International Research Journal of BIOLOGICAL SCIENCES

Volume 01 | Issue 02 | 2019

SciRange PUBLICATIONS
www.scirange.com

SciRange

Open Access

International Research Journal of Biological Sciences

Research Article

pISSN: 2663-5968, eISSN: 2663-5976

Epidermal Features and Phytochemical Analysis of Leaves of *Gmelina arborea* and *Tectona grandis* in Kogi State, Nigeria: A Comparative Study

O.E. Matthew, P.R.O. Edogbanya and O.S. Ayegba

Department of Biological Sciences, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria

ARTICLE INFORMATION

Received: August 31, 2019

Accepted: October 21, 2019

Corresponding Author:
P.R.O. Edogbanya,
Department of Biological Sciences,
Faculty of Natural Sciences,
Kogi State University, Anyigba, Nigeria

ABSTRACT

Correct identification of medicinal plants and quantification of their bioactive constituents are of utmost concern in the field of pharmacognosy and herbal medicine. The use of crude morphology in identification of medicinal plants though being rampant and easier may not be altogether scientific, hence are ineffective, especially where two or more plant species share close resemblance. In this research, the epidermal features and phytochemical analysis of leaves of *Gmelina arborea* (Roxb.) and *Tectona grandis* (Linn.) were studied in an attempt to show their scientific distinctiveness for proper identification. For the epidermal studies, several fresh leaves of the two plant species were collected separately, rinsed in clean water and bleached in 3.5% sodium hypochlorite for about 18-24 h. They were then stained in dilute glycerol for about 5-10 min and epidermal slides were made from them for microscopic observation. Also, the air dried samples of the two plant species were subjected to quantitative phytochemical analysis using standard methods. Results revealed that there were significant differences in the epidermal features and phytochemical constituents of leaves of *Gmelina arborea* and *Tectona grandis.* From this study it can be concluded that the differences observed are of taxonomic significance for further distinguishing between these two plants.

Key words: Comparative study, epidermal features, phytochemical analysis, herbal medicine, *Gmelina arborea, Tectona grandis*

INTRODUCTION

Plants exhibit natural variation in forms and structures. The study of these forms and structures in plants is called morphology[1]. General or crude morphology deals with macro structures of plants such as size, texture and colour of leaves, stems, roots, fruits, seeds etc. while leaf epidermal studies (epi-morphology) deals with anatomical or microscopic features of leaves such as trichomes, stomata, cuticle, epidermis, veins etc.[2]. The use of leaf epidermal studies could be a useful tool in identifying and differentiating one plant species from the other even if they are of the same family sharing resemblance morphologically[2]. This knowledge is very vital in the proper identification of medicinal plants.

Correct identification of a plant intended for use, is the first process in quality control of botanical preparations. Though several methods of identification are available,

macroscopic identification is the most used, due to its simplicity and reduced financial implication. In this method, identification and classification is done based on external morphological structures such as shapes, number and colour of leaves, flowers, fruits, seeds, etc. However, some of these structures are seasonal in production and crude extracts such as powder forms may prove difficult or even impossible during identification; hence the need for a better means of identification and classification. The use of epidermal studies and structures is one of such means[3].

Leaf epidermal study (epi-morphology) is the study of micro morphological structures in plant organs. Such micro-morphological structures commonly found in leaves include cuticles, epidermis, stomata, trichomes etc. The structures vary from one plant to another in shape, number, length and width depending on the plant species involved. Epidermal characters have been proved to be of great use not only in identifying plants but also the fossil remains of angiosperms and in studying relationships between extant taxa[4].

Phytochemicals are secondary metabolites that are responsible for the colour and flavor of plant or its parts[5]. Just like the functions of antibodies in animals, phytochemicals are the defense mechanism of plants which help in protecting plants against diseases and harsh environmental conditions[6]. The essence of phytochemical screening in plants was to check for any non-nutritive chemical(s) (phytochemicals) found in the plants that may be of therapeutic use to man. Further modification or transformation of these chemicals into patent drugs by suitable biological and chemical means has been of great value to man[7]. Phytochemical screening of medicinal plants is done with the purpose of ascertaining the efficacy of its curative claims. The users may require accuracy and efficiency with regards to the identity of the plants and the parts involved[3]. Method of screening and analysis of these chemicals vary from one to another depending on nature of plant material (part) involved[8].

Gmelina arborea Roxb commonly called Malina, the most widely cultivated species of the genus *Gmelina* in the disputed families-Lamiaceae and Verbenaceae is among the most common medicinal plants of India and Africa[9-12]. Extracts from the leaves, fruits and seeds of *Gmelina arborea* Roxb. has been reported from various sources to contain bioactive chemicals called phytochemicals. These include alkaloids, steroids, anthrax quinones, glycosides, triterpenoids, saponins, phenolic compounds, flavonoids, proteins, carbohydrates etc.[13,14].

Similarly, *Tectona grandis* commonly called Teak, a member of the disputed families Lamiaceae and Verbenaceae show the presence of several classes of phytochemicals such as alkaloids, glycosides, saponins, steroids, flavonoids, proteins and carbohydrates[15].

This research seeks to use epidermal studies as well as phytochemical analysis to further differentiate these two plant species (*Gmelina arborea* and *Tectona grandis*) which are commonly disputed among the two families, Lamiaceae and Verbenaceae. This would be go long way to help in their identification even when in crude form such as powder. The objectives of study were:

- To evaluate the difference in epidermal features (epidermal cell, trichome and stomata) of the leaves of *Gmelina arborea* and *Tectona grandis*
- To evaluate the difference in the phytochemical composition of leaves of and *Tectona grandis*

The Hypotheses of the study were:

- There was no significant difference in the epidermal features of *Gmelina arborea* and *Tectona grandis*
- There was no significance difference in the phytochemical composition of leaves of *Gmelina arborea* and *Tectona grandis*

METHODOLOGY

Collection of plant samples: The fresh samples (leaves of *G. arborea* and *T. grandis*) were collected within the Faculty of Management Sciences, Kogi State University, Anyigba (Latitude: 7°28' 51.39" N and Longitude: 7°11' 14.86" E), Kogi State, North central (Middle belt) of Nigeria[16]. The leaves were then authenticated by Mr. Sulein the herbarium of the department of Plant Science and Biotechnology, Kogi State University, Anyigba for the research studies.

Epidermal studies: Some of the collected leaf samples were washed and kept separately in two different containers. Commercial bleach (3.5% sodium hypochlorite) was added to the two containers until the samples were completely immersed. These were then kept for about 18 to 24 h. The resulted achlorophyllus (non-greenish) leaf structure were cut into several sections and kept in four petri dishes, two carrying abaxial (lower) and the other two carrying adaxial (upper) surfaces of the samples separately. These were labelled 'adaxial for *G. arborea*', 'abaxial for *G. arborea*', 'adaxial for *T. grandis*' and 'abaxial for *T. grandis*'.

The labeled samples were stained with 1% safranin solution for about 5 to 10 min; the sections were then rinsed carefully

in several changes of water to remove excess stain. These were then mounted in dilute (10%) glycerol solution on clean glass slides for further microscopic observation. The assessed leaf epidermal features were viewed, counted, measured (both in length and width) per field of view and captured with the aid of the Olympus BH-2 compound microscope fitted with the JVC KYF70B digital camera. Some of the captured images were then imported as bitmaps to Corel Draw 12 (Corel Corporation, Ottawa, Canada 2003). Stomata index (SI) was calculated using the formula:

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

where, S= Number of stomata per field of view and E = Number of subsidiary (epidermal) cells in the same surface area.[2,17,18].

Phytochemical studies: The remaining washed samples were shade dried at room temperature for about two (2) weeks, pulverized into powder with the aid of pestle and mortar. Powdered samples were stored separately in airtight containers and labeled appropriately.

Five grams (5 g) each for the two samples was extracted separately using 25 mL of methanol for 24 h. The resultant filtrates were then subjected to phytochemical analysis following standard methods[19-23].

Determination of cardiac glycosides: It was determined according to the approach of Solich *et al.*[20] with some modifications. Ten percent extract of the sample was mixed with 10 mL freshly prepared Balject's reagent (95 mL of 1% Picric acid + 5 mL of 10% NaOH). The mixture was left to stand for an hour, after which it was diluted with 20 mL distilled water and its absorbance was measured at 495 nm.

Standard curve was prepared using 10 mL of different concentrations (12.5-100 mg L^{-1}) of securidaca extract. Total Glycosides were expressed as mg of Securidaside per gram of the sample used or based on the calibration curve of Securidaside:

$$Y = 2.285X - 0.012, R^2 = 0.984$$

Hence, X = (Y+0.012)/2.285
Where, Y = Absorbance
X = Concentration

Determination of tannin content: The Tannins were determined by following Folin-Ciocalteu method[24]. About 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of the distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent. One millilitre (1 mL) of 35% Na_2CO_3 solution was diluted to 10 mL with distilled water. The mixture was then shaken and kept at room temperature for 30 min.

A set of standard solution of gallic acid 20, 40, 60, 80 and 100 µg mL^{-1} were prepared in the same manner as described earlier. Absorbance for the test and standard solution measured against the blank at 725 nm with an UV visible spectrophotometer. The tannin content was expressed in terms of mg of Gallic Acid Equivalent (GAE) g^{-1} of extract. It was determined using the relationship below:

$$Y = 0.057X - 0.071$$

Where, Y = Absorbance
X = Concentration

Determination of total phenolic content: Total phenolic content was determined by the Folin-Ciocalteu method[24].

A 20 µL aliquot of extract solution was mixed with 1.16 mL distilled water and 100 µL of Folin-Ciocalteu reagent followed by addition of 300 µL of Na_2CO_3 solution (20%). The mixture was then incubated in a shaking incubator at 40°C for about 30 min and the absorbance was taken at 760 nm. The total phenolic content was expressed as Gallic Acid Equivalent (GAE). This was calculated using the linear relationship based on the calibration curve as shown below:

$$A = 0.98C + 9.925 \times 10^{-3} (R^2 = 0.9997)$$

Where, A = Absorbance
C = Concentration in mg $GAEg^{-1}$ dry weight

Determination of total saponin content: Total saponins content was determined by the method described by Makkar *et al.*[21] based on Vanillin-Sulphuric acid calorimeter reaction with some modifications.

About 50 µL of sample extract was added with 250 µL distilled water. To this, about 250 µL of Vanillin reagent (800 mg of Vanillin in 10 mL 99.5% ethanol) was added. Then 2.5 mL of 72% sulphuric acid was added and mixed well. This solution was kept in a water bath at 60°C for 10 min.

After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The value was expressed as Diosgenin equivalent in mg g^{-1} extract derived from a standard curve.

The concentrations in mg g^{-1} were calculated using the relation:

$$Y = 0.0005X - 0.0052$$

Where, Y = Absorbance
X = Concentration

Determination of total flavonoid content: About 1 g of sample in 10 mL of 95% ethanol was kept for 1 h[22].

Total flavonoid content was measured by the Aluminum chloride calorimetric assay. The reaction mixture consists of 1 mL of extract and 4 mL of distilled water was taken into a 10 mL volumetric flask. To the flask 0.30 mL of 5% sodium nitrite was treated and after 5 min, 0.30 mL of 10% Aluminum chloride was mixed with the solution.

After 5 min, 2 mL of 1 M Sodium hydroxide was treated and diluted to 10 mL with distilled water. A set of reference standard solution of Quercetin (20, 40, 60, 80 and 100 µg mL^{-1}) were prepared in the same manner as described earlier. The absorbance for test and standard solution were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer.

The total flavonoid content was expressed as mg of QE g^{-1} of extract using the following based on calibration curve:

$$Y = 0.009X - 0.006$$

Where, Y = Absorbance
X = Concentration

Determination of percentage composition of alkaloids: Fifty millilitres (50 mL) of 20% acetic acid was added to 5 g of sample in 250 mL beaker and covered to stand for four (4) hours. The mixture containing solution was filtered and the volume was reduced to one quarter using water bath.

Concentrated ammonium hydroxide was to the sample in a drop-wise manner until it was completely precipitated. The solution was allowed to settle, after which it was filtered and the precipitate collected and weighed. The percentage of total alkaloids was calculated as follow:

$$\text{Weight of Alkaloids (\%)} = \frac{\text{Residual} \times 100}{\text{Weight of sample}}$$

$$\text{i.e. Alkaloid (\%)} = \frac{(w2 - w1) \times 100}{\text{Weight of sample}}$$

Where, W_2 = Weight of paper + alkaloids (g)
W_1 = Weight of filter paper (g)

Statistical analysis: The means of the corresponding parameters obtained for both plants were compared using student T-test. Means were expressed as Mean±SD. The level of significance was taken at $p<0.05$. All computations were done using Statistical Package for Social Sciences (SPSS) Version 20.

RESULTS

Result interpretation:

Abaxial epidermal features: The quantitative epidermal features on the abaxial (lower) surface of leaves of the plant species assessed revealed that that there were significant differences in abaxial trichome number (TN), stomata number (SN), number of epidermal cells (EN), length of epidermal cells (EL) and width of epidermal cells (EW), whereas there were no significant differences in abaxial trichome length (TL), trichome width (TW), stomata length (SL), stomata width (SW) and stomata index (S.I) per field of view ($p<0.05$) (Table 1).

Abaxial trichome numbers per field of view were significantly higher ($p<0.05$) in *Tectona grandis* compared to that of *Gmelina arborea*. There was significantly more abundance of stomata per field of view on the abaxial of *G. arborea* than on *T. grandis* ($p<0.05$).

Also, the numbers of epidermal cells per field of view were significantly higher ($p<0.05$) in *G. arborea* than in *T. grandis*. Furthermore, the result indicates that the length and width of epidermal cells in *T. grandis* were significantly longer and wider ($p<0.05$) than that of *G. arborea* (Table 1).

Adaxial epidermal features: The quantitative epidermal features on the adaxial (upper) surface of leaves of the plant

Table 1: Epidermal features of the abaxial surface of leaves of *G. arborea* and *T. grandis*

Species	TN	TL (µm)	TW (µm)	SN	SL (µm)	SW (µm)	EN	EL (µm)	EW (µm)	S.I (%)
AbG1	1.67±1.15	11.60±3.63	0.63±0.15	9.00±3.61	1.83±0.12	1.43±0.23	134.00±24.33	2.83±0.59	0.80±0.00	6.60±3.47
AbT1	6.00±1.00	10.07±2.21	0.70±0.30	1.00±0.00	1.80±0.10	1.80±0.10	11.33±1.53	7.70±0.61	6.20±3.47	8.19±0.98
p-value	0.008*	0.572	0.754	0.018*	0.725	0.065	0.001*	0.010*	0.000*	0.494

Mean±SD: *Values are significant at $p<0.05$, abG1 = Abaxial surface of *Gmelina arborea,* abT1 = Abaxial surface of *Tectona grandis*, TN = Trichomenumber, TL = Trichome length, TW = Trichome width, SN = Stomata number, S = Stomata length, SW = Stomata width, EN = Epidermal cell number, EL = Epidermal cell length and EW = Epidermal cell width

Table 2: Showing quantitative epidermal features on the adaxial surface of leaves of *G. arborea* and *T. grandis*

Species	TN	TL (μm)	TW (μm)	SN	SL (μm)	SW (μm)	EN	EL (μm)	EW (μm)	S.I (%)
AdG2	9.33±2.89	3.17±0.29	0.30±0.00	24.0±2.65	1.57±0.06	1.27±0.21	9.67±3.21	11.27±1.17	7.50±0.62	71.39± 9.14
AdT2	2.67±0.58	8.70±1.47	0.77±0.15	0.00±0.00	0.00±0.00	0.00±0.00	17.33±4.73	2.40±0.00	0.70±0.00	0.00±0.00
p-value	0.017*	0.020*	0.034*	0.000*	0.000*	0.000*	0.090	0.006*	0.000*	0.000*

Mean±SD: *Values are significant at p<0.05, adG2 = Adaxial surface of *Gmelina arborea,* adT2 = Adaxial surface of *Tectona grandis*, TN = Trichome number, TL = Trichome length, TW = Trichome width, SN = Stomata number, SL = Stomata length, SW = Stomata width, EN = Epidermal cell number, EL= Epidermal cell length, EW = Epidermal cell width and S.I = Stomata index

Table 3: Showing quantitative phytochemical composition of methanolic extracts of *G. arborea* and *T. grandis* leaves

	Concentration (mg g^{-1})		
Phytochemicals	*G. arborea*	*T. grandis*	p-value
Tannins	5.260±0.00	4.220±0.014	0.006*
Total phenol	720.970±0.721	517.400±1.443	0.000*
Saponins	44.540±0.141	37.840±0.283	0.057
Flavonoids	81.500±0.085	137.835±0.078	0.000*
Cardiac glycosides	4.575±0.007	7.535±0.007	0.000*
Alkaloids	7.000±0.000	2.300±0.141	0.014*

Mean±S.D: * Values are significant at p<0.05

species assessed revealed that there were significant differences in adaxial TN, TL, TW, SN, SL, SW, EL, EW and SI whereas, there was no significant difference in adaxial EN (p<0.05).

The result showed that the adaxial trichome number was significantly higher (p<0.05) in *G. arborea* than in *T. grandis*, whereas the trichome length of *T. grandis* was significantly longer (p<0.05) than that of *G. arborea*. Trichome width was significantly wider (p<0.05) on the upper epidermis of leaves of *T. grandis* compared to the upper epidermis of leaves of *G. arborea*. The result also revealed that there were significantly more stomata (p<0.05) in the adaxial surface of leaf of *G. arborea* compare to that of *T. grandis* on the same surface.

In addition, the result revealed that length and width of stomata on the adaxial surface of leaf of *T. grandis* were significantly longer and wider (p<0.05) than that of *G. arborea* in the same surface, whereas the length and width of epidermal cells in the adaxial surface of *G. arborea* were significantly longer and wider (p<0.05) than that of *T. grandis* respectively (Table 2).

Quantitative phytochemical analysis: The quantitative phytochemical analysis carried out revealed that the quantity of tannins, total phenols and alkaloids were significantly higher (p<0.05) in *G. arborea* than in *T. grandis* leaves respectively.

From the result, the quantity (mg g^{-1}) of flavonoids and cardiac glycosides in *T. grandis* were significantly higher (p<0.05) than in *G. arborea* leaves respectively. Statistically,

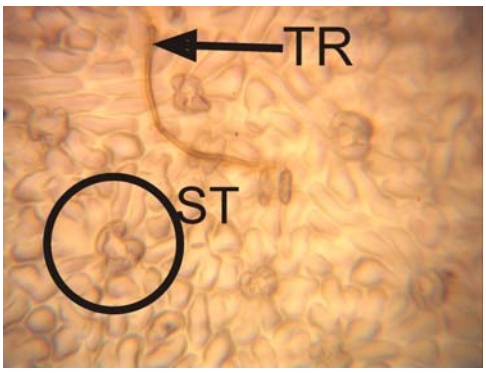

Fig. 1: Showing epidermal features on the abaxial surface of leaf of *G. arborea* (Mg ×40), ST = Stomata, TR = Trichome

there was no significant difference between the quantity of saponins in *G. arborea* and *T. grandis* leaves (p<0.05) (Table 3).

DISCUSSION

Abaxial epidermal features: The variations in some of the abaxial epidermal features are of great taxonomic importance in distinguishing the plant species[25]. From the study, the result revealed that trichomes (hairs and papillae) were significantly more on the abaxial surface (lower epidermis) of *T. grandis* than on that of *G. arborea* (Table 1). It has been reported that the occurrence of hairs and papillae (collectively called trichomes) and cellular structures in leaves can be used extensively by taxonomist as an aid to identify medicinal plants since there was wide range of form[26]. In form and structure, the presence of uniseriate multicellular, non-glandular and long trichrome on the lower epidermis of leaves of *G. arborea* compared to the uniseriate, unicellular, non-glandular, short and branched trichomes found on *T. grandis* (Fig. 1-5) supported the work of Haruna and Ashir[18] that observed and reported the same in the two plant species. The results also revealed that there were more stomata on the lower epidermis of leaf of *G. arborea* compared to that of *T. grandis*. This was in conjunction with the study of Haruna and Ashir[18] which reported that in terms of stomata breadth (width), *G. arborea* was wider compared to *T. grandis* at

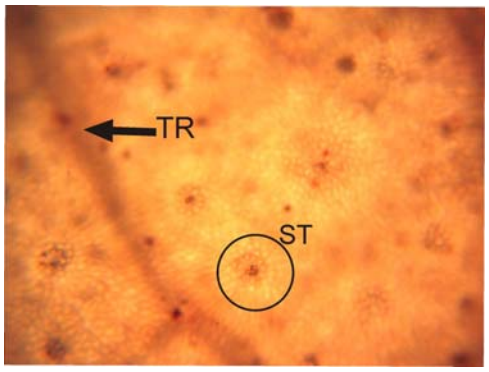

Fig. 2: Part used showing epidermal features on the abaxial surface of leaf *T. grandis* (Mg ×40), ST = Stomata, TR = Trichome

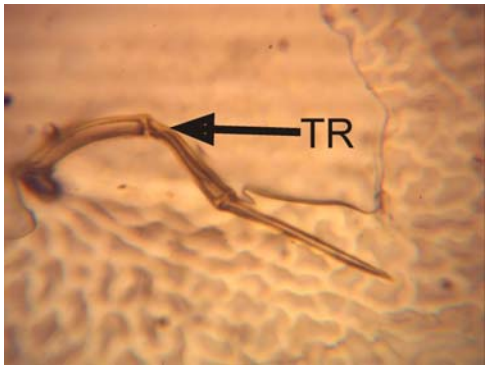

Fig. 3: Part used showing simple multicellular trichome on the adaxial surface of leaf of *G. arborea* (Mg ×40), TR = Trichome

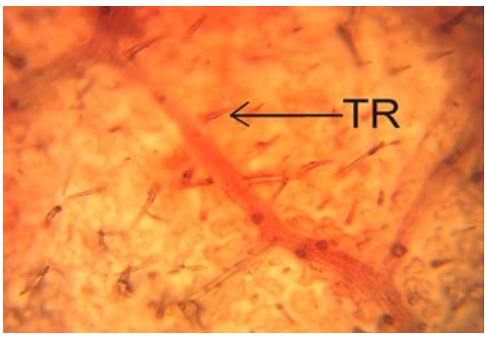

Fig. 4: Part used showing uniseriate, non-glandular, short and branched trichomes on the adaxial surface of leaf of *T. grandis* (Mg ×40)

p<0.05. However, this study shows no significant differences in stomata length and width at p<0.05.

The significantly higher number of epidermal cells recorded in *G. arborea* as against in *T. grandis* on the lower epidermis of

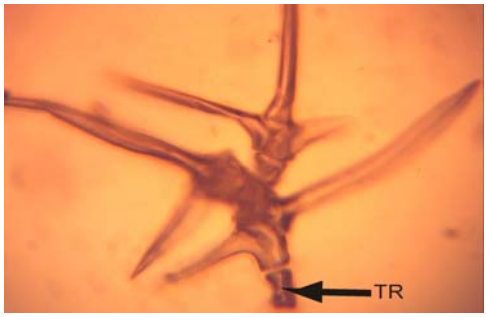

Fig. 5: Part used showing multiseriate, unicellular, glandular, long and branched trichome on the adaxial surface of leaf of *T. grandis* (Mg ×40)

leaves also supported the work of Haruna and Ashir[18] which reported a similar result that showed that the number of epidermal cells in *G. arborea* were significantly higher (p<0.05) than that of *T. grandis.*

Furthermore, the significant variation in the length and width (µm) of epidermal cells in the abaxial surface of the leaves of the two plant species are of taxonomic significance in delimiting plant species[2]. *Tectona grandis* possesses epidermal cells whose length and width were significantly longer and wider respectively than the length and width of *G. arborea* (p<0.05). It was opinioned by Ogundare and Saheed[2] that the shape of epidermal cells, types and arrangement of stomata as well as the size and shape of trichomes are important taxonomic characters which is supported by this current study.

Adaxial epidermal features: The result from this research revealed that the number of trichomes per field of view on the upper epidermis of leaves of *G. arborea* were significantly higher than that on *T. grandis*. This was in line with the work of Haruna and Ashir[18]. However, the length of trichomes were longer on the upper epidermis of leaf of *T. grandis* compared to that of *G. arborea*, hence Rick[27] reported that trichomes were reliable taxonomic markers as they are of diverse types and forms. Trichomes are said to be diagnostic characters not only helpful in identification of particular plant species but also of crude drugs and detections of adulterants.

The presence of stomata on the adaxial surface of leaves of *G. arborea* and its corresponding absence on the adaxial surface of leaves of *T. grandis* was significantly of high diagnostic value for identifying the two plant species Ogundare and Saheed[2]. It was reported that in terms of presence of stomata, *G. arborea* was amphistomatic (having stomata on both lower and upper epidermis of the

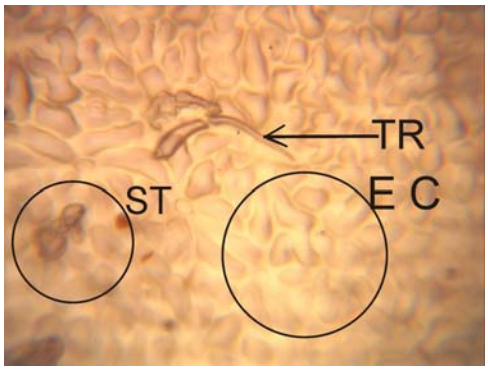

Fig. 6: Part used showing epidermal features on the adaxial surface of *G. arborea* (Mg ×40), ST = Stomata, TR = Trichome, EC = Epidermal cells

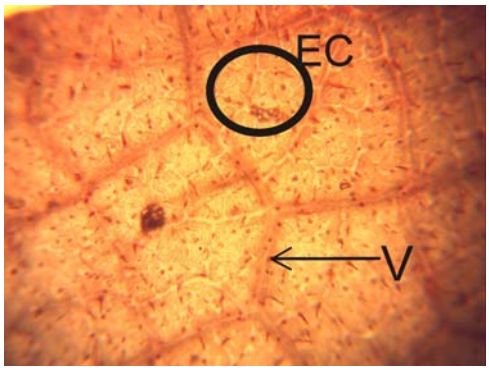

Fig. 7: Part used showing epidermal features on the adaxial surface of *T. grandis* (Mg ×40), EC = Epidermal cells, V = Vein

leaf) while *T. grandis* was hypostomatic (having stomata only on the abaxial surface of the leaf)[18]. In forms and structures, *G. arborea* was reported to have anomocytic and anisocytic types of stomata while *T. grandis* has paracytic type of stomata[18,28,29]. These were also observed and supported by this research (Fig. 6, 7).

Longer and wider epidermal cells as observed in *G. arborea* compared to that of *T. grandis* respectively were also useful in distinguishing the two plant species[30].

Quantitative phytochemical compositions: The result of the quantitative phytochemical analysis revealed the presence of the secondary metabolites: tannins, total phenols, flavonoids, saponins, cardiac glycosides and alkaloids in the methanolic extracts of leaves of the two plant species. Total phenols and flavonoids were highly present in the two plant species compared to other secondary metabolites. These support the findings of Offor[31], Sangram *et al.*[32], Bishwanath *et al.*[33] and Ogunmefun *et al.*[34]. Osunlana and Johnson[35] reported that flavonoids were found most abundant in many plants, hence it was suggested that such plants are justified in their pharmacological potentials. Iswarya and Mayavel[36] proposed that *Gmelina arborea* is one of the trees with significant pharmacological supplements that can be used to remedy a variety of ailments, due to the number of phytochemicals it contains. The significant differences in the phytochemical constituents of leaves of *G. arborea* and *T. grandis* were also of great chemo-taxonomical importance[37]. According to Nadine and Paul[37], the studies of micro molecular taxonomy (an aspect of biochemical taxonomy that deals with the distribution and biosynthetic interrelationships of small molecular weight compounds e.g., free amino acids and secondary metabolites e.g. tannins, total phenols, flavonoids, terpenes, alkaloids, etc.) was a good approach and useful tool in resolving systematic problems.

CONCLUSION

It was concluded that there were significant differences in the epidermal features and phytochemical constituents of leaves of *Gmelina arborea* and *Tectona grandis*, which are of taxonomical significance in further distinguishing between these two plants.

RECOMMENDATIONS

Molecular studies can be carried out to further distinguish between *Gmelina arborea* and *Tectona grandis*. To avoid any acute toxicological or other side defects, it is also recommended that acute and sub-acute toxicity studies should be carried out on these two plant species as phytochemical analysis only and cannot confer their safe uses.

REFERENCES

1. Peter, H.R., F.E. Ray and S.E. Eichorn, 2005. Biology of plants. 7th Edn., New York: W.H. Freeman Publishers, pp: 9.
2. Ogundare, C.S. and S.A. Saheed, 2012. Foliar epidermal characters and petiole anatomy of four species of *Citrus* L. (Rutaceae) from southwestern Nigeria. Bangladesh J. Plant Taxonomy, 19: 25-31.
3. Applequist, W., 2006. The identification of medicinal plants. A Handbook of the Morphology of Botanical in Commerce. Texas American Botanical Council Publishers, pp: 87.
4. Inyama, C.N., V.U.N. Osuoha, F.N. Mbagwu, and C.M. Duru, 2015. Comparative morphology of the leaf epidermis in six *Citrus* species and its biosystematic importance. J. Med. Aromat. Plants, 4: 194.
5. Craig, W.J., 2009. Health effects of vegan diets. Am. J. Clin. Nutr., 89: 1627-1633.

6. Sangeeta, S., D. Priyanjali, B. Sujata, T.P.A.D. Devasagayam and G. Saroj, 2006. Bark and fruit extracts of *Gmelina arborea* protect liver cells from oxidative stress. Int. J. Pharm. Biol., 44: 237-243.
7. Guinder, J.K. and S.A. Daljik, 2009. Antibacterial activity and phytochemical screening of *Anethum graveolens, Framcrutumvulgara* and *Trachyspermum ammi.* J. Phytochem., 30: 182-186.
8. Gulcinet, I., E. Bursal, M.H. Shitoglu, M. Bilsel and A.C. Groen, 2010. Polyphenol of lyophilized aqueous extracts of propolis from Erzurum, Turkey. J. Food Biochem., 48: 227-238.
9. Sedgley, M. and J.A. Gardner, 1990. International survey of underexploited tropical and subtropical perennials with edible fruits or seed. J. Int. Soc. Horticult. Sci., 250: 26-30.
10. Conn, B.J., 2001. *Gmelina*-New South Wales flora. An online Plant Information Network System, 2.0. Sydney, Australia: The Royal Botanic Gardens and Domain. Retrieved from: http://plantnet.rbgsyd.NSW.gov.au/cgi-bin/NSWfl.pls?-
11. Hyland, B.P.M., T. Whiffin and F.A. Zich, 2010. Australian Tropical Rainforest Plants. 6.1 Edn. The Centre for Australian National Biodiversity Research, Australian Tropical Herbarium, James Cook University, Australia. Retrieved from: http://end.m.wikipedia.org/wiki/lomatia-fraxinifolia.
12. Akyala, I.A., I. David and A. Simon, 2013. Phytochemical screening and antibacterial activity of *Gmelina arborea* fruit extracts. Int. J. Microbiol. Immunol. Res., 1: 21-31.
13. Adegbehin, J.O., J.O. Abayomi and L.C. Nwaigbo, 1988. *Gmelina arborea* in Nigeria. Common Wealth Forestry Review, 67: 159-166.
14. Nayak, B.S., P.K. Jena, S.C. Dinda and P. Ellaiah, 2012. Phytochemical investigation and *in vitro* evaluation of anthelmintic activity of *Gmelina arborea* Roxb. fruit extracts. Asian J. Chem., 24: 3444-3448.
15. Neha, K. and B. Sangeeta, 2013. Phytochemical and pharmacological evaluation of *Tectona grandis* Linn. Int. J. Pharm. Pharm. Sci., 5: 923-927.
16. Global Positioning System (GPS), 2019. Satellite map of Kogi State University, Anyigba. Retrieved from: https://googleweblight.com/i?...k-s-u&hl=en-NG.
17. Ogbonna, C.E., F.I. Nwafor, E.A. Ugbogu and K. Nwazue, 2018. Assessment of foliar parameters and air pollution tolerance of broad leaved trees in Ugwuele Quarry site, Uturu, Nigeria. J. Applied Environ. Sci., 6: 48-56.
18. Haruna, H. and H.I. Ashir, 2017. Leaf epidermal structure and stomata ontogeny in some members of the Lamiaceae family. Bayero J. Pure Applied Sci., 10: 670-675.
19. Ashvin, G.G. and S.S. Rajaram, 2014. Phytochemical analysis of leaves of *Tectona grandis* Linn. Int. J. Pharm. Biosci., 5: 355-359.
20. Solich, P., V. Sedliakova and R. Karlicek, 1992. Spectrophotometric determination of cardiac glycosides by flow-injection analysis. J. Anal. Chim. Acta, 269: 199-203.
21. Makkar, H.P.S., M. Bluemmel, N.K. Borowy, and K. Becker, 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric., 61: 161-165.
22. Kaviarasan, S., G.H. Naik, R. Gangabhagirathi and C.V. Anuradha, 2007. *In vitro* studies on antiradical and antioxidant activities of Fenugreek (*Trigonella foenum graecum*) seeds. J. Food Chem., 103 : 31–37.
23. Trease, G.E. and W.C. Evans, 2002. Pharmacognosy. 15th Edn., London: Saunders Publishers, pp: 42-44, 221-229, 391-393.
24. Andressa, B., G.L. Gisely, and C.P.M. Joao, 2013. Application and analysis of the Folin-Ciocalteu method for the determination of the total phenolic content from *Limonium brassiliense* L. J. Mol., 18: 6852-6865.
25. Chisom, F.I., U.O. Clement and C.O. Nkumah, 2015. Leaf epidermal studies in the species of *Stachytarpheta* found in Awka, Nigeria and its taxonomic implications. Int. J. Applied Biol. Pharmaceut. Technol., 6: 299-305.
26. Cutler, E.G., 1978. Applied plant anatomy. J. Exp. Bot., 29: 1481.
27. Rick, G.K., 1984. Glandular trichomes: A helpful taxonomic character for *Artemisia nova* (Black Sagebrush). J. Range Manage., 37: 370-372.
28. Abdulrahaman, A.A., 2013. Cooling effects and humidification potentials in relation to features in some shade plants. Int. J. Applied Sci. Technol., 3: 144.
29. Chothani, D.L. and N.M. Patel, 2012. Preliminary phytochemical screening, pharmacognostic and physicochemical evaluation of leaf of *Gmelina arborea*. Asian Pacific J. Trop. Biomed., 2: S1333-S1337.
30. Kuntala, N.B., P.B. Papori and R. Raja, 2018. Study on foliar micromorphology of *Gmelina arborea* Roxb.: A clonal identification against *Craspedonta leayana*. J. Med. Plants Stud., 6: 235-239.
31. Offor, C.E., 2014. Phytochemical and proximate analysis of dry *Gmelina arborea* leaves. Int. J. Curr. Res. Acad. Rev., 2: 101-105.
32. Sangram, K.P., D. Debajot and K.T. Niraj, 2015. Phytochemical investigationand anthelmintic activity of various root extracts of *Gmelina arborea* Roxb. Asian J. Plant Sci. Res., 5: 54-58.
33. Bishwanath, M., M.K. Durga, M. Laxmidhar and P.M. Ganesh, 2016. Physiochemical and phytochemical investigation of different fractions from hydrochloric extract of *Tectona grandis* (Linn) barks. J. Res. Library, Der Pharmacia Lettre, 8: 80-85.

34. Ogunmefun, O.T., E.A. Ekundayo, F.C. Akharaiyi and D. Ewhenedoro, 2017. Phytochemical screening and antibacterial activities of *Tectona grandis* L.f. (Teak) leaves on microorganism isolated from decayed food samples. Int. J. Soc. Trop. Plant Res., 4: 376-382.
35. Osunlana, O.R., M.O. Bello and J.A. Johnson, 2018. Nutritive values and bioactive compounds content of three commonly used blood pressure regulating plant leaves. J. Pharmacol., 1: 128-136.
36. Iswarya, S., M. Sridevi and A. Mayavel, 2017. Comparative study on phytochemical and anti-oxidant properties of *Gmelina arborea* Roxb. from four different geographical refions. Int. Pharm. Clin. Res., 9: 275-280.
37. Nadine, Z. and R.J. Paul, 2015. Phylogenetic approaches to natural product structure prediction. Res. J. Project Manage. Consultant, 517: 161-182.