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Histochemical Analysis of *Gmelina arborea* Roxb. and its Implication as Forage for Goats

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ABSTRACT

Background: *Gmelina arborea* Roxb. is a fast-growing plant that has been utilized as feed resource for livestock production particularly for goat production. However, this plant was noted to contain toxic compounds which can affect the production and performance of livestock animals. These contradictory results is the primary reason of determining the localization of secondary products which is commonly noted as toxic compounds in plants. Thus, this study aims to provide baseline information on cell and tissue localization of different plant secondary products present in *Gmelina arborea*.

Methods: Field-laboratory investigation was conducted in the last quarter of 2021. Localization of these compounds were determined by sectioning and histochemical analysis. Stains and reagents were used to detect the location of these compounds.

Result: Most of the secondary products were detected in the epidermis, parenchyma and collenchyma in the cortex and near vascular tissues and mesophyll cells of the lamina. The results of this study support the results of different phytochemical studies conducted using this plant. This is an indicator that plant secondary products are widely distributed in the different parts of the plant. Considering plants as forage for livestock, especially for goats, necessitates the consideration on the presence and localization of these phytochemicals as these may have nutritional and toxic effects.

Key words: *Gmelina arborea,* Histochemical analysis, Phytochemicals, Plant secondary products.

INTRODUCTION

Gmelina arborea is a fast-growing deciduous tree belonging to family Lamiaceae. It is an important plantation and timber species. It is also used in reforestation projects due to its rapid growth and abundance in tropical and subtropical region. This species produces large numbers of fertile fruits that are easily dispersed by birds and bats, spreading seedlings quite far from the parent tree (Warrier *et al.*, 2020). In addition, this plant has been utilized as a feed resource for livestock as indicated by Ubani and Tewe (2001). The availability of plant parts all year round is one of the characteristics of utilizing *G. arborea* as non-conventional feedstuff as stated by Lamidi *et al.* (2009). However, in the study of Augustine *et al.* (2018), this plant species has been noted as toxic to goats and associated this toxicity to plant secondary products present in this species. Most cases of toxicosis recorded in this study is commonly attributed to overconsumption of *Gmelina arborea* leaves. Toxicosis caused by *G. arborea* can be attributed to anti-nutritional components. This was confirmed by the study of Augustine *et al.* (2018) wherein *G. arborea* leaves contain anti-nutritional components which include tannins, oxalates, phytates and saponins.

There have been several studies conducted on the phytochemistry of the different parts of *G. arborea* and little is known on the tissue localization of plant secondary products. Plant histochemistry is another way of detecting plant secondary products in cells and tissues of plants. It is one of the initial and cost-effective procedures in the identification and localization of compounds. It is successfully applied to detect active cell constituents such as proteins, carbohydrates, lipids, nucleic acids and plant secondary metabolites (Dhale, 2011). Furthermore, the reliability of this qualitative tests has been proven by various research studies which also looks into the localization of target compounds in plant tissues. This study aims to provide a baseline information on the localization of plant secondary products in *Gmelina arborea*. The results also provide the whole picture of phytochemical constituents and their localization of this plant which can be associated to toxicity issues and implications in utilizing this species as a forage for goats.

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MATERIALS AND METHODS

Field and laboratory investigation was done from October to December 2021. Fresh *Gmelina arborea* leaf and stem

samples were collected in a plantation from Brgy. Pedtad, Kabacan, North Cotabato, Philippines. Mature and juvenile samples were both collected for comparison. Samples were soaked in water and were prepared for sectioning. Laboratory investigation was conducted in the Biology Central Laboratory, University of Southern Mindanao, Kabacan, North Cotabato, Philippines. Qualitative histochemical analysis of selected forages was conducted following the procedures of Dhale (2011). Cross-sections of fresh mature and juvenile leaf and stem samples were prepared. One (1) to two (2) drops of specific stain were added to these sections to detect the type and tissues localization of major plant secondary products. A summary of reagents and positive results for alkaloids, saponins, flavonoid, tannins and cardiac glycosides is provided in Table 1. Examination of cross section with stain or reagent were done using a compound binocular microscope. Photomicrographs were generated with scale using Olympus CX41 with camera.

RESULTS AND DISCUSSION

Alkaloids inyoung and mature *G. arborea* petiole and stem samples were observed in cortical parenchyma cells surrounding the vascular tissue, epidermis and sieve-tube elements. Alkaloids were also observed in the epidermis and cortical collenchyma of the midrib and mesophyll cells of the lamina in both samples (Fig 1).

Epidermis and cortical collenchyma cells in the stem and the midrib, cortical parenchyma cells of the petiole of *G. arborea* young and mature samples showed positive result for saponins. Localization of saponins were also observed in the mesophyll cells of the lamina in young samples (Fig 2).

Cortical parenchyma cells were positive for flavonoids in the stem, petiole and midrib of *G. arborea* young and mature samples. Positive results were also observed in the petiole of young samples, some trichomes of the midrib and, epidermis and trichomes in the abaxial portion in the lamina of mature samples (Fig 3).

In the stem of *G. arborea*, tannins were observed in the cortical collenchyma cells of the mature samples and some parenchyma cells between vascular tissues of young samples. Epidermis, cortical parenchyma cells and phloem parenchyma in the petiole, the epidermis, cortical collenchyma cells and some trichomes in the midrib and epidermis and mesophylls cells in the lamina of young and mature sample showed positive results (Fig 4).

Cardiac glycosides were observed cortical collenchyma and parenchyma cells and some sieve-tube elements in the stem of both samples and trichomes in the stem of mature samples (Fig 5).

The above findings coincide with the histochemical findings of other researchers. The distribution of alkaloids, as observed by Hashimoto *et al.* (1992), are common in active tissues near the vegetative points, ovule, epidermis and the layer just inside of it, trichomes, peripheral layers of fruits and seeds, vascular bundles, cork cambium, cork tissues and laticifers. Some observations are similar with histochemical analysis of *Catharanthus roseus* wherein the distribution of alkaloids is observed in phloem-associated parenchyma and epidermis (Yamamoto *et al.*, 2016). The epidermis and outer cortical layer, the parenchyma within and adjacent to the phloem and the periphery of the pith near xylem of *Atropa belladonna* was also observed to contain abundant alkaloids (James, 1950). Detection of alkaloids in young and mature samples may vary as plant age affects the production of these compounds and, more often than not, young samples have higher concentration than mature samples (Koomson *et al.*, 2018). Although with slow histochemical reaction due to low concentration, detection of alkaloids in the trichomes of mature samples is still possible (Jing *et al.*, 2014).

Localization of saponins was commonly observed in the epidermis and cortex of the stem and the midrib and mesophyll cells of the lamina in *G. arborea*. This observation are similar to other histochemical studies of *Alphonsea zeylanica*, *Ruta graveolens* (Dhale, 2011), *Polygala sibirica* (Teng *et al.*, 2009a) and *Polygala tenuifolia* (Teng *et al.*, 2009b). Detection of saponins in the leaves may indicate its concentration as Chen *et al.* (2019) observed highest accumulation of saponins in the palisade layer of *Cyclocarya paliurus*leaves. Francis *et al.* (2002) indicated that there is a trend of higher concentration of saponins in young as compared to mature plants but these compounds occur in both samples and has no significant difference according to Koomson *et al.* (2018) which supports the results of this study.

Tissue localization of flavonoids in *G. arborea* were observed mostly in parenchyma cells. Parenchyma cells in the stem of *Morinda citrifolia* contain flavonoids as observed by Mownika *et al.* (2020). Flavonoids are also localized in the parenchyma cells and hypodermal layers of the stem and petiole of *Centratherum punctatum* (Chitra *et al.*, 2014). Flavonoids can be detected in the leaves as they largely accumulate in the adaxial epidermal layer and sub-epidermal cells (Agati *et al.*, 2002) and palisade parenchyma

Table 1: Major compounds, stains and indicators used for the histochemical tests.

Compound group	Stains	Positive indicator*
Alkaloids	Dragendorff's reagent	Chocolate brown precipitate
Saponins	Concentrated H_2SO_4	Violet-blue green coloration
Flavonoids	10% NaOH	Yellow coloration
Tannins	Vanillin-Hydrochloric acid	Bright red condensates
Cardiac glycosides	Keller-Killani's reagent	Reddish brown coloration
	Kedde's reagent	Red violet coloration

(Tattini *et al.*, 2004). Localization of flavonoids can be observed in young and mature samples as these compounds occur in juvenile stage of plants and continuously synthesized as the plant mature. The concentration of flavonoids may vary across different developmental stages but can still be detected through histochemical analysis (Chang *et al.*, 2018; Luengas-Caicedo *et al.*, 2007).

Localizations of tannins in the stem, petiole and midrib observed in this study is in agreement with the observations of Dhale (2011), Mahendra *et al.* (2017) and Salatino *et al.* (1993). The detection of tannins in parenchyma cells around or near vascular tissues of the stem can be supported by the study of Robil and Tolentino (2015) on *Medinilla magnifica*stem as tannins specifically accumulate in these tissues. On the other hand, tannins in the leaves of *Struthantus vulgaris* (Salatino *et al.*, 1993) and *Medinilla magnifica* (Robi and Tolentino, 2015) are more abundant in the palisade parenchyma and in the more compact spongy parenchyma adjacent to the lower epidermis which is also similar to the result of this study. Some epidermal structures, like trichomes, are also known to contain tannins. Sudipta *et al.* (2014) observed tannin localization in the leaf

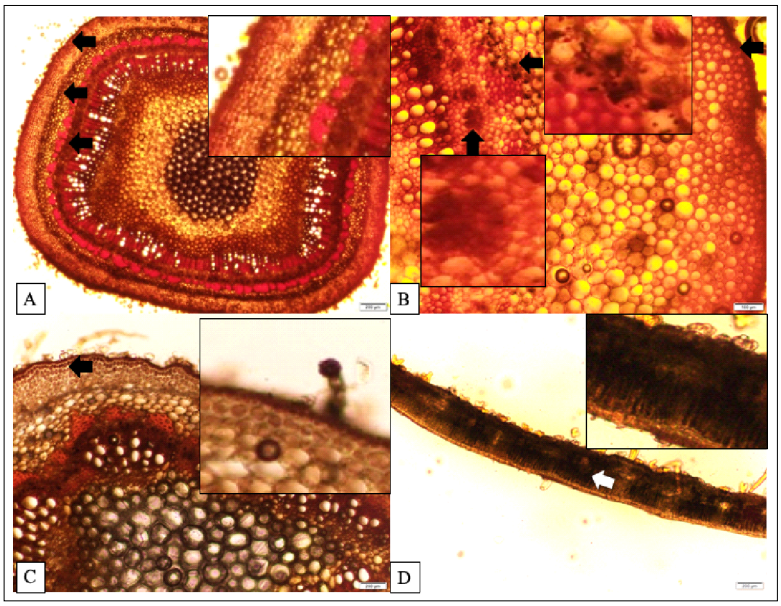


Fig 1: Localization of alkaloids in *G. arborea* mature stem (A); petiole (B), midrib (C) and lamina (D) of a mature leaf visualized using Dragendorff's reagent. (Arrows indicate the localization of alkaloids).

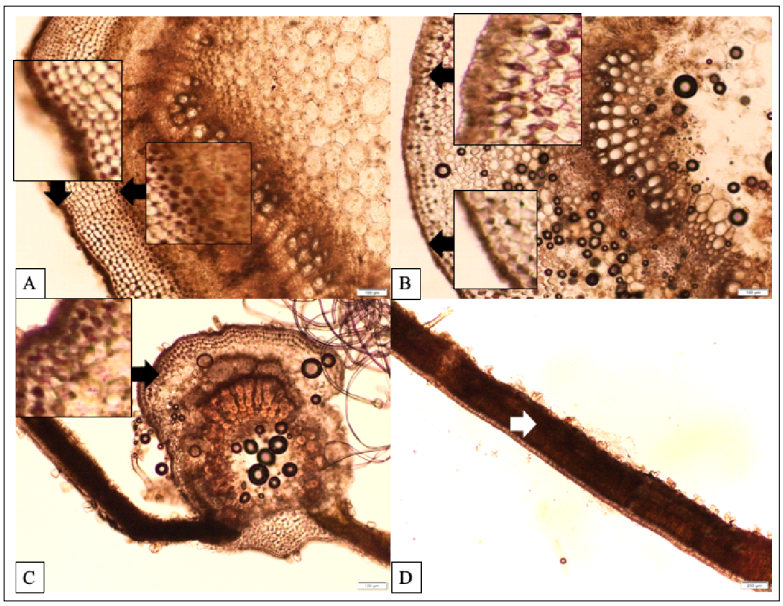


Fig 2: Localization of saponins in *G. arborea* mature stem (A); petiole (B) and midrib (C) of a mature leaf and lamina of a young leaf (D) visualized using Conc. H_2SO_4. (Arrows indicate the localization of saponins).

trichomes in some members of Combretaceae including *Terminalia chebula, Terminalia catappa, Quisqualis indica, Terminalia bellirica* and *Combretum decandrum*. Occurrence of tannins in some tissues of young and mature samples was also observed by Fleurat-Lessard *et al.* (2016) in different cellular compartments of the pulvinus of young and mature *Mimosa pudica*. Different types of condensed tannins also occur in the young and mature branchlets of *Casuarina equisetifolia* as observed by Zhang *et al.* (2009).

Cardiac glycosides were detected in both *G. arborea* only in the cortical collenchyma and parenchyma cells of the stem and petiole and some glandular trichomes of young and mature samples. It implies that these substances occur in few tissues of these plants. Localizations observed in this study can be supported by the study of Morsy (2017) as these compounds occur in the seeds, leaves, stem, roots and bark of plants in small amount. The study of Hollman (1985) enumerated some plant families with representatives known to exhibit cardiac glycosides, however, Lamiaceae and Malvaceae are not included. Moreover, the observed localization of cardiac glycosides in both young and mature samples indicate that age is not a determining factor on the detection of this compound in both species. This is in congruent with the study of Agrawal *et al.* (2012) wherein cardenolides of *Asclepias syriaca* were detected by in both mature plant and seedlings but of different concentrations.

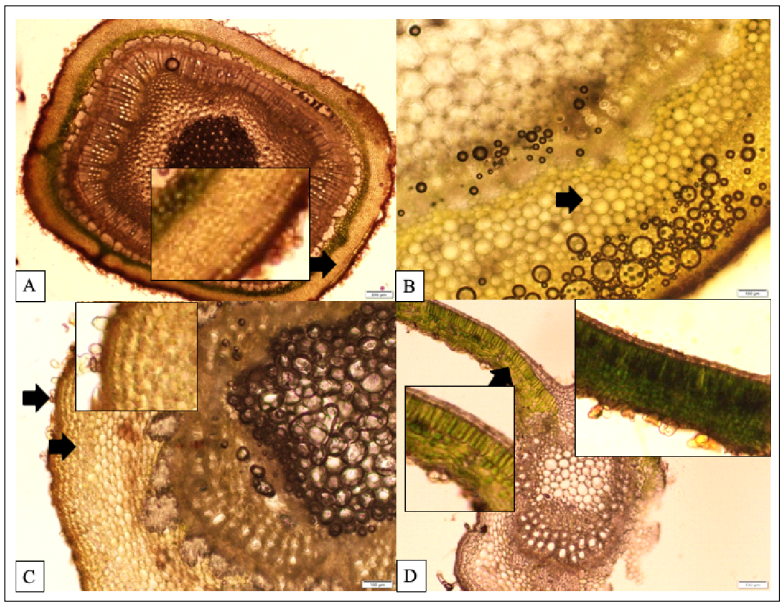


Fig 3: Localization of flavonoids in *G. arborea* mature stem (A); petiole of a young leaf (B); midrib of a mature leaf (C) and a young leaf (D) using 10% NaOH. (Arrows indicate the localization of flavonoids).

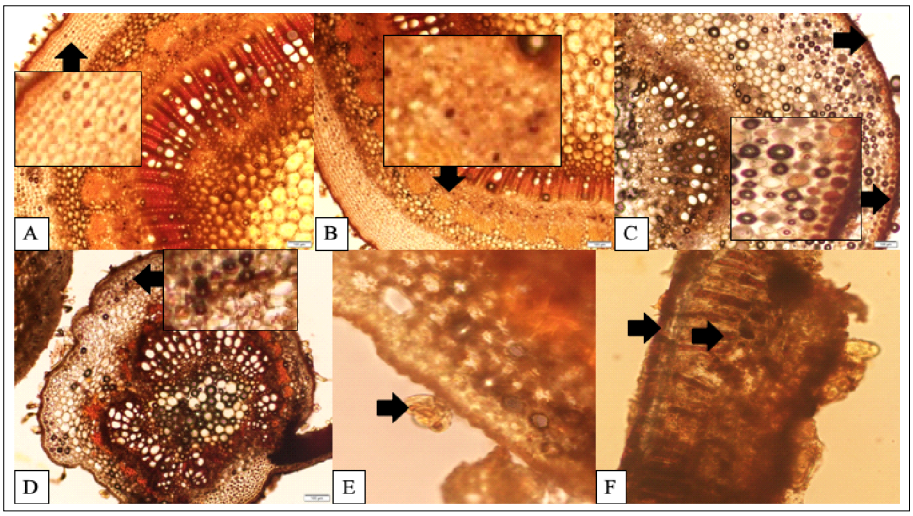


Fig 4: Localization of tannins in *G. arborea* mature stem (A); petiole of a young leaf (B); petiole (C) and midrib (D) with trichomes (E) of a mature leaf and lamina of a young leaf showing the mesophyll cells (F) using Vanillin-HCl. (Arrows indicate the localization of tannins).

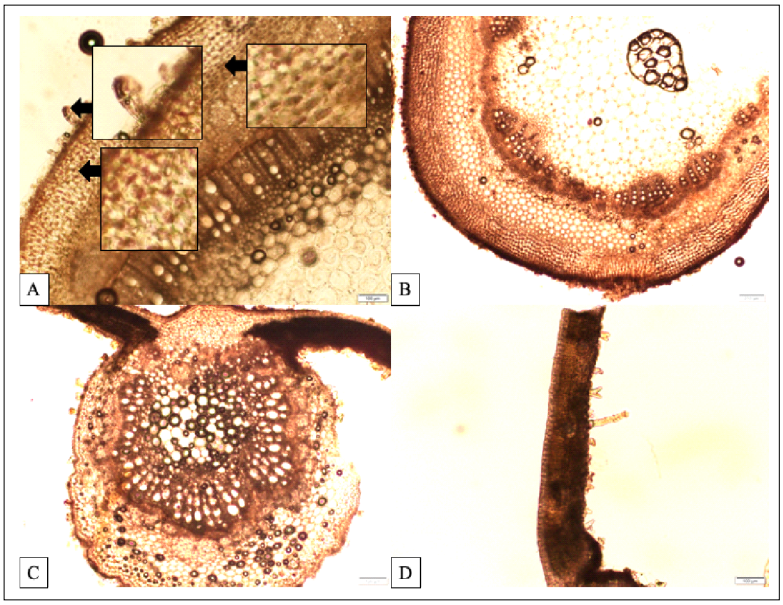


Fig 5: Localizaton of cardiac glycosides in *G. arborea* mature stem (A); petiole (B), midrib (C) and lamina (D) of a mature leaf visualized using Keller-Killiani's and Kedde's Reagent. (Arrows indicate the localization of cardiac glycosides).

Based on the results of histochemical analysis, plants secondary products are localized in the different cells and tissues of different part of *G. arborea*. This implies that these secondary products can be consumed by livestock, particularly goats when this plant species are utilized as forage. Both nutritional and toxic effects to goats can be attributed to the phytochemical constituents of this plant species. These effects are dependent on the amount of these phytochemicals consumed which is one of the main considerations in using *G. arborea* as sole forage for goats.

CONCLUSION

Most of the secondary products were detected in the epidermis, parenchyma and collenchyma in the cortex and near vascular tissues and mesophyll cells of the lamina. This is an indicator that plant secondary products are widely distributed in the different parts of the plant. Considering plants as forage for livestock, especially for goats, necessitates the consideration on the presence and localization of these phytochemicals as these may have nutritional and toxic effects. Further investigation on the estimated amount of these plant secondary products detected in this study is important to determine the amount that can be consumed by animals when this plant species is utilized as a feed resource for livestock.

Conflict of interest: None.

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