



Morphoanatomical and preliminary phytochemical studies of two specimens of *Dorstenia contrajerva* native to Guatemala

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Abstract

This work presents a descriptive study of the morphoanatomical characters and preliminary phytochemical data, of the vegetative organs and the inflorescences of *Dorstenia contrajerva* L. and *D. contrajerva* var. *tenuiloba* (Blake) Standl. & Steyerl. that grows in Guatemala, useful to establish identity characters for quality control standards, especially when they are dry and/or fragmented. The techniques used were freehand cutting, leaf diaphanization, maceration and screening of secondary metabolites by histochemical and thin layer chromatography methods. Both specimens of *D. contrajerva* showed widely simple lobed leaves, actinodromous reticulate venation, hypostomatic leaves with anisocytic and anomocytic stomata at level and sunk. Glandular hairs with 1-2 cellular head and unicellular stalk, uncinata, large and small simple hairs. The stem and rhizome, circular and irregular, present simple uncinata hairs, laminar collenchyma and open collateral vascular bundles, arranged in a eustele. A polyarch root and evident cuticle was found. Inflorescence shows simple uncinata and hispidule large and small hairs, glandular ones with unicellular stalk and 1-2 cellular head and papillary hairs. The phytochemical screening showed the presence of starch grains, fats and oils, mucilages, saponins and coumarins. Most of the findings in this study, matches the ones reported for other authors to other species of *Moraceae* family, but many of them were no previously reported for other species of *Dorstenia* genus. Original photomicrographs show the main characters.

Keywords: Micrograph analysis, quality control, psoralen analogs, plants drugs

INTRODUCTION

Dorstenia contrajerva and *Dorstenia contrajerva* var. *tenuiloba*, belong to *Moraceae* family, and are popularly known as “contrahierba”, “contrayerba”, “mano de león”, “hierba de sapo”, “cresta de gallo” or “contauíl”. Geographically distributed from the Caribbean to Peru and Guyanas. In Guatemala, this genus is present in Petén, Alta Verapaz, Chiquimula, Jalapa, Santa Rosa, Escuintla, Guatemala, Sacatepéquez, Chimaltenango, Retalhuleu, Quetzaltenango and Huehuetenango. *Dorstenia* is widely utilized in folk medicine due to its medicinal properties: antiophidic, anti-diarrheal, antimicrobial, and anti-inflammatory in eruptive skin diseases. The whole plant is used in a simple decoction process for intestinal pathologies like diarrhea, dysentery or indigestion, and also for snake bite treatment, eruptions, fever, and photosensitizing effect.^{1,2,3}

Furanocoumarins are present in *Dorstenia* species and due to the increased search for psoralen analogs as photochemotherapeutic agents, *Dorstenia* species has acquired more interest.^{4,5}

When whole or relatively whole botanical material is as-

sessed, it is easy to detect the adulteration, however, when powdered materials are assessed, microscopy is one of the primary tools used to detect the presence and identity of any other botanical material.⁶

This observational, cross-sectional, descriptive study was performed with the main purpose of establishing morphoanatomical characters and histochemical preliminary data of the vegetative organs and the inflorescences of *Dorstenia contrajerva* L. and *D. contrajerva* var. *tenuiloba* (Blake) Standl. & Steyerl. that grows in Guatemala in order to contribute to its quality control and its rationale use, due to the fact that there are no controlled cultures of this species so the raw material came from wild collection.

MATERIALS AND METHODS

Plant material

Whole and fresh wild plants of two specimens of *D. contrajerva* were collected from “Ecoparcela el Kakawatal”, Suchitepéquez, Guatemala; localized at 539 meters under the sea level at 14°33'6.66"N; 91°21'53.19"O. Herbarium specimens were done according to Giberti.⁷ The identifica-



tion and deposit of the specimens were made at the Herbario de Biología de Guatemala (BIGU) from the Faculty of Chemical & Pharmaceutical Sciences of the San Carlos of Guatemala University.

Anatomical studies

Fresh and dried plant materials were used for the macroscopic, organoleptic and micromorphologic studies. The macroscopical description was made on specialized literature basis. The morphologic aspects were compared with the ones described in the *Flora of Guatemala* in order to establish the minimum characteristics for its identification. For microscopical analysis, reference material of freehand transverse sections from leaf, stem, root and flowers of the two specimens was performed and stained with Safranin according to Gattuso & Gattuso. Sections were mounted with gelatin-glycerin and observed with a Leica® CME optical microscope.

Leaves, stems and roots were macerated. Leaves were cleared, stained and mounted by conventional methods,⁸ and microscopic descriptions of tissues were supplemented with photomicrographs wherever necessary. The images of different magnifications were observed with the Micromaster® microscope, visualized and photographed using a Westover™ camera, and digitalized with Micron (USB) program.

Phytochemical preliminary screening

Secondary metabolites screening by histochemical techniques,⁸ were performed for each part of the plants on freehand transverse sections of fresh material.

Coumarin screening was performed on the crude MeOH extract by two methods. In the first, two different spots of extract were placed on a filter paper, one was covered with one drop of KOH 0.5N and then observed under UV light. In the second, a thin layer chromatography by standard methods was performed and exhibited on a TLC Silica gel 60 F254 film and toluene-ethyl acetate mobile phase.⁹

Physicochemical analysis

From dried material, total and acid ashes were determined by standard laboratory methods and moisture percent was determined by the thermogravimetric process.⁹

RESULTS

Dorstenia contrajerva specimens collected from Suchitepéquez, Samayac, Chiguaxté, Ecoparcela el Kakagual, were deposited as herbarium specimens and, according to the curators and experts who collaborated with the study, the specimens were identified as *Dorstenia contrajerva* L. Guatemala, Suchitepéquez, Ecoparcela el Kakawatal, 539 msnm, boucher 067412, 23/VI/13, collected by C. Díaz and K. Flores (Figure 1A) and *Dorstenia contrajerva* var. *tenuiloba* (Blake) Standl. & Steyerl. Guatemala, Suchitepéquez, Ecoparcela el Kakawatal, 539 msnm, Boucher 067413, 23/VI/13, collected by C. Díaz y K. Flores (Figure 1B).

Botanical description of plant material

D. contrajerva L. (Figure 1C), and *D. contrajerva* var. *tenuiloba* (Blake) Standl. & Steyerl. (Figure 1D) are evergreen perennial plants with latex that grows up to 40 cm



Figure 1: Macroscopical and microscopical main characters. A: herbarium specimen of *D. contrajerva* L. B: herbarium specimen of *D. contrajerva* var. *tenuiloba*. C: live specimen of *D. contrajerva* L. D: live specimen of *D. contrajerva* var. *tenuiloba*. E: inflorescence of *D. contrajerva* var. *tenuiloba*. F: dry material of *D. contrajerva*. G: blade transverse section of *D. contrajerva* L. H: blade transverse section *D. contrajerva* var. *tenuiloba*.

in height, with herbaceous habits, short and rhizomatous stems (brevicaule), alternating phyllotaxis, with mostly short internodes and green axillaries inflorescence. For *D. contrajerva* L., leaf has a decurrent widely lobated base and an acuminate or shaped apex, and a smooth-shining surface. *D. contrajerva* var. *tenuiloba* shows leaves with deep, long and narrow lobes and a smooth-matte surface. Both of them show rough texture, crenate-dentate margin and pubescent back side, especially over the veins. Inflorescence with flowers imbedded in a fleshy, quadrangular and green or green with purple margin receptacle (Figure 1E), with deep lobes in *D. contrajerva* L. and more regular in var. *tenuiloba*.

Raw material is conformed by the whole plant. Dried and fragmented drug became greenish brown, and contains parts of leaves, flowers, stems and rhizome, with sweet herbal smell, thin and fragmental consistency, and both specimens look alike (Figure 1F). The root has a peculiar aromatic smell and a light astringent warm bitterish taste.

Diagnostic micromorphological characteristics

Blade transverse section shows a dorsiventral leaf in both specimens, with evident cuticle, thicker in *D. contrajerva* L. (Figure 1G) than in var. *tenuiloba* (Figure 1H). Both, adaxial and abaxial epidermis are unistratified, show various shapes and sizes of cells, larger in *D. contrajerva* L. than in var. *tenuiloba*, both with flat aniclinial walls and convex tangential ones; short palisade cells in one layer and 1-3 cellular spongy mesophyll. At the midrib level, transverse section shows laminar collenchyma under both epidermal layers (Figure 2A), and 1-3 cellular sclerenchyma (Figure 2B), surrounding the open collateral vascular bundles, these are arranged in one open arch to almost circular near the base of the leaf, with one or two accessory bundles (Figures 2C). Both, adaxial and abaxial epidermis show non-glandular uncinuate hairs, around 100 µm in length (Figure 2D), short unicellular stiff hairs, large and unicellular covering hairs (Figure 2E), glandular hairs with a globular 1-2 cellular head (25 µm in diameter) and a unicellular stalk (Figure 2F and 3A); papillary hairs are seen on the blade edge (Figure 2G). On midrib some large hairs can be seen on the epidermis, at level and elevated above (Figure 2H). Adaxial surface of *D. contrajerva* shows reduced amount of hairs.

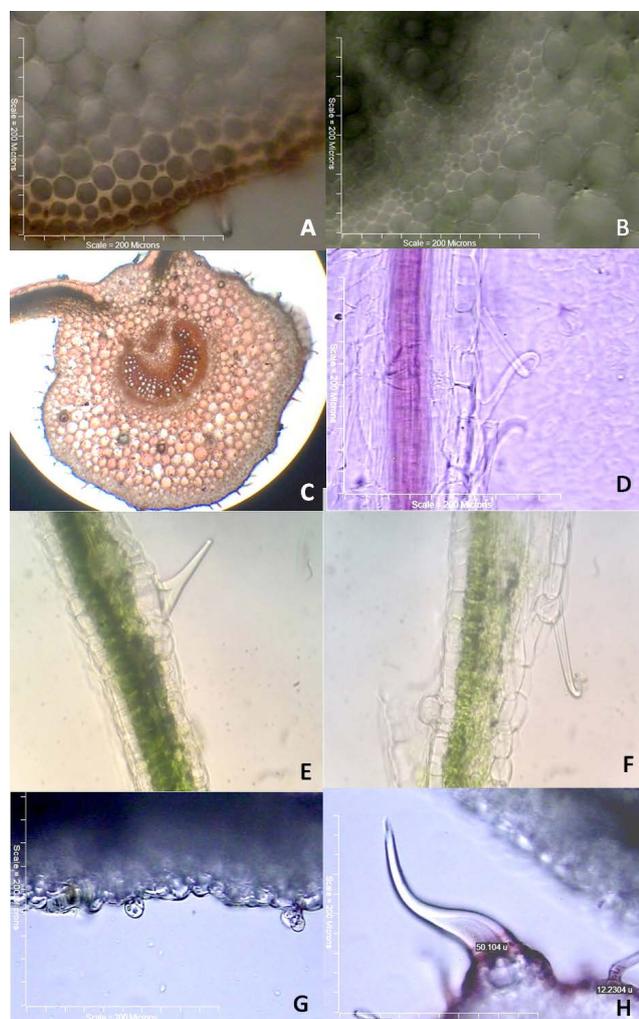


Figure 2. Microscopical characters of *Dorstenia*. A: laminar collenchyma. B: 1-3 cellular sclerenchyma. C: midrib transverse section. D: non-glandular uncinuate hairs. E: short unicellular stiff hair. F: glandular hair with globular head. G: papillary hairs. H: covering hairs at level and elevated above.

On diaphanized leaf, both specimens of *D. contrajerva* showed actinodromous reticulate venation, hypostomatic with aniscytic and anomocytic stomata at epidermis level and sunk (Figure 3A), polygonal shape of epidermal cells in adaxial surface (Figure 3B) and irregular on abaxial one, bulbous base prickly shape and uncinated covering hairs, glandular hairs with a unicellular stalk and a 1-2 cellular head. Unicellular and multicellular rosette-like bases of hairs are seen (Figure 3C). The macerated leaves show xylem tracheas with annular, helicoidal and scalariform wall thickening vessels, sclereids, and fragments of epidermis with and without stomata.

Transversal section of stem and rhizome (Figure 3D), are round or oval shaped, with open collateral vascular bundles in a broken ring (eustele). A polyarch root with wide epidermal cells and uncinated hairs are seen (Figure 3E). Some laticifers can be found between amiliferous parenchyma cells.

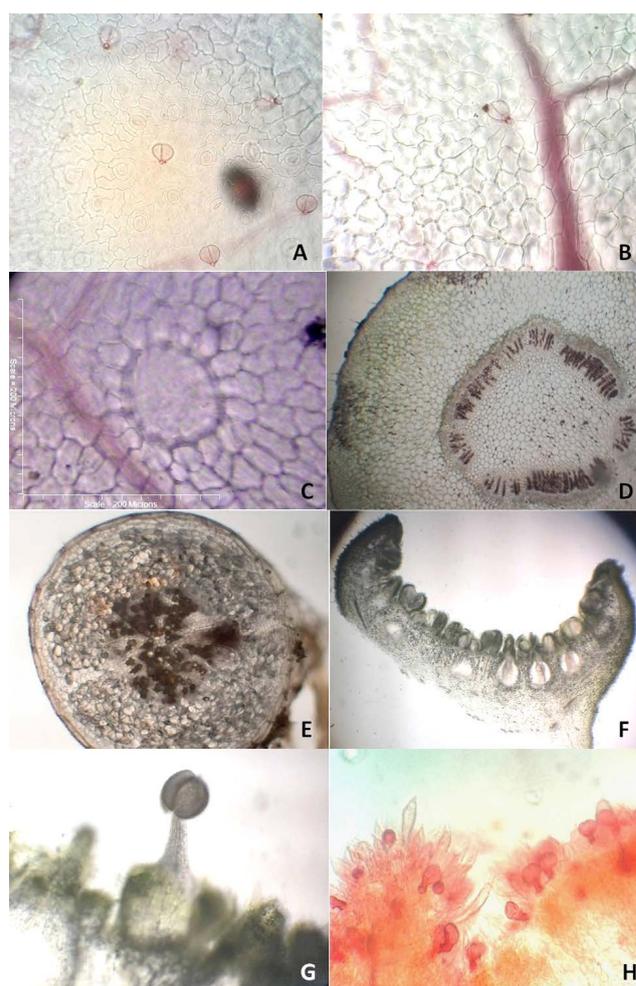


Figure 3. Microscopical features of *Dorstenia*. A: aniscytic and anomocytic stomata at epidermis level and sunk; glandular hairs. B: polygonal shape of epidermal cells in adaxial surface, and glandular hairs. C: multicellular rosette-like bases of hairs. D: transversal section of rhizome. E: polyarch root with wide epidermal cells and uncinated hairs. F: transversal section of the reproductive structure. G: male flowers. H: papillary hairs and pollen grains.

Microscopically, the striking reproductive structure in both specimens is composed of an androgynous receptacle (Figure 3F), where clusters of staminate and carpellate flowers are found. The male ones show two dark tepals (Figure 3G). The inflorescence in longitudinal section shows one

flat, disk-like inflorescence with sunken ovaries of the female flowers, the 2-branched styles, and the anthers of the male flowers. The inflorescence also shows chlorophyll parenchyma and a very hairy epidermal surface with unicellular covering hairs, glandular hairs with a globular 1-2 cellular head and unicellular stalk, and in back side a large amount of unicellular papillary hairs. Pollen grains had globular appearance; verrucose surface and looks polyporate (Figure 3H).

Phytochemical Screening

Table 1 shows a synthesis of the histochemical screening for secondary metabolites on the different structures analyzed for each specimen: alkaloids and tannins were absent, and saponins, starch, fats and oils and mucilage were demonstrated. All the organs analyzed show the presence of the same metabolites.

Metabolite	Reagent	<i>D. contrajerva</i> L.					<i>D. contrajerva</i> var. <i>tenuiloba</i>					
		Leaf	Stem	Rhizome	Root	Inflorescence	Leaf	Stem	Rhizome	Root	Inflorescence	
Alkaloids	Dragendorff	-	-	-	-	-	-	-	-	-	-	-
Saponins	Concentrated Sulfuric Acid	+	+	+	+	+	+	+	+	+	+	+
Tannins	Ferric Sulfate	-	-	-	-	-	-	-	-	-	-	-
Starch (Amylum)	Lugol's Iodine	+	+	+	+	+	+	+	+	+	+	+
Fats and Oils	Sudan IV	+	+	+	+	+	+	+	+	+	+	+
Mucilages	Cresyl Blue 1 %	+	+	+	+	+	+	+	+	+	+	+

Table 1: Histochemical demonstration of metabolites for each plant organ studied.

Coumarin analysis by thin layer chromatography				
Reference solution	<i>D. contrajerva</i> L.	<i>D. contrajerva</i> var. <i>tenuiloba</i>	R _f ^a <i>Dorstenia contrajerva</i> L	R _f ^a <i>Dorstenia contrajerva</i> var. <i>tenuiloba</i>
Coumarins: dark Green fluorescent zone	A dark Green fluorescent zone (coumarin)	A dark Green fluorescent zone (coumarin)	0,6489	0,6489
Coumarins: light Green fluorescent zone	A light Green fluorescent zone (coumarin)	A light Green fluorescent zone (coumarin)	0,2802	0,2802
Umbelliferone: aqua fluorescent zone	An aqua fluorescent zone (Umbelliferone)	An aqua fluorescent zone (Umbelliferone)	0,2138	0,2138

Table 2: Thin layer analysis of coumarins in *D. contrajerva* L. and *D. contrajerva* var. *tenuiloba*.

Coumarin analysis shows the presence of at least three different coumarins, but the lack of specific reference solutions does not allow the identification of each one. Table 2 shows the results of the TLC analysis.

Purity Tests

Moisture and total acid ashes were made by quintuplicate, and the results demonstrated quality of plant materials used in this study, considering that all were between the OMS standards.

Discussion

This study was performed with the main purpose of establishing morphoanatomical characters and histochemical preliminary data of the vegetative organs and the inflorescences of *Dorstenia contrajerva* L. and *D. contrajerva* var. *tenuiloba* (Blake) Standl. & Steyerl. that grow in Guatemala, useful to establish identity quality control standards, especially when they are dry and/or fragmented.

Macroscopic characteristics found for both specimens like herbaceous habits, short stems (brevicaule), alternating phyllotaxis, lobated leaves and green axillaries inflorescences matches those reported for different species of *Dorstenia* from Mexico to South America.¹⁰ Macroscopically, both varieties can be differentiated by the morphology of their leaves and inflorescences. In *D. contrajerva* var. *tenuiloba*, the lobes of the leaf are more pronounced than in *D. contrajerva*; on the contrary, the lobes of the inflorescence are more pronounced in *D. contrajerva*.

Microscopically, the presence of unistratified epidermis with various shapes and sizes of cells and an evident cuticle, 1-cellular palisade parenchyma, and 2-3 cellular spongy parenchyma, as well as the shape of midrib with open collateral bounds surrounded by angular collenchyma, found in this study had been reported by González De García and coworkers for several Moraceae species¹¹ and for *Dorstenia* species by Valente & Carauta.¹² The presence of idioblastic cells in petiole, midrib, stem and root was previously described for other members of the Moraceae family.¹³ The presence of laticifers was also described¹⁴ and in this study they were seen only in rhizome.

Both *Dorstenia* specimens studied show a large number of different hairs, non glandular ones: large and unicellular covering hairs, short unicellular stiff hairs and uncinated hairs. Glandular hairs with a globular 1-2 cellular head, and unicellular stalk and papillary hairs. Most of these hairs had been reported previously for members of Moraceae family,^{15,16} but they were better described by Jacomassi, Moschetti and Machado.¹⁴ Elevated above epidermis hairs seen in this study were not previously reported for this genus. Also, there is no previous report of pluricellular rosette-like bases of hairs, probably because most of the studies do not include diaphanized leaf, but were previously described for other Moraceae species.¹⁵ According to W.Z. Faust and S.B. Jones, Jr., the morphology, and the wide occurrence of trichomes among different taxa, makes them the most useful of all anatomical features for systematic comparisons of Angiosperms.¹⁷ According to Cardona-Peña, Fuentes & Cayola (2005), uncinated and hispidule (stiff) hairs are widespread between Moraceae species.¹⁸

A hypostomatic leaf with anomocytic and anisocytic stomata were found as previously described,^{19,20} and the presence of sunk stomata was also reported by Chantarasuwan *et al.*²¹ The venation pattern founded, matches the actinodromous venation reported for *Dorstenia* genus by Dos Santos (2012).²²

In rhizome and stem, the round shape, the 1-cellular epidermis with evident cuticle and the presence of laticifers between amiliferous parenchyma cells, was previously described by Pilon, Tamaio, & Leo (2013) for this genus,¹ like Morales Martínez (2008)¹³ reported for other Moraceae species, both specimens of *Dorstenia* show polyedric crystals, which occupy nearly the entire lumen of parenchymal cells. The only difference between stem and rhizome was the presence of chlorophyllic parenchyma in the stem. According to Jacomassi and coworkers,¹⁴ the open collateral vascular bundles in a eustele found in rhizome, stem and petiole, have taxonomic relevance in Moraceae family. This arrangement was also described by Valla²³ for other members of Moraceae.

Inflorescence composed by male and female flowers look like other members of *Dorstenia*.²⁴ The flowers show a hairy epidermis with short and large non-glandular hairs and capitate glandular ones. On top, the receptacle shows papilla-like hairs and under it, uncinated and simple ones. No previous report was found. Chlorophyll parenchymas were observed around carpellate feminine flowers and staminate ones were previously described by Datwyer & Weiblen for other Moraceae species.²⁶ The arrangement of the flowers in the receptacle matches the ones described for other members of *Dorstenia* genus.²⁴

Our findings about pollen structure matches with Barth (1985),²⁶ however more detailed studies are needed for better observation of pollen grains from both specimens. We observed a polyporate structure and according to other authors is at least 6-pantoporate.²⁷

Histochemical analysis reveals the presence of starch grains in parenchyma cells of the total of the structures analyzed, especially the leaves. The presence of starches in the leaves matches that observed by Chicas, Paredes, Paz, & Cáceres, (2013), for *Litsea guatemalensis*,²⁸ and according to other authors this is a common finding in evergreen plants and is useful for the photosynthesis process.¹¹ The starch plays an important role in the day-to-day carbohydrate metabolism of the leaf. Thus, leaf starch can be seen as a short-term carbohydrate reservoir and is often termed 'transitory starch', according to Zeeman, Smith and Smith (2007). Plants grown in short days partition relatively more of their photo-assimilates into starch than those grown in long days. Thus the longer the night, the higher the proportion of photo-assimilates stored as starch to provide a supply of sugars during this period.²⁹

A large amount of fats and oils was evidenced in all the structures analyzed like droplets. This matches the observation made by Jacomassi and coworkers who also reported a moderate amount of mucilage in all the structures analyzed, as we observed.¹⁴ A moderate amount of saponins was demonstrated. These can be related to its antiophidic medicinal properties.³⁰

The presence of coumarins was confirmed by TLC, but the lack of specific standards was a limitation for the identification of these. Because of their biological activities, coumarins are very interesting compounds and are widely investigated. Some furanocoumarins have pharmacological activity as Ca-channels blockers, anticoagulants, cytostatics, antitumor, anti-inflammatory, and antifungal drugs.^{30,31} Some substances from this group have photosensibilizing properties and are important drugs in leucodermy therapy. Psoralene derivatives also have the ability to retard DNA synthesis, which is advantageous in the therapy of psoriasis.^{4,5} Coumarins can also be markers for the chemotaxonomic identification of plant species.³² The presence of coumarins in both investigated specimens of *Dorstenia* are consistent with some of the common uses of this herb in traditional medicine.

D. contrajerva L. and *D. contrajerva* var. *tenuiloba* shows morphoanatomical characteristics that can be useful for their quality control, as much as when they are in their natural habitat, like when they are dry or fragmented. Among them we can mention: being small herbs, the curious shape

of their inflorescences, widely simple lobed leaves, actinodromous reticulate venation, as well as their microscopical structures, like the microscopical flowers, the presence of idioblast, large crystals, hypostomatic leaves with anisocytic and anomocytic stomata at level and sunk. Glandular hairs with 1-2 cellular head and unicellular stalk, uncinata, large and small simple hairs, laminar collenchyma and open collateral vascular bundles, simple uncinata and hispidule large and small hairs, and papilla-like hairs found in the studied materials.

The preliminary phytochemical studies are consistent with other authors and more detailed studies are needed to establish correlation between metabolites and medicinal properties of *Dorstenia* Guatemalan specimens.

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