JOURNAL OF TROPICAL LIFE SCIENCE
2023, Vol. 13, No. 3, 453 – 460
http://dx.doi.org/10.11594/jtls.13.03.04

Research Article

Morpho-anatomy, *Ex-situ* Conservation and Haemolytic Activity of *Pentaphragma grandiflorum* Kurz. (Pentaphragmataceae)

Victor B. Amoroso [1]*, Lalaine Grace M. Maghanoy [1], Cristine Mea A. Aser [2]

[1] Division of Plant Biology and Microbiology, Institute of Biological Sciences, College of Arts and Sciences, Central Mindanao University, Maramag 8714, Bukidnon, Philippines
[2] Natural Sciences Department, College of Arts and Sciences, Bukidnon State University, Malaybalay City 8700, Bukidnon, Philippines

Article history:
Submission July 2022
Revised January 2023
Accepted January 2023

**Corresponding author:*
E-mail: victorbamoroso@gmail.com

ABSTRACT

Pentaphragma grandiflorum Kurz. is one of the five (5) species of *Pentaphragma* that is found in the Philippines. It is an edible flowering plant consumed as a vegetables and utilized for its medicinal value by the locals. It is categorized as one of the Other Threatened Species with a high economic value. Despite the many uses of *P. grandiflorum*, there is still inadequate information on its morpho-anatomical characteristics conservation and lack of scientific evidence to support the claim of its safe utilization by the locals. The collected wildlings of the *P. grandiflorum* were nurtured and monitored in the canopy greenhouse of Central Mindanao University, Philippines. The safe utilization of the leaf extract of *P. grandiflorum* was assessed through a haemolytic assay. The following are the morpho-anatomical features described in this study: fleshy erect shrub with primary and aerial roots; stem puberulous at a young stage and glabrous upon maturity with dictyostele type of stele; petiole fleshy, puberulous, dissected vascular bundle with dictyostele arrangement; leaf finely puberulous adaxial and glabrous abaxial, dictyostele arrangement, midrib amphibrical arrangement, with tri- to tetracytic stomates; inflorescence arises singly in axil, bisexual, elongated, with yellow and/or purple corolla. Fifty-three out of 130 wildlings survived and abundantly produced bisexual flowers in the succeeding months. The 1 mg/mL concentration of ethanolic and methanolic leaf extracts of *P. grandiflorum* were found to have an average haemolysis percentage of 0.32 ± 6.78 and 0.45 ± 0.65, respectively. The result of the haemolysis assay revealed that the ethanolic and methanolic leaf extract of *P. grandiflorum* is safe to utilize and do not pose any toxic effects to humans.

Keywords: Cytotoxicity, Development, Edible angiosperm, Native species

Introduction

Pentaphragmataceae has a single genus that is referred to as genus *Pentaphragma* [1, 2]. Thirty species of *Pentaphragma* are found in the whole south-eastern Asia, while five species of which are found in the Philippines [2]. The 5 species of *Pentaphragma* plants found in the Philippines include *Pentaphragma grandiflorum* Kurz., *P. mindanaense* Merr., *P. philippinensis* Merr., *P. platyphyllum* Merr. and *P. pulgarense* Elmer [3]. *P. grandiflorum* is an edible flowering plant [1,2] that is commonly known as “wild pechay” and is locally known in the Philippines as “biga-ok” and “pitsay-pitsay”.

The taxonomic position of *P. grandiflorum* was established in 1941 [4]. It is categorized as Other Threatened Species [5] that thrives at low and medium elevation that is found in primary forests that ascends up to 1,700 masl and narrowly distributed and native to both the Philippines and New Guinea [3]. The local people of Surigao del Sur and Zamboanga del Sur, Philippines collect the healthy leaves of *P. grandiflorum* as

How to cite:
Amoroso VB, Maghanoy LGM, Aser CMA (2023) Morpho-anatomy, *Ex-situ* Conservation and Haemolytic Activity of *Pentaphragma grandiflorum* Kurz. (Pentaphragmataceae). Journal of Tropical Life Science 13 (3): 453 – 460. doi: 10.11594/jtls.13.03.04.

vegetables, which are served as salads and as an egg dish. Reports on ethnomedicinal importance mentioned that various plant parts are used by indigenous people in Mindanao to treat common illnesses through decoction, infusion, maceration, juice extraction and poultice [6].

However, despite the mentioned important characteristics and high economic value of *P. grandiflorum*, there are no existing studies on its morpho-anatomical characteristics and *ex-situ* conservation. Also, there is a need for scientific evidence to support the claim of safe utilization of *P. grandiflorum* by the locals. Thus, this paper elucidates new insights into the morpho-anatomy conservation and assesses the safety of the leaf extract of *P. grandiflorum*.

Material and Methods

Collection of the plant samples

A gratuitous permit was obtained to collect wildings in three different accessions in the Philippines, including the following: 1. tropical lowland forest in Mt. Hamiguitan Range Wildlife Sanctuary in San Isidro, Davao Oriental, 2. Concepcion Valencia City, and 3. San Fernando, Bukidnon. The collected wildlings were secured in plastic bags, sealed and carefully brought to the Natural Science Research Center, Central Mindanao University, for morpho-anatomy examination and to the greenhouse for *ex-situ* conservation.

Morphology and Anatomy Examination

Collected plant samples were examined and measured for the qualitative and quantitative characteristics of *P. grandiflorum.* The following are the chemicals used for the detailed anatomy examination of *P. grandiflorum*: alcohol series (70%, 85% and 95% ethanol), 1% safranin stain, xylene series (25%, 50%, 75%, 100% xylene), fast green counterstain, eukit media and sodium hypochlorite as clearing chemical.

Free-hand transections of vegetative and reproductive organs of *P. grandiflorum* were fixed for thirty minutes with 70% ethanol, washed thoroughly with distilled water, and fixed. Ten minutes of dehydration of *P. gradiflorum* was then followed with 85% and 95% ethanol. Transection samples of *P. grandiflorum* were stained for 3 hours with 1% safranin and were then washed thoroughly with 95% ethanol. Further dehydration of the samples was performed with a xylene series. Lastly, transections were mounted using eukit media for examination and documentation.

Ex-situ conservation

Wildlings of *P. grandiflorum* were found and collected at the following sites: 1. tropical lowland forest in Mt. Hamiguitan Range Wildlife Sanctuary in San Isidro, Davao Oriental, 2. Concepcion Valencia City, and 3. San Fernando, Bukidnon, and were designated with respective accessions each. One hundred thirty (130) wildlings were transplanted in pots for 13 months in a canopy greenhouse condition in Central Mindanao University, Philippines. The growth and development of *P. grandiflorum* was consistently monitored.

Haemolysis Activity

Preparation of P. grandiflorum leaf samples

The preparation of 1mg/ml concentration of *P. grandiflorum* leaf sample was carried out as described [7] with few modification. Two kilograms of *P. grandiflorum* healthy leaves were collected and placed inside a clean plastic bag. The fresh leaves were washed first with tap water and were finally rinsed with distilled water. The leaves were air-dried to constant weight for 14 days at room temperature. The dried samples were ground to a fine powder using a mechanical blender and set aside for ethanolic and methanolic extraction.

Methanolic and ethanolic leaf extraction

The ethanolic and methanolic extraction were carried out following the method described by [8] with few modifications. The 0.5 g of pulverized leaf samples of *P. grandiflorum* were used for ethanolic and methanolic extraction procedures, respectively. The leaf samples were solvent-extracted separately in conical tubes with 100% ethanol and 100% methanol. The mixtures were mixed in a vortex mixer for 20 seconds and were forwarded in an orbital shaker at 300 rpm under an ambient temperature for an hour. The mixtures were sonicated for 10 minutes and were centrifuged at 5,000 rpm for 5 minutes. A 5 ml supernatant of each leaf extract was collected in a separate conical tube with a pre-recorded empty tube weight. The ethanolic and methanolic crude leaf extracts were processed in a rotational vacuum concentrator (Martin RVC 2-25 Rotational-Vacuum-Concentrator).

Haemolysis assay

The cytotoxicity of the *P. grandiflorum* ethanolic and methanolic leaf extracts were determined by haemolytic assay as described by [9, 10, 11] with slight modifications. Proper ethical procedure for the extraction of human red blood cells (RBC) was followed. The cytotoxicity level of the ethanolic and methanolic leaf samples were determined by the following percentage of haemolysis: non-toxic (9.4%), slightly toxic (9.54%-49.44%), toxic (49.45%-89.44%), and highly toxic (89.45%-100%). The percentage of hemolysis was calculated through the method:

$$\% \text{ hemolysis} = \frac{A - B}{C - B} \times 100$$

Note:
A: Absorbance of test sample
B: Absorbance of negative control
C: Absorbance of positive control

Results and Discussion

Morphology and anatomy

Erect fleshy shrub, up to 1.5 meters tall (Figure 1A & 2B). **Roots** fleshy; white color primary roots, up to 3-8 mm thick; green color aerial roots, up to 2-6 mm (Figure 2B). **Stem** robust, up to 1.5 meter tall, 2.5 cm thick, fleshy to woody, pubescent when young, glabrous at maturity, abundant lenticels, dark green color (Figure 1G-I). **Leaf** alternate, obliquely elliptic, up to 30 by 25 cm, oblique to cuneate base, acuminate to apiculate apex, shallowly dentate margin, finely

Figure 1. (A) Habit of *P. grandiflorum*, (B) young and mature flowers, (C) longitudinal section of flowers, (D-E) upper and lower surface of leaves, (F) tip and margin of leaf, (G) hairy stem, (H) stem with lenticels (l), (I) transectioned stem (c- cortex, ph- phloem, x- xylem, p-pith).

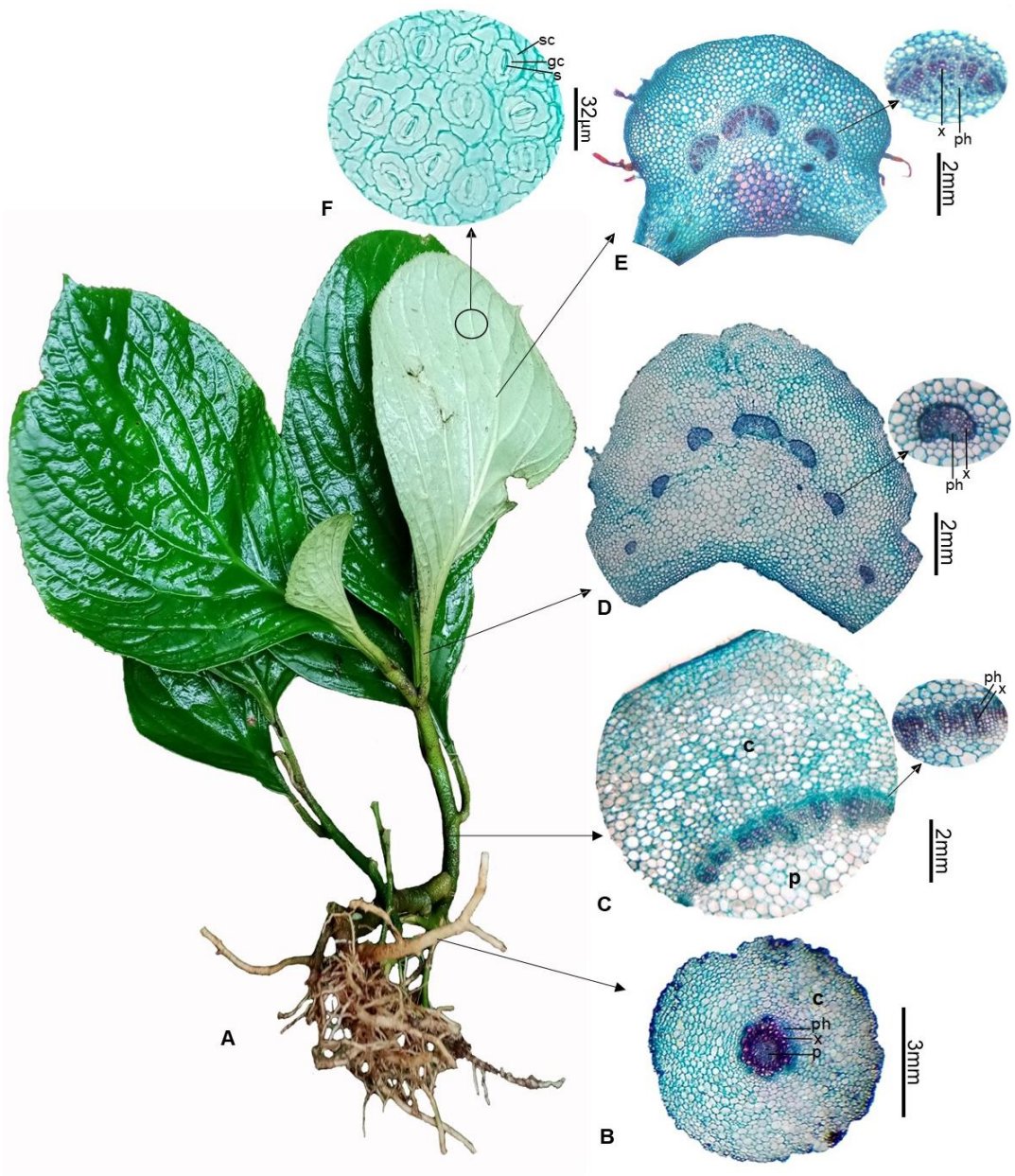

Figure 2. (A) Habit of *Pentaphragma grandiflorum*, (B) transections of the primary root showing the siphonostele and tissue arrangement, (C) stem with dictyostele arrangement, (D) petiole with dissected stele (dictyostele), (E) vein with 3 distinct vascular bundles, xylem surrounded with phloem tissue, and (F) surface view of the lower epidermis showing tri- to tetracytic stomates. (c-cortex, ph- phloem, x- xylem, p- pith, sc- subsidiary cell, gc- guard cell, s- stomata).

puberulous to glabrescent lower surface light green color, glabrous upper surface dark green color, 6-7 paired nerves (Figure 1D-F); petiole fleshy, up to 8 by 1 cm, puberulous when young, glabrous when fully mature. **Inflorescence** arises singly from 1-4 axils, bisexual, shortly compound to scorpioid cyme arrangment, elongated anthesis, up to 5-6 cm, long- or short pedicel, erect, up 12 flowers each. Bracts paired green lanceolate are very variable, small and/or large, up to 2-3 by 1 cm. Calyx-tube narrowly obconic, 4-5 cm by 1-4 mm, sometimes with 5 ridges, glabrous. Calyx very variable, 4-5 sepals (2 larger and 3 smaller), up to 3 by 1.5 cm, linear to elliptic, glabrous, acuminate to acute apex, with obvious 3-5 shallow canals, white color. Corolla fleshy, 5 petals, up to 12 by 5 mm, almost or choripetalous, segments elliptic-obovate, rounded to acute apex, recurved apex, glabrous, purple color and/or yellow color when mature. Pedicel fleshy with hairs, 1-3 cm by

0.5-1 cm, light to dark green color. Stamen connected to style; filament 1-2mm, white color; anther oblong, 1 mm, yellow color. Pistil fleshy, 3-5 cm; style 2-3 mm; stigma round, erect 2-3 by 1-2 mm, yellow color; ovary covered with calyx-tube, large, 3-4 cm by 1-3 mm (Figure 1B-C). Unlike the previously discovered two (2) new species, the P. bicolor and P. pendula, which are only 20 cm tall [12], *P. grandiflorum* is one the largest species of Pentaphragmataceae, reaching up to 1.5 m tall. Also, the elongated glabrous calyx tube, calyx and longer recurved corolla apex of of the *P. grandiflorum* is very distinct from other species of *Pentaphragma* that composed of stout, puberulous calyx and reduced inflorescence such as *P. philipinensis, P. mindanaense, P. platyphyllum, P. pulgarense, P. bicolor, P. pendula* and many other species [1, 3, 12].

Transectioned roots of *P. grandiflorum* showed siphonostele type of stele in which the vascular tissues form a small ring (Figure 2B). Also, the ring of the vascular bundle is visible in stem transection in which vascular tissues are dissected (in between gaps), which shows the dictyostele type of stele (Figure 2C). The protoxylem tissues of both root and stem transection face towards the axis, showing endarch differentiation. In petiole transection, vascular bundles are bean-shape and arranged in an inverted v-shape manner in which the vascular tissues are dissected (in between gaps) that shows dictyostele type of stele. In addition, three (3) distinct bean-shaped vascular bundles in midrib transection are also dissected, showing a dictyostele type of stele (Figure 2D). Midrid transection showed amphicribal arrangement (Figure 2D and E). The lower epidermis of *P. grandiflorum* has tri- to tetracytic stomates (Figure 2F).

Similarly, petiolar accesory vascular bundles were observed in some Asteraceae species such as *Tridax pricumbens, Acmella uliginosa* and *Spilanthes costata*. has accessory observed on its petiole. The dissected v-shaped outline vascular bundle of *P. grandiflorum* is also *A. uliginosa* and *S. costata* [13].

Distribution and ecology

P. grandiflorum is native to the Philippines and New Guinea and was reported to be present in Surigao del Sur and Zamboanga del Sur (Pelser, 2021). However, in the current research *P. grandiflorum* was found and was collected in Mt. Hamiguitan Range Wildlife Sanctuary, Davao del Sur (N 06 44.161° E 126° 08.625', 450m); Concepcion, Valencia City (N 07 88380° E125.25881', 464m); and in Natampod, San Fernando, Bukidnon (N 07° 521.06' E 125° 2515.33', 1,145 m) respectively. *P. grandiflorum* were found along the stream in steep, moist, clayish soil and shaded areas and were often congregated. It is surrounded by *Angiopteris*, *Selaginella* and Alocasia.

Ex-situ conservation

One hundred thirty wildlings of *P. grandiflorum* were nurtured in greenhouse conditions for 13 months, and after one month, a new leaf arose (Figure 3A). The proper protocol for *ex-situ* conservation is shown in Figure 4. There were 53 plants that survived (Figure 3D and E) and the flowers appeared after 6 months of transplant (Figure 3G-H) and continually produced abundant flowers in the succeeding months (Figure 3-I). An invasion of pest such as *Planaria* sp. and African snail observed caused the wilting and casualties of some nurtured plants of *P. grandiflorum*.

Haemolytic activity

The red blood cells (RBCs) of mammals exemplify a good model to assess the cytotoxicity of an organic or inorganic compound [11]. In connection, a cytotoxicity test is important to assess the safety and toxicity of a plant extract that may have a potent biological activity to consumers. Ethanol was used as one of the extraction solvents for natural substances in both food and natural products with medicinal purposes [14]. On the other hand, methanol was found to be effective in the extraction of lower molecular weight polyphenols [15] and is used as an efficient extraction solvent for various plant samples [16].

The 1mg/ml concentration of ethanolic and methanolic leaf extracts of *P. grandiflorum* were found to have an average haemolysis percentage of 0.32 ± 6.78 and 0.45 ± 0.65, respectively. In this study, the ethanolic and methanolic leaf extracts of *P. grandiflorum* showed no toxic effects to human red blood cells. Leaf extracts with percent haemolysis that is above 30% are considered toxic [17], while the degree of *in vitro* cytotoxicity to haemolytic activity with mortality rate of 0-9% indicates a non-toxic action [11]. Thus, the result in this study indicates the non-toxicity of ethanolic and methanolic leaf extracts of *P. grandiflorum* to

Figure 3. *Ex situ* conservation of *P. grandiflorum* procedure: (A) select, (B) Earth ball, (C) secure in zip lock the healthy *P. grandiflorum* wildlings, (D) transplant to clay pots in canopy greenhouse, (E) put sticks beside wildlings and cover with cellophane for 1 week, (F) new leaf arises after 1 month, (G-H) emerging flower after 6 months, (I) plants nurtured in canopy greenhouse after 13 months.

human RBCs. The low percentage haemolysis value of *P. grandiflorum* ethanolic and methanolic leaf extracts can be attributed to the resistance of human RBCs to haemolysis against the compound. The findings in this study imply that *P. grandiflorum* exhibits no cytotoxic effect on human RBCs. Treating cells with a cytotoxic compound can pose serious effects to consumers that would lead to
various diseases [18]; thus, if the leaf extracts are toxic to the cells, the RBCs would lose their membrane integrity due to the toxic effect, leading to rapid death of RBCs due to cell lysis. Consequently, the result in the haemolysis assay for *P. grandiflorum* suggests that the ethanolic and methanolic leaf extract of *P. grandiflorum* is safe to utilize and does not pose toxic effects to humans.

Conclusion

The morphology and ecological information are useful for the *ex-situ* conservation of the economically important *P. grandiflorum*. Haemolysis assay revealed that the ethanolic and methanolic leaf extracts of *P. grandiflorum* is safe to utilize and is not harmful to humans. The ethnobotanical use of the species as edible plant is supported by data on haemolytic activity.

Acknowledgment

We gratefully extend our thanks to the Department of Agriculture- Bureau of Agricultural Research (DA-BAR) and Department of Science and Technology- Grants-In-Aid (DOST-GIA) for funding our research and to the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) for monitoring our project. Likewise, thanks to the

indigenous people of Natampod, San Fernando, Bukidnon and logistic support of the Center for Biodiversity Research and Extension in Mindanao (CEBREM) and Central Mindanao University Administration.

References

1. Airy Shaw, HK (1941) Additions to the flora of Borneo and other Malay Islands: the Pentaphragmataceae of the Oxford University expedition of Sarawak. Kew Bull 1941: 233 - 236. Doi: 10.2307/4102526
2. Lamers TG (2007) Pentaphragmataceae. The Families and Genera of Vascular Plants, Springer, Berlin 8: 605 - 607. doi: https://doi.org/10.1007/978-3-319-93605-5.
3. Pelser PB (2021) Pentaphragmataceae. Co's Digital Flora of the Philippines.
4. Dunbar A (1978) Pollen Morphology and Taxonomic Position of the Genus *Pentaphragma* Wall. (Pentaphragmataceae). Grana 17: 141 - 147. doi: https://doi.org/10.1080/00173137809431959
5. DENR Administrative Order (2017) Updated National List of Threatened Philippine Plants and Their Categories. DAO 2012-11.
6. Lagunday NE, Cabana VG (2013) Taxonomy of Ethnomedicinal Botanicals and Documentation of Ethnomedicinal Practices Traditionally Used by Three Selected Ethnolinguistic Communities in Mindanao, Philippines. Asian Journal of Health 3: 67. doi: 10.7828/ajoh.v3i1.486.
7. Ademiluyi AO, Aladeselu OH, Oboh G, Boligon AA (2018) Drying Alters the Phenolic Constituents, Antioxidant Properties, α-amylase, and α-glucosidase Inhibitory Properties of Moringa (*Moringa oleifera*) Leaf. Food Science & Nutrition 6 (8): 2123 – 2133. doi: 10.1002/fsn3.770.
8. Padda MS, Picha DH (2007) Antioxidant Activity and Phenolic Composition in 'Beauregard' Sweet Potato are Affected by Root Size and Leaf Age. Journal of the American Society for Horticultural Science 132 (4): 447-451. doi: 10.1002/fsn3.770.
9. Greco I, Molchanova N, Holmedal E, Jenssen H, Hummel BD, Watts JL, Svenson J (2020) Correlation between Hemolytic Activity, Cytotoxicity and Systemic In Vivo Toxicity of Synthetic Antimicrobial Peptides. Scientific Reports 101: 1 - 13. doi: 10.1038/s41598-020-69995-9.
10. Riaz M, Rasool N, Bukhari IH, Shahid M, Zubair M, Rizwan K, Rashid U (2012) In Vitro Antimicrobial, Antioxidant, Cytotoxicity and GC-MS Analysis of *Mazus goodenifolius*. Molecules 17 (12): 14275 - 14287. doi: 10.3390/molecules171214275.
11. Pagano M, Faggio C (2015) The Use of Erythrocyte Fragility to Assess Xenobiotic Cytotoxicity. Cell Biochemistry and Function, 33 (6): 351 - 355. doi: 10.1002/cbf.3135.
12. Lin CW (2016) Two new species of *Pentaphragma* (Pentaphragmataceae) from Sarawak, Borneo. Taiwania 61 (4): 355 - 361. doi: 10.6165/tai.2016.61.355
13. Ekeke C, Ogazie CA (2020) Systematic Significance of Petiole Anatomical Characteristics in Some Members of Asteraceae from Some Parts of Nigeria. Singapore Journal of Science Research 10: 387-399. doi: 10.3923/sjsres.2020.387.399
14. 14. Hikmawanti NPE, Fatmawati S, Asri AW (2021) The Effect of Ethanol Concentrations as the Extraction Solvent on Antioxidant Activity of Katuk (*Sauropus androgynus* (L.) Merr.) Leaves Extracts. In IOP Conference Series: Earth and Environmental Science 755 (1): 012060. doi: 10.1088/1755-1315/755/1/012060.
15. Sultana B, Anwar F, Ashraf M (2009) Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules (Basel, Switzerland) 14 (6): 2167 – 2180. Doi: 10.3390/molecules14062167.
16. Truong DH, Nguyen DH, Ta NT, Bui AV, Do TH, Nguyen HC (2019) Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and *In Vitro* Anti-inflammatory Activities of *Severinia buxifolia*. Journal of Food Quality 1 – 9. doi: 10.3390/molecules14062167.
17. Kundishora A, Sithole S, Mukanganyama S (2020) Determination of the Cytotoxic Effect of Different Leaf Extracts from *Parinari curatellifolia* (Chrysobalanaceae). Journal of Toxicology. doi: https://doi.org/10.1155/2020/8831545.
18. Baillie JK, Thompson AAR, Irving JB, Bates MGD, Sutherland AI, Macnee W, Webb DJ (2009). Oral Antioxidant Supplementation Does Not Prevent Acute Mountain Sickness: Double Blind, Randomized Placebo-controlled Trial. QJM: An International Journal of Medicine 102 (5): 341 - 348. doi: https://doi.org/10.1155/2020/8831545.

This page is intentionally left blank.