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Taxonomic value of the leaf micro-morphology and quantitative phytochemistry of *Clitoria ternatea* and *Centrosema pubescens* (*Papilionoideae, Fabaceae*)

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Abstract. The foliar epidermal and phytochemical characteristics of *Clitoria ternatea* and *Centrosema pubescens* are studied in this work. Results have revealed minor differences between the examined species. A microscopic study has shown that both species are hypoamphistomatic, have irregular cells and simple trichomes on their abaxial and adaxial surfaces. *C. pubescens* has more stomata, trichomes and epidermal cells than *C. ternatea*, but its stomata and epidermal cells are smaller in size. The stomata index ranges between 5.1–21.0 %. On the other hand, a phytochemical analysis has shown little difference in the amount of bioactive compounds present in both species, except for in the flavonoid content which was significantly different between the two. Although phytosteroidal content in the two species is also not very significant, its presence may be linked to the species medicinal value as an aphrodisiac.

Key words: *Centrosema, Clitoria,* micromorphology, Papilionoideae, phytochemistry

Introduction

Papilionoideae is a large, homogenous and easily recognized leguminous group comprising trees, shrubs, climbers, or herbs; with generally compound leaves, often imparipinnate or tri-foliate, or simple, usually stipulate, and leaflets occasionally stipulate (Hepper 1958). The members of this group have very distinct flowers (zygomorphic, mostly hermaphrodite), and most of them obviously having in general the same form as the bean flower (Hepper 1958, Keay 1989). Hepper (1958) recorded nine tribes and 80 genera in the West Tropical Africa, but recently Soladoye & Lewis (2003) reported 15 tribes and 83 genera, each genus represented by at least one collection in Nigeria deposited at the Forest Herbarium, Ibadan (FHI).

As described by Hepper (1958), *Clitoria ternatea* is a perennial herbaceous climber with pinnate leaves; leaflets 5–7, elliptic and rounded at the tip; vivid deep-blue solitary flowers, occasionally pure white. This plant is native to many African countries, including Nigeria. It has been used for centuries in the traditional ayurvedic medicine as memory enhancer, antidepressant, anticonvulsant, tranquilizing and sedative agent (Mukherjee & al. 2008). Ac-

cording to Ravishankar & al. (2012), its roots have ophthalmic, laxative and aphrodisiac medicinal value, and also it is used as tonic for weakened bodily conditions.

Centrosema pubescens Benth. is described as a perennial creeper, with slender pubescent stems. Leaves pinnately 3-foliolate; stipules ovate to ovate-lanceolate, 2–3 mm, elliptical, rounded at the base, rounded to acuminate at the apex, dark-green, slightly hairy, especially on the lower surface; petiole up to 5.5 cm long, stipules 2–4 mm long; flowers pink-mauve or white with purple markings (Hepper 1958, Burkill 1995). Both species belong to the tribe *Phaseoleae* (Soladoye & Lewis 2003) of the *Papilionoideae*, and phylogenetically are positioned very close to each other.

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. Although *C. ternatea* and *C. pubescens* are easily recognized by their external morphology when in flowering, the two species may look confusingly similar, while in their sterile condition. Owing to this morphological similarity, it is important to provide alternative characters for their delimitation. Therefore this work, while examining the resemblance between the aforementioned legumes, also takes into consideration their foliar epidermal and phytochemical properties, with a view of finding additional diagnostic characters that may be used to distinguish the taxa.

Material and methods

Plant material

Fresh specimens of *C. ternatea* and *C. pubescens* were collected from the arboretum of the Forestry Research Institute of Nigeria, Ibadan and were carefully identified by comparing with the existing collections deposited at the Forest Herbarium Ibadan (FHI) of the same Institute. Voucher specimens for the two species were prepared and deposited at the same Herbarium: *Centrosema pubescens* Benth. – FHI 1096454 and *Clitoria ternatea* L. – FHI 109645. The Forest Herbarium, Ibadan (FHI) is an International Herbarium listed in Holmgren et al. (1990).

Leaf epidermal preparations

Pieces of 1–5 cm^2 of the leaves of each specimen were cut and soaked in concentrated trioxonitrate (v) acid (HNO_3) in well covered Petri dishes for about two to four hours to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermises were transferred into clean Petri dishes and adequately rinsed with distilled water, before the abaxial and adaxial layers were separated with forceps. Tissue debris was carefully cleared off the epidermises with fine Carmel hair brush, and the isolated epidermal layers were adequately rinsed in water. The epidermises were then transferred into another Petri dish containing 50 % ethanol for 1–2 minutes, thereby allowing hardening of cells. Afterwards, tissues were transferred unto a clear-glass microscopic slide and stained with Safranin O for five minutes and then rinsed again in distilled water to remove excess staining. They were mounted thereafter in 25 % glycerol on a microscopic slide, covered with cover-slips and the edges of the cover slip were ringed with nail varnish to prevent dehydration and thus the slips were sealed to the slides. Five slides were prepared for each epidermis of the two species. Methods followed those of Radford & al. (1974), Khatijah & Zaharina (1998), Adedeji (2004), and Metcalfe & Chalk (2004) for leaf epidermal descriptions.

All slides were labeled appropriately and examined under Fisher light microscope with ×40 objective. Photomicrographic images of each specimen were taken with an Olympus digital camera mounted on Olympus photomicroscope at the Department of Botany, University of Ibadan, Ibadan, Nigeria. Microscope observations and measurements were made with a micrometer eyepiece. For each micro-morphological character, measurements were randomly taken from all slides prepared for each specimen. The mean value and standard error for all microscopic parameters were also calculated on the basis of occurrence of each examined character in a total of 20 fields of view, as mentioned above. The stomata index (SI) for the epidermises was calculated using the formula reported by Salisbury (1927):

$$\text{Stomatal Index (SI)} = \frac{S}{E + S} \times 100\,\%,$$

where S = number of stomata per unit area, and
E = number of epidermal cells on the same area.

Phytochemical screening

Fresh specimens were collected and dried for about 15 days, until they were completely void of moisture. The dried specimens were ground into fine powder and then subjected to phytochemical screening conducted at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. This screening was done to determine the biologically active compounds present in the plant parts and procedures were adapted from earlier works on plant analysis as outlined by Sofowora (1993), Rahila & al. (1994), Trease and Evans (2005), Adaramola & al. (2012), and Soladoye & Chukwuma (2012). The detailed method of extraction and purification techniques for active plant constituents followed Harborne (1973) so as to yield accurate result.

Results and discussion

The micro-morphological characteristics of the species are summarized in Tables 1 and 2 and illustrated in Plates 1 and 2. Phytochemical examination results are presented in Table 3 and Fig. 1 respectively. The foliar epidermal cells were generally irregular, with a few linear to polygonal ones on both surfaces of the examined species. The anticlinal walls were undulate to cuneate and the stomata were rather anomocytic than paracytic and anisocytic (Table 1). The species were hypostomatic, but considering the number of stomata on the adaxial epidermises, the leaves were hypoamphistomatic, with *C. pubescens* having the highest number of stomata. Interestingly, the stomata index was the lowest and the highest in *C. pubescens* (5.1 % – adaxial; 21.0 % abaxial).

Trichomes were simple, unicellular, short and long, and located on coastal and intercoastal surfaces on the abaxial and adaxial sides of both species. It was also evident that trichomes prevailed on both surfaces of *C. pubescens* rather than in *C. ternatea*. The glandular trichomes observed on the abaxial surface of *C. pubescence* were short, sessile and unicellular. Metcalfe & Chalk (1965) reported earlier that in the genera *Clitoria* and *Centrosema* the non-glandular trichomes type had "hooked hairs with short basal cells and a larger bent terminal cell". The simple unicellular, short or long trichomes evidenced in this work could be regarded as some extra trichome types exhibited by the studied taxa.

Table 1. Qualitative leaf micro-morphological characteristics of the studied species.

	Clitoria ternatea		*Centrosema pubescens*	
Characters	Abaxial	Adaxial	Abaxial	Adaxial
Cell shape	Irregular, rectangular	Irregular, rectangular	Irregular, rectangular	Irregular, rectangular
Anticlinal wall	Undulate-cuneate, straight, curve	Undulate, straight-curve	Undulate, straight	Undulate-cuneate
Stomata type	Paracytic, anomocytic	Paracytic, anomocytic, anisocytic	Paracytic, anomocytic	Paracytic, anomocytic
Trichome	Simple	Simple	Simple	Simple
Micro-crystal	Present	Present	Present	Present

Table 2. Quantitative leaf micro-morphological characteristics of the studied species.

	Clitoria ternatea		*Centrosema pubescens*	
Characters	Abaxial	Adaxial	Abaxial	Adaxial
Stomata index	12.7%	10.3%	21.0%	5.1%
Cell (per mm2)	172(211.3±7.5)241	98(139.7±8.5)186	243(302.0±10.9)350	242(279.5±7.3)309
Cell length (µm)	40.5(53.7±3.7)85.0	40.0(58.2±2.8)75.0	24.3(33.0±2.3)48.6	37.8(47.7±2.0)62.1
Cell width (µm)	16.2(25.7±1.8)35.0	20.0(26.0±1.6)35.0	10.0(17.9±1.2)25.0	17.5(25.1±1.2)32.4
Cell wall thickness (µm)	2.7(3.0±0.3)5.4	2.7(3.1±0.3)5.4	1.4(1.0±0.0)1.4	2.7(3.1±0.3)5.4
Stomata (per mm2)	14(30.7±3.3)51	11(16.1±0.8)21	40(80.1±8.9)130	5(15.1±1.5)24
Stomata length (µm)	17.5(22.5±0.9)27.0	13.5(18.2±0.9)24.3	12.5(16.6±1.0)21.6	13.5(18.7±1.3)24.3
Stomata width (µm)	10.0(12.3±0.7)18.9	5.4(9.2±0.9)16.2	5.0(8.8±0.6)10.8	7.5(8.8±0.4)10.8
No. of trichomes (per mm2)	1(1.8±0.2)4	1(2.2±0.9)4	1(4.1±0.7)10	3(5.5±0.9)12

All data are arranged as follows: minimum (mean±standard error 5 maximum).

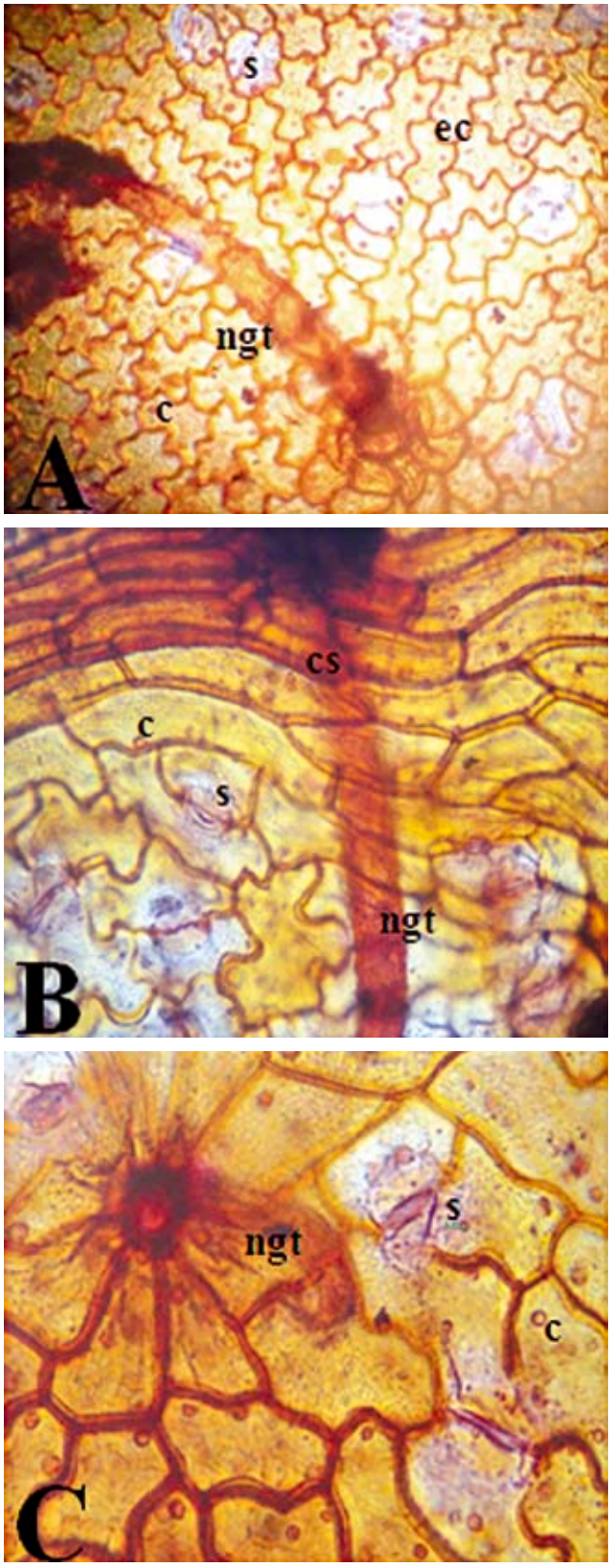

Plate 1. Photomicrographs of the epidermal layers of *Clitoria ternatea* ×400. (**A** – abaxial; **B**, **C** – adaxial). **A** – trichomes on intercoastal surface; **B** – trichomes on coastal surface; and **C** – trichomes on intercoastal surface. **ec** = epidermal cell; **ngt** = non-glandular trichome; **s** = stoma; **c** = crystals; **cs** = coastal cells.

Table 3. Quantitative phytochemical content of the examined species.

Phytochemicals	*Clitoria ternatea*	*Centrosema pubescens*
Phenol	5.58±0.09	5.33±0.07
Flavonoid	1.02±0.13	1.47±0.09
Anthraquinone	0.53±0.02	0.42±0.02
Saponin	0.34±0.02	0.21±0.01
Phytosteroid	0.43±0.05	0.35±0.04
Cardiac glycosides	0.01±0.00	0.01±0.00
Cyanogenic glycosides	0.12±0.01	0.10±0.01
Alkaloids	0.22±0.02	0.13±0.02
Tannins	2.81±0.10	2.60±0.03

All measurements are in mg/g and are expressesed as folows: mean±standard error.

Further epidermal studies have also shown that the average number of epidermal cells ranged from 98 on the adaxial surface of *C. ternatea* to 350 on the abaxial surface of *C. pubescens*. In contrast, the stomata length and width ranged from 12.5 µm and 5.0 µm on the abaxial surface of *C. pubescens* to 27.0 µm and 18.9 µm respectively also on the abaxial surface of *C. ternatea*. The smallest in size cells occurred on the abaxial surface of *C. pubescens* (length: 24.3 µm; width: 10.0 µm) and the largest on the abaxial surface of *C. ternatea* (length: 85.0 µm; width: 35.0 µm). It can therefore be assumed that the higher the number of epidermal cells and stomata, the smaller is their respective length and width. In other words, the cell length and width are inversely proportional to the frequency of cells, and the same applies to the stomata length, width and frequency (Table 2).

This work supports the fact 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, as emphasized already by Salisbury (1927).

A phytochemical analysis has shown that the two examined species are very rich in phenol and tannin content. While the content of phenol was greater (>50 %), tannin was also on the high side (>20 %) in each species. However, only traces of cardiac glycosides and cyanogenic glycosides were noticed (Fig. 1). Although the presence of phytosteroids in the examined plants was not very significant, it suggested their importance in drug production as sex hormones (Okwu 2001) in support of Ravishankar & al. (2012), who earlier reported that the

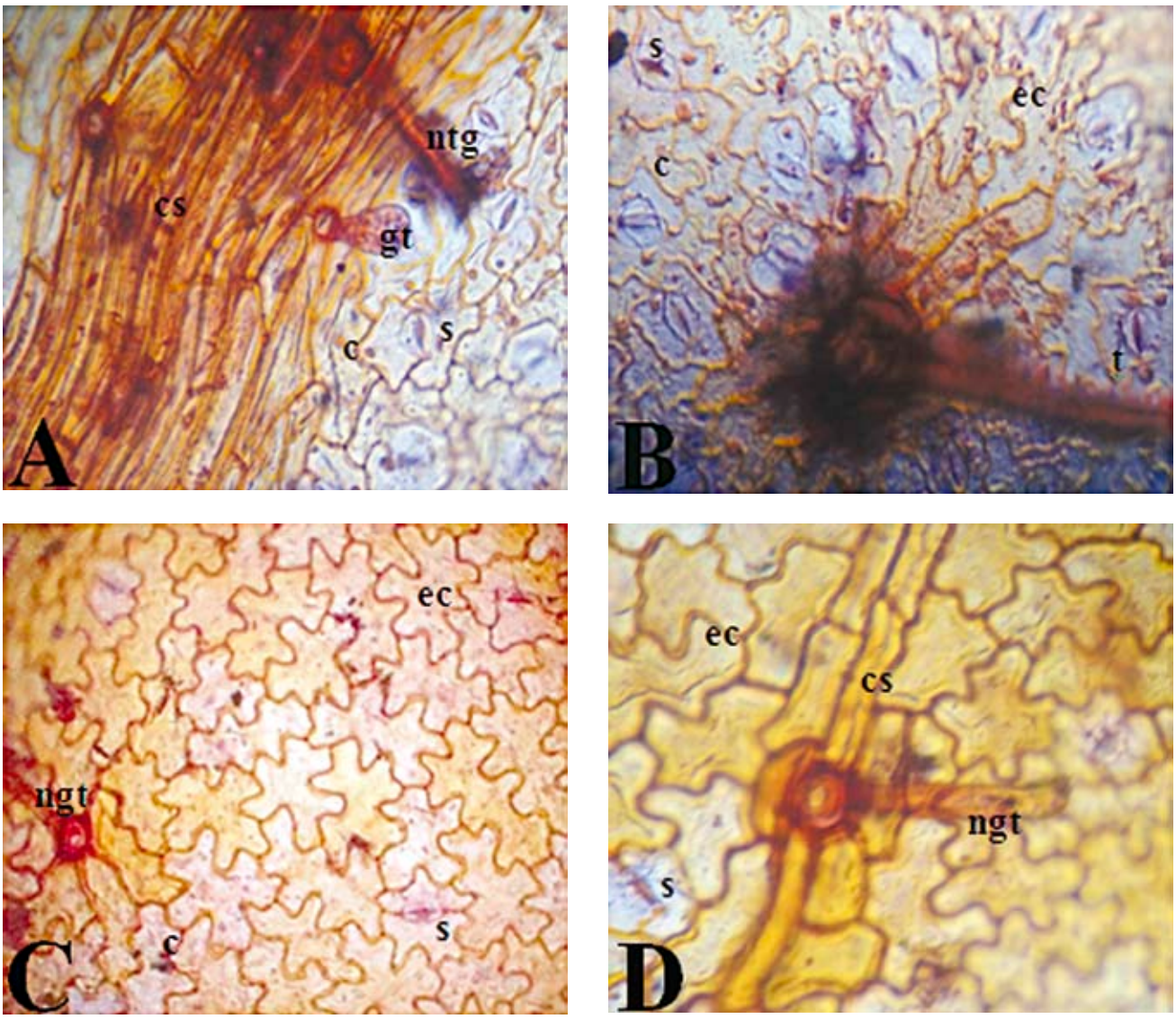

Plate 2. Photomicrographs of the epidermal layers of *Centrosema pubescens* x400. (**A**, **B** – abaxial; **C**, **D**– adaxial). **A** – trichomes on coastal surface; **B** – trichomes on intercoastal surface; and **C** – irregular cells, stomata and trichomes, **D** – trichomes on coastal surface. **ec** = epidermal cell; **gt** = glandular trichome; **ngt** = non-glandular trichome; **s** = stoma; **c** = crystals; **cs** = coastal cells.

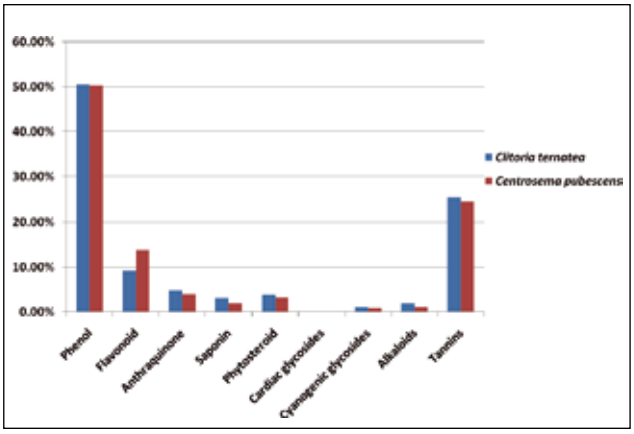

Fig. 1. Comparative graphical illustration of phytochemical contents (%) of the examined species.

root of *C. ternatea* is medicinally important as an aphrodisiac. Alan & Miller (1996) have also reported earlier that flavonoids have antiviral, antiallergic, antithrombotic, antibacterial, antimultagic, and anti-inflammatory properties. The large amounts of this essential compound (1.02±0.13 mg/g in *C. ternatea* and 1.47±0.09 mg/g in *C. pubescens*) observed in this study (Table 3) could also lead to the assumption that the examined species may have potent anti-oxidant properties.

Generally, the phytochemical contents of *C. ternatea* exceeded those of *C. pubescens,* except for flavonoids, which exceeded in amount the latter. The large amount of tannin compounds also indicated the spe-

cies importance in bacterial and viral drugs production (Soladoye & Chukwuma 2012). An earlier study (Ekpo & al. 2011) has also shown the presence of such bioactive compounds as saponin and tannins in *C. pubescens* and has reported the antimicrobial and wound healing properties of the plant. Although leaf micro-morphological and phytochemical characteristics have contributed immensely to the taxonomy of angiosperms, much is needed still to be done on this subject matter. However, findings in the present work have shown the need in constant incorporation of micro-morphology and phytochemical screenings into plant taxonomic studies.

Conclusion

The present study has shown that the examined plant species are very similar in their phytochemical and leaf epidermal features. Nonetheless, the presence of glandular trichomes on the abaxial surface of *C. pubescens* can serve as a diagnostic character in distinguishing this species from *C. ternatea*. While this work supports the earlier studies and reports of amphistomatic leaf epidermises exhibited by members of the *Papilionoideae*, is has also contributed to the existing taxonomic information about *C. ternatea* and *C. pubescens* and may be used in distinguishing the species in the absence of their inflorescences or in fragmentary state, as well as in their taxonomic description. Hopefully, the results of the phytochemical analysis will form a basis for carrying out further tests to ascertain the potencies of the bio-active compounds present in the species.

References

Adaramola, T.F., Ariwaodo, J.O. & Adeniji, K.A. 2012. Distribution, phytochemistry and antioxidant properties of the genus *Parkia* R.Br. (*Mimosaceae*) in Nigeria. – Int. J. Pharm. Phyt. Res., **4**(4): 172-178.

Adedeji, O. 2004. Leaf epidermal studies of *Emilia* Cass. (*Senecioneae, Asteraceae*) in Nigeria. – Bot. Lithuan., **10**(2): 12-133.

Alan, L. & Miller, N.D. 1996. Antioxidant flavonoids: structure, function and clinical usage. – Alt. Med. Rev., **1**: 103-111.

Burkill, H.M. 1995. The Useful Plants of West Tropical Africa, vol. **3**, 2nd ed. Royal Botanic Gardens, Kew, Richmond, UK.

Ekpo, M., Mbagwu, H., Jackson, C. & Eno, M. 2011. Antimicrobial and wound healing properties of *Centrosema pubescens* (*Leguminosae*). – J. Pharm. Clin. Sci., **1**: 1-6.

Harborne, J.B. 1973. Phytochemical Methods. Chapman & Hall Ltd, London.

Hepper, F.N. 1958. *Papilionaceae*. – In: **Hutchinson, J. & Dalziel, J.M (eds)**, Flora of West Tropical Africa, Vol. **1**, part 2. Crown Agents for Oversea Governments and Administration, London, UK, pp. 505-587.

Holmgren, P.K., Holmgren, N.H. & Barnett. L.C. 1990. Index herbariorum. Part 1: The Herbaria of the World. 8th ed. New York Bot. Gard., NY.

Keay, R.W.J. 1989. Trees of Nigeria. Oxford Univ. Press, NY..

Khatijah, H. N. & Zaharina, M. S. 1998. Comparative leaf anatomical studies of some *Sterculia* L. species (*Sterculiaceae*). – Bot. J. Linn. Soc., **127**: 159-174.

Metcalfe, C.R. & Chalk, L. 1965. Anatomy of the Dicotyledons, Vol. **I**, Oxford Univ. Press, London.

Metcalfe, C.R. & Chalk, L. 1972. Anatomy of the Dicotyledons, Vol. **1**, 1st ed. Oxford Univ. Press, London.

Metcalfe, C.R. & Chalk, L. 2004. Anatomy of the Dicotyledons, Vol. **1**, 2nd ed. Clarendon Press, Oxford.

Mukherjee, P.K., Kumar, V., Kumar, N.S. & Heinrich, M. 2008. The ayurvedic medicine *Clitoria ternatea*: From traditional use to scientific assessment. – J. Ethnopharm., **120**(3): 291-301.

Okwu, D.E. 2001. Evaluation of the chemical composition of indigenous spices and flowering Sagents. – Glob. J. Pure Appl. Sci., **7**: 455-459.

Radford, A.E., Dickson, W.C., Massey, J.R. & Bell, C.R. (eds). 1974. Vascular Plant Systematics. Harper and Row Publishers, NY.

Rahila T., Rukhsandra N., Zaidi A.A. & Shamishilia, R. 1994. Phytochemical screening of medicinal plants belonging to *Euphorbiaceae*. – Pak. Vet. J., **14**: 160-162.

Ravishankar, V.M., Praful, S.J. & Rajashree, R. 2012. Effect of *Clitoria ternatea* Linn. plant root extract on the hippocampal area Ca 3 and pancreas of juvenile diabetic rats: A preliminary investigation. – Spatula DD., **2**(1): 9-16.

Salisbury, E.J. 1927. On the causes of and ecological significance of stomatal frequency, with special reference to the woodland flora. – Phil. Trans. R. Soc., **216**B: 1-65.

Sofowora, A. 1993. Medicinal Plants and Traditional Medicine in Africa 2nd ed. Spectrum Books Ltd., Ibadan, Nigeria.

Soladoye, M.O. & Chukwuma, E.C. 2012. Phytochemical analysis of the stem and root of *Cissus populnea* (*Vitaceae*) – an important medicinal plant in Central Nigeria. – Phytol. Balcan., **18**(2): 149-153.

Soladoye, M.O. & Lewis, G.P. 2003. A Checklist of Nigerian Legumes. CENRAD Natural Resources Research Assessment and Conservation Series 03. Ibadan, Nigeria, West Africa..

Trease, G.E. & Evans, M.C. 2005. Pharmacognosy. 14th ed., pp. 53, 431 and 512. Elsevier, New Delhi, India.