The entire mixture was allowed to stand at room temperature for 30 min after which the absorbance was measured at 420 nm. The total flavonoid content in each plant part

was expressed in terms of standardized quercetin equivalent (QE) per 100mg of each extracted compound (Aiyegoro & Okoh 2010).

Determination of phytosteroids

For this purpose, the crude extract of each leaf was mixed with 2 ml of chloroform and concentrated sulphuric acid (H_2SO_4) was added sidewise. The presence of steroids was noticed from the red colour produced in the lower layer of chloroform. To confirm further the presence of this phytochemical, another test was performed by mixing each crude extract of the plant materials with 2 ml of chloroform. Two ml of concentrated H_2SO_4 and of acetic acid were then poured into the mixture and the development of greenish coloration indicated the presence of steroids (Soladoye & Chukwuma 2012).

Determination of cardiac glycosides

Buljet's reagent (El-Olemy *et al.* 1994) was used to evaluate the cardiac glycoside content in the examined *Jatropha* species. For this purpose, 1 g of each powdered sample was soaked in 100 ml of 70 % ethanol for 2 hrs before filtration. Using lead acetate and Na_2HPO_4 solution, the obtained extracts were purified before the addition of freshly prepared Buljet's reagent. The difference between the intensity of colours of the experimental and blank samples (distilled water and Buljet's reagent) gave the absorbance, which is proportional to the concentration of glycosides.

Determination of alkaloids

A weighed amount (5 g) of each powdered sample of the *Jatropha* leaves was transferred into a 250 ml beaker. 200 ml of 10 % acetic acid was added and then covered to stand for 4 hrs. Filtration was done, and concentration of the extracted content to one quarter of original volume was applied using a water bath. Drop-wise addition of concentrated ammonium hydroxide to the extract followed until the precipitate was complete. The entire solution was allowed to settle and collection of the precipitate was done by filtration (Harborne 1997, Obadoni & Ochuko 2001) and then weighed.

Determination of saponins

The method described by Obadoni & Ochuko (2001) was used for the determination of saponin. 100 cm^3 of 20% aqueous ethanol was added into a conical flask containing 20 g of the powdered samples. The mixture was properly shaken together, and then heated over a hot water bath for 4 hours with continuous stirring at 55 ºC. It was filtered and re-extraction of residue was performed with 200 ml of 20 % aqueous ethanol. The combined extracts were then reduced to 40 ml over water bath at 90 ºC after which the concentrate was transferred into a 250 ml separating funnel with the addition of 20 ml diethyl ether, and shaken vigorously. The aqueous layer of the solution was recovered while the ether layer was discarded. The purification process was then repeated and thereafter, 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride and the remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight, the saponin content was calculated as percentage at a wavelength of 380 nm.

Determination of phenol

Total phenol content in each plant sample was determined using Folin-Ciocalteu's method as modified by Olajire & Azeez (2011). 0.5 mL of the extract was added to 10 mL of deionized distilled water and 2.5 mL of 0.2 N Folin-Ciocalteu's phenol reagent. The mixture was allowed to stand at room temperature for 5 min and then 2 mL of sodium carbonate solution was added. The absorbance of the solution was measured at 760 nm after 2 hours of incubation using a spectrophotometer. Quercetin was used as standard for calibration curve.

All tests were carried out in triplicate and mean values were recorded appropriately.

Results and discussion

Results from this work showed that the leaves of *J. integerrima* and *J. podagrica* have important diagnostic features that can be useful in taxonomic studies. The leaves of the examined species are also rich in plant compounds. These characteristics exhibited by these important angiosperms are summarised in tables 1 and 2 respectively.

Epidermal cells were generally polygonal and numerous on the surfaces, while stomata paracytic in both species. Trichome is simple, long, multicellular and non-glandular on the adaxial surface of *J. integerrima*, and completely absent on abaxial surface and both surfaces of *J. podagrica* (Fig. 2). Similarity of the stomata complex in the two species is noteworthy, although stomata are larger in the former than in the latter. Salisbury

Species	Leaf surface	Stomatal type (s)	Stomatal frequency (%)	Stomatal index (%)	Stomatal size (µm)	Anticlinal cell wall pattern	Trichome
Jatropha integerrima	Adaxial	-	-	-	-	Straight, polygonal	Present
	Abaxial	Paracytic	100	14.50[b]	47.69[b]	Straight, polygonal	Absent
Jatropha podagrica	Adaxial	-	-	-	-	Straight, polygonal	Absent
	Abaxial	Paracytic	100	8.06[a]	30.56[a]	Straight, polygonal	Absent

Tabla 1. Características epidérmicas foliares de las especies estudiadas de *Jatropha*.
Table 1. Foliar epidermal characteristics of *Jatropha* species studied.

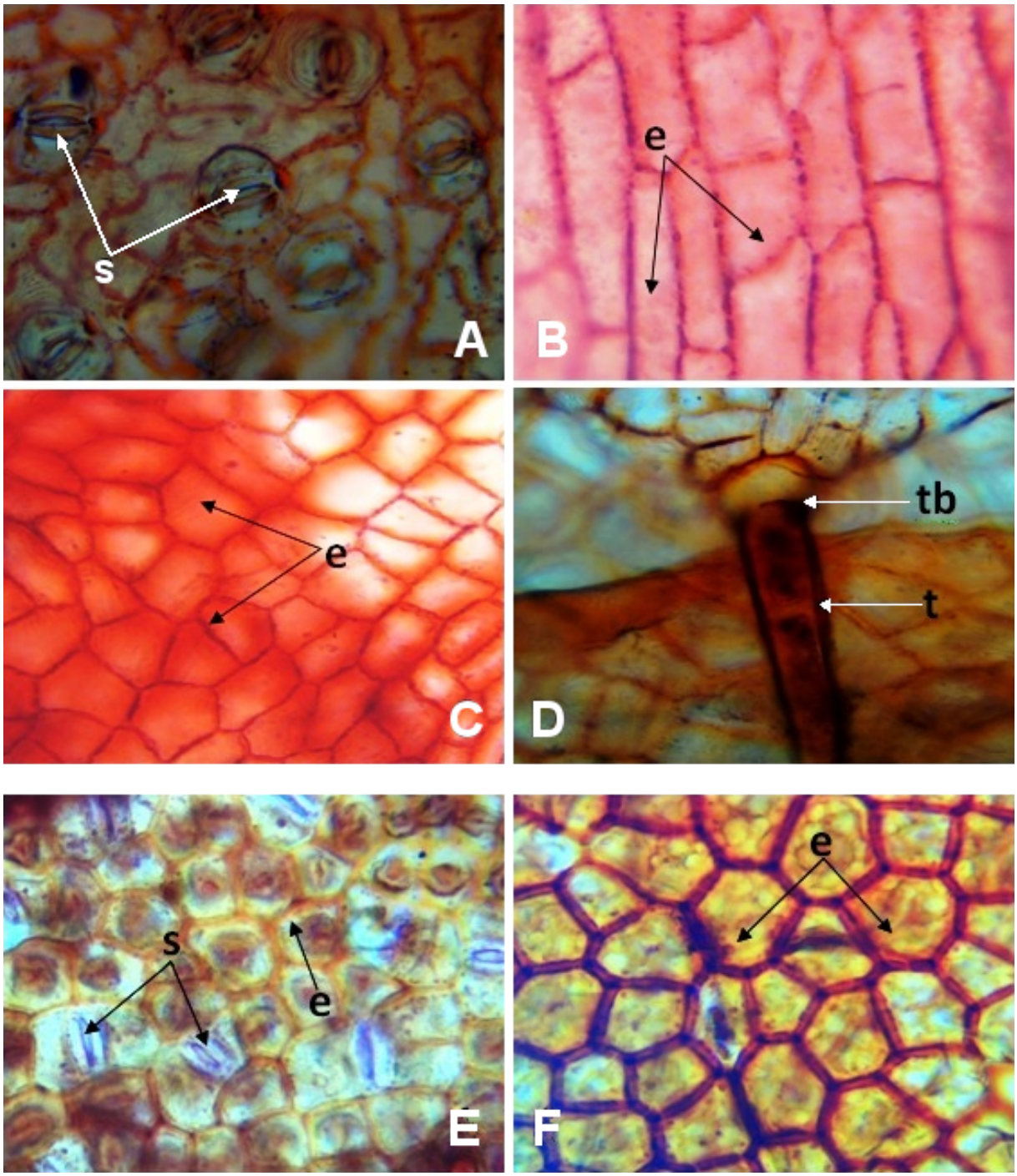

Figura 2. Fotografía de las superficies epidémicas x400. **A, B:** *J. integerrima*, superficie abaxial; **C, D:** *J. integerrima*, superficie adaxial; **E:** *J. podagrica*, superficie abaxial; **F:** *J. podagrica*, superficie adaxial. **e:** célula epidérmica; **s:** estoma; **t:** tricoma; **tb:** base del tricoma.
Figure 2. Photomicrographs of epidermal surfaces x400. **A, B:** *J. integerrima*, abaxial surface; **C, D:** *J. integerrima*, adaxial surface. **E:** *J. podagrica*, abaxial surface; **F:** *J. podagrica*, adaxial surface. **e:** epidermal cell; **s:** stomata; **t:** trichome; **tb:** trichome base.

Phytocompounds	*Jatropha integerrima*	*Jatropha podagrica*
Alkaloids	1.21±0.07[b]	0.98±0.04[b]
Cardiac glycosides	2.35±0.12[c]	1.27±0.05b
Flavonoids	11.61±0.55[e]	6.29±0.23[d]
Phenols	14.90±0.84[f]	7.93±0.55[e]
Phytosteroids	0.42±0.01[a]	0.42±0.01[a]
Saponins	0.81±0.35[a]	0.49±0.02[a]
Tannins	8.13±0.60[d]	4.43±0.05[c]

All measurements are in mg/g and expressed as mean ± standard error.
Values with the same letters are not significantly different at P<0.05

Tabla 2. Contenido cuantitativo fitoquímico de las hojas de las especies estudiadas de *Jatropha*.

Table 2. Quantitative phytochemical content of the leaves of *Jatropha* species studied.

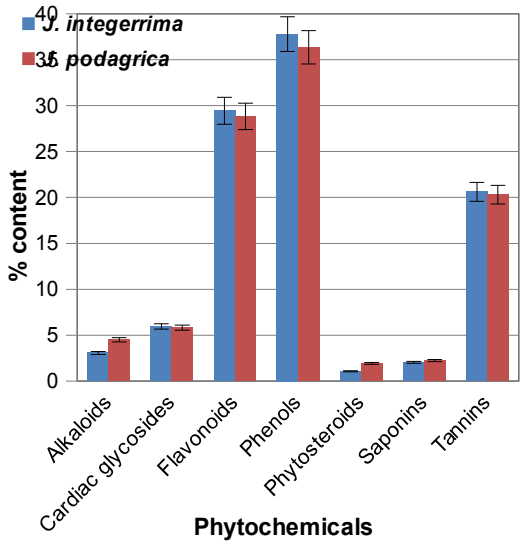

Figura 3. Porcentaje de contenido e fitocompuestos en las hojas de las especies estudiadas de *Jatropha*.

Figure 3. Percentage content of phytocompounds in the leaves of *Jatropha* species studied.

(1927) stressed that the number of stomata is higher, when the size of epidermal cells is low and the number of stomata is lower, when the size of the cells is large. This fact was also observed in this work. However, the taxonomic use of leaf epidermal characters had earlier been reviewed by some workers (Wilkinson 1979, Stace 1989), while the advantages and disadvantages of using such characters as taxonomic indicators have also been earlier conversed by others (Van Staveren & Bass 1973, Soladoye, 1982).

Further findings from the phytochemical screening showed that the phenolic and flavonoid contents in both species where higher that other phytocompounds (Table 2). This was closely followed by tannin with 8.13 ± 0.60 mg/g in *J. integerrima* and 4.43 ± 0.05 mg/g in *J. podagrica* respectively. The large amounts of flavonoids (11.61 ± 0.55 mg/g in *J. integerrima* and 6.29 ± 0.23 mg/g in *J. podagrica*) could lead to the assumption that the examined species may have potent anti-oxidant properties. The tannin content as observed is an indication that the species are important in antibacterial, antiviral and antiparasitic drugs production (Akiyama *et al.* 2001, Kolodziej & Kiderlen 2005, Soladoye & Chukwuma 2012). Interestingly, the contents of phytosteroids is the same in both species. Although the presence of this compound as well as saponins in the examined plant species was not very significant, it also suggests their usefulness in drug industry, especially phytosteroids due to its relationship with sex hormones as noted by Okwu (2001). Asl & Hossein (2008) also noted that there is evidence of saponins in traditional medicine preparations, where oral administration might be expected to lead to hydrolysis of glycoside from terpenoids.

As generally observed the epidermal cell sizes, cell wall thickness and the stomata size were variable and overlapping between the two *Jatropha* species, and as such, they may not be useful in the characterization of these two species even though they have added to existing taxonomic information regarding them. Also, the phytochemical content in the examined species can be summarised thus: phenols> flavonoids> tannins> cardiac glycosides> alkaloids> saponins> phytosteroids, as illustrated in figure 3.

As observed in this work, it is noteworthy that the non-availability of species is a great hindrance to taxonomic studies. It is therefore imperative to encourage the sustainable collection and use of medicinally important species, not only of the Nigerian flora but of the world at large. By so doing, species extinction can be mitigated.

Conclusión

This study has further revealed that members of the genus *Jatropha* could be relied upon for several medicinal properties. The two species examined in this work (*J. integerrima* and *J. podagrica*) both possess a number of useful phytocompounds in appreciable amount, thus suggesting that they could serve as potential sources of useful drugs in the near future if they are further screened for pharmacognostic properties. Nonetheless, the foliar epidermal studies also conduc-

ted showed that they share similar anatomical features, yet some of these characters can be useful in delimiting the studied taxa. Such include the presence of trichome only on the adaxial surface of *J. integerrima* and totally absent on its abaxial surface as well as both surfaces of *J. podagrica.* Although this study has added to the existing information about the two species, it however recommends that practical conservation strategies be adopted for the protection of important plant species such as those examined in this work.

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