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Metabolite profiling, distribution of secretory structures, and histochemistry in *Curculigo orchioides* Gaertn. and *Curculigo latifolia* Dryand. ex W.T.Aiton

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Abstract: *Curculigo orchioides* and *Curculigo latifolia* (Hypoxidaceae) have been widely used as traditional medicines in Indonesia and other Asian countries for antihyperglycemic, aphrodisiac, antioxidant, and antimicrobial treatments. This work aimed to determine the distribution of secretory structures and metabolites. Metabolite profiling approach of the plant organs was determined by UHPLC-Q-Orbitrap-HRMS. Histochemical-based techniques on rhizome, root, petiole, and leaf, with transversal sections on fresh samples, were prepared using a razor blade to determine the secretory structures of the plants, followed by plant anatomy procedures. Histochemical analysis was carried out using several reagents to detect the metabolites. Metabolite profiling revealed several classes of compounds, i.e. phenolics, alkaloids, terpenes, essential oils, and lipophilic. Secretory cavities and idioblast cells, were detected in both species and localize a diverse metabolites. Additionally to the specialized structures, hypodermis, epidermis, intercellular spaces, and cuticle also contain some of those compounds. The secretory structures spread over the different organs. This discovery may be useful to distinguish particular organs as medicine source, which does not only depend on the availability of rhizome. The secretory structures and the chemical compounds of the two species, described herein for the first time, can be used further in plant identification purposes based on chemical markers.

Key words: Hypoxidaceae, intracellular secretory structures, medicinal plant, metabolomics, metabolites resources, secondary metabolite

1. Introduction

Two medicinal plants, known as *Curculigo orchioides* Gaertn. and *C. latifolia* Dryand. ex W.T.Aiton, are widely distributed in China, Japan, Malaysia, India, and Australia (Zuo et al., 2010; Ishak et al., 2013). In Indonesia, both species grow from Sumatera to Papua islands. *C. orchioides* is more familiar to people who collect it's rhizome as the source of medicinal compounds. It inflicts to the species extinction in the future while people do not cultivate the plant, whereas *C. latifolia* grows more extensively. These species have been known as traditional medicinal plants that are widely used also by people in India, China, and Pakistan (Nagesh and Shanthamma 2016), with diverse pharmacological activities, such as antihistamines, anti-carcinogenic, antihyperglycemic, aphrodisiac, immunosuppressant, antioxidant, hepatoprotective, antidiabetic, hypolipidemic, antimicrobials (Ishak et al., 2013; Nie et al., 2013; Murali and Kuttan, 2015; Sharma and Singh, 2017). This recognition is due to their secondary compounds. Plants produce secondary metabolites for defense and protection purposes, phytohormones, and many others. Some of those secondary metabolites have been extracted to produce drugs, vaccines, food preservatives, coloring agents, fragrances, and other purposes (Calvo et al., 2020).

Metabolomics studies revealed that the rhizome of both species contains many compounds, such as phenolic and phenolic-glycosides, lignans and lignan-glycosides, triterpenes and triterpenoid-glycosides, flavones, eudesmanes, alkaloids, and aliphatic compounds (Zuo et al., 2010; Nie et al., 2013; He et al., 2015). Several in vitro and in vivo assays with chemical compounds extracted

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from the rhizome of *C. orchioides* show pharmacological effects. The metabolomics analysis (non-targeted) could identify rapidly and accurately metabolites in a complex biological sample. The analytical instrumentation; GC, GC-MS, LC-MS/MS, is often used to identify the metabolites of medicinal plants to obtain both qualitative and quantitative data (Liu et al., 2015; Li et al., 2020).

Medicinal plants can be characterized through physiological and histochemical studies to investigate the types and distribution of secretory structures in the plant organs and the major chemical compounds they acquired. The results will give us knowledge on which organs the sites of their accumulation are, mainly if the medicinal plants will be used in the fragmented form. Also, it helps in determining the authenticity of a bioactive containing plant. The known plant secretory structures are trichome, idioblast, secretory cavities, secretory canal, laticifer, and osmophores (Kuster and Vale 2016; Naidoo and Naidoo 2018; De Godoy et al., 2019; Almeida et al., 2020). Studies related to the distribution of secretory structures and the histochemical analysis of *Curculigo* species (Hypoxidaceae) are still limited. For the first time, we have performed a metabolomics analysis to identify the metabolites and their distribution more comprehensively.

This work aimed to analyze the major secondary metabolites contained in the rhizome, root, petiole, and leaf organs of *C. orchioides* and *C. latifolia* by metabolites profiling technique. At the same time, we studied the distinctive secretory structures in those organs, their distribution and histochemistry, provide an overview of the accumulation sites of those compounds.

2. Materials and methods

2.1. Plant material

Plant samples were collected from two regencies (Barru and Sinjai) of South Sulawesi (Celebes Island), Indonesia. The sampling locations of *Curculigo* spp. (Supplement Figure 1) and the plant morphology of *Curculigo orchioides* Gaertn. (Figures 1a–1c) and *Curculigo latifolia* Dryand. ex W.T. Aiton (Figures 1d–1f) are presented. All samples were identified based on the published flora and voucher specimens. The plant specimens were deposited at the Herbarium Bogoriense, Research Center for Biology, the Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia, as voucher specimen number 184 (*C. latifolia*) and number 288 (*C. orchioides*).

2.2. Putative identification of metabolites by UHPLC-Q-Orbitrap-HRMS

This qualitative analysis referred to the method developed by He et al. (2015) with some modifications. About 50 mg of dried extract was ultrasonically extracted (Branson Ultrasonic Corporation, Connecticut, USA) with 1.5 mL methanol (Merck, Darmstadt, Germany) 30 °C for 15 min. The mixture was then filtered through a 0.2 μm syringe

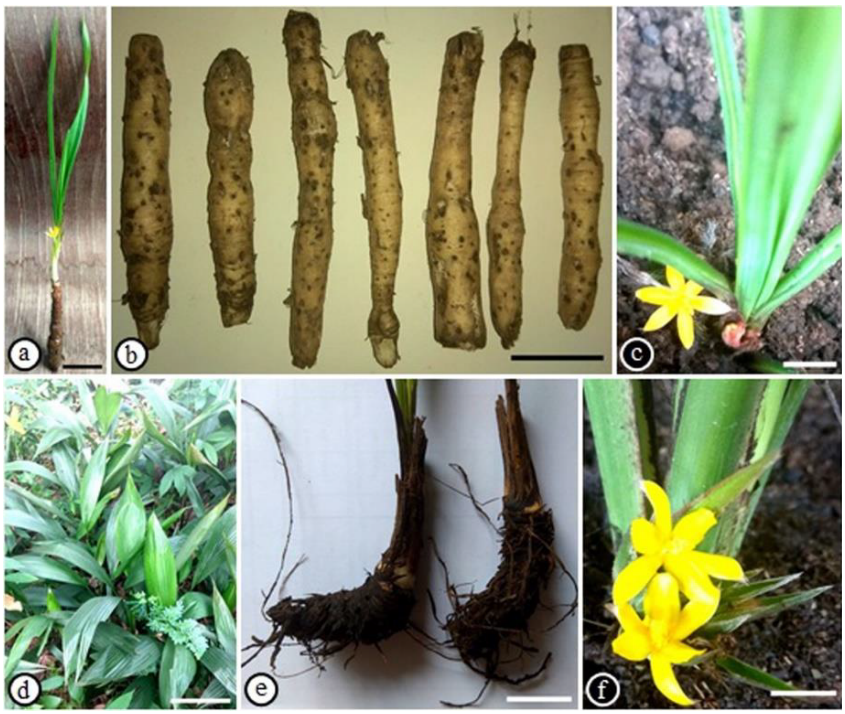

Figure 1. Plant morphology, rhizomes and flowers of *C. orchioides* (a, b, c) and *C. latifolia* (d, e, f). Bars ≈ 1.2 cm (a, f), ≈ 1 cm (c, d), ≈ 1.5 cm (e), ≈ 2 cm (b).

filter membrane (SY25TF PTFE mdi), and the filtrate was collected in a vial. The analysis of chemical compounds was conducted using a UHPLC-Q-Orbitrap-HRMS (Thermo Fisher Scientific, Kandel, Germany) and ESI sources. The separation was performed using 2.6 µm LC columns (2.1 mm × 100 mm), with a UV detector at 254 nm. The flow rate from the delivery system was set at 0.300 mL min^{-1}, the autosampler temperature was maintained at 10 °C, and the sample injection volume was 0.5 µL. The mobile phase consisted of (A) 0.1% fFormic acid (Merck, Darmstadt,Germany) in water and (B) acetonitrile (Merck, Darmstadt, Germany). A linear gradient elution program applied was as follows: 0–1.5 min (5% B), 1.5–9 min (5–10% B), 9–13 min (10–20% B), 13–17 min (20–28% B), 17–23 min (28–78% B), 23–26 min (70–95% B), 26–29 min (95% B), and 29–32 min (5% B). Putative compounds were analyzed using the MS-DIAL ver. 4.16 (Tsugawa et al., 2015). A heatmap was constructed by applying MetaboAnalyst 4.0. (https://www.metaboanalyst.ca/) (Chong et al., 2019).

2.3. Anatomical characterization

Secretory structures were identified on fresh materials and fixed in 70% ethanol. Transversal and longitudinal sections of the rhizome, root, petiole, and leaf were made manually using a razor blade. The sections were stained with 1% safranin O (CI 50240; Merck, Darmstadt, Germany) (Bukatsch 1972), and with 1% methylene blue plus 1% Azur II in a 1% aqueous solution of sodium tetraborate (Merck, Darmstadt, Germany) (Sulborska 2013). All samples were then mounted in 1:1 (v/v) water/glycerin on glass slides with a coverslip. Observations and photos were made using an Olympus microscope CX21Led and an OptiLab Viewer ver:2.1 (Olympus Corporation, Tokyo, Japan).

2.4. Histochemical characterization

The histochemical specimen was also examined using light microscopy. Various histochemical tests were applied to investigate the metabolite compounds that exist in the secretory cells, and elsewhere. Phenolic compounds were investigated using 10% ferric chloride (Merck, Darmstadt, Germany), and the positive reaction would be indicated by blackish-green or black color (Johansen 1940). The samples were placed in a freshly made reagent for 15–20 min.

Alkaloids were analyzed using Dragendorff's reagent (Merck, Darmstadt, Germany), which generates a gold/orange-colored product with alkaloids (Brechú-Franco et al., 2016), and Wagner reagent (Merck, Darmstadt, Germany) gives a positive result when reddish-brown color appears (Furr and Mahlberg, 1981). The samples were placed for 20–30 min in each of the reagents.

Essential oils and terpenes were detected by NADI reagent [α-naphtol (Merck & Co., Darmstadt, Germany) and N,N-Dimethyl-*p*-phenylendiamine (FUJIFILM Wako Chemicals, Osaka, Japan)]. The terpenes stained purple and the essential oil blue in color. The samples were placed for 14–45 min in a freshly prepared reagent (David and Carde, 1964). To reconfirm the existence of terpenoid compounds, we also used 5% cupric acetate (Merck, Darmstad, Germany), and the positive result would give reddish-brown or brownish-yellow color in the tissue (Harborne, 1998).

Lipophilic (steroids, oils, grease, and fats) were tested using Sudan III reagent (Merck, Darmstadt, Germany), and positive results appeared when the color turned to orange (Sass 1951); Sudan IV reagent (Merck, Darmstadt, Germany) gave positive results (with lipids, triglycerides, and lipoprotein) when it produced red, yellow or orange color (Boix et al., 2011). The samples were mixed with each of the reagents, at 40 °C in a water bath for 30–40 min.

3. Results

3.1. Metabolites profiling

Putative identification of metabolites in both species was performed to determine the chemical classes contained in the plant, and also the similarities and differences in their composition and concentration levels. Chromatogram profiles showed different patterns in each species and organ, and the compounds identified in the rhizome (ROB) and leaf (LOB) of *C. orchioides*, and in the rhizome (RLS), leaf (LLS), and petiole (PLS) of *C. latifolia* are presented in Supplement Table 1. The results of positive and negative modes were 77, 88, 49, 86, and 47, respectively. These detected compounds were of the group aliphatic hydroxy ketones, alkaloids, alkanes hydrocarbons, benzyl benzoate glucosides, cycloartane (triterpene glycosides), furans, geranyl acetone, lignans, norlignans, norlignan glycosides, phenolics, phenolic glycosides, sitosterols, steroids, and triterpene glycosides. The most abundant chemical classes, namely phenolics and phenolic glycosides, were accumulated in the rhizome (ROB) and leaf (LOB) of *C. orchioides*, respectively; *C. latifolia* contained phenolics in high amount in the rhizome (RLS) and in the leaf (LLS) (Figure 2). The compounds found in positive (a) and negative (b) ions of the heatmaps model (Figure 3) varied in the intensity in regard of plant parts of both species.

The compounds identified from the phenolic class in both samples were orcinol glucoside, curculigoside B, curculigine A, syringic acid, and vanillin. Orcinol glucoside (m/z 287.1125, ion mode $[M+H]^+$), was fragmented into m/z 269.1019 $[M+H-H_2O]^+$,which indicates the release of H_2O molecules, followed by m/z 125.0597 ($[M+H-C_6H_8O_4]^+$) and m/z 109.0647 $[M+H-O^+]^+$ due to the release of $C_6H_8O_4$ and O^+ atom, respectively (Supplement Figure 2a). Curculigoside B (m/z 453.1391, ion mode $[M+H]^+$) belongs to the phenolic compound

Table 1. Histochemical identification of secondary substances in *Curculigo orchioides* and *Curculigo latifolia*.

Chemical compounds	Reagent	*Curculigo orchioides*		*Curculigo latifolia*	
		Location	Organ	Location	Organ
Phenolics	Ferric chloride	Epithelium cells of secretory cavities and intercellular spaces	Rhizome	Epitheliumcells of secretory cavities and intercellular spaces Epithelium cells of secretory cavities and intercellular spaces Idioblast and hypodermis	Rhizome Petiole Leaf
Alkaloids	Dragendorff's	Idioblast and lumen of secretory cavities	Rhizome	Idioblast, epithelium of secretory cavities and lumen of secretory cavities Epithelium of secretory cavities and lumen of secretory cavities	Petiole Rhizome
	Wagner's	-	-	Lumen of secretory cavities Idioblast	Rhizome Root
Terpenoids	Cupric acetate	Idioblast	Rhizome	Idioblast	Rhizome
	NADI	Cuticle, idioblast (adaxial epidermis), and idioblast (hypodermis)	Leaf	Idioblast (bulliform), idioblast (abaxial epidermis) Cuticle, idioblast, and intercellular spaces	Leaf Petiole
Essential oils	NADI	Idioblast	Rhizome	Epithelium of secretory cavities Cuticle and idioblast	Rhizome Petiole
Lipophilics	Sudan III	Idioblast (epidermis and hypodermis)	Leaf	-	-
	Sudan IV	Idioblast and intercellular spaces	Rhizome	-	-

Note: - negative reaction

class. It was fragmented into m/z 435.128) $[M+H-H_2O]^+$, which indicates the release of H_2O molecules, followed by m/z 291.0863 $[M+H-C_6H_8O_4]^+$, and m/z 273.0757 $[M+H-H_2O]^+$ indicates the loss of $C_6H_8O_4$ and H_2O molecules (Supplement Figure 2b). Curculigine A with m/z 527.1092 in ion mode $[M-H]^-$, which was fragmented at m/z 511.0779 $[M-H-CH_4]^-$, indicates the loss of CH_4. Then it was fragmented again into m/z 376.0356 [M–H–C6H8O4]$^-$ and m/z 204.9828 $[M-H-C_6H_{10}O_5]^-$, indicating the release of $C_6H_8O_4$ and $C_6H_{10}O_5$, respectively (Supplement Figure 2c). Syringic acid (m/z 199.0600, ion mode $[M+H]^+$) was fragmented at m/z 181.0495 $[M+H-H_2O]^+$ and m/z 153.0546 $[M+H-CO]^+$, indicating the release of H_2O and CO molecules (Supplement Figure 2d); vanillin compounds (m/z 153.0546, ion mode $[M+H]^+$) were fragmented into m/z 135.0440 $[M+H-H_2O]^+$ and then into m/z 123.0440 $[M-H-C]^+$, which proves the release of H_2O and C atom (Supplement Figure 2e).

The compound identified from the norlignan class was curcapital (m/z 313.0706, ion mode $[M+H]^+$), which was fragmented at m/z 293.0444 $[M+H-CH_4]^+$ and then at 281.0444 $[M+H-C]^+$, indicating the release of CH_4 and C atom (Supplement Figure 2f). Crassifoside A (m/z 475.1234, ion mode $[M+H]^+$) belongs to the norlignan class. It was fragmented at m/z 457.1129 $[M+H-H_2O]^+$ and m/z 313.0706 $[M+H-C_6H_8O_4]^+$, which indicates the release of H_2O and $C_6H_8O_4$. Then, it was fragmented again at m/z 295.0600 $[M+H-H_2O]^+$ with the loss of H_2O molecule (Supplement Figure 2g).

The substance identified from the alkaloids class was lycorine (m/z 288.1230, ion mode $[M+H]^+$), fragmented at m/z 270.1124 $[M+H-H_2O]^+$, which indicates the release of H_2O molecule. Then, it was fragmented again at m/z 258.1124 $[M+H-H_2O]^+$ and 252.1019 $[M+H-C]^+$, indicating the release of H_2O and C atom (Supplement Figure 2h).

One of the compounds identified from the triterpenoids class was curculigosaponin (C, G, and M). Fragmentation patterns representing these compounds are summarized in Supplement Figure 2i, m/z 963.5523, ion mode $[M+H]^+$ was fragmented at m/z 801.4999, 639.4466 and became 459.3832 $[M+H-C_6H_{12}O_5]^+$ with the loss of three $C_6H_{12}O_5$ molecules.

Lipophilic (steroids) compound is stigmasterol (m/z 413.3777, ion mode $[M+H]^+$), which was fragmented at m/z 395.3672 $[M+H-H_2O]^+$, which was indicated by the

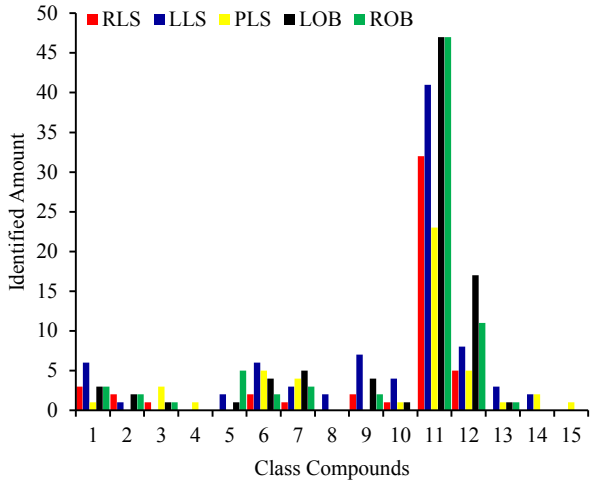

Figure 2. Metabolites detected in the extract of rhizomes, leaves, and petioles of *C. orchioides* and *C. latifolia*. ROB (rhizome orchioides from Barru), RLS (rhizome latifolia from Sinjai), LOB (leaves orchioides from Barru), LLS (leaves latifolia from Sinjai), PLS (petiole latifolia from Sinjai). Class compounds: aliphatic hydroxy ketones (1), alkaloid (2), alkane (hydrocarbon) (3), benzyl benzoate glucosides (4), cycloartane (triterpene glycosides) (5), furan (6), geranyl acetone (7), lignan (8), norlignan (9), norlignan glycosides (10), phenolic (11), phenolic glycosides (12), sitosterol (13), steroid (14), and triterpene glycosides (15).

loss of H_2O molecules. Then, it was fragmented again at m/z 313.2525 $[M+H–C_7H_{14}]^+$ through the release of C_7H_{14} (Supplement Figure 2j). Daucosterol compound (m/z 575.4317, ion mode $[M–H]^-$) was fragmented at m/z 557.4211 $[M–H–H_2O]^-$ and 413.3788 $[M–H–C_6H_8O_4]^-$ by the release of H_2O and $C_6H_8O_4$, and then it became 395.3682 $[M–H–H_2O]^-$ due to the release of H_2O molecules (Supplement Figure 2k).

3.2. Secretory structures and distribution

3.2.1. Rhizome

Transversal sections of the rhizomes of both species (Figures 4a and 4b) show secretory cavities with ellipsoidal or rounded in shape, outlined by slightly thickened cell walls. The secretory cavities of *C. orchioides* (Figure 4a) were located among the vascular bundles, while those of *C. latifolia* (Figure 4b) were found surrounding the vascular bundles in various sizes, and form a circular pattern. This type of secretory structure in *C. latifolia* was more numerous and distributed in a regular pattern, distinctive from that in *C. orchioides*. The epithelium lining the cavities was composed of single and double layer(s) of cells in these two species. The cells were identified as flattened and tightly packed around the lumen (Figures 4c–4f). The secretory cavities in the rhizome of both species can be categorized as lysigenous type.

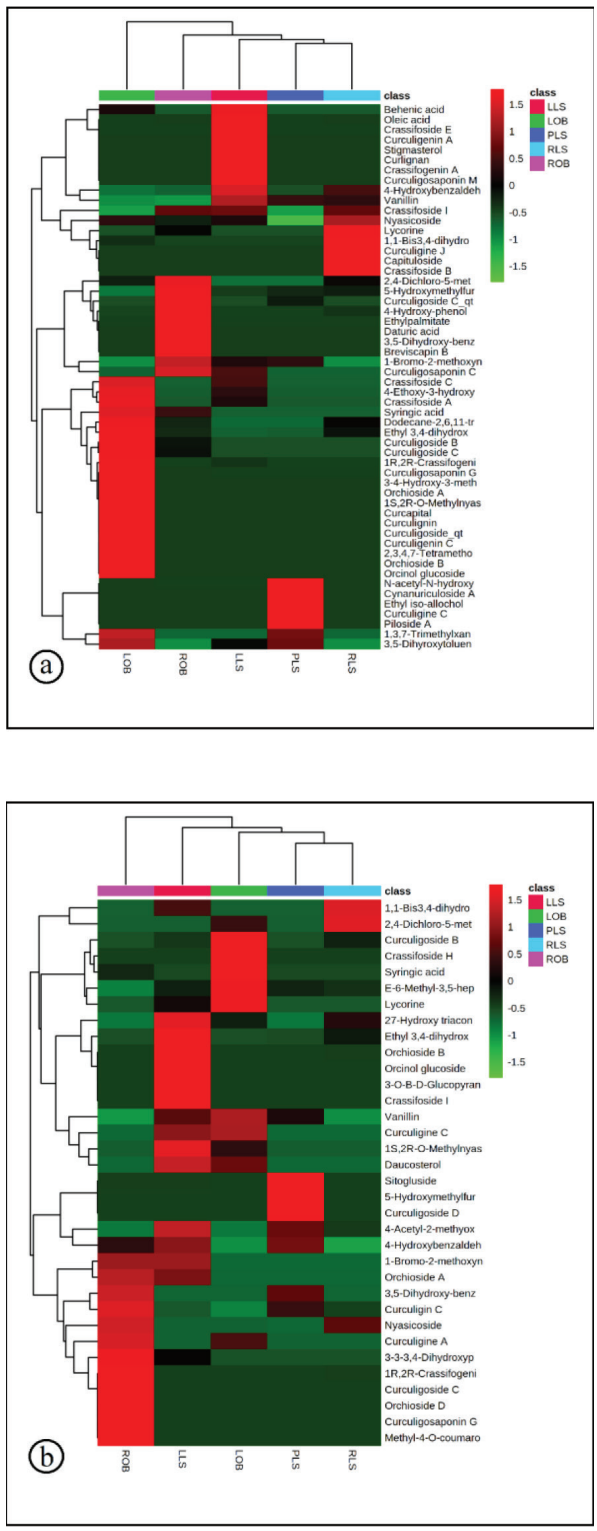

Figure 3. Heatmaps demonstrating the correlation among chemical compounds in the extracts of *C. orchioides* and *C. latifolia* in 70% ethanol and their relative abundance. Positive mode (a) and negative mode (b). The color scale differentiates the values: high (red), moderate (black), and low (green).

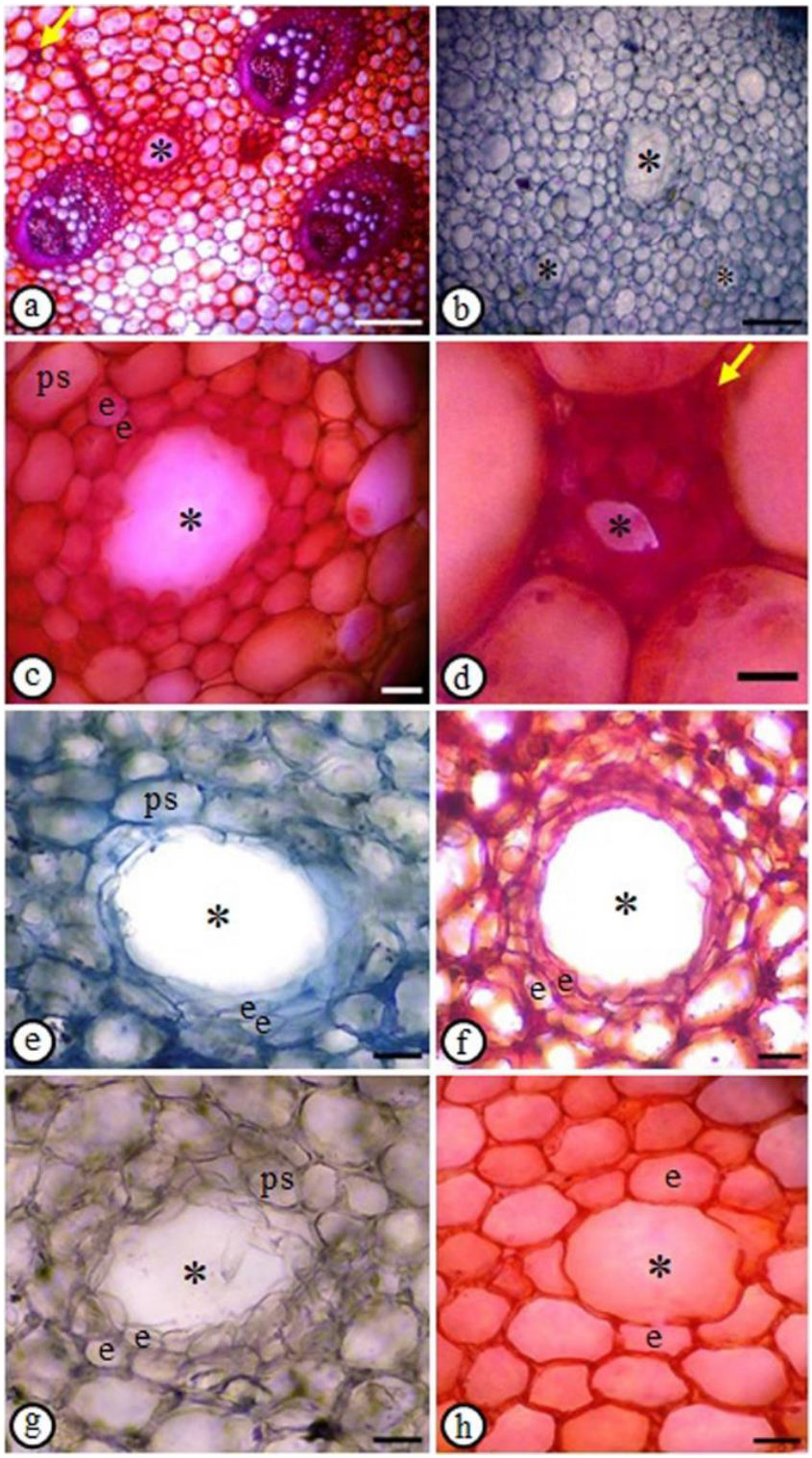

Figure 4. Transversal sections on rhizome with secretory cavities (asterisk and arrow) in *C. orchioides* (a) and in *C. latifolia* (asterisks) (b). Detailed view of the secretory cavity in the rhizome of *C. orchioides* with two (c) and single (d) layered epithelium cells. Detailed secretory cavities of *C. latifolia* with two-layered epithelium cells (e and f). Secretory cavity in *C. latifolia* root, with two (g) and single (h) layered epithelium cells. Samples in (a, c, f, h) were stained with safranin O; (b) and (e) were stained with methylene blue with Azur, and (g) without staining. Bars ≈ 160 µm (a, b), ≈ 40 µm (c, h), ≈ 65 µm (d), ≈ 50 µm (e, f, g). Abbreviations: e = epithelium cell, ps = parenchyma sheath, vb = vascular bundle.

3.2.2. Root

The secretory cavities were also found in the roots of *C. latifolia*, with double (Figure 4g) and single (Figure 4h) layer(s) of epithelium cells. The secretory cavities in the root can be categorized as lysogenous.

3.2.3. Petiole

The petioles of *C. orchioides* and *C. latifolia* present a uniseriate epidermis, covered by a thick cuticle (Figures 5a and 5b). The transversal section of *C. orchioides* demonstrates secretory cavities in different sizes, rounded and oval shapes, surrounded by single epithelial cells (Figures 5c and 5d). They look as lysigenous type. The round-shaped cells are more numerous than the ovals. The secretory structures were also found in *C. latifolia* (Figures 5e and 5f). They scatter among the vascular bundles and are categorized as lysogenous type.

3.2.4. Leaf

The leaves of *C. orchioides* (Figure 6a) and *C. latifolia* (Figure 6b) present a uniseriate epidermis, covered by a thick cuticle. We observed numerous secretory cavities in the lamina transversal sections of *C. orchioides* (Figure 6c) and

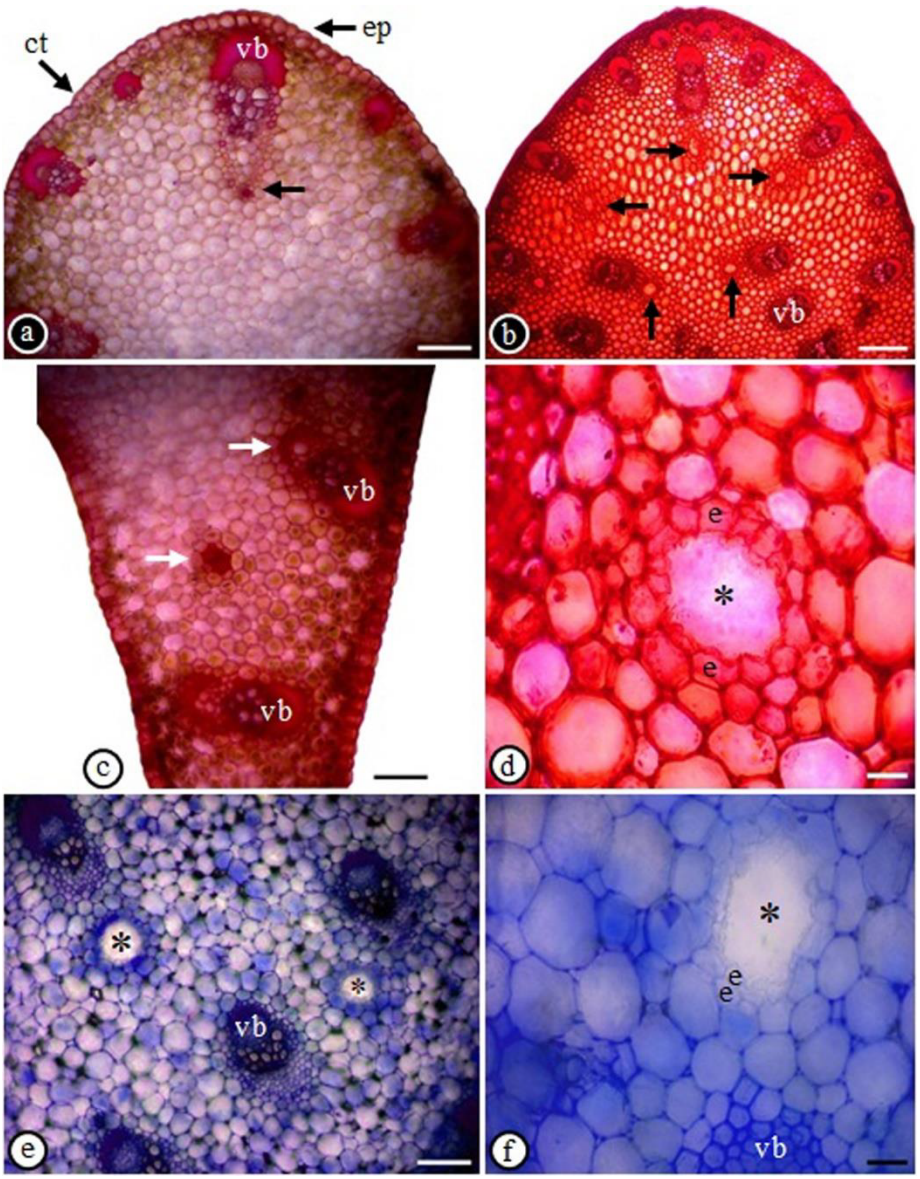

Figure 5. Transversal sections on petiole of *C. orchioides* (a) and of *C. latifolia* (b), showing secretory cavities (arrows). Secretory cavities in *C. orchioides* (arrows) (c) were viewed in details surrounded by single epithelial cells (asterisk) (d). Secretory cavities in *C. latifolia* (asterisks) (e) were observed in detail (f), having two-layers of epithelium cells. Figures (a-d) were stained with safranin O, and (e, f) with methylene blue and Azur. Bars ≈ 100 µm (a, b, c, e), ≈ 75 µm (d, f). Abbreviations: ct = cuticle, e = epithelium cell, ep = epidermis, vb = vascular bundle.

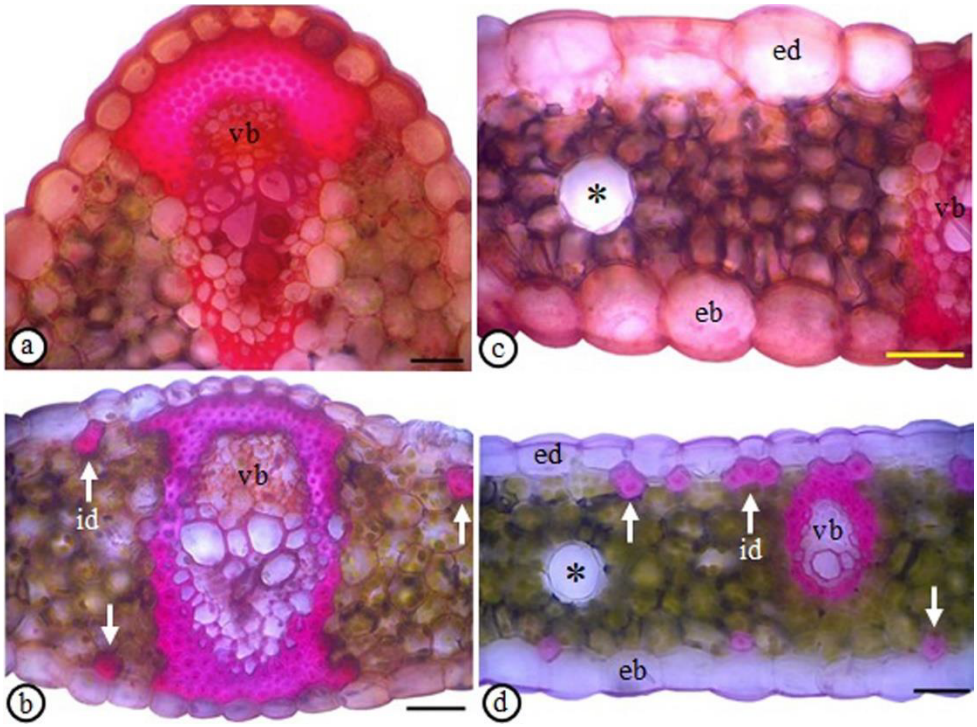

Figure 6. Transversal sections of *C. orchioides* and *C. latifolia* leaves. Detailed view of vascular bundle in the midvein of *C. orchioides* (a), and *C. latifolia* (b) with idioblast cells (arrows). Secretory cavity (asterisk) in the lamina of *C. orchioides* (c), and secretory cavity (asterisk) and idioblasts in the lamina of *C. latifolia* (d). All samples were stained with safranin O. Bars ≈ 90 µm (a), ≈ 125 µm (b), ≈ 145 µm (c), ≈ 100 µm (d). Abbreviations: eb = abaxial epidermis, ed = adaxial epidermis, id = idioblast cell, vb = vascular bundle.

C. latifolia (Figure 6d), which are outlined by a single layer of epithelium cell and round in shape. Idioblast cells were detected only in *C. latifolia* (Figures 6b and 6d).

3.2.5. Histochemical analysis

Phenolic compounds were identified in the transversal section of *C. orchioides* rhizome, i.e. in some secretory cavities along with the epithelium cells around them and in the intercellular spaces (Figure 7a), indicated by the blackish-green or black color after being treated with ferric chloride reagent. The color was absent in the sample without reagent that served as control (Figure 7b). Meanwhile, we did not find phenolic compounds in the petiole nor the leaf. The application of this reagent on *C. latifolia* demonstrated that phenolic compounds reside in the secretory cavity, in the epithelium cells, and in the intercellular spaces of the rhizome (Figure 7c), while the control tissues are shown in Figure 7d. In *C. latifolia*, these substances were also detected in the epithelium cells and intercellular spaces of the petiole (Figure 7e); Figure 7f was the control for this section. In addition, phenolics were detected in idioblast cells (Figure7g) and also in hypodermis tissue (Figure 7h) of the leaves. The control tissues without reagents is shown in Figure 7i.

Alkaloids in *C. latifolia* were identified using Dragendorff's reagent, and they were detected in the petiole, i.e. in the idioblast cells (Figures 8a–8d), in the lumen (Figure 8b) and in the epithelium cells around the secretory cavities (Figures 8a, 8b, and 8e). The control of the petiole section is presented in Figure 8f. These substances were also found in *C. latifolia* rhizome, i.e. in the epithelium cells (Figures 9a and 9b) and in the lumen of the secretory cavity (Figure 9b); Figures 9c and 9d represent the controls. In *C. orchioides*, alkaloids were detected only in the rhizome, where the idioblast cells (Figures 9e and 9f) and cavities lumen (Figure 9g) resulted in yellowish-brown colour. The control for *C. orchioides* rhizome is presented in Figure 9h. The detection of alkaloids was also carried out by adding Wagner reagent as an alternative. This reagent reconfirmed the presence of alkaloid substances in the lumen of the secretory cavity of *C. latifolia* rhizome (Figures 10a and 10b) and unveiled the substances in the idioblast cells of its roots (Figure 10c). Figures 10d and 10e represent their control. However, this reagent did not detect alkaloids neither in the petiole or leaf of *C. latifolia* nor in all parts of *C. orchioides*.

Treatment with cupric acetate revealed that terpenes were present in the rhizomes, i.e. in the idioblast cells of *C.*

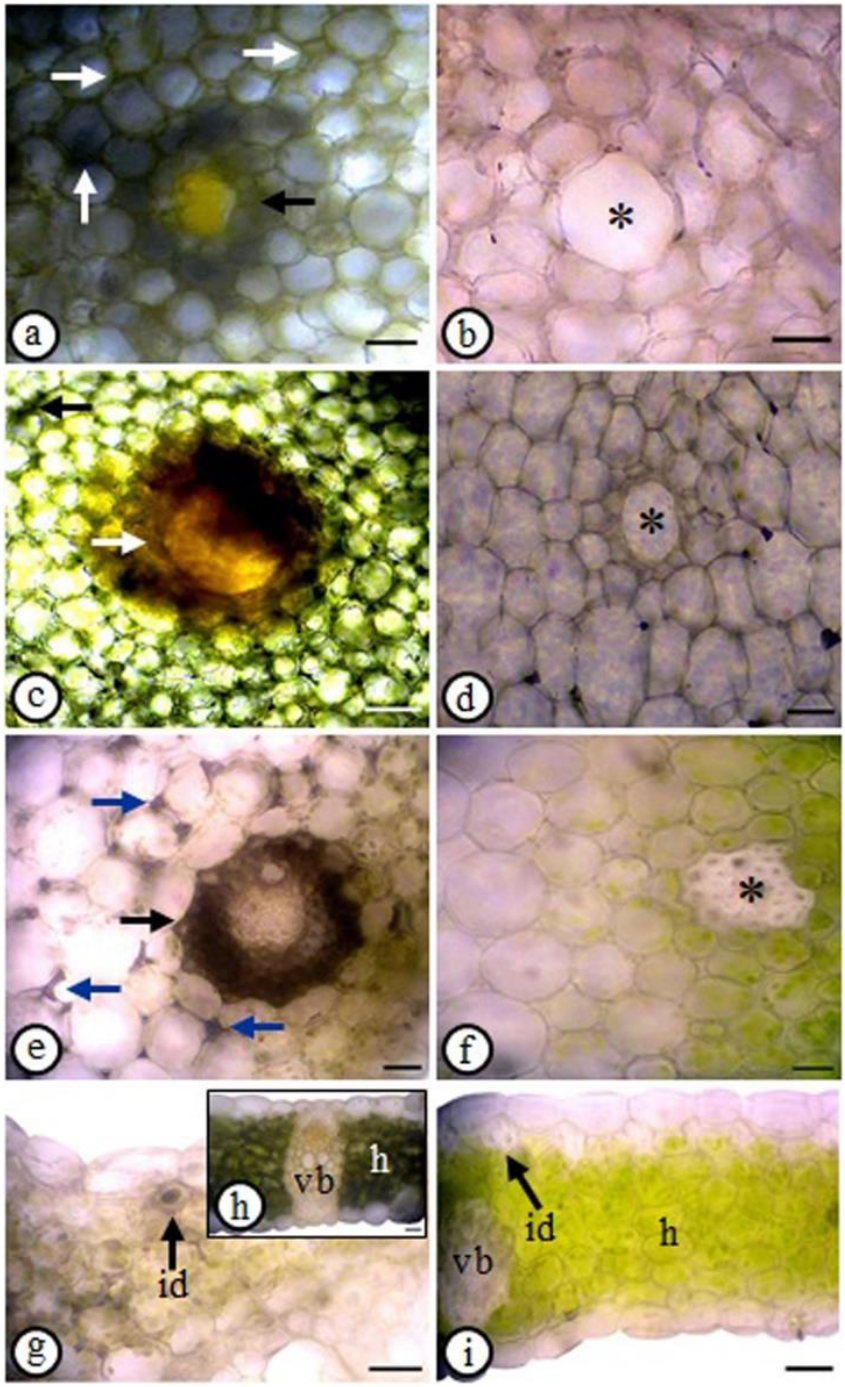

Figure 7. Phenolics evidence in transversal sectionsof rhizome, petiole and leaf treated with ferric chloride. Rhizome of *C. orchioides*: in the intercellular spaces (white arrows) and in the epithelium cells of secretory cavity (black arrow) (a), and (b) the control without reagent. Rhizome of *C. latifolia*: in the secretory cavity (white arrow) and in the intercellular spaces (black arrows) (c), and (d) the control without reagent. Petiole of *C. latifolia*: in the secretory cavity (black arrow) and in the intercellular spaces (blue arrows) (e), and (f) the control for this section. Leaf of *C. latifolia*: in the idioblast cells and hypodermis tissue (g and h), and (i) the control without reagent. Bars ≈ 50 µm (a, d, e, f, i), ≈ 75 µm (b, c, g), ≈ 15 µm (h). Abbreviations: h = hypodermis, id = idioblast cell, is = intercellular spaces, vb = vascular bundle.

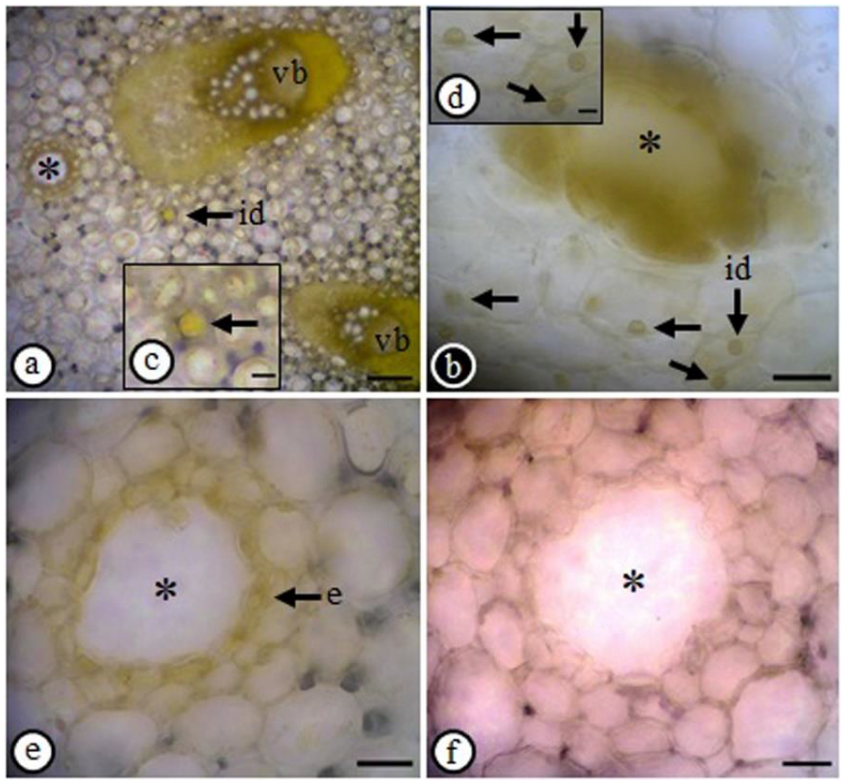

Figure 8. Alkaloids evidence in the transversal sectionsof *C. latifolia* petiole. Alkaloids were detected in idioblast cells (arrows) (a and b) with detailed views in (c and d), in the epithelium of the secretory cavities (asterisk) (a, b, e), and in the lumen of the secretory cavity (asterisk) (b). The yellowish-brown color indicates alkaloids with Dragendorff's reagent. (f) represents the control without Dragendorff's reagent. Bars ≈ 85 µm (a, b), ≈ 20 µm (c), ≈ 15 µm (d), ≈ 50 µm (e, f). Abbreviations: e = epithelium cell, id = idioblast cell, vb = vascular bundle.

latifolia (Figures 11a and 11b) and of *C. orchioides* (Figure 11c). The control tissues without reagent are presented in Figures 11d and 11e, respectively. When NADI reagent was used, it was apparent that idioblasts and cuticle layers, mainly at the leaf adaxial site of *C. orchioides* (Figure 12a), and idioblasts situated among the leaf hypodermal tissue (Figures 12b and 12c) contained terpenoid compounds. In *C. latifolia*, those substances were also found in the idioblasts among the leaf bulliform cells (Figure 12d) and among the abaxial epidermal tissues (Figures 12e and 12f). They also exist in the cuticle of the leaf margin (Figure 12f). Additionally, we found terpenoids in idioblast cells, spreading around the vascular bundle and in the intercellular spaces of *C. latifolia* petioles (Figures 12g and 12h). The color was absent in the samples without reagent (Figures 12i and 12j).

Essential oils were detected after reacting with NADI reagent. The essential oils in the rhizome were accumulated in the idioblast cells of *C. orchioides* (Figure 13a) and in the epithelium cells of *C. latifolia* (Figure 13b); their controls are shown in Figures 13c and 13d, respectively. We also detected the essential oils in the idioblast cells, which are sparsely distributed below the epidermal layer and in the cuticle (Figure 13e) of *C. latifolia* petiole.

Assays to detect lipophilic compounds using Sudan IV showed positive results only in the rhizome of *C. orchioides*, and the substances accumulated in the idioblast cells which are spread over the cortex parenchyma and in the intercellular spaces (Figures 14a–14d), indicated by a reddish-brown color. With Sudan III reagent, we observed the presence of lipophilic compounds in the idioblast cells at the abaxial and adaxial leaf epidermal layers of *C. orchioides* (Figure 14e). These substances were not detected in *C. latifolia*. Figure 14f represents the control for rhizomal tissue without Sudan IV, and Figure 14g is that of leaf tissue without Sudan III.

Table 1 presents a resume of the histochemical identification, the specialized cells, and their localization in the organs of both species.

4. Discussion

The secondary compounds identified in the rhizome of *C. orchioides* have been reported to have pharmacological effects. Phenolics, such as orcinol glucoside has an

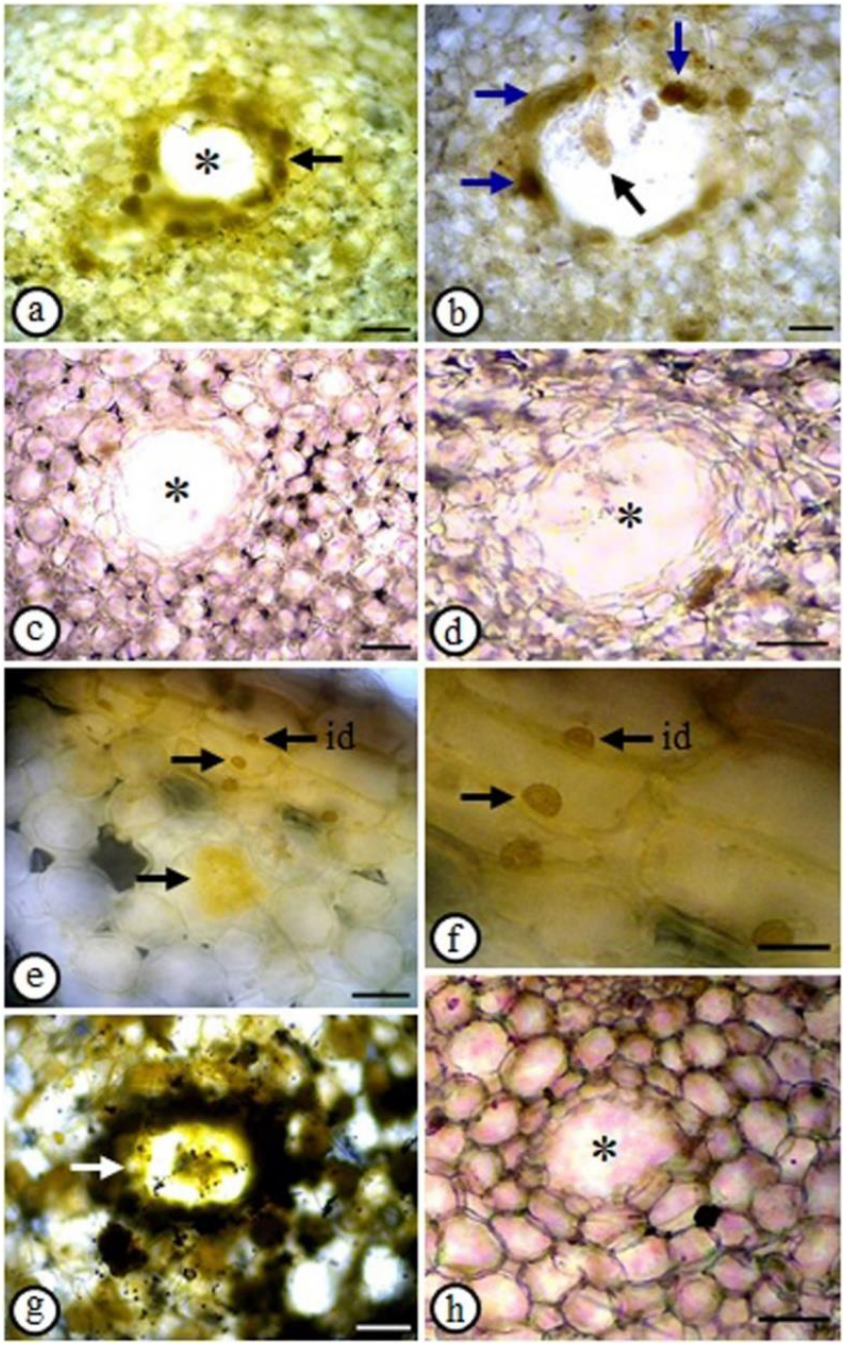

Figure 9. Transversal sections of *C. latifolia* rhizome with alkaloid substances in the epithelium cells (arrows) (a, b), and in the lumen of the epithelium cells (black arrow) (b); the control for (a) and (b) are presented in (c) and (d), respectively. Transversal sections of *C. orchioides* rhizome with alkaloids in the idioblast cells (arrows) (e)with detailed view in (f) and in the lumen (arrow) of the secretory cavity (g); and (h) the control for (e, g). They were resulted by Dragendorff's reagent and control without the reagent. Bars ≈ 75 µm (a, c, e, g), ≈ 45 µm (b), ≈ 150 µm (d, f), ≈ 125 µm (h). Abbreviation: id = idioblast cell.

immunostimulatory effect (Lakshmi et al., 2003); curculigoside demonstrates an anti-arthritic effect (Tan et al., 2019); curculigoside B and curculigine A function as antiosteoporotic (Ma et al., 2011; Wang et al., 2012); syringic acid and vanillin are used as antioxidant and as standards in several antioxidant studies (Xu et al., 2020). Norlignan class, i.e. curcapital, crassifoside A, acts as an antioxidant (Wang and Li 2008; Wang et al., 2010). Lycorine, an alkaloid, has antioxidant and α-glucosidase inhibition activities (Ghane et al., 2018). Triterpene,

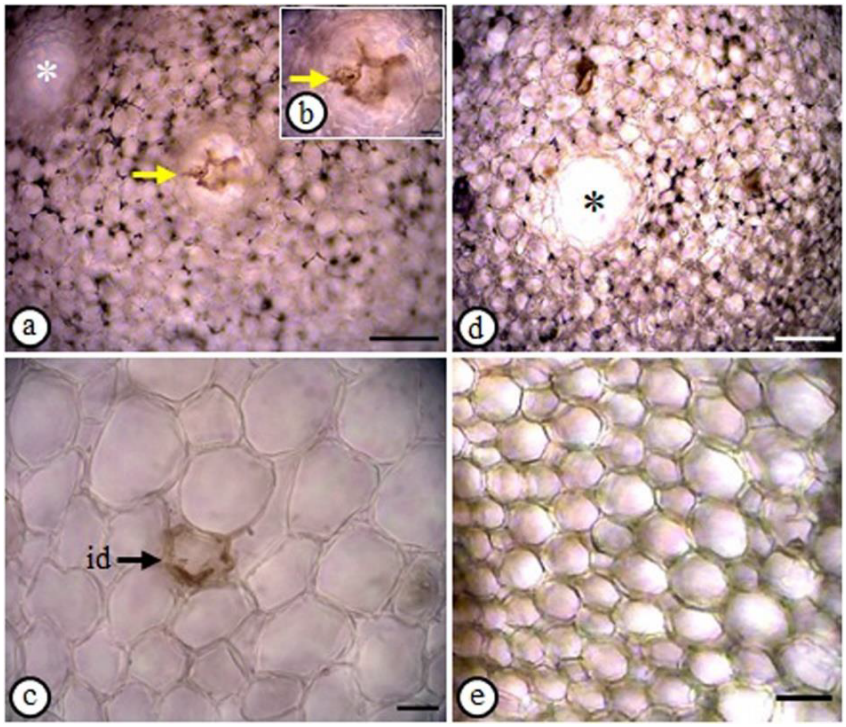

Figure 10. Alkaloids revelation by Wagner's reagent in the transversal sections of *C. latifolia* rhizome: in the lumen of a secretory cavity (arrow) (a) and its detailed view (b). Transversal section of root: alkaloids in the idioblast cell (arrow) (c). Rhizome and root tissues (d, e), respectively, treated with tartaric acid as the controls. Bars ≈ 100 µm (a), ≈ 25 µm (b), ≈ 50 µm (c), ≈ 75 µm (d, e). Abbreviation: id = idioblast cell.

such as curculigosaponin, has been reported to increase the proliferation of spleen lymphocytes significantly (Nie et al., 2013). Lipophilic compounds stigmasterol (steroids) function as antiparasite (Bansal et al., 2020), and daucosterol has been proven to block prostate cancer growth (Gao et al., 2019). Those substances were also identified in *C. latifolia* through metabolites profiling in this study.

Based on the compounds classes identified, Figure 2 revealed that *C. latifolia* is richer in secondary compounds than *C. orchioides*. It has aliphatic hydroxy ketones, alkane (hydrocarbon), benzyl benzoate glucosides, furan, lignan, norlignan, and steroid triterpene glycosides.

Rudall et al., (1998) first published a report on the anatomical structures of *Curculigo* (Hypoxidaceae) on five species, i.e. *C. latifolia*, *C. orchioides*, *C. villosa*, *C. pilosa*, and *C. recuvata*. It suggested that the species of *C. orchioides* has mucilage canals in the rhizome and petiole. It has also been reported by Tiwari and Gupta (2018) that *C. orchioides* possesses secretory cavities of lysigenous type in the rhizomes. This experiment ensures that *C. latifolia* and *C. orchioides* have secretory structures in the forms of secretory cavities and idioblasts. These structures were generally found near the vascular tissues and demonstrated distinct shapes from the other cells surrounding them. The secretes (metabolites) may be released to the surface of the gland, intercellular spaces, or stored in the secretory cells themselves (Demarco 2017). The secretory cells may consist of a small single cell to a large group of cells. The study also shows that the secretory structures of both species are predominated by the cavities, with two layers of epithelium cells. The presence of epithelium cells in both species indicates that lysigenous development occurred to construct the cavity (Sulborska 2013; Retamales, Scherson, and Scharaschkin 2014; Bartoli et al., 2015). Epithelium cells may have the role of an envelope and protect the secretory cavities from damages (Sulborska 2013).

The histochemical approach was carried out using several reagents because the same gland or even the same secretory structure may store up several different types of metabolites simultaneously (Demarco 2017; da Silva et al., 2019), and different secretory structures in the same organ may produce or accumulate different substances too (Kuster and Vale 2016). Histochemical tests carried out on the rhizome, petiole, leaf, and root organs revealed several substances in the secretory cavities, in idioblasts, and even in common tissues such as mesophyll and epidermis, and in intercellular spaces. The substances include phenolics,

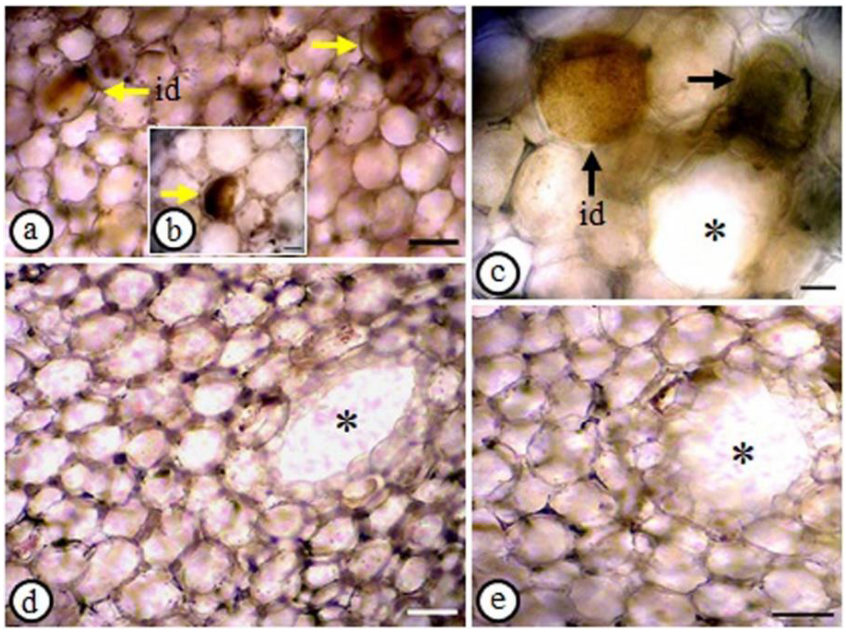

Figure 11. Terpenoids evidence in the transversal sections of rhizome treated with cupric acetate: in the idioblast cells (arrows) (a) and its detailed view (b) of *C. latifolia*, and in the idioblast cells (arrows) (c) of *C. orchioides*. (d) and (e) the control for (a) and (c), respectively. Bars ≈ 50 µm (a), ≈ 15 µm (b), ≈ 45 µm (c, d), ≈ 75 µm (e). Abbreviation: id = idioblast cell.

alkaloids, and terpenes. In *C. orchioides*, Kamalam and Nithya (2019) found those compounds mainly in leaves, namely epidermal tissue, palisade, and leaf vascular bundles, while, in rhizome, they were detected only in vascular bundles. Those compounds are well known as secondary metabolites, which are normally used in the defense and adaptation mechanisms of the plants to the environment, and for human beings, they are essential as medicinal ingredients (Palermo et al., 2017; Silva et al., 2017; Genovese et al., 2020).

Phenolic substances were detected in the epithelial cells around the secretory cavities, idioblasts, intercellular spaces, and hypodermis tissue and identified at the specific organs of both species. The phenolic compounds are variable, generally found in almost all plant parts and resided in the vacuole, cytoplasm or in the cell wall of the vascular bundle and ducts cells, cavities, trichomes, idioblast, laticifers, colleters, nectaries, osmophores and stigma (Castro and Demarco, 2008). Phenolic compounds are secondary metabolites consisting of phenol groups, containing one or more hydroxyl groups on one or several aromatic benzene rings (Castro and Demarco, 2008). It explains that phenolic compounds are synthesized through several different routes. Even though phenolic compounds exist in almost all plant tissues, only few idioblast cells were observed containing these substances. Histochemical tests may not always result as expected because specific reagents recognize only certain groups of compounds. Ferric chloride will recognize well certain flavonoid groups in the form of chelates with hydroxyl groups at the positions 3, 5, 3', and 4'. These sites are between the 3-hydroxyl and 4-oxo, the 5-hydroxyl and 4-oxo, and the ortho-hydroxyl group in the B-ring (Mira et al., 2002), just like catechol type phenols (Rodriguez et al., 2018). Thereby, the phenolic compounds identified in this study may be only of certain phenolic group.

Dragendorff's reagent's positive reaction indicates the existence of tertiary amine of alkaloids in the secretory structures (Takagi et al., 1980). Dragendorff's reagent presents an excellent resolution for identifying alkaloid substances compared to the other reagents (Gomez et al., 2019). While Wagner's reagent interacts with the nitrogen atom of alkaloid compounds through a covalent bond with potassium ion of this reagent (McMurry and Fay 2003), the alkaloids detected in the lumen of secretory cavities and idioblast in the rhizome of *C. latifolia* may form such a covalent bond. Alkaloids were detected in these species and localized in the epithelium and lumen of secretory cavities and idioblast cells. The epithelial cells of the secretory cavities probably have an important role in secreting materials into the lumen that serves as the accumulation site (Rodrigues and Machado, 2012; Sá et al., 2016). Alkaloids are composed of nitrogen and several other groups, and some are very toxic (da Silva et al.,

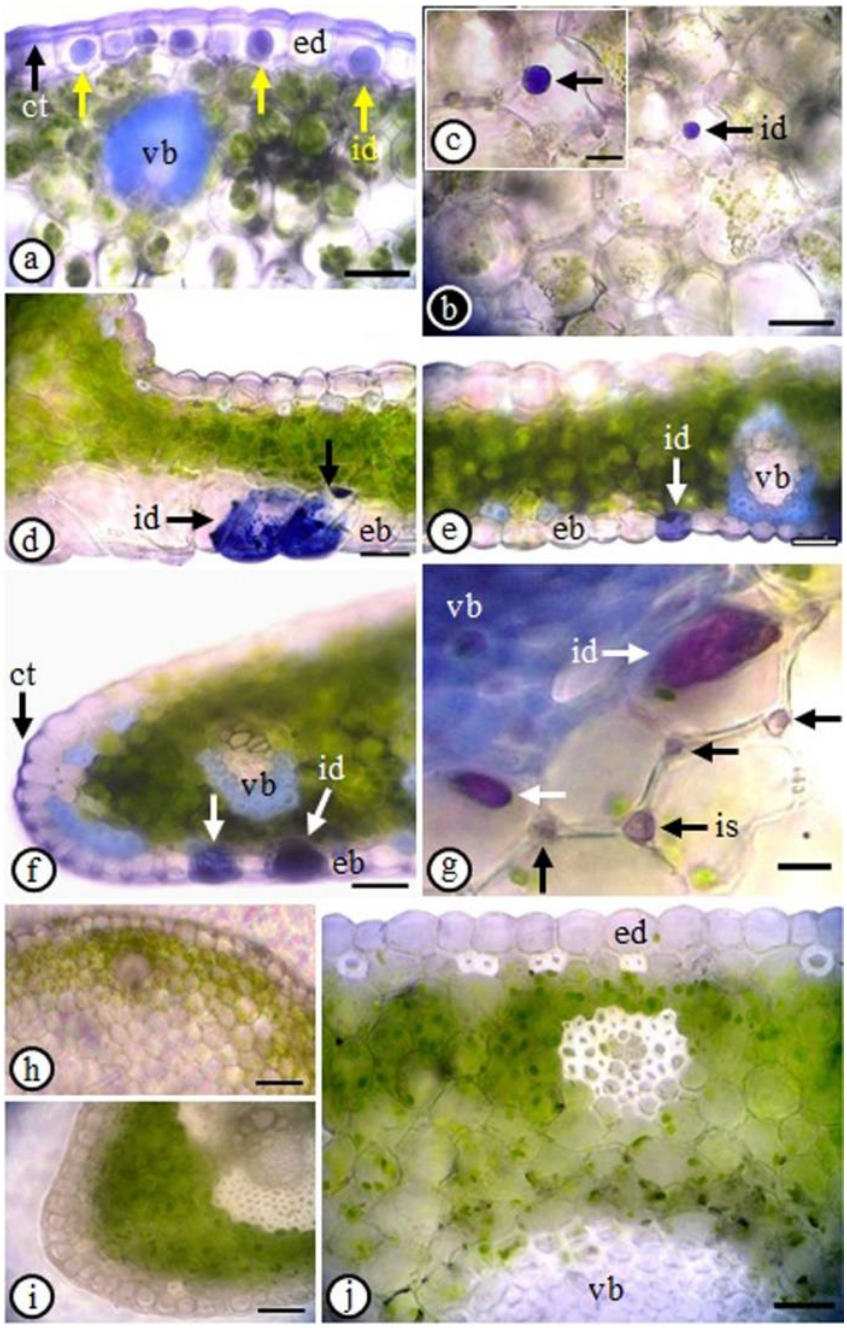

Figure 12. Terpenoids in transversal sections of leaves and petioles treated with NADI reagent. *C. orchioides* leaves: in the cuticle layer (black arrow), and in the idioblasts (yellow arrows) among the adaxial epidermal cells (a), in idioblast cell (arrow) among the hypodermal tissue (b) and its detailed view (c). *C. latifolia* leaves: in the idioblasts among the bulliform cells (d), among the abaxial epidermis (e, f), in the cuticle layer (arrow) at the leaf margin (f). *C. latifolia* petiole: in the idioblast cells (white arrows) and in the intercellular spaces (black arrows) (g). (h) the control without NADI reagent for *C. orchioides* leaves, (i) the control for *C. latifolia* leaves and its petiole (j). Bars ≈ 90 µm (a, b), ≈ 50 µm (c, g), ≈ 80 µm (d, f), ≈ 65 µm (e), ≈ 40 µm (h, i), ≈ 85 µm (j). Abbreviations: ct = cuticle, eb = abaxial epidermis, ed = adaxial epidermis, id = idioblast cell, is = intercellular spaces, = vb vascular bundle.

2019). Alkaloids are generally used as self-defense against predators and have been known to cause many cases of intoxication in animals, including humans (da Silva et al., 2019).

NADI reagent produces a blue or purple color due to the presence of oxygenated or lipophilic (terpenoid) compounds in the essential oils, located in the secretory structures, such as channels, trichomes, secretory cavities,

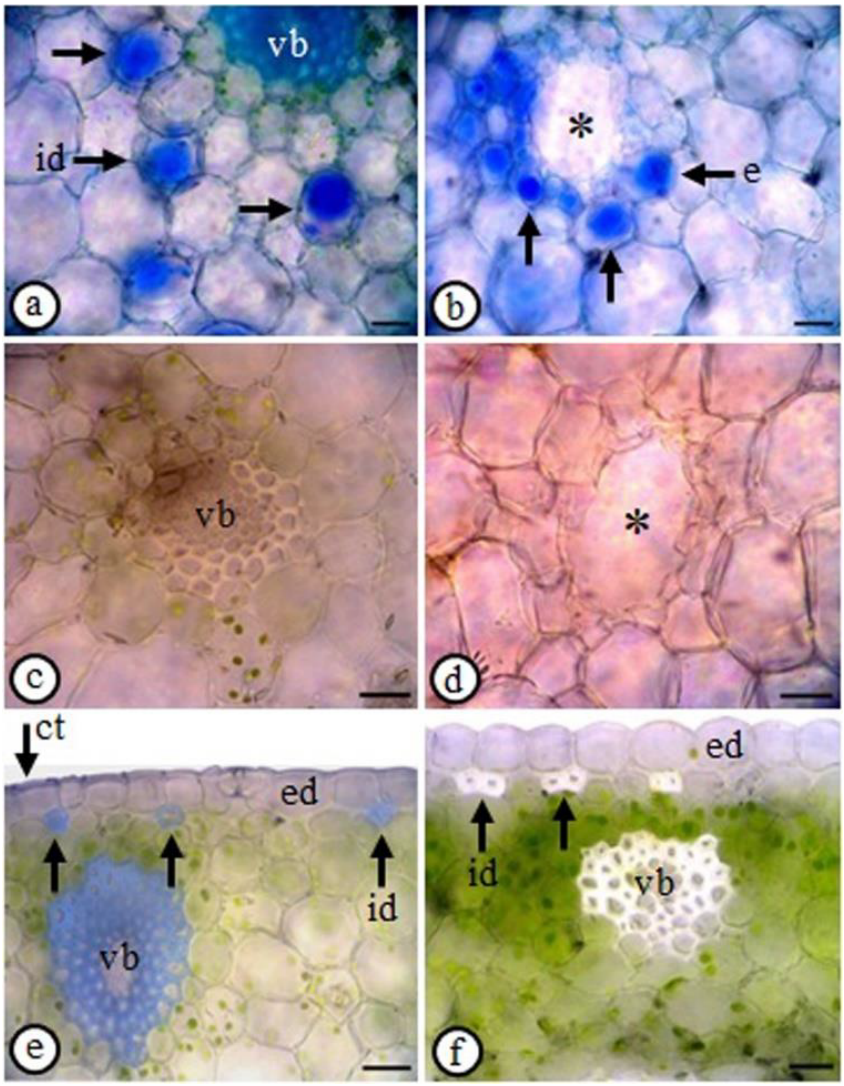

Figure 13. Essential oils in the transversal sections of rhizome and petiole treated with NADI. *C. orchioides* rhizome: in the idioblast cells (arrows) (a); *C. latifolia* rhizome: in the epithelium cells (arrows) around the secretory cavity (b). (c, d) are sections without NADI reagent as the control for (a, b), respectively. *C. latifolia* petiole: in the idioblast cells and in the cuticle (arrows) (e), and (f) the control for this section without NADI reagent. Bars ≈ 50 µm (a, b, f), ≈ 75 µm (c, d), ≈ 100 µm (e). Abbreviations: ct = cuticle, e = epithelium cell, id = idioblast cell, vb = vascular bundle.

secretory ducts, idioblast and others (Bezerra et al., 2018; Rodriguez et al., 2018). This study revealed the existence of terpenoids in idioblast cells, spreading over some parts of the rhizomes, leaves, and petioles, in cuticle and hypodermis of the leaves and petioles.

Idioblast is an intracellular secretory structure, which is a place of secretion and accumulation of essential oils or resinous oils, phenolics, and terpenoids (Garcia et al., 2014; Lange, 2015; Kuster and Vale, 2016; da Cunha Neto et al., 2017). Furthermore, terpenes and essential oils in this study were also detected in common leaf tissues, such as cuticle and hypodermis, and even in the intercellular spaces. In general, the components of essential oils can be divided into two distinct groups of chemical constituents, i.e. hydrocarbon terpenes (monoterpenes, sesquiterpenes, and diterpenes) and oxygenated terpenoids (mainly aldehydes, alcohols, esters, phenols, ketones and oxides) (Zengin and Baysal, 2014). Essential oils can consist of a large quantity (more than 100) terpenoids and hydrocarbon terpenes, whereas terpenes only refer to single form of monoterpenes, sesquiterpenes, or diterpenes (Máthé, 2015). Previous studies (Ibanez et al., 2010; Jezler et al., 2013) reported that *Trollius europaeus* and *Alpinia zerumbet*, accumulate terpenes and essential oils in those tissues. Those reconfirming facts explain the functions of terpenoid substances in direct and indirect defenses

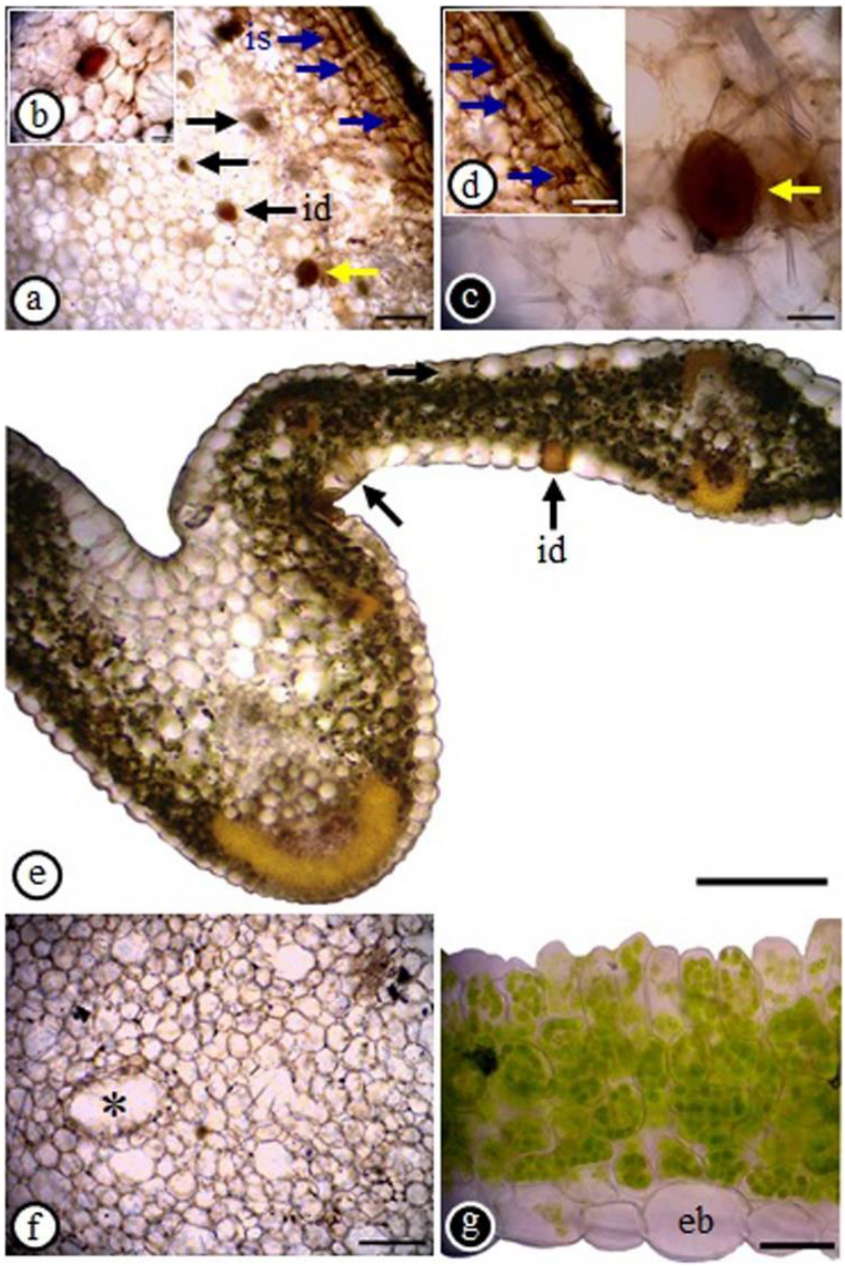

Figure 14. Lipophilic substances in the transversal sections of rhizome and leaf of *C. orchioides.* In the rhizome's cortex parenchyma, lipophilics were found in the idioblast cells (black arrows) (a) with their detailed view in (b and c), and in the intercellular spaces (blue arrows) (a) with the detailed view in (d), after treatment with Sudan IV reagent. Reaction with Sudan III shows lipophilic in the idioblast cells (arrows) (e) among the leaf adaxial and abaxial epidermis. (f) and (g), the controls without Sudan IV in rhizomal tissue and without Sudan III in leaf tissue, respectively. Bars ≈ 75 µm (a), ≈ 15 µm (b), ≈ 65 µm (c), ≈ 45 µm (d), ≈ 150 µm (e), ≈ 135 µm (f, g). Abbreviations: eb = abaxial epidermis, id = idioblast cell, is = intercellular spaces, vb = vascular bundle.

against herbivores, as wound signaling and resistance to abiotic stress (Kromer et al., 2016), while essential oils serve as insect antifeedants, allelochemicals, phytoalexins, and pheromones (Żuraw et al., 2014).

Lipid compounds in the plant might occur in the form of sterols, fats, waxes, mono-, di- and triglycerides, phospholipids, and others, which have specific functions, such as storing energy, signaling, and acting as structural components of the cellular membrane (Kromer et al., 2016). Lipophilic were found in the idioblast cells in the rhizome and leaf. Several factors may influence the qualitative and quantitative lipophilic compounds, i.e. environmental

factors, stressors related to climate change (drought, UV-B radiation, and heat). These substances play an immediate defense against UV-B in leaf. Also, lipophilic are related to plant oxidative damage and protection against pathogens (Dias et al., 2019).

Morphologically, *C. latifolia* has a bigger posture than *C. orchioides*. The former plant is more adaptive to lowland and more frequently found than the other one. Table 1 proves that *C. latifolia*'s organs, rhizome, leaf, and petiole, are rich in phenolics, alkaloids, terpenoids, and essential oils (except lipophilic) that widespread over those organs, in the specialized structures and non-specialized ones. Through metabolites profiling, the data indicate that its leaves contain curculigoside B, vanillin, crassifoside A, lycorine, curculigosaponin C and M, stigmasterol, and daucosterol; petioles are rich in vanillin, and rhizomes have vanillin, curculigoside B, and lycorine. Leaves are the organ accumulating those compounds more abundantly. These facts suggest that the exploitation of rhizome, petiole, and root for medication can be avoided, for the sake of the plant conservation.

In conclusion, the predominant secretory structures of *C. orchioides* and *C. latifolia* are secretory cavities (including the lumen and epithelium cells) and idioblast cells. They are located in the root, rhizome, petiole, and leaf organs. Those secretory structures accumulate secondary metabolites, i.e. phenolics, alkaloids, and terpenoids, essential oils, and lipophilic compounds. Some substances are stored in common tissues, i.e. leaf cuticle, epidermis, and hypodermis, as well as in the leaf and rhizome's intercellular spaces. Apart from the rhizome, leaves are richer in secondary compounds that can be used as alternative sources for medicinal raw materials.

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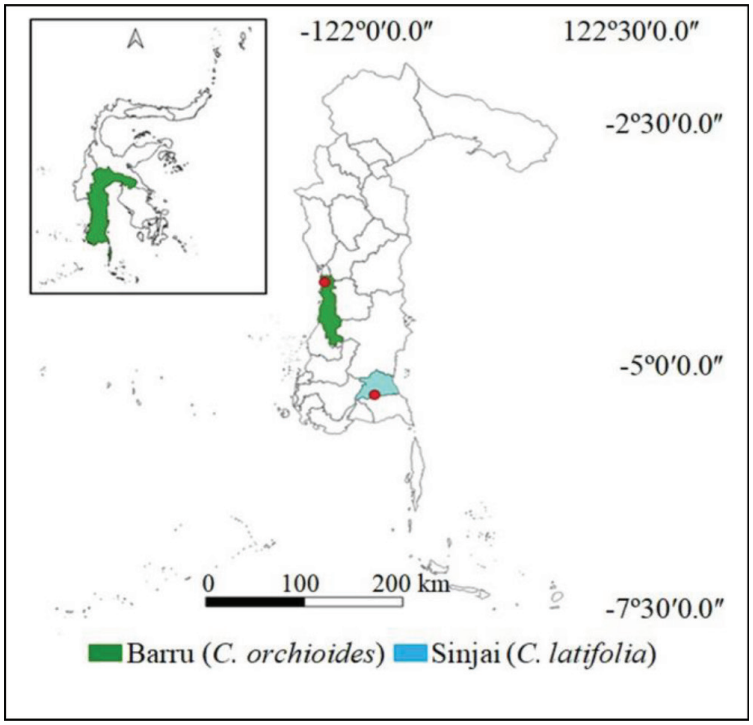


Supplement Figure 1. Location map of South Sulawesi Province, Indonesia, and two districts where the samples were collected.

Supplement Table 1. Metabolites detected in the extract of rhizomes, leaves, and petioles of the *C. orchioides* and *C. latifolia*. ROB (rhizome orchioides from Barru), RLS (rhizome latifolia from Sinjai), LOB (leaves orchioides from Barru), LLS (leaves latifolia from Sinjai), and PLS (petiole latifolia from Sinjai).

ROB

ID	RT [min]	m/z	Type	Metabolite name	Formula	Chemical type
1835	22.390	111.1167	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
282	0.981	111.1168	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
28	0.377	111.1169	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
29	0.377	111.1170	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
417	3.358	111.1170	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
1825	22.143	111.1170	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
2433	25.022	111.1170	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
397	8.108	121.0285	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
1170	20.971	121.0285	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
3156	31.189	121.0285	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
11	0.395	121.0286	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
286	1.095	121.0286	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
373	5.661	123.0077	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
382	6.019	123.0077	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
662	13.780	123.0078	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
65	0.756	123.0405	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
854	13.765	123.0437	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
578	11.259	123.0438	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
1077	16.200	123.0439	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
944	15.200	123.0442	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
298	1.047	125.1326	$[M+H]^+$	3,5-Dihyroxytoluene	$C_7H_8O_2$	Phenolic
3646	31.311	127.1114	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
32	0.377	127.1116	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
117	0.887	134.1174	$[M+H]^+$	N-acetyl-N-hydroxy-2-carbamic acid methylester	$C_4H_7NO_4$	Alkaloid
3161	31.189	151.0392	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
3305	30.898	153.0440	$[M+2H]^{2+}$	Vanillin	$C_8H_8O_3$	Phenolic
3306	30.897	153.0444	$[M+H]^+$	Vanillin	$C_8H_8O_3$	Phenolic
1350	21.846	153.1276	$[M-H]^-$	3,5-Dihydroxy-benzoic acid	$C_7H_6O_4$	Phenolic
1397	22.266	153.1276	$[M-H]^-$	3,5-Dihydroxy-benzoic acid	$C_7H_6O_4$	Phenolic
3550	31.033	155.1176	$[M+H]^+$	3,5-Dihydroxy-benzoic acid	$C_7H_6O_4$	Phenolic
3551	31.032	155.1177	$[M+H]^+$	3,5-Dihydroxy-benzoic acid	$C_7H_6O_4$	Phenolic
1956	22.807	155.1178	$[M+H]^+$	3,5-Dihydroxy-benzoic acid	$C_7H_6O_4$	Phenolic
612	12.014	183.0647	$[M+H]^+$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
374	5.661	197.0448	$[M-H]^-$	Syringic acid	$C_9H_{10}O_5$	Phenolic
663	13.780	197.0448	$[M-H]^-$	Syringic acid	$C_9H_{10}O_5$	Phenolic
375	5.661	197.0453	$[M-H]^-$	Syringic acid	$C_9H_{10}O_5$	Phenolic
381	5.984	197.0453	$[M-H]^-$	Syringic acid	$C_9H_{10}O_5$	Phenolic
858	13.765	199.0594	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic

Supplement Table 1. (Continued).

713	13.161	199.0598	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic
516	5.715	199.0599	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic
529	6.002	199.0600	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic
620	12.748	204.9824	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8C_{l2}O_2$	Phenolic
1370	21.916	204.9824	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8C_{l2}O_2$	Phenolic
3123	31.084	204.9927	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8C_{l2}O_2$	Phenolic
3787	31.556	206.9943	$[M+2H]^{2+}$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8C_{l2}O_2$	Phenolic
1386	21.985	236.1051	$[M-H]^-$	1-Bromo-2-methoxynaphthalene	$C_{11}H_9BrO$	Phenolic
131	0.887	238.0916	$[M+H]^+$	1-Bromo-2-methoxynaphthalene	$C_{11}H_9BrO$	Phenolic
900	14.805	271.5063	$[M+2H]^{2+}$	Daturic acid	$C_{17}H_{34}O_2$	Aliphatic hydroxy ketones
1002	15.560	283.5283	$[M+H]^+$	Oleic acid	$C_{18}H_{34}O_2$	Aliphatic hydroxy ketones
1263	17.525	283.5460	$[M+H]^+$	Dodecane-2,6,11-trimethyl-	$C_{15}H_{32}$	Alkane (Hydrocarbon)
958	15.200	285.5305	$[M+2H]^{2+}$	Ethylpalmitate	$C_{18}H_{36}O_2$	Aliphatic hydroxy ketones
3454	30.931	288.1113	$[M+H]^+$	Lycorine	$C_{16}H_{17}NO_4$	Alkaloid
2303	24.527	319.3186	$[M+H]^+$	Breviscapin B	$C_{17}H_{18}O_6$	Phenolic
2697	26.898	321.3140	$[M+H]^+$	Curculigoside C_qt	$C_{16}H_{16}O_6$	Phenolic glycosides
773	15.506	329.1035	$[M-H]^-$	(1R,2R)-Crassifogenin-D	$C_{18}H_{18}O_6$	Norlignan
2177	25.819	351.2904	$[M-H]^-$	Methyl-4-O-coumaroylquinate	$C_{17}H_{20}O_8$	Norlignan
2162	25.426	353.3065	$[M-H]^-$	3-[[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxy-cyclohexanecacarboxylic acid	$C_{16}H_{18}O_9$	Phenolic
977	15.200	453.1381	$[M-H]^-$	Curculigoside B	$C_{21}H_{24}O_{11}$	Phenolic glycosides
2081	25.039	459.4277	$[M-H]^-$	Orchioside D	$C_{23}H_{24}O_{10}$	Phenolic glycosides
2143	25.249	459.4277	$[M-H]^-$	Orchioside D	$C_{23}H_{24}O_{10}$	Phenolic glycosides
2144	25.249	461.1581	$[M-H]^-$	Orchioside B	$C_{23}H_{26}O_{10}$	Phenolic glycosides
1051	19.763	465.1401	$[M-H]^-$	Orchioside A	$C_{22}H_{26}O_{11}$	Phenolic glycosides
826	16.646	465.1404	$[M-H]^-$	Orchioside A	$C_{22}H_{26}O_{11}$	Phenolic glycosides
937	15.164	477.1247	$[M+H]^+$	Crassifoside I	$C_{23}H_{24}O_{11}$	Phenolic
439	11.276	477.1397	$[M-H]^-$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
642	12.480	479.1542	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
548	8.449	479.1543	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
642	13.107	481.1362	$[M-H]^-$	Curculigoside C	$C_{22}H_{26}O_{12}$	Phenolic glycosides
1231	17.345	483.1504	$[M+H]^+$	Curculigoside C	$C_{22}H_{26}O_{12}$	Phenolic glycosides
1904	24.475	530.3336	$[M-H]^-$	Curculigine A	$C_{20}H_{28}Cl_2O_{12}$	Phenolic glycosides
2949	30.880	534.7917	$[M-H]^-$	Curculigin C	$C_{19}H_{25}Cl_3O_{11}$	Phenolic glycosides
2620	26.950	575.9548	$[M-H]^-$	Sitogluside	$C_{35}H_{60}O_6$	Sitosterol
1487	20.635	769.4671	$[M+H]^+$	Curculigosaponin C	$C_{41}H_{68}O_{13}$	Cycloartane (Triterpene Glycosides)
1886	24.439	781.4753	$[M-H]^-$	Curculigosaponin G	$C_{42}H_{70}O_{13}$	Cycloartane (Triterpene Glycosides)

Supplement Table 1. (Continued).

2104	25.039	781.4753	$[M-H]^-$	Curculigosaponin G	$C_{42}H_{70}O_{13}$	Cycloartane (Triterpene Glycosides)
1488	20.635	783.4864	$[M+H]^+$	Curculigosaponin G	$C_{42}H_{70}O_{13}$	Cycloartane (Triterpene Glycosides)
1243	17.381	783.4869	$[M+H]^+$	Curculigosaponin G	$C_{42}H_{70}O_{13}$	Cycloartane (Triterpene Glycosides)

RLS

ID	RT [min]	m/z	Type	Metabolite name	Formula	Chemical type
4440	31.827	111.1161	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
435	1.317	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
3087	25.076	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
2712	22.173	111.1163	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
2739	22.449	111.1163	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
3560	31.369	121.0277	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
83	0.621	121.0278	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
944	7.798	121.0278	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
231	1.035	121.0279	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
217	1.004	123.0070	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
65	0.737	123.0394	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
66	0.737	123.0396	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
1275	10.973	123.0431	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
425	1.285	127.1110	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
36	0.637	127.1111	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
196	0.895	134.1167	$[M+H]^+$	N-acetyl-N-hydroxy-2-carbamic acid methylester	$C_4H_7NO_4$	Alkaloid
87	0.621	151.0382	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
647	3.772	151.0382	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
912	6.658	151.0382	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
1905	15.193	151.0382	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
3506	31.134	151.0385	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
3907	30.915	153.0433	$[M+2H]^{2+}$	Vanillin	$C_8H_8O_3$	Phenolic
178	0.973	181.0581	$[M-H]^-$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
1430	11.703	183.0637	$[M+H]^+$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
2301	16.217	183.0638	$[M+H]^+$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
2364	21.916	204.9813	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
3466	31.066	204.9916	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
4258	31.118	206.9935	$[M+H]^+$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
2034	15.245	283.5269	$[M+H]^+$	Oleic acid	$C_{18}H_{34}O_2$	Aliphatic hydroxy ketones
2295	16.182	283.5491	$[M+H]^+$	Dodecane-2,6,11-trimethyl-	$C_{15}H_{32}$	Alkane (Hydrocarbon)

Supplement Table 1. (Continued).

213	0.957	288.1130	$[M+H]^+$	Lycorine	$C_{16}H_{17}NO_4$	Alkaloid
2167	19.219	297.0746	$[M-H]^-$	1,1-Bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane	$C_{17}H_{14}O_5$	Phenolic
1289	10.973	299.0891	$[M+H]^+$	1,1-Bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane	$C_{17}H_{14}O_5$	Phenolic
2068	16.513	329.1011	$[M-H]^-$	(1R,2R)-Crassifogenin-D	$C_{24}H_{28}O_{11}$	Norlignan glycosides
2277	20.536	451.1365	$[M-H]^-$	Curculigoside B	$C_{21}H_{24}O_{11}$	Phenolic glycosides
3746	31.640	451.7986	$[M-H]^-$	27-Hydroxy triacontan-6-one	$C_{30}H_{60}O_2$	Aliphatic-Hydroxy Ketones
2628	24.228	461.1568	$[M-H]^-$	Orchioside B	$C_{23}H_{26}O_{10}$	Phenolic glycosides
3641	28.081	461.4350	$[M+H]^+$	Crassifoside B	$C_{23}H_{24}O_{10}$	Phenolic
722	4.523	465.1480	$[M-H]^-$	Orchioside A	$C_{22}H_{26}O_{11}$	Phenolic glycosides
274	0.988	477.1348	$[M+H]^+$	Crassifoside I	$C_{23}H_{24}O_{11}$	Phenolic
1099	10.475	477.1382	$[M-H]^-$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
1983	15.177	479.1499	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
276	0.988	479.1502	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
1643	12.885	479.1503	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
3642	28.081	479.4415	$[M+H]^+$	Capituloside	$C_{23}H_{26}O_{11}$	Norlignan
2076	15.279	493.1639	$[M+H]^+$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides
3112	25.249	502.3136	$[M+H]^+$	Curculigine J	$C_{19}H_{26}Cl_2O_{11}$	Phenolic glycosides
958	8.0379	508.0269	$[M-H]^-$	4-Acetyl-2-methyoxy-5-methyltriacontane	$C_{34}H_{68}O_2$	Aliphatic hydroxy ketones
3719	31.539	534.7844	$[M-H]^-$	Curculigin C	$C_{19}H_{25}Cl_3O_{11}$	Phenolic glycosides

LOB

ID	RT [min]	m/z	Type	Metabolite name	Formula	Chemical type
2857	22.175	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
2883	22.450	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
3575	25.051	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
4186	27.062	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
4757	31.626	121.0275	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
22	0.455	121.0276	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
567	7.205	121.0276	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
1261	16.099	121.0276	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
393	4.919	123.0061	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
941	13.538	123.0068	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
4240	31.187	123.0069	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
222	1.402	123.0070	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
394	4.919	123.0071	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranylacetone
56	0.710	123.0395	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
57	0.710	123.0396	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
1679	15.049	123.0429	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
1483	13.556	123.0430	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
239	1.255	123.0433	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic

Supplement Table 1. (Continued).

2068	16.082	123.0433	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
4191	27.062	125.1316	$[M+H]^+$	3,5-Dihyroxytoluene	$C_7H_8O_2$	Phenolic
202	0.963	127.1108	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
2663	20.206	127.1108	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
26	0.610	127.1109	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
313	1.723	127.1109	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
146	0.838	134.1165	$[M+H]^+$	N-acetyl-N-hydroxy-2-carbamic acid methylester	$C_4H_7NO_4$	Alkaloid
616	8.175	151.0383	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
431	6.031	151.0384	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
4687	30.933	153.0433	$[M+2H]^{2+}$	Vanillin	$C_8H_8O_3$	Phenolic
4207	27.062	169.1936	$[M+H]^+$	4-Ethoxy-3-hydroxymethyl-phenol	$C_9H_{12}O_3$	Phenolic
4209	27.062	179.1783	$[M+H]^+$	3-(4-Hydroxy-3-methoxyphenyl) acrylaldehyde	$C_{10}H_{10}O_3$	Phenolic
2072	16.082	183.0636	$[M+H]^+$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
2286	17.483	183.0638	$[M+H]^+$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
2514	19.721	183.0638	$[M+H]^+$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
389	4.886	197.0433	$[M-H]^-$	Syringic acid	$C_9H_{10}O_5$	Phenolic
943	13.539	197.0434	$[M-H]^-$	Syringic acid	$C_9H_{10}O_5$	Phenolic
547	4.937	199.0582	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic
548	4.937	199.0583	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic
1418	13.523	199.0583	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic
336	2.203	199.0584	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic
500	4.522	199.0584	$[M+H]^+$	Syringic acid	$C_9H_{10}O_5$	Phenolic
1980	21.883	204.9808	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
1950	21.642	204.9809	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
4193	31.085	204.9918	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
5125	31.711	206.9935	$[M+H]^+$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
5126	31.711	206.9935	$[M+H]^+$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
5001	31.170	206.9937	$[M+H]^+$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
2086	16.082	283.5488	$[M+H]^+$	Dodecane-2,6,11-trimethyl-	$C_{15}H_{32}$	Alkane (Hydrocarbon)
1931	20.844	286.1069	$[M-H]^-$	Lycorine	$C_{16}H_{17}NO_4$	Alkaloid
1602	14.532	287.1003	$[M+H]^+$	Orcinol glucoside	$C_{13}H_{18}O_7$	Phenolic glycosides
1593	14.497	299.0894	$[M+H]^+$	1,1-Bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane	$C_{17}H_{14}O_5$	Phenolic
1047	12.064	299.0896	$[M+H]^+$	1,1-Bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane	$C_{17}H_{14}O_5$	Phenolic

Supplement Table 1. (Continued).

4237	27.062	305.3178	$[M+H]^+$	Curculigoside_qt	$C_{16}H1_6O_6$	Phenolic glycosides
3643	25.717	311.2559	$[M+H]^+$	Curcapital	$C_{17}H_{10}O_6$	Norlignan
3607	25.259	317.3212	$[M+H]^+$	2,3,4,7-Tetramethoxyxanthone	$C_{17}H_{16}O_7$	Phenolic
3467	24.221	317.3227	$[M+H]^+$	2,3,4,7-Tetramethoxyxanthone	$C_{17}H_{16}O_7$	Phenolic
3711	25.821	321.3164	$[M+H]^+$	Curculigoside C_qt	$C_{16}H_{16}O_6$	Phenolic glycosides
4336	27.563	321.3164	$[M+H]^+$	Curculigoside C_qt	$C_{16}H_{16}O_6$	Phenolic glycosides
2293	17.483	331.1130	$[M+H]^+$	(1R,2R)-Crassifogenin-D	$C_{18}H_{18}O_6$	Norlignan
1517	13.799	341.5906	$[M+H]^+$	Behenic acid	$C_{22}H_{44}O_2$	Aliphatic hydroxy ketones
1677	14.918	341.5920	$[M+H]^+$	Behenic acid	$C_{22}H_{44}O_2$	Aliphatic hydroxy ketones
4339	27.563	349.3440	$[M+H]^+$	Curculignin	$C_{18}H_{20}O_7$	Norlignan
761	11.345	451.1221	$[M-H]^-$	Curculigoside B	$C_{21}H_{24}O_{11}$	Phenolic glycosides
4680	31.558	451.7973	$[M-H]^-$	27-Hydroxy triacontan-6-one	$C_{30}H_{60}O_2$	Aliphatic-Hydroxy Ketones
1724	15.049	453.1359	$[M+H]^+$	Curculigoside B	$C_{21}H_{24}O_{11}$	Phenolic glycosides
2974	22.894	459.7256	$[M+H]^+$	Curculigenin C	$C_{19}H_{25}Cl_3O_{11}$	Norlignan
2671	24.412	461.1556	$[M-H]^-$	Orchioside B	$C_{23}H_{26}O_{10}$	Phenolic glycosides
2672	24.412	461.1558	$[M-H]^-$	Orchioside B	$C_{23}H_{26}O_{10}$	Phenolic glycosides
1868	20.497	461.1577	$[M-H]^-$	Orchioside B	$C_{23}H_{26}O_{10}$	Phenolic glycosides
1236	13.320	463.1501	$[M+2H]^{2+}$	Orchioside B	$C_{23}H_{26}O_{10}$	Phenolic glycosides
2519	19.721	467.1508	$[M+H]^+$	Orchioside A	$C_{22}H_{26}O_{11}$	Phenolic glycosides
2112	16.082	467.1513	$[M+H]^+$	Orchioside A	$C_{22}H_{26}O_{11}$	Phenolic glycosides
4346	27.563	475.4277	$[M+H]^+$	Crassifoside A	$C_{23}H_{22}O_{11}$	Phenolic
2888	24.895	477.1492	$[M-H]^-$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
1041	12.029	479.1518	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
679	6.669	479.1520	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
779	7.257	479.1523	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
1439	13.523	483.1459	$[M+H]^+$	Curculigoside C	$C_{22}H_{26}O_{12}$	Phenolic glycosides
1869	20.497	491.1674	$[M-H]^-$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides
2450	23.895	491.8653	$[M-H]^-$	Crassifoside H	$C_{23}H_{21}ClO_{10}$	Phenolic
1842	15.150	493.1651	$[M+H]^+$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides
1941	15.496	493.1656	$[M+H]^+$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides
4455	28.039	493.4360	$[M+H]^+$	Crassifoside C	$C_{23}H_{24}O_{12}$	Phenolic
2543	24.344	530.3275	$[M-H]^-$	Curculigine A	$C_{20}H_{28}Cl_2O_{12}$	Phenolic glycosides
2890	24.895	530.3304	$[M-H]^-$	Curculigine A	$C_{20}H_{28}Cl_2O_{12}$	Phenolic glycosides
1874	20.497	530.3392	$[M-H]^-$	Curculigine A	$C_{20}H_{28}Cl_2O_{12}$	Phenolic glycosides
4713	31.558	534.7861	$[M-H]^-$	Curculigin C	$C_{19}H_{25}Cl_3O_{11}$	Phenolic glycosides
3526	26.656	575.8508	$[M-H]^-$	Daucosterol	$C_{35}H_{60}O_6$	Sitosterol
2595	19.787	783.4826	$[M+H]^+$	Curculigosaponin G	$C_{42}H_{70}O_{13}$	Cycloartane (Triterpene Glycosides)

Supplement Table 1. (Continued).

LLS

ID	RT [min]	m/z	Type	Metabolite name	Formula	Chemical type
645	0.900	111.1160	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
6451	22.446	111.1161	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
8364	25.059	111.1161	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
9199	27.051	111.1161	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
130	0.611	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
6250	22.176	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
662	1.009	121.0275	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
7	0.559	121.0276	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
2740	14.282	121.0277	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
9477	31.815	121.0277	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
9478	31.815	121.0277	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
1573	7.153	121.0278	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
647	0.979	123.0070	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranyl acetone
9077	31.134	123.0071	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranyl acetone
9078	31.134	123.0072	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranyl acetone
217	0.742	123.0394	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
9202	27.051	125.1315	$[M+H]^+$	3,5-Dihyroxytoluene	$C_7H_8O_2$	Phenolic
104	0.576	127.1108	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
729	0.932	127.1108	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
4964	17.370	127.1108	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
4060	14.957	127.1109	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
2132	7.378	127.1110	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
5299	18.313	127.1110	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
9431	31.711	151.0381	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
1453	5.909	151.0382	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
2793	14.455	151.0383	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
2794	14.455	151.0383	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
10040	30.916	153.0436	$[M+H]^+$	Vanillin	$C_8H_8O_3$	Phenolic
2496	9.817	153.0534	$[M+H]^+$	Vanillin	$C_8H_8O_3$	Phenolic
9217	27.051	169.1939	$[M+H]^+$	4-Ethoxy-3-hydroxymethyl-phenol	$C_9H_{12}O_3$	Phenolic
859	1.407	181.0523	$[M-H]^-$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
8954	31.067	204.9916	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
920	1.640	236.0911	$[M-H]^-$	1-Bromo-2-methoxynaphthalene	$C_{11}H_9BrO$	Phenolic
4982	21.988	236.1032	$[M-H]^-$	1-Bromo-2-methoxynaphthalene	$C_{11}H_9BrO$	Phenolic
468	0.869	238.0905	$[M+H]^+$	1-Bromo-2-methoxynaphthalene	$C_{11}H_9BrO$	Phenolic
1229	1.589	238.1060	$[M+H]^+$	1-Bromo-2-methoxynaphthalene	$C_{11}H_9BrO$	Phenolic
4219	15.343	283.5269	$[M+H]^+$	Oleic acid	$C_{18}H_{34}O_2$	Aliphatic hydroxy ketones
1232	3.857	285.0965	$[M-H]^-$	Orcinol glucoside	$C_{13}H_{18}O_7$	Phenolic glycosides
1466	6.291	285.1090	$[M-H]^-$	Orcinol glucoside	$C_{13}H_{18}O_7$	Phenolic glycosides
4984	21.988	286.1070	$[M-H]^-$	Lycorine	$C_{16}H_{17}NO_4$	Alkaloid

Supplement Table 1. (Continued).

4335	19.031	297.0747	$[M–H]^-$	1,1-Bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane	$C_{17}H_{14}O_5$	Phenolic
365	0.885	297.0809	$[M–H]^-$	1,1-Bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane	$C_{17}H_{14}O_5$	Phenolic
8384	25.059	313.2707	$[M+H]^+$	Crassifogenin A	$C_{17}H_{12}O_6$	Norlignan
6623	22.614	313.2708	$[M+H]^+$	Crassifogenin A	$C_{17}H_{12}O_6$	Norlignan
9160	26.802	313.2708	$[M+H]^+$	Crassifogenin A	$C_{17}H_{12}O_6$	Norlignan
9272	27.121	313.2708	$[M+H]^+$	Crassifogenin A	$C_{17}H_{12}O_6$	Norlignan
4083	15.133	341.5917	$[M+H]^+$	Behenic acid	$C_{22}H_{44}O_2$	Aliphatic hydroxy ketones
5720	19.885	341.6037	$[M+H]^+$	Behenic acid	$C_{22}H_{44}O_2$	Aliphatic hydroxy ketones
1566	6.949	353.3000	$[M–H]^-$	3-[[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxy-cyclohexanecacarboxylic acid	$C_{16}H_{18}O_9$	Phenolic
1077	3.162	353.3020	$[M–H]^-$	3-[[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxy-cyclohexanecacarboxylic acid	$C_{16}H_{18}O_9$	Phenolic
9968	30.849	361.3631	$[M+H]^+$	Curlignan	$C_{19}H_{20}O_7$	Lignan
9969	30.849	361.3631	$[M+H]^+$	Curlignan	$C_{19}H_{20}O_7$	Lignan
2329	9.120	413.6920	$[M+H]^+$	Stigmasterol	$C_{29}H_{48}O$	Steroid
8134	24.816	413.7057	$[M+H]^+$	Stigmasterol	$C_{29}H_{48}O$	Steroid
2144	11.016	451.1351	$[M–H]^-$	Curculigoside B	$C_{21}H_{24}O_{11}$	Phenolic glycosides
2249	11.333	451.7993	$[M–H]^-$	27-Hydroxy triacontan-6-one	$C_{30}H_{60}O_2$	Aliphatic hydroxy ketones
1398	2.940	453.1238	$[M+H]^+$	Curculigoside B	$C_{21}H_{24}O_{11}$	Phenolic glycosides
2148	11.016	461.1624	$[M–H]^-$	Orchioside B	$C_{23}H_{26}O_{10}$	Phenolic glycosides
2722	14.248	465.1493	$[M–H]^-$	Orchioside A	$C_{22}H_{26}O_{11}$	Phenolic glycosides
464	0.885	475.1273	$[M–H]^-$	Crassifoside I	$C_{23}H_{24}O_{11}$	Phenolic
9369	27.519	475.4277	$[M+H]^+$	Crassifoside A	$C_{23}H_{22}O_{11}$	Phenolic
2643	10.477	475.7182	$[M+H]^+$	Curculigenin A	$C_{30}H_{50}O_4$	Norlignan
2389	9.504	475.7183	$[M+H]^+$	Curculigenin A	$C_{30}H_{50}O_4$	Norlignan
2492	9.782	475.7184	$[M+H]^+$	Curculigenin A	$C_{30}H_{50}O_4$	Norlignan
716	0.900	477.1349	$[M+H]^+$	Crassifoside I	$C_{23}H_{24}O_{11}$	Phenolic
466	0.885	477.1417	$[M–H]^-$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
6944	24.901	477.1488	$[M–H]^-$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
773	0.932	479.1508	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
2947	12.621	479.1513	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
1888	6.551	479.1520	$[M+H]^+$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
4596	20.491	491.1671	$[M–H]^-$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides
2366	9.224	493.1641	$[M+H]^+$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides
2442	9.713	493.1659	$[M+H]^+$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides

Supplement Table 1. (Continued).

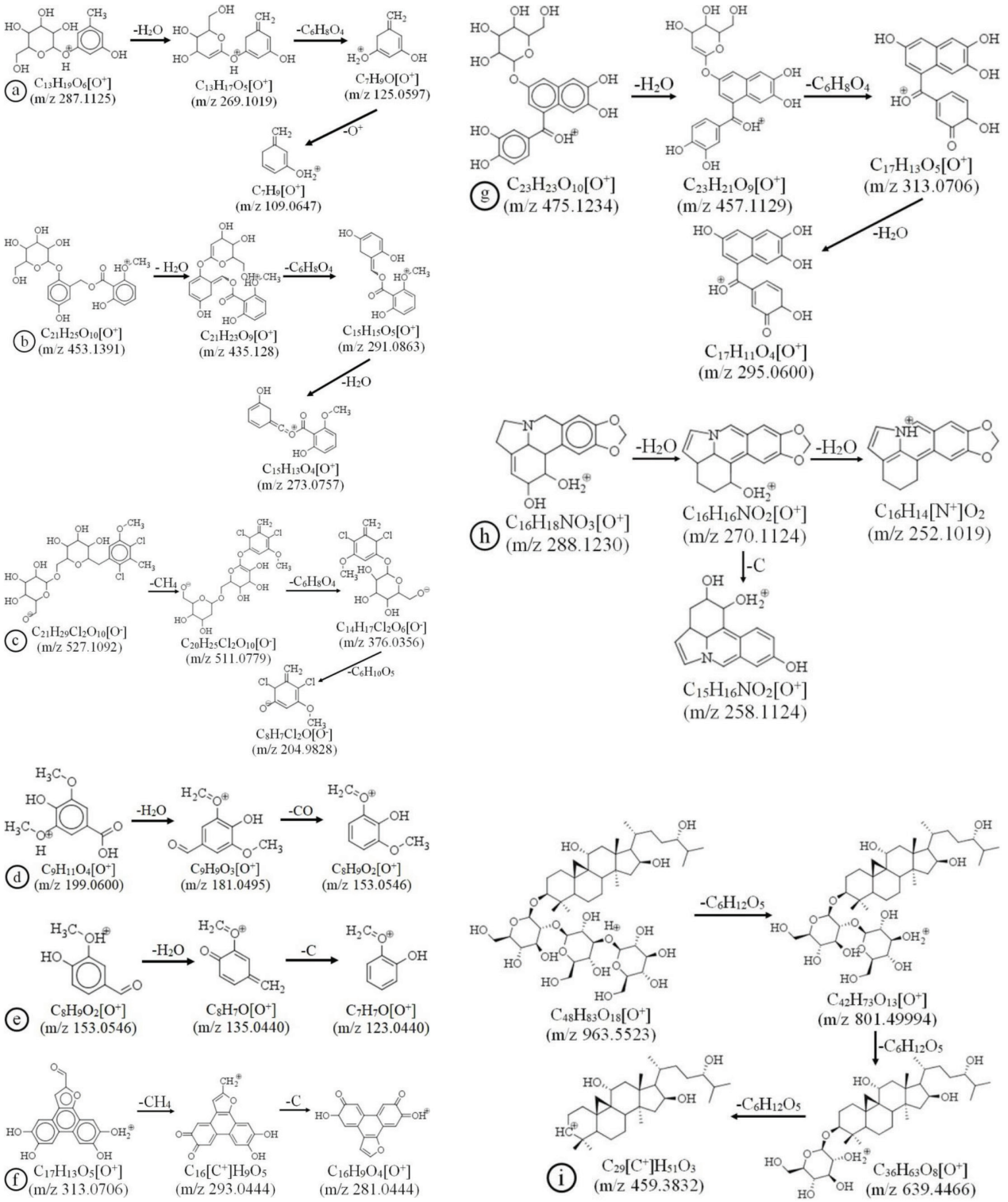
2476	9.748	493.1662	$[M+H]^+$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides
9166	26.802	493.4375	$[M+H]^+$	Crassifoside C	$C_{23}H_{24}O_{12}$	Phenolic
9505	28.178	503.4637	$[M+H]^+$	Crassifoside E	$C_{25}H_{26}O_{11}$	Phenolic
1489	6.464	508.0273	$[M-H]^-$	4-Acetyl-2-methyoxy-5-methyltriacontane	$C_{34}H_{68}O_2$	Aliphatic hydroxy ketones
1108	3.162	508.0283	$[M-H]^-$	4-Acetyl-2-methyoxy-5-methyltriacontane	$C_{34}H_{68}O_2$	Aliphatic hydroxy ketones
9439	31.711	534.7062	$[M-H]^-$	Curculigine C	$C_{19}H_{25}Cl_3O_{11}$	Phenolic glycosides
114	0.758	534.7859	$[M-H]^-$	Curculigin C	$C_{19}H_{25}Cl_3O_{11}$	Phenolic glycosides
8390	28.526	575.4299	$[M-H]^-$	3-O-B-D-Glucopyranosyl sitosterol	$C_{35}H_{60}O_6$	Sitosterol
8494	28.999	575.4301	$[M-H]^-$	3-O-B-D-Glucopyranosyl sitosterol	$C_{35}H_{60}O_6$	Sitosterol
7720	26.678	575.8502	[M-H]-	Daucosterol	$C_{35}H_{60}O_6$	Sitosterol
7943	26.926	575.9502	$[M-H]^-$	Sitogluside	$C_{35}H_{60}O_6$	Sitosterol
8336	24.954	769.4648	$[M+H]^+$	Curculigosaponin C	$C_{41}H_{68}O_{13}$	Cycloartane (triterpene glycosides)
9787	29.309	1078.255	$[M+H]^+$	Curculigosaponin M	$C_{53}H_{88}O_{22}$	Cycloartane (triterpene glycosides)

PLS

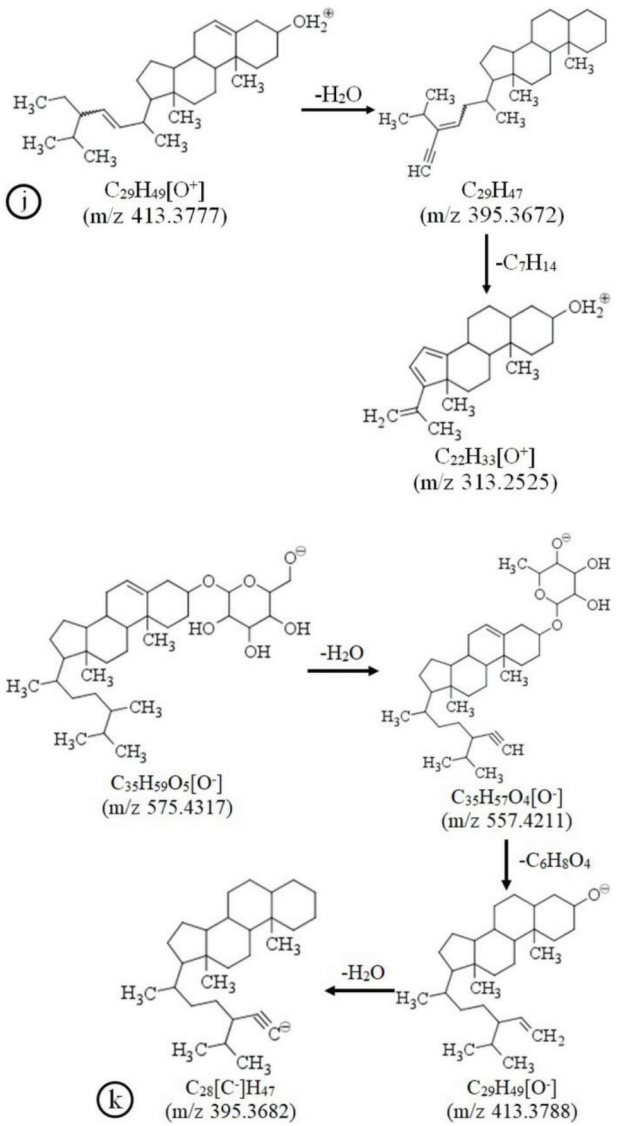
ID	RT [min]	m/z	Type	Metabolite name	Formula	Chemical type
15	0.269	111.1161	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
918	0.942	111.1161	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
3707	25.037	111.1161	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
4623	27.045	111.1162	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
2838	22.145	111.1163	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
2907	22.425	111.1163	$[M+H]^+$	4-Hydroxy-phenol	$C_6H_6O_2$	Phenolic
375	1.741	121.0276	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_6H_6O_2$	Phenolic
519	7.063	121.0277	$[M-H]^-$	4-Hydroxybenzaldehyde	$C_6H_6O_2$	Phenolic
4605	31.492	123.0068	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranyl acetone
4194	31.119	123.0070	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranyl acetone
4195	31.119	123.0070	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranyl acetone
235	0.958	123.0072	$[M-H]^-$	(E)-6-Methyl-3,5-heptadien-2-one	$C_8H_{12}O$	Geranyl acetone
149	0.714	123.0395	$[M+H]^+$	4-Hydroxybenzaldehyde	$C_7H_6O_2$	Phenolic
549	8.399	125.1148	$[M-H]^-$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
4628	27.045	125.1316	$[M+H]^+$	3,5-Dihyroxytoluene	$C_7H_8O_2$	Phenolic
69	0.338	127.1109	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
5480	31.338	127.1109	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
1012	1.179	127.1110	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
2183	18.319	127.1110	$[M+H]^+$	5-Hydroxymethylfurfural	$C_6H_6O_3$	Furan
1018	1.179	134.1165	$[M+H]^+$	N-acetyl-N-hydroxy-2-carbamic acid methylester	$C_4H_7NO_4$	Alkaloid
441	0.816	134.1166	$[M+H]^+$	N-acetyl-N-hydroxy-2-carbamic acid methylester	$C_4H_7NO_4$	Alkaloid

Supplement Table 1. (Continued).

979	15.972	151.0381	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
4617	31.526	151.0383	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
538	8.013	151.0384	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
461	4.480	151.0385	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
721	12.028	151.0385	$[M-H]^-$	Vanillin	$C_8H_8O_3$	Phenolic
5156	30.901	153.0435	$[M+H]^+$	Vanillin	$C_8H_8O_3$	Phenolic
1913	21.815	153.1267	$[M-H]^-$	3,5-Dihydroxy-benzoic acid	$C_7H_6O_4$	Phenolic
444	3.437	181.0486	$[M-H]^-$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
1412	11.130	183.0642	$[M+H]^+$	Ethyl 3,4-dihydroxybenzoate	$C_9H_{10}O_4$	Phenolic
970	1.008	197.2082	$[M+H]^+$	1,3,7-Trimethylxanthine	$C_8H_{10}N_4O_2$	Alkaloid
4137	31.051	204.9915	$[M-H]^-$	2,4-Dichloro-5-methoxy-3-methylphenol	$C_8H_8Cl_2O_2$	Phenolic
1955	21.988	236.1037	$[M-H]^-$	1-Bromo-2-methoxynaphthalene	$C_{11}H_9BrO$	Phenolic
1299	4.708	238.1033	$[M+H]^+$	1-Bromo-2-methoxynaphthalene	$C_{11}H_9BrO$	Phenolic
4613	26.904	321.3122	$[M+H]^+$	Curculigoside C_qt	$C_{16}H_{16}O_6$	Phenolic glycosides
2270	18.873	437.6302	$[M+H]^+$	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	Steroid
2311	19.152	437.6318	$[M+H]^+$	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	Steroid
656	11.431	461.1457	$[M-H]^-$	Orchioside B	$C_{23}H_{26}O_{10}$	Phenolic glycosides
3653	27.749	465.4269	$[M-H]^-$	Curculigoside D	$C_{22}H_{26}O_{11}$	Phenolic glycosides
1842	21.501	477.1512	$[M-H]^-$	Nyasicoside	$C_{23}H_{26}O_{11}$	Phenolic
2421	23.832	491.1723	$[M-H]^-$	(1S,2R)-O-Methylnyasicoside	$C_{24}H_{28}O_{11}$	Norlignan glycosides
683	11.643	508.0268	$[M-H]^-$	4-Acetyl-2-methyoxy-5-methyltriacontane	$C_{34}H_{68}O_2$	Aliphatic hydroxy ketones
4430	31.289	534.7850	$[M-H]^-$	Curculigin C	$C_{19}H_{25}Cl_3O_{11}$	Phenolic glycosides
298	0.781	536.6946	$[M+H]^+$	Curculigine C	$C_{19}H_{25}Cl_3O_{11}$	Phenolic glycosides
3294	27.028	575.9503	$[M-H]^-$	Sitogluside	$C_{35}H_{60}O_6$	Sitosterol
334	0.781	629.5770	$[M+H]^+$	Piloside A	$C_{28}H_{36}O_{16}$	Benzylbenzoates diglucosides
5069	30.427	924.2644	$[M+H]^+$	Cynanuriculoside A_qt	$C_{28}H_7O_{42}$	Triterpene Glycosides



Supplement Figure 2. The proposed fragmentation pattern of representative orcinol glucoside (a), curculigoside B (b), curculigine A (c), syringic acid (d), vanillin (e), curcapital (f), crassifoside A (g), lycorine (h), curculigosaponin (i), stigmasterol (j), and daucosterol (k) at ion mode $[M+H]^+$) and $[M–H]^-$) in *C. orchioides* and *C. latifolia*.



Supplement Figure 2. (Continued).