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HISTOCHEMICAL SCREENING AND MEDICINAL POTENTIALS OF *GARUGA FLORIBUNDA* IN MINDANAO ISLAND, PHILIPPINES

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ABSTRACT
This study was conducted to determine the bioactive components of Garuga floribunda (Burseraceae) used by the villagers of Bukidnon, Mindanao Island, Philippines, used in various ethno-veterinary practices as well in traditional medicine in other Asian region. Histochemical analyses of the stem of Garuga floribunda showed presence of alkaloids, saponins, tannins, oxalic acid, formic acid, tartaric acid, fats and oils localized in various tissues. This includes sclerenchymatous peridem, collenchyma cells in the cortex, phloem, xylem and vascular cambium and parenchymatous pith tissue of the stem. This study confirms the presence of alkaloids, arbutin, fats and oils, saponins, tannins as well as organic acids such as oxalic, formic and tartaric acid. The presence of bioactive compounds such as tannins suggests the potential anti-helmintic properties of the plant as reported in traditional medicine.
KEY WORDS: *histochemical*, *Garuga, Burseraceae, ethno-veterinary, Mindanao, Philippines*

INTRODUCTION

Garuga floribunda Decne locally known as "bogo" in Mindanao Island, Philippines is a small deciduous tree belonging to the Burseraceae family. It is found in Southeast Asia, Northern Australia (Northern Queensland, Cape York Peninsula, Northern Western Australia) and the Western Pacific at altitudes from sea level to about 400 m (Kalkman, 1953; Pooma, 1999; Williams, 2011). It grows in landlocked forests on clay-loam and sandy-loam soil and also in coastal forests on sandy-loam soil with lime and weathered corals. It is deciduous, shedding its compound leaves from February to April, small to medium sized or occasionally fairly large tree up to 30 meters tall (Fig. 1). The trunk is straight and has flaking fissured grey bark, while the more juvenile ones have non-flaking and relatively smoother bark. The leaves are fugacious (regularly shedding leaves), imparipinnate (odd-pinnate), alternately arranged along the branches, crowding at the apex of each branch. Stipules of mature leaves are attached at the base of petioles, while those of the juvenile ones are attached at the base of the petioles. Leaflets (young and mature) are oppositely arranged along the rachis, oblong-lanceolate, crenate - serrate. Venation is reticulate or netted.

In literatures, this species of *Garuga* was reported to be traditionally used in India, the leaf astringent for the treatment of asthma; the fruit is used for dysentery; the bark is applied for eye disorder and wounds; whereas the root is used for skin and venereal disorders (Hazarika *et*

al., 2012). Through focus group discussion with local villagers in the province of Bukidnon, Philippines, it was recounted that locals use the bark of *Garuga floribunda* for ethno-veterinary practices such as deworming swine and cattle. Moreover, bark is likewise applied to the cattle's leg that could not be bent (Amoroso *et al.,* 2008) hence, "bogo" tree is considered locally as a valuable species. The rural folks made use of these medicinal plants due to the plant's availability and expensive cost of veterinary drugs. These practices play a vital role especially in rural areas as a primary form of medicine being used to cure livestock particularly in many developing countries (Abbasi *et al.,* 2013; Ul Hassan *et al.,* 2014). Ethno-veterinary practices are not only cost effective but are socially compatible and generally comprise of easily available local flora or ingredients (Das & Tripathi, 2009).

Morphological, anatomical and histochemical characterization of medicinal plants have been regarded to be important in quality control exams of samples of certain species (Ianovici, 2011; Pacheco-Silva & Donato, 2016; De Melo Silva *et al.,* 2016) as well as for detecting toxic heavy metals in plant samples (Seregin & Kozhevnikova, 2011). Morpho-histochemical study of these medicinal plants help establish the scientific basis on their reported medicinal values. This study presents the histochemical screening of *Garuga floribunda* to determine the bioactive components detected in the tissues and its potentials in Philippine traditional medicine.

FIG. 1. Habit and form of *Garuga floribunda*

MATERIALS AND METHODS

Fresh samples of *Garuga floribunda* were collected within the province of Bukidnon. For the identification of main classes of compound, histochemical analyses were performed using fresh transverse sections of the stems of *Garuga floribunda* following the protocol of Johansen (1940). Fresh sections were treated with iodo-potassium iodide for the detection of alkaloids; 10% nitric acid for arbutin; Sudan IV for fats and oils; mercuric chloride, hydrochloric acid and 1% potassium hydroxide for formic acid; ferrous phosphate and phosphoric acid for oxalic acid; sulfuric acid for saponins; 10% aqueous ferric chloride and

sodium carbonate for tannins; and 4% aqueous solution of ferrous salt and 10% potassium permanganate for tartaric acid. Sections were then examined under light microscopy (100X magnification) and documented using a SONY digital camera.

RESULTS AND DISCUSSIONS

The presence of bioactive principles in the histochemical screening may provide valuable insights to the reported medicinal utilization of the plant and claims of its usefulness in various cultures (Ianovici *et al*, 2010). Histochemical investigations revealed that various substance groups were localized in the stem of *Garuga floribunda*. General information is given in Table 1. Some of the reactions are also presented in Figures 2 & 3. Chocolate brown colour reaction was observed indicating the presence of alkaloids in the tissues of the bark and the wood (Fig.2 A). Arbutin (Fig.2 B) which stained red to orange and saponins (Fig.2 C) which stained red were also observed to be concentrated in the bark of the plant. Blue-green colour reactions for tannins were localized in the cortex, vascular cambium, and vascular bundles of the stem (Fig. 2 D). The different bioactive principles were localized in diverse cellular structures. This includes sclerenchymatous peridem, collenchyma cells in the cortex, phloem, xylem and vascular cambium and parenchymatous pith tissue of the stem. Experimental studies suggest direct anti-helminthic efficacy of tannins derived from plant sources towards gastrointestinal parasites (Athanasiadou *et al.,* 2001; Alonso-Díaz *et al.,* 2011; Williams *et al.,* 2014). The abundant tannins in the bark may explain the traditional anti-helminthic uses of *G. floribunda.*

The presence of polyphenolic compounds in *G. floribunda* which include tannins have been likewise detected through thin layer chromatography method (Hafid *et al.,* 2014; Widyawaruyanti *et al.,* 2014). Formic acid which stained black in colour was present all throughout the stem tissues (Fig.3 E). Violet colour reaction for tartaric acid (Fig.3 F) were likewise concentrated mainly in the bark of the plant. Intense yellow colour reaction for oxalic acid was also observed in the bark (Fig.3 G). Moreover, red colour reaction for fats and oils (Fig.3 H). Recent studies also report the presence of linoleic acid, palmitic and the combination of the two C18:1 fatty acids (oleic and asclepic) in the seeds of *G. floribunda* (Knothe *et al.,* 2017) as well as flavonoid, terpenoids and polyphenols in both the plant's leaves and stem which also showed free-radical scavenging activity (Hafid *et al.,* 2014) and anti-malarial properties (Widyawaruyanti *et al.,* 2014). The presence of essential oils like terpenoids was also reported in other asian *Garuga* species (Rahman *et al.,* 2008).

Alkaloids and saponins was also detected in other species within Burseraceae family like *Commiphora molmol (*Al-Daihan *et al.,* 2013) as well as tannins and glycosides like arbutin in *Commiphora berryi* (Selvamani *et al.,* 2009). The abundance of biologically active compounds in the bark have been likewise observed in other tropical members of Burseraceae which were used in traditional medicine such *Bursera simaruba* (Maldini *et al.,* 2009) and *Canarium patentinervium* (Mogana *et al.,* 2011) and *Protium* (Rüdigera *et al.,* 2007).

CONCLUSIONS

This study shows the morphological, histochemical, and ultrastructural diversity of the production sites of bioactive principles in the stem of *G. floribunda*, as well as its ethnobotanical importance. Histochemical investigations confirm the presence of alkaloids,

arbutin, fats and oils, saponins, tannins as well as organic acids such as oxalic, formic and tartaric acid. The present work also opens new perspectives for screening other ethno-verterinary plants used in the province of Bukidnon, Philippines. The use of ethnobotanical knowledge provides valuable insight in discovering important compounds of economic and medicinal values. New approaches however are necessary to investigate the potential applications of other bioactive compounds detected in the plant.

TABLE 1. Histochemical localization of active principles in the stem of *Garuga floribunda*

Active principles	Localizations in the tissues of the stem`							
			Bark			**wood**		
	periderm	cortex	Phloem		vascular cambium	xylem		pith
			primary phloem	secondary phloem		primary xylem	secondary xylem	
Alkaloids	+	+	+	+	+	+	+	+
Arbutin	+	-	+	-	-	-	-	-
Fats & Oils	+	+	+	+	+	+	+	+
Formic acid	+	-	+	+	+	+	+	+
Oxalic acid	+	-	-	+	+	-	-	-
Saponins	+	-	-	-	-	-	-	-
Tannins	+	-	-	+	+	-	-	-
Tartaric acid	+	-	-	-	+	+	+	-

Legend: (+) positive, (-) negative

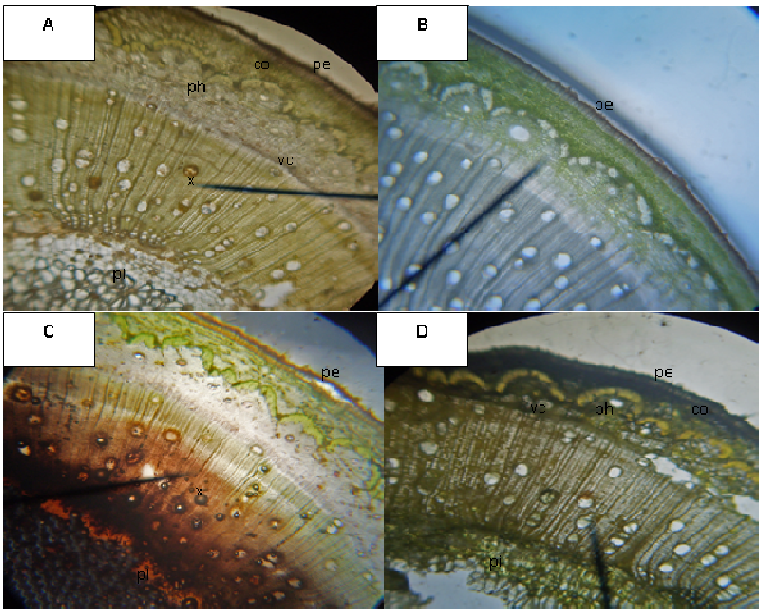

FIG. 2. A. Transverse section of *Garuga floribunda* stem showing chocolate brown colour in the periderm, vascular cambium, phloem, xylem and pith for alkaloids. B. Red-orange colour in the bark for arbutin. C. Red colour reaction for saponin localized in the periderm, phloem, xylem and pith. D. Blue-green colour reaction in the cortex, phloem, vascular cambium and pith for the presence of tannins (magnification: 100X).

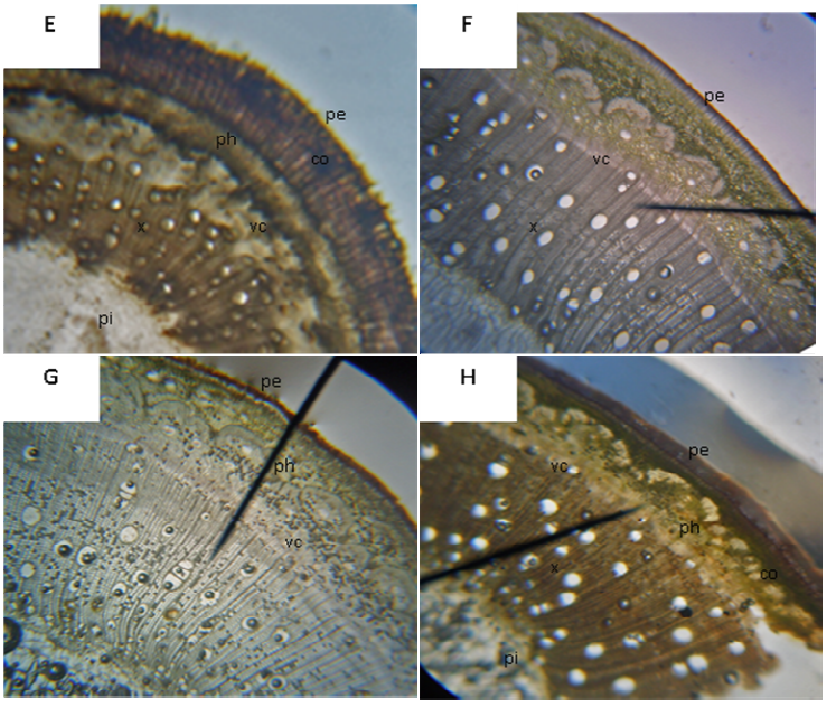

FIG. 3. E. Black colour reaction in the periderm, cortex, vascular cambium, xylem and phloem and pith tissues indicating the presence of formic acid. F. Violet colour reaction in the periderm, vascular cambium and xylem indicating the presence of tartaric acid. G. Intense yellow colour reaction in the periderm and seconday phloem and vascular cambium for oxalic acid. H. Reddish colour reaction in the periderm, vascular cambium, phloem, xylem and pith tissues for fats and oils. (Legend: pe – periderm; co – cortex; ph- phloem; vc – vascular cambium; x – xylem; pi – pith (magnification: 100X)

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