Free Radicals and Antioxidants. 2022;12(1):27-32
A Multifaceted Journal in the field of Free Radicals and Antioxidants
www.antiox.org | www.journalonweb.com/fra | www.phcog.net

Original Article

Microscopic Evaluation and Antioxidant Activity of *Glyphaea brevis* (Spreng.) Monach. (Family Tiliaceae)

Mubo Adeola Sonibare[1,*], Taiwo Rukayat Onifade[1], Akingbolabo Daniel Ogunlakin[2], Opeyemi Josephine Akinmurele[3], Samuel Abayomi Adebodun[1]

ABSTRACT

Background: Glyphaea brevis has been used traditionally to treat a variety of ailments, some of which are linked to oxidative stress. Despite the numerous studies on the biological activities of G. brevis, no literature was found on the microscopic study of this plant, which is essential for preparation of its monograph. As a result, the antioxidant activity of *G. brevis* and its qualitative microscopic characteristics were investigated in this study. **Methods:** The adaxial and abaxial epidermal layers of the G. brevis leaf, as well as transverse sections of the midrib and petiole, were obtained, cleared, and examined under a light microscope at X100 and X400 magnifications. The antioxidant activity of the crude methanol extract and fractions of *G. brevis* was evaluated via bioautographic method using 1,1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH) free radical scavenging activity (FRSA), total phenolic content (TPC) and the total flavonoid content (TFC). **Results:** Microscopic examination of the leaf revealed the presence of straight and few wavy thin-walled polygonal epidermal cells in the adaxial and abaxial layers, numerous stomata cells ranging from anisocytic, anomocytic, to paracytic in the abaxial layer, and various types of trichomes in both adaxial and abaxial layers. The FRSA and TFC were highest in the crude extract. The ethyl acetate fraction of G. brevis had highest FRSA, whereas the hexane fraction had the least. **Conclusion:** The microscopic study revealed characters that can be used to identify Glyphaea brevis, while the free radical scavenging activity supports its use in the treatment of many oxidative stress related diseases.

Keywords: *Glyphaea brevis*, Microscopy, Antioxidants, DPPH.

Mubo Adeola Sonibare[1,*], Taiwo Rukayat Onifade[1], Akingbolabo Daniel Ogunlakin[2], Opeyemi Josephine Akinmurele[3], Samuel Abayomi Adebodun[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, NIGERIA.
[2]Department of Biochemistry, Bowen University, Iwo, NIGERIA.
[3]Department of Pharmacognosy, Faculty of Pharmacy, Madonna University, Elele, Rivers NIGERIA.

Correspondence

Prof. Mubo Adeola Sonibare,

Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, 200132, Ibadan, NIGERIA.

E-mail: sonibaredeola@gmail.com

History
- Submission Date: 07-06-2022;
- Review completed: 23-06-2022;
- Accepted Date: 26-06-2022

DOI : **10.5530/fra.2022.1.5**

Article Available online
http://www.antiox.org

INTRODUCTION

Microscopic analysis of crude drugs involves the examination of the size, shape, relative position of different cells and tissues, the form and nature of cell contents as well as the chemical nature of the cell walls. In Pharmacognosy, the authenticity and quality of medicinal plants can be assessed using microscopy.[1] The first step towards establishing the purity and identity of plant materials is to carry out the microscopic evaluation.[2] Microscopic analysis of medicinal drugs involves the evaluation of both qualitative and quantitative properties. In qualitative microscopic analysis, the transverse sections of leaf, root bark and the longitudinal section of root bark are studied under photomicrograph. Parameters such as the stomata, trichomes, starch grains, calcium oxalate crystals and xylem vessels are the microscopic features usually examined under qualitative microscopy.[1]

Oxidative stress has been considered to be the primary cause of many diseases such as cardiovascular diseases, cancer, atherosclerosis, hypertension, ischemia, neurogenerative diseases such as Alzeimer's and Parkinson's, diabetes mellitus, rheumatoid arthritis and ageing.[3-6] Antioxidants break radical chain reactions, preventing oxidative stress-related damage.[7] They prevent and destroy the presence of reactive oxygen species, which at high concentrations generate oxidative stress that can damage many cellular components and cause diseases in living cells.[8-9] Plant polyphenols have been considered to have potent antioxidant properties and a marked effect in the prevention of various oxidative stress related diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes and cancer.[10-11] Flavonoids, phenolic acids, tannins (hydrolysable and condensed), lignans and stilbenes are the main groups of polyphenol.[12]

Glyphaea brevis (Spreng.) Monach. family Tiliaceae, is either a spreading shrub, tree up to 8 m high or rarely a herb. It is widely spread in tropical Africa and South America.[13] When fully grown, it is a small tree and it is majorly present in forest regrowth, swampy places, rocky savanna, forest galleries and fallow land.[14-17] The leaf decoction of the plant is used for the treatment of sexually transmitted infections, as an abortifacient, antiemetic, eye treatment, pain killers, treatment of pulmonary disorders and gout.[13] *In vivo* biological investigations revealed the antimicrobial and antioxidant effects of the leaves of *G. brevis*.

Cite this article: Sonibare MA, Onifade TR, Ogunlakin AD, Akinmurele OJ, Adebodun SA. Microscopic Evaluation and Antioxidant Activity of *Glyphaea brevis* (Spreng.) Monach. (Family Tiliaceae). Free Radicals and Antioxidants. 2022;12(1):27-32.

Despite so many researches on the biological activities of *Glyphaea brevis*, no literature search was found on the microscopic study of this plant. Hence, for proper identification and monograph preparation of this plant, an evaluation of its microscopic characters is required. Furthermore, phyto-oxidants have been found to be beneficial in the prevention and control of diseases related to oxidative stress. Therefore, this study was designed to investigate the qualitative microscopic characters of *Glyphaea brevis* and to assess its antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent.

MATERIALS AND METHODS

Plant Material

The leaves of *G. brevis* were collected from the wild in Gbandu area, Oluponna, Osun State (7°35'34.7"N4°11'27.5"E) on 13th October, 2019. The leaves were identified and authenticated at the Forest Herbarium, Ibadan (FHI) by Mr. K. A. Adeniyi, Dr O. A. Ugbogu and Mr A.J Egunjobi with a voucher specimen number of FHI: 110067.

Epidermal Layer Preparation for Microscopy

The epidermal layer of the abaxial and adaxial layer of the leaf of *G. brevis* was obtained through hand scrapping method. The epidermal layers obtained were cleared in 2% sodium hypochlorate and stained in safranin O, counter stained in Toluidine blue and passed through graded ethanol (50%, 70% and 100%) after which they were mounted on a glass microscope slide with dilute glycerol and observed under a light microscope at different magnifications. Photomicrographs of the epidermal layers were taken with a digital microscope camera and a smart phone camera.

Transverse Section Preparation

The transverse sections of the leaves were obtained by free hand sectioning using a scalpel, while the leaf midrib (1 cm x 1 cm) was cut. The transverse sections were cleared in 2% sodium hypochlorate for 2-5 min, rinsed severally in water, stained in Safranin O and counter-stained in methylene blue. Each section was passed through graded ethanol (50%, 70% and 100%) for effective clearing and mounted on a glass microscope slide with dilute glycerol. The distribution of tissue through the mid rib was observed under the light microscope at different magnifications.

Qualitative Phytochemical Screening

Alkaloids, saponin, tannin, flavonoids, sugar and anthraquinone were screened qualitatively using the standard procedures.[18]

Preparation of plant extracts

The leaves were dried using the air-drying method, pulverized into powder, weighed and stored in a dry, clean, sterile plastic container ready for extraction. The dried pulverized plant material was extracted with methanol at room temperature for 72 hr with occasional stirring. The extract was filtered and the filtrate concentrated *in vacuo* using rotary evaporator (Bibby Sterlin Ltd, London). The extract obtained was refrigerated for subsequent use. Using a separating funnel, the extract (35g) was re-constituted in methanol: water (3:l) and mixed with aliquots (50 mL per aliquot) of *n*-hexane, dichloromethane (DCM), ethyl acetate and *n*-butanol successively. Each aliquots of *n*-hexane, DCM, ethyl acetate and *n*-butanol fractions was pooled and evaporated *in vacuo* to give residues, which were kept in air tight containers for subsequent preliminary assays. The weights of the partitioned extracts were recorded and used to calculate the percentage yield for each fraction.

Qualitative Phytochemical Screening
Determination of Total Phenols

Following the method described by Nabavi *et al.*, total phenolic content (TPC) was measured using Folin–Ciocalteu.[19] Folin–Ciocalteu reagent (2.5 mL) was introduced into 0.5mL each aliquots of the extracts (100 µg/mL) and was allowed to stand for 3 min after which 2 mL of 7.5% Na_2CO_3 solution in distilled water was added to the mixture. Experiment was prepared in triplicates. Content of the mixture was thoroughly mixed and incubated at room temperature for 30 min. Blank was set up with 0.5mL methanol, 2.5 mL of Folin Ciocalteu reagent and 2mL of 7.5% Na_2CO_3. After 30 min of incubation, absorbance of mixture was read at 765 nm using UV–VIS spectrophotometer (Spectrumlab 752S). Using absorbance reading of gallic acid (12.5– 0.39 µg/mL) at wavelength of 765 nm, a linear dose response regression curve was generated. Result of TPC was expressed as mg of Gallic Acid Equivalent (GAE) per gram of dry weight of extracts.

The TPC in the plant extract was calculated using the formula below:

$$TPC = \frac{CV}{M}$$

TPC is the total phenolic contents in mg of GAE per gram of dry weight of extracts, where C is the concentration of equivalent gallic acid established from calibration curve µg/mL, V is the volume of extract (mL) and M is the weight of plant extract (0.03 g).

Determination of total Flavonoids

The aluminum chloride colorimetric method used in the study was adopted from the method described by Ebrahimzadeh *et al.*[20] The test sample (1mL, 200µg/mL) in methanol was mixed with 1mL methanol, 0.1mL 1% aluminium chloride, 0.1mL 1M potassium acetate and 2.8mL of distilled water. The extract remained at room temperature (28°C – 30°C) for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a 752s spectrum lab UV/Visible spectrophotometer. Quercetin was used as standard for the calibration curve. Quercetin solution was prepared at concentrations 12.5 to 0.39 µg/mL in methanol. All determinations were performed in triplicates. A yellow colour indicated the presence of flavonoids. Based on the measured absorbance, the concentration of flavonoids was read from the calibration curve; then the content of the flavonoid in extract was expressed in terms of quercetin (mg/quercetin).

Antioxidant Activity

Thin Layer Chromatography – Bioautography Technique

Analytical thin layer chromatography for the crude extract and the partitioned fractions of *G. brevis* was carried out using Kieselgel 60 F_{254} according to the method described by Wang *et al.*[21] with slight modifications. Small quantity of each of the fractions and the crude extract (about 0.2g) was re-dissolved in distilled methanol. A capillary tube was used to spot the extracts carefully on a pre-coated plate and allowed to dry. The plate was then carefully developed in a glass tank containing Methanol: Hexane: Dichloromethane: Ethylacetate (0.5: 2.0: 1.0: 0.5) after allowing the solvent to saturate the tank. The developed plate was allowed to dry and visualized in daylight and under the UV lamp. The developed plate was then sprayed with a solution of DPPH. The same procedure was repeated for the standard (Gallic acid) using the solvent system; Methanol: Ethylacetate (2.5: 2.5) with a drop of acetic acid.

In vitro Antioxidant Activity

The free radical scavenging activity of the fractions (Crude, *n*-hexane, dichloromethane, ethylacetate, *n*-butanol and aqueous) of the methanol extracts of *G. brevis* was evaluated according to the methods described by Sonibare and Adeniran[22] with slight modifications. One mL methanol solution of test samples and standard (gallic acid) at different concentrations (200, 100, 50, 25, 12.5, 6.25 and 3.125µg/mL) were mixed separately with 3 mL (0.004%) of freshly prepared 1,1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH-Sigma Aldrich). In the control, 1 mL methanol replaced the test sample. The reaction mixtures were incubated at room temperature and allowed to react for 30 min in the dark. After 30 min, the absorbance was measured at 517 nm using UV–VIS spectrophotometer (Spectrumlabm752S) and converted to percentage free radical scavenging activity (%FRSA). The results were recorded in triplicate.

RESULTS

Microscopic Investigation of *Glyphaea brevis* Leaf

The adaxial layers are characterised with absence of stomata cells, numerous glandular trichomes with multicellular head and unicellular stalk and uniserate glandular trichome (Figure 1). The abaxial layers are characterised with coastal cells, numerous stomata cells (anisocytic, anomocytic and paracytic), uniserate glandular trichomes, glandular trichomes with multicellular head and unicellular stalk and stellate trichomes (Figure 2). The epidermal layer also shows the vein islet and the vein islet termination (Figure 3). The leaf midrib of *G. brevis* revealed bicollateral vascular bundles, a thin cuticle, collenchyma cells, secretory gland, unicerate glandular trichome, stellate trichome and sclerenchyma sheath (Figure 4). The transverse section of the petioles is circular in outline and revealed a thick cuticle, uniserate glandular trichome and bicollateral vascular bundles (Figure 5).

Phytochemical Screening

The phytochemical studies carried out on the powdered leaf sample of *G. brevis* revealed the presence of anthraquinones, flavonoids, cardiac glycosides, saponins and tannins (Table 1).

From the results of the total phenolic content and total flavonoid contents (Figures 6 and 7) obtained from this investigation, there is no linear correlation between the antioxidant activity of the fractions of *G. brevis* investigated in terms of IC_{50} and their total phenolic and total flavonoid results. Dichloromethane fraction had total flavonoid (17.23 mg/GAE) and total phenolic (51.34 mg/QE) contents, while ethyl acetate showed insignificant total flavonoid content (2.62 mg/GAE).

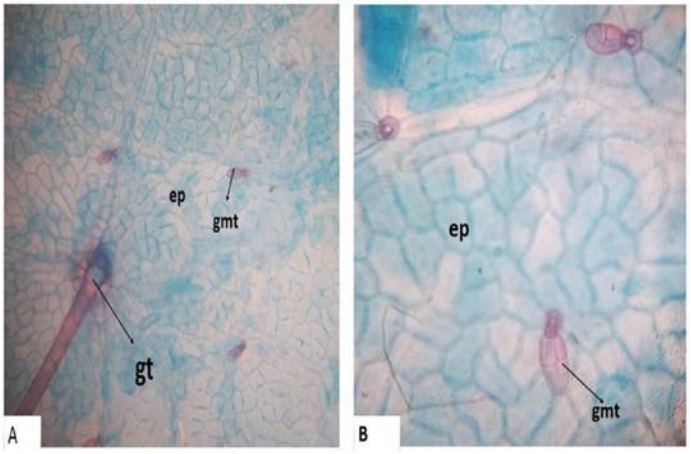

Figure 1: Adaxial layer of *Glyphaea brevis* stained with Safranin O and counter stained with toluidine blue; (A) x100; (B) x400.
ep: epidermal cells; gt: uniserate glandular trichome; gmt:glandular trichome with multicellular head and unicellular stalk.

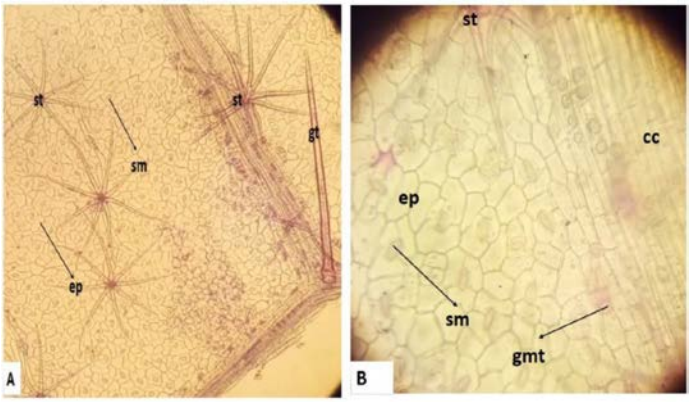

Figure 2: Abaxial layer of *Glyphaea brevis* stained with Safranin O; (A) x100, (B) x400.
cc: coastal cells; ep: epidermal cells; gt: uniserate glandular trichome; st: stellate trichome; sm: stomata cells; gmt:glandular trichome with multicellular head and unicellular stalk.

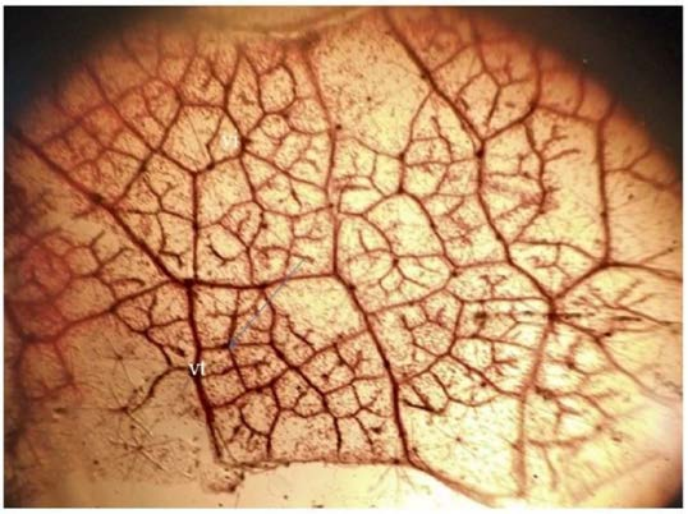

Figure 3: Photomicrograph of the venation pattern of *Glyphaea brevis* stained with Safranin O; x40.
vi: vein-islet, vt: vein termination

Bioautographic Assessment for Evaluation of Antioxidant Activity

The result obtained from the bioautographic assessment of the crude and partitioned fractions using the DPPH reagent for the evaluation of antioxidant activity showed yellow spots against a purple background of DPPH reagent (Figure 8).

In vitro Antioxidant Activity

The crude and other partitioned fractions (*n*-hexane, aqueous and *n*-butanol) also showed significant free radical scavenging activity at various concentrations as shown in Figure 9. Ethyl acetate fraction had the highest %FRSA.

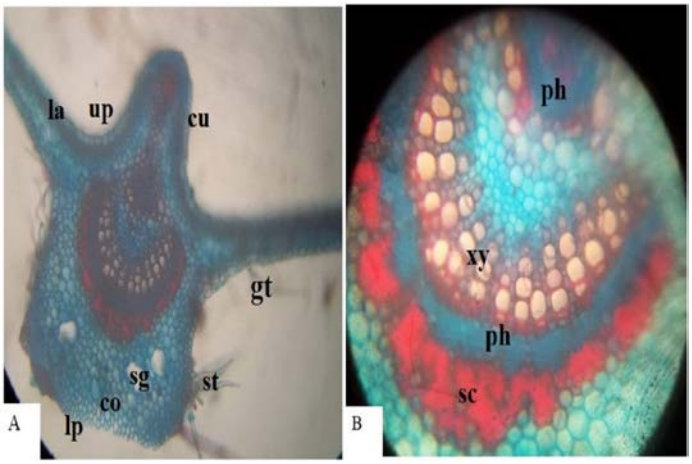

Figure 4: Photomicrograph of Transverse section (TS) of *Glyphaea brevis* stained with Safranin O and counter stained with toluidine blue; A x100, B x400.
co: collenchyma; cu: cuticle; gt: unicellular glandular trichome; la: lamina; lp: lower epidermis; sc: sclerenchyma sheath; sg: secretory gland; st: stellate trichome; up; upper epidermis; ph: phloem; st: stellate trichome; xy: xylem

Table 1: **Phytochemical screening results of *Glyphaea brevis*.**

Tests	*Glyphaea brevis* leaf powder sample
1. Alkaloids	
a) Dragendoff	-
b) Mayer	-
c) Wagner	-
2. Anthraquinones	
a) Borntrager test	+
3. Flavonoids	++
4. Glycosides	
a) Keddes test	+
b) Keller Killiani test	++
5. Saponin	
a) Foam test	+
6) Tannins	
a) Ferric chloride test	-

Keys: ++ = Moderately present; + = detected; - =Absent

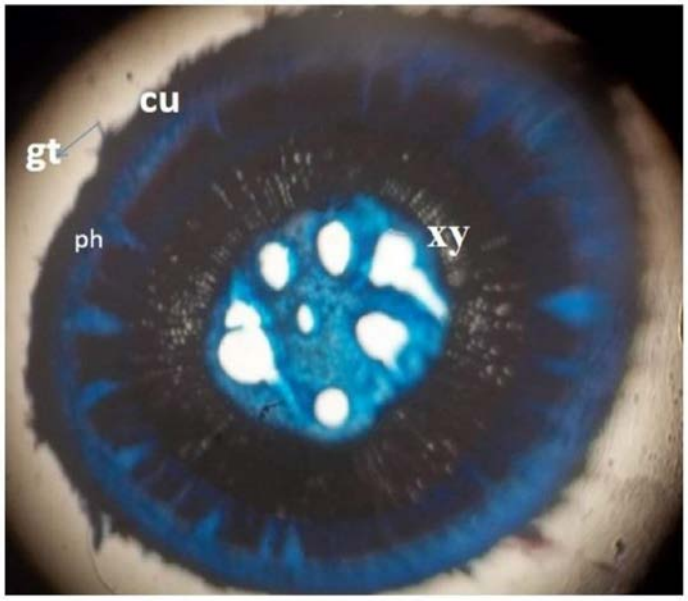

Figure 5: Photomicrograph of the petiole transverse section of *Glyphaea brevis* stained with Safranin O and counter stained with Toludine blue; x100.
cu: cuticle; gt: unicellular glandular trichome; ph: phloem; xy: xylem.

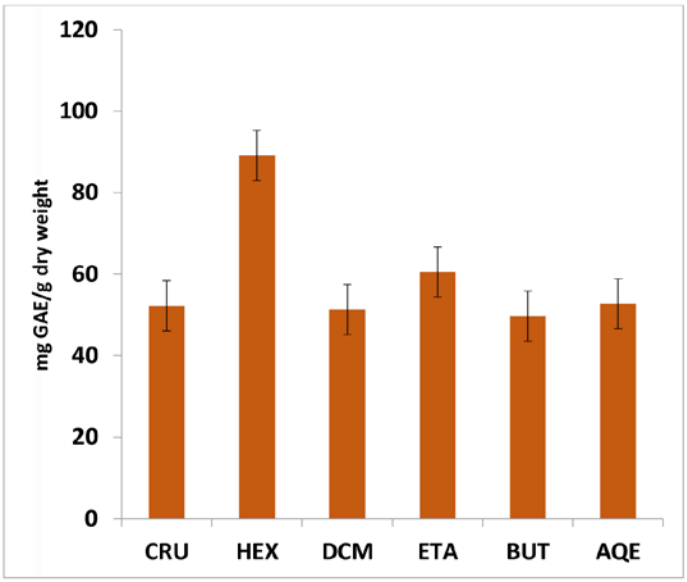

Figure 6: Total Phenolic Content values for antioxidant activity of *Glyphaea brevis*.
CRU: Crude; Hex: Hexane; DCM: Dichloromethane; ETA: Ethyl acetate; BUT: Butanol; AQE: Aqueous; Gallic acid: Standard

DISCUSSION

Straight and few wavy thin-walled polygonal epidermal cells were found in the adaxial and abaxial layers of Glyphaea brevis epidermal cells. The abaxial layer's coastal cells are similar to those found in the adaxial and abaxial epidermal layers of Alstonia boonei and Alstonia congensis, respectively (Apocyanaceae).[23] As a result, the epidermal cell differences in G. brevis (Tiliaceae) are distinctive and can be used to distinguish between species in this family.[24-25] In the abaxial layer, stomata types ranged from anisocytic to anomocytic to paracytic. In a study conducted by Chung, he reported that majority of the stomata found on some species of *Grewia* L. (Tiliaceae) and *Microcos* L. (Tiliaceae) were the anomocytic, anisocytic and paracytic stomata.[26] The leaf of *G. brevis* can be said to be hypostomatic due to the presence of stomata cells on the abaxial layer only. This can be a distinguishing factor between *G. brevis* and some species of the *Grewia* L., such as *G. occidentalis* and *G. polygama* which are amphistomatic. The adaxial layer has more glandular trichomes with multicellular head and unicellular stalk than the abaxial. Chung reported the presence of non-glandular trichomes on the abaxial and adaxial leaf surfaces of *G. huluperakensis, G. laevigata, G. multiflora* and *G. polygama.*[26] The absence of glandular trichomes in some of the species of *Grewia* can be a distinguishing factor between *G. brevis* and other members of this genus.[26]

The phloem is situated on the peripheral and inner side of the xylem and the vascular bundle has a humpy shape at the upper part. In a study carried out by Shokefun *et al.* on some species of *Microcos* L. Section

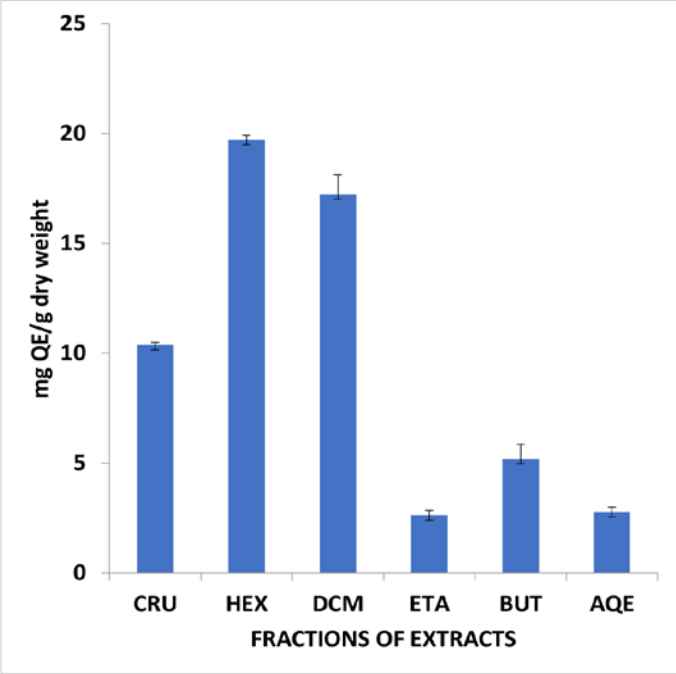

Figure 7: Total Flavonoid Content values for antioxidant activity of *Glyphaea brevis*.
CRU: Crude; Hex: Hexane; DCM: Dichloromethane; ETA: Ethyl acetate; BUT: Butanol; AQE: Aqueous; Gallic acid: Standard

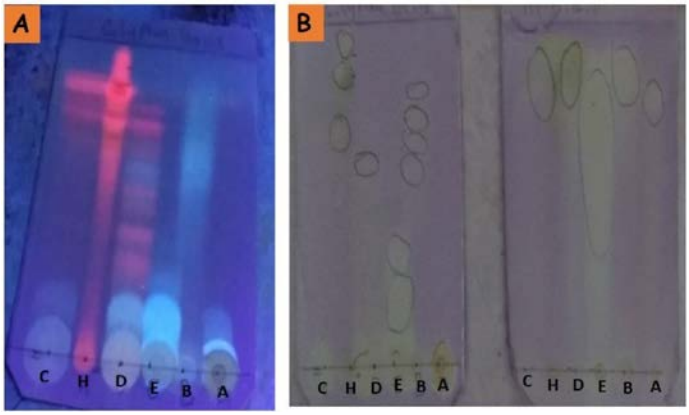

Figure 8: (A): Thin Layer Chromatography (TLC) plate of the crude extract and partitioned fractions of *Glyphaea brevis* using MeOH: Hexane: Ethyl-acetate: Dichloromethane (0.5: 2.0: 0.5: 1.0). (B): Bioautographic detection of the crude and partitioned fractions of *Glyphaea brevis* using DPPH spray. Key: C: Crude extract; H: *n* hexane fraction; D: Dichloromethane; E: Ethyl acetate; B: *n* butanol, A: Gallic acid (Standard)

Eumicrocos in Nigeria, he reported the presence of a large u-shaped incurved vascular bundle in *M. oligoneura*.[27] The sclerenchyma sheath surrounds the vascular bundle and it located directly on top of the phloem. This observation is in accordance with parameters reported for some plants in Tiliaceae family.[24-25] Furthermore, the presence of stellate trichomes in the leaf mid rib of *G. brevis* and circular petiole with trichomes emerging from all over the surface can be another factors which differentiate this plant in the family Tiliaceae.[24-25]

Bioautography is a useful method for separation and detection of the active antioxidants in a mixture of compounds.[28] On the Thin Layer

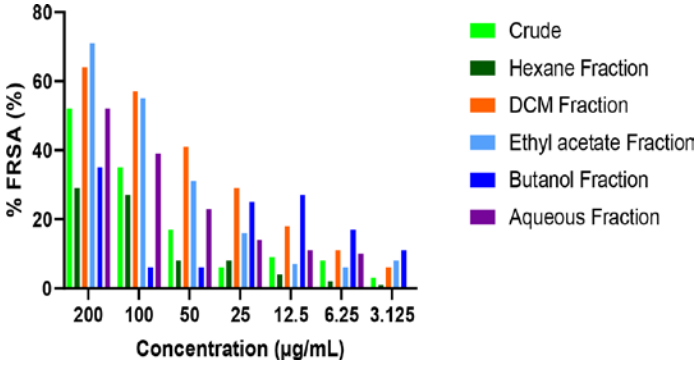

Figure 9: Antioxidant Activity of crude and solvents fractions of the *G. brevis* leaf.

Chromatographic (TLC) plate, antioxidant compounds were seen as white or yellow spots on a purple background. This was in line with the results previously obtained from bioautographs of medicinal plants.[29-31]

The highest free radical scavenging activity was exerted by the ethyl acetate fraction of the plant extract, followed closely by dichloromethane fraction. The effect of antioxidants on DPPH is due to their hydrogen donating ability.[32] The reduction of the purple colour of DPPH to light yellow by the extracts indicates the capacity of the extracts to scavenge free radicals.[33] Phenolic acids and flavonoids, which are widely found in plants, possess significant antioxidant activities that could decrease the incidence of certain human disease.[20,34] Compounds such as flavonoids which contain hydroxyl functional groups are responsible for antioxidant effect in plants.[35] The results obtained showed that there is a significant difference in the antioxidant properties of the various fractions of *G. brevis* investigated due to the differences in their %FRSA values. Furthermore, the extracts showed dose-dependent DPPH radical scavenging activity.

Different literature reported a linear correlation of total phenolic and flavonoid contents with antioxidant capacity.[36] The maximum amount of total phenolic content and total flavonoid content obtained from *n* hexane does not correlate with its %FRSA obtained for this fraction. The highest antioxidant activity, expressed in %FRSA, was recorded in ethyl acetate despite the highest TPC and TFC in *n* hexane fraction. Ethyl acetate fraction exerted the highest antioxidant activity, hence its antioxidant activity cannot be related to its flavonoid content. The presence of some phenolic compounds rather than flavonoids in ethyl acetate may be responsible for its antioxidant activity. It could also be because of reducing power of phenolic hydroxyl groups in which different phenolic compounds respond differently to the Folin–Ciocalteu reagent.[37] Since cinnamic acid derivatives are more effective antioxidants than benzoic acid derivatives mostly in *n* hexane, higher concentration of benzoic acid derived phenolics in *n* hexane fraction of *G. brevis* could have contributed to its low free radical scavenging activity.[38]

CONCLUSION

The anatomical characters such as the shape of the epidermal cells, stomata arrangement, type of trichomes and vascular bundle arrangement obtained from the qualitative microscopic investigation of the leaves of *Glyphaea brevis* in this study in this study are useful in identifying and distinguishing *G. brevis* from the other members of the Tiliaceae family. The crude extract and partitioned fractions of *Glyphaea brevis* leaf exhibited antioxidant activities *in vitro*, with the ethyl acetate fraction of the extract exerting the best antioxidant activity as compared to the other fractions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Alamgir AN. Microscopy in pharmacognosy. In therapeutic use of medicinal plants and their extracts. Prog Drug Res. 2007;1:497-513.
2. Kumar D, Buge PV, Bhat ZA, Dhumal JS. Macroscopical and microscopical evaluation of leaves of *Clerodendrum inerme* Gaertn. Int J Biol Med Res. 2011;2(1):404-8.
3. Papiya B, Sourabh V, Alok S, Chandra Shekhar SC. Pharmacognostic and Physico-chemical standardization of *Macrotyloma uniflorum* (Lam.) verdc. edible seed. Phcog Commn. 2014;4(1):16-24. doi: 10.5530/pc.2014.1.4.
4. Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. J Parkinsons Dis. 2013;3(4):461-91. doi: 10.3233/JPD-130230, PMID 24252804.
5. Andonova L, Georgieva M, Zlatkov A. Free radicals, oxidative stres, and diseases associated with them. Pharmacia. 2015 January 1;62(2):26.
6. Haghi Aminjan HH, Abtahi SR, Hazrati E, Chamanara M, Jalili M, Paknejad B. Targeting of oxidative stress and inflammation through ROS/NF-kappaB pathway in phosphine-induced hepatotoxicity mitigation. Life Sci. 2019;232:116607. doi: 10.1016/j.lfs.2019.116607, PMID 31254582.
7. Da Pozzo E, De Leo M, Faraone I, Milella L, Cavallini C, Piragine E, *et al*. Antioxidant and antisenescence effects of bergamot juice. Oxid Med Cell Longev. 2018;2018:9395804. doi: 10.1155/2018/9395804, PMID 30116497.
8. Vlahos R, Stambas J, Selemidis S. Suppressing production of reactive oxygen species (ROS) for influenza A virus therapy. Trends Pharmacol Sci. 2012;33(1):3-8. doi: 10.1016/j.tips.2011.09.001, PMID 21962460.
9. Sharma V, Nagar A, Chhipa A. Roles of antioxidants in biological system: A review. Mintage J. Pharm. Med Sci. 2017;6:7-12.
10. El Gharras H. Polyphenols: Food sources, properties and applications – a review. International Journal of Food Science and Technology. 2009;44(12):2512-8. doi: 10.1111/j.1365-2621.2009.02077.x.
11. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-52. doi: 10.3390/molecules15107313, PMID 20966876.
12. Khan MK, Zill-E-Huma, Dangles O. A comprehensive review on flavanones, the major citrus polyphenols. J Food Compos Anal. 2014;33(1):85-104. doi: 10.1016/j.jfca.2013.11.004.
13. Burkill HM. The useful plants of West Tropical Africa. Vol. 686. London: Royal Botanical Gardens, Kew; 2008. p. 2(5).
14. Okafor JC. Agroforestry development in the Boshi Okwango. Park: Division of the Cross River National; 1990.
15. Dickson RA, Ekuadzi E, Annan K, Komlaga G. Antibacterial, anti-inflammatory, and antioxidant effects of the leaves and stem bark of *Glyphaea brevis* (Spreng) Monachino (Tiliaceae): A comparative study. Pharmacognosy Res. 2011;3(3):166-72. doi: 10.4103/0974-8490.85001, PMID 22022164.
16. Obiri DD, Osafo N, Abotsi RE. Antiallergic and antiarthritic effects of stem bark extract of *Glyphaea brevis* (Spreng.) Monachino (Tiliaceae) in Murine Models-Research Article. Pharmacol. 2013;2013:1-13.
17. Hyde M, Wursten B. Ballings, P. *Glyphaea flora* of Mozambique website. [accessed Mar 14, 2020].
18. Banu KS, Cathrine L. General techniques involved in phytochemical analysis. IJARCS. 2015;2(4):25-32.
19. Nabavi S, Ebrahimzadeh M, Nabavi SF, Bahramian F. *In vitro* antioxidant activity of Phytolacca americana Berries. Pharmacologyonline. 2009;1:81-8.
20. Ebrahimzadeh M, Nabavi S, Eslami B. Antioxidant and antihemolytic potentials of *Physospermum cornobiense* (L.) DC. Pharmacologyonline. 2009;3:394-403.
21. Wang J, Yue YD, Tang F, Sun J. TLC screening for antioxidant activity of extracts from fifteen bamboo species and identification of antioxidant flavone glycosides from leaves of Bambusa. textilis McClure. Molecules. 2012;17(10):12297-311. doi: 10.3390/molecules171012297, PMID 23085665.
22. Adeniran AA, Sonibare MA. *In vitro* antioxidant activity, brine shrimp lethality and assessment of bioactive constituents of three wild Dioscorea species. J Food Meas Charact. 2016;11(2):685-95.
23. Omitola J, Sonibare M, Elujobs A. Providing some pharmacopoeial standards for the leaves of two closely related Alstonia species. Niger. J Pharm Sci. 2019;15(2):237-47.
24. Kaur S, Singh B, Kaur N, Kaur S. Pharmacognostic Investigations on Leaves of *Grewia asiatica* Linn. Int Res J Pharm. 2018;9(5):85-90. doi: 10.7897/2230-8407.09578.
25. Kataria S, Rao SK, Bhandari A, Kaur D. Pharmacognostical standardization of *Corchorus depressus* (L.) Stocks (Tiliaceae)-A promising ethnomedicinal plant. Indian.
26. Chung RC. Leaf epidermal micromorphology of *Grewia* L. and *Microcos* L. (Tiliaceae) in Peninsular Malaysia and Borneo. Gard Bull Singapore. 2002;54:263-86.
27. Shokefun EO, Ayodele AE, J. Akinloye A. Systematic Importance of Leaf Anatomical Characters in Some Species of Microcos Linn. Section Eumicrocos Burret. in Nigeria. Am J Plant Sci. 2016;07(1):108-17. doi: 10.4236/ajps.2016.71012.
28. Jothy SL, Zuraini Z, Sasidharan S. Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of *Cassia fistula* seeds extract. J Med Plants Res. 2011;5(10):1941-7.
29. Wang T, Jónsdóttir R, Liu H, Gu L, Kristinsson HG, Raghavan S, *et al*. Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*. J Agric Food Chem. 2012;60(23):5874-83. doi: 10.1021/jf3003653, PMID 22612266.
30. Enas MA. Phytochemical composition, antifungal, antiaflatoxigenic, antioxidant, and anticancer activities of *Glycyrrhiza glabra* L. and *Matricaria chamomilla* L. essential oils. J Med Plants Res. 2013;7(29):2197-207. doi: 10.5897/JMPR12.5134.
31. Nickavar B, Adeli A, Nickavar A. TLC-bioautography and GC-MS analyses for detection and identification of antioxidant constituents of *Trachyspermum copticum* essential oil. Iran J Pharm Res. 2014;13(1):127-33. PMID 24734063.
32. Ghimeray AK, Jin CW, Ghimire BK, Cho DH. Antioxidant activity and quantitative estimation of Azadirachtin and nimbin in *Azadirachta indica* A. Juss grown in foothills of Nepal. Afr J Biotechnol. 2009;8(13):3084-91.
33. Ahmad N, Fazal H, Ahmad I, Abbasi BH. Free radical scavenging (DPPH) potential in nine Mentha species. Toxicol Ind Health. 2012;28(1):83-9. doi: 10.1177/0748233711407238, PMID 21646282.
34. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 2003;81(3):321-6. doi: 10.1016/S0308-8146(02)00423-5.
35. De Oliveira EB, Humeau C, Chebil L, Maia ER, Dehez F, Maigret B, *et al*. A molecular modelling study to rationalize the regioselectivity in acylation of flavonoid glycosides catalyzed by *Candida antarctica* lipase B.J. Journal of Molecular Catalysis B: Enzymatic. 2009;59(1-3):96-105. doi: 10.1016/j.molcatb.2009.01.011.
36. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem. 2003;51(8):2144-55. doi: 10.1021/jf020444+, PMID 12670148.
37. Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999;299:152-78). doi: 10.1016/S0076-6879(99)99017-1.
38. Zielinski H, Achremowicz B, Przygodzka M. Antioxidants in cereal grains. Food. Sci Technol Qual. 2012;19(1):5-26.

Cite this article: Sonibare MA, Onifade TR, Ogunlakin AD, Akinmurele OJ, Adebodun SA. Microscopic Evaluation and Antioxidant Activity of *Glyphaea brevis* (Spreng.) Monach. (Family Tiliaceae). Free Radicals and Antioxidants. 2022;12(1):27-32.