

IDENTIFICATION OF SECRETORY STRUCTURE, HISTOCHEMISTRY AND PHYTOCHEMICAL COMPOUNDS OF MEDICINAL PLANT *Hyptis capitata* Jacq.

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ABSTRACT

Hyptis capitata Jacq. (common name: Knobweed or False Ironwort) belongs to Lamiaceae family and is among known traditional medicine. The Anak Dalam Tribe of Jambi Province uses the leaves of *H. capitata* to cure external and internal wounds. This study was aimed at identifying and analyzing secretory structure, histochemistry and phytochemical content of *H. capitata* leaves. The results showed that *H. capitata* leaves have secretory structures in the form of peltate, capitate and uniseriate glandular trichomes on the upper and lower leaf surfaces, with idioblast cells scattered throughout the leaf mesophyll. Histochemical tests indicated that the peltate trichomes have four head cells, containing alkaloids and terpenoids. This study classified capitate trichomes into two types: 1. type I has one stalk cell and two head cells, all containing alkaloids, terpenoids and lipophilic compounds; 2. type II has long stalk cells consisting of seven to ten cells with one head cell, all containing alkaloids and terpenoids. Uniseriate glandular trichomes of *H. capitata* leaves consist of four to eight cells containing alkaloids and terpenoids. The idioblast cells are round-shaped and contain lipophilic compounds. GC-MS analysis showed that *H. capitata* leaves contain terpenoid compounds assumed to serve as anti-infective agents, including l-limonene, eugenol, farnesol isomers A, d-nerolidol, hexahydrofarnesol and neophytadiene.

Keywords: Glandular trichome, *Hyptis capitata*, idioblast, medicinal plants, phyto-compounds

INTRODUCTION

Rapid deforestation in Jambi area will cause a great loss of medicinal plants. It is, therefore, important to preserve the tribal knowledge on herbal medicine. *Hyptis capitata* is one of various plant species used as herbal medicine by Anak Dalam Tribe, which is native to Jambi Province in Sumatera, Indonesia.

Hyptis capitata is an erect herbaceous plant, native to Central and tropical South America. The plant is usually abundantly found in open fields and roadsides. In Indonesia, particularly in Jambi Province, the leaves of *H. capitata* (Fig. 1) are used by the Anak Dalam tribe as herbal medicine to cure external and internal wounds.

The tribe crushes or squeezes the leaves prior to using them to cure wound. The leaf sap can be consumed to treat inner injuries (Tumenggung Tarip, Anak Dalam Tribe, pers. comm. 2012). This plant is also used as traditional medicine in India, Jamaica and Bangladesh (Michell & Ahmad 2006; Biswas *et al.* 2010). A phytochemical study conducted by Okach *et al.* (2013) reported that several other species of the Lamiaceae family, such as *Becium obovatum* (E.Mey. Ex. Benth) N.E.Br., *Leucas calostachys* Oliv, *Ocimum kilimandscharicum* Baker Ex. Gurke, *Plectranthus barbatus* and *Satureja biflora* (Ham Ex. Andrews D. Don) Brig., contain sterols, terpenoids, alkaloids, saponins, glycosides, flavonoids and tannins.

Dickison (2000) reported that most medicinal plants have secretory structures that play a part in metabolite production. Various chemical

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Figure 1 *H. capitata* grows wildly in the jungle-rubber agroforest at Bukit Duabelas National Park, Jambi Province

compounds, such as essential oils, resins, latex, mineral salts, alkaloids and glycosides, are produced by secretory structures. Werker (1993) stated that phytochemical substance of terpenoids, alkaloids and various volatile compounds are generally produced by the glandular trichomes contained in the species of Lamiaceae. Scientific study to identify the secretory structures which might be the site of accumulation and/or biosynthesis of metabolite and phytochemical compounds in *H. capitata* will improve the production of its desirable phytochemical compounds through tissue or cell culture. This study was aimed at identifying the secretory structure, analyzing the accumulated metabolite compounds in the secretory structure and analyzing the phytochemical content in *H. capitata* leaves. This research also examined the efficacy of *H. capitata* plant, so that the natural substance contained in the plant can be scientifically justified for its pharmaceutical properties.

MATERIALS AND METHODS

Plant Material Collection

The study was carried out in tropical lowland rain forest of Bukit Dua Belas National Park in Jambi Province, Sumatera, Indonesia (01°96'109" S, 102°58'630" E, 87 m asl). Leaf samples were collected from areas of jungle-rubber agroforest,

which is an agroforestry system that combines rubber trees with natural wild plant species grown surrounding the rubber trees. Leaves were harvested in the dry season of July 2013 at average temperature of 29 °C and average relative humidity of 84%.

Microscopic observation and histochemical analysis were performed on leaves from the third node of the plant, with three replications. A composite of leaves taken from several plant samples was subjected to phytochemical analysis. For microscopic observation purpose, leaves were preserved in 70% ethanol. For observation using Scanning Electron Microscope (SEM), the leaves were preserved in FAA solution (formaldehyde, glacial acetic acid and 70% ethanol; 1 : 1 : 18). Fresh leaves were collected for the histochemical analysis. For GC-MS analysis the samples were sun-dried for three days and then oven-dried at 60 °C for three days.

Observation using Light Microscope

Paradermal leaf sections were prepared as semi-permanent slides, following a procedure suggested by Sass (1951). The preserved leaves were washed with distilled water for five minutes, soaked in 50% HNO₃ solution for five minutes, rinsed with distilled water for one minute, and then the adaxial and abaxial surfaces of the leaves were peeled. The samples were observed using a light microscope (Olympus CX21) at 100x and 400x magnifications.

Observation using Scanning Electron Microscope (SEM)

The leaf samples were washed in caccodylate buffer for two hours using an ultrasonic cleaner (Sibata SU-6THE, Japan), pre-fixed in a 2.5% glutaraldehyde solution for two days at 4 °C, and then fixed in 2% tannic acid solution for 6 hours. The samples were then washed twice in caccodylate buffer for 10 minutes each time, rinsed with distilled water for 15 minutes, and dehydrated in a graded ethanol series of 70%, 80% and 90% for 10 minutes each, followed by twice dehydration in 100% ethanol for 10 minutes each time. The samples were then soaked twice in tert-butanol for 10 minutes each time, dried in vacuum drier for 20 minutes, coated with gold, and finally observed using a Scanning Electron Microscope (JEOL JSM 5310 LV Hitachi) at 20 kV (200x, 750x and 1,000x magnifications).

Histochemical Analysis

For histochemical analysis, fresh leaves were transversely sectioned at 15 - 20 µm, using a dual purpose microtome (Yamato RV-240). A small piece of fresh leaf lamina was inserted in the specimen holder. The specimen holder was then filled with water and frozen using the freezing system. The specimen embedded in the frozen water was then sectioned directly using a microtome blade. Leaf sections were then treated with specific reagents to identify the presence and specific locality of terpenoids, alkaloids and lipophilic compounds. Terpenoid presence in the leaf tissues was identified by soaking a leaf section in 5% cupric acetate solution, as suggested in Harbone (1993). A positive test for terpenoids was indicated by the appearance of yellow or brownish-yellow color in the tissue. Alkaloid presence was tested by soaking leaf lamina sections in Wagner reagent. A positive result was indicated by the presence of reddish-brown or yellow deposits. For negative control, the leaf sections were pre-treated with 5% tartaric acid in 95% ethanol for 48 hours at room temperature, as suggested in Furr & Mahlberg (1981). The presence of lipophilic compounds was tested using sliced samples washed in 70% ethanol for one minute, which were then soaked in 0.03% sudan IV for 30 minutes at 40 °C in a water bath. The sliced samples were then washed quickly in

70% ethanol and observed using light microscope at 400x magnification. The presence of lipophilic compounds was indicated by the production of red, yellow, or orange colors in the leaf tissue, following Boix *et al.* (2011).

Phytochemical Analysis

The presence of phytochemical components in the leaf tissue was identified using GC-MS technique. Two milligrams of powdered sample was placed in the chamber of the GC-MS instrument (Shimadzu-QP2010). To analyze the chemical composition, the sample went through the following processes: pyrolysis temperature 400 °C, oven temperature 50 °C, injector temperature 280 °C, inter-surface temperature 280 °C and ion source temperature 200 °C. The carrier gas was helium. The spectrogram mass resulting from those processes was then automatically calculated by the GC-MS instrument. The calculation was based on the similarity of mass number/charge number pattern referring to the GC-MS instrument's database NIST/Wiley databases.

RESULTS AND DISCUSSION

In the leaves of *H. capitata*, there were two types of secretory structure observed i.e. glandular trichomes and idioblast cells. Glandular trichomes were found on the upper (adaxial) surface and lower (abaxial) surface of the leaves. Idioblast cells were found throughout the leaf mesophyll, from the palisade to the spongy parenchyma. There were one peltate, two types of capitate and one uniseriate glandular trichome identified in this species (Fig. 2).

Members of the Lamiaceae family, such as *Ocimum canum*, *Mentha spicata* as well as *Scutellaria linearis* Benth., *S. baiacalensis*, *S. gallericulata* L. and *S. agrestis* (Shang *et al.* 2010; Venkateshapp & Sreenath 2013; Oliveira *et al.* 2013), generally have glandular trichomes. Several species have both peltate and capitate trichomes, while others have either only peltate or only capitate trichomes (Huang *et al.* 2008). Species of Lamiaceae family having various types of glandular trichomes include *Salvia chrysophylla*, *Egyptian ballota*, *Isodon rubescens*, *Pogostemon cablin* Benth., *Scutellaria agrestis* and *Rosmarinus officinalis* (Kahraman *et al.* 2009;

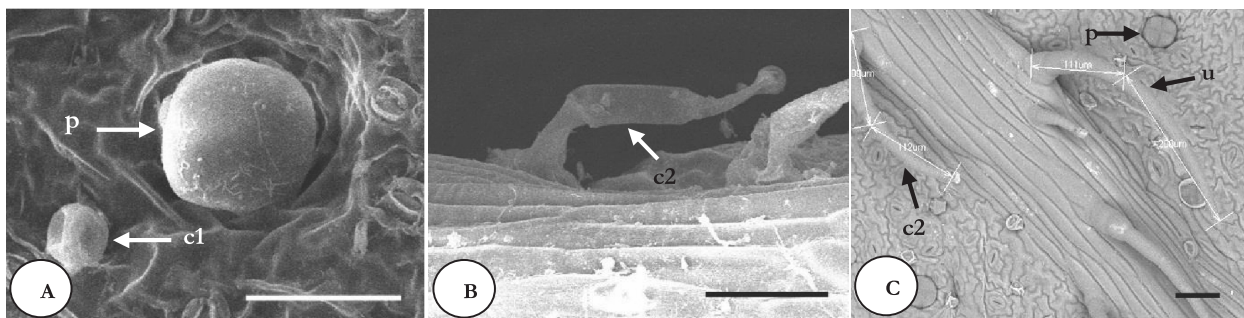


Figure 2 Glandular trichome types of *H. capitata*; images obtained using SEM (A, B, C)

p = peltate, c1 = capitulate glandular trichome type I, c2 = capitulate glandular trichome type II and u = uniseriate
(Note: Bar = 50 μm)

Salmaki *et al.* 2009; Liu & Liu 2012; Rusydi *et al.* 2013; Oliveira *et al.* 2013; Boix *et al.* 2011). Species belong to genus *Hyptis* having peltate and capitulate glandular trichomes include *H. caespitosa* and *H. suaveolens* (Rudall 2007; Jelani & Prabhakar 1991). *H. capitata* has peltate glandular trichomes in a submerged (sunken) position in the epidermal tissue. This type of glandular trichomes was also found in several species, such as *Ocimum basilicum*, *Ocimum irvinei* and *H. caespitosa* (Ogunkule & Oladele 2000; Rudall 2007).

Glandular Trichomes

Peltate glandular trichomes, found on the leaves of *H. capitata*, consisted of four head cells and one basal cell. The head cells of peltate glandular trichomes in Lamiaceae family vary widely in terms of cell numbers. Some species have three to six head cells, for example those of *Isodon rubescens* (Liu & Liu 2012). Several species of genus *Ziziphora* have 12 to 18 head cells, namely *Ziziphora clinopodioides*, *Z. tenuior*, *Z. taurica* and *Z. taurica* subsp. *cleonioides* (Kaya *et al.* 2013). There are 4 head cells in the peltate glandular trichomes of both *H. capitata* and *H. caespitosa* (Rudall 2007).

Capitate glandular trichomes in Lamiaceae family usually consisted of one to two head cells with short or long stalks consisted of one to three cells. These are found in many species, such as *Lavandula pinnata*, *Thymus lykae*, *Isodon rubescens* and *Rosmarinus officinalis* (Huang *et al.* 2008; Marin *et al.* 2008; Liu & Liu 2012; Boix *et al.* 2011). All three types of glandular trichome were found scattered on both leaf surfaces of *H. capitata*. Type I of capitulate glandular trichomes in *H. capitata* had a short stalk with one cell and one head consisted of two cells. Type II of capitulate glandular trichomes had a long stalk consisted of seven to ten cells and one head cell. Uniseriate glandular trichomes consisted of four to eight cells and had a tapered tip (Fig. 2).

Peltate glandular trichomes were generally shorter than other trichomes. However, their head size was much larger than that of capitulate glandular trichomes. The size of each trichome in the adaxial and abaxial surfaces was similar (Table 1). In fact, most trichome dimensions in adaxial and abaxial surfaces showed no significant differences, except for the width of type I capitulate glandular trichome which was greater in the abaxial surface than that in adaxial surface.

Table 1 Size and density of glandular trichomes of *H. capitata* leaf

Trichome types	Length of trichome stalk (μm)		Trichome head (μm)				Density (mm^{-2})	
			Length		Width			
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Peltate	-	-	27.7 \pm 0.3	28 \pm 0.5 ns	43.6 \pm 0.3	44.4 \pm 1.2 ns	9.6 \pm 0.7	32.3 \pm 1.9 s
Capitate type I	7.5 \pm 0.5	7.8 \pm 0.9 s	12.8 \pm 0.5	13.3 \pm 0.9 ns	25.1 \pm 0.5	27.5 \pm 0.9 s	12.6 \pm 3.3	18.9 \pm 1.3 s
Capitate type II	351 \pm 14.7	354.7 \pm 8.1 ns	27.5 \pm 0.7	28.3 \pm 0.3 ns	19.1 \pm 0.5	19.4 \pm 0.9 ns	3.9 \pm 0.3	4.5 \pm 0.2 s
Uniseriate	343.7 \pm 32.6	342.7 \pm 44.2 ns	-	-	-	-	3.9 \pm 0.7	4.4 \pm 0.3 ns
Idioblast	Diameter (μm)				Diameter (μm)			
	Palisade	Sponge			Palisade	Sponge		
	5.9 \pm 0.4	4.2 \pm 0.3 s			261.2 \pm 15.4	271.7 \pm 33.2 ns		

Note: s = significant; ns = non-significant (based on t-test at $\alpha = 5\%$)

Peltate and capitate glandular trichomes were randomly scattered in the adaxial and abaxial surfaces of leaves. The density of peltate glandular trichomes was much higher in the abaxial than that in the adaxial surface. The distribution densities of both types of capitate glandular trichomes were similarly higher in abaxial than in adaxial surfaces. The uniseriate trichomes were distributed equally in the adaxial and abaxial surfaces (Table 1).

Studies on various species showed that glandular trichomes are usually found to be more densely distributed in abaxial surface of the leaf. Only in certain species that glandular trichomes are more abundant in the adaxial side, such as in *H. suaveolens* and *O. basilicum* (Ogunkule & Oladele 2000).

The peltate and capitate glandular trichomes found in the leaves of *H. capitata* are more abundant in abaxial than in adaxial surface. This distribution pattern is also observed in several species belong to Lamiaceae family i.e. *Mentha piperita*, *Salvia chrysophylla* and *Rosmarinus officinalis* (Turner *et al.* 1999; Kahraman *et al.* 2010; Boix *et al.* 2011). The same pattern is also observed in several other families, such as Dipterocarpaceae (*Parashorea* spp); Malvaceae (*Gossypium hirsutum* L.) and Fabaceae (*Phaseolus vulgaris* L.) (Dahlin *et al.* 1992; Noraini & Cutler 2009; Bondada & Oosterhuis 2000).

Idioblast cells

Idioblast cells in the leaves of *H. capitata* were round-shaped and found in the mesophyll of the leaf, from the palisade to the spongy parenchyma. Idioblast cells in the leaves of different plant species are not always found throughout the mesophyll. In some cases idioblast cells are found only in specific tissues. In *Litsea euosma*, *Litsea praecox* and *Actinodaphne trichocarpa* the idioblast cells were only present in the palisade parenchyma, while in the leaves of *Phoebe forrestii*, they were found in the spongy parenchyma (Gang & Hai 1999).

Idioblast cells found in the palisade parenchyma of *H. capitata* were larger than similar cells located in the spongy parenchyma, while the cell densities showed no significant difference (Table 1). Different species exhibit a variety of sizes and densities of idioblast cell. For example, in several species of *Machilus* and *Persea* genera,

various sizes and densities of idioblast cells have been previously reported i.e. the size of idioblast cells in palisade and spongy parenchyma of *Machilus leptophylla* were 30 - 40 μm with a density of 3 mm^{-2} ; in *M. yunnanensis* the idioblast cells were 25 - 50 μm with a density of 60 mm^{-2} ; in *M. salicoides* the idioblast cells were 30 - 40 μm with a density of 24 mm^{-2} ; the largest idioblast cells were found in *Persea americana* at 35 - 45 μm with a density of 89 mm^{-2} (Gang & Hai 1999). Referring to these data, the size of idioblast cells in the palisade and spongy parenchyma of *H. capitata* were much smaller in size than those in *Machilus* and *Persea* genera, while the density was much higher than those of the two genera.

Histochemical Analysis of Glandular Trichomes and Idioblast Cell

Histochemical testing of type I capitate glandular trichomes gave a positive result for terpenoids, as confirmed by the yellow-colored response to a cupric acetate reagent. The presence of alkaloids was indicated by the formation of a brownish yellow deposit, when treated with Wagner's reagent. Type II capitate glandular trichomes were shown to contain terpenoids, as indicated by the formation of a yellow color in the resultant mixture. This type II also contained alkaloids as indicated by brown color response as well as lipophilic compounds as indicated by yellow-colored reaction. Peltate glandular trichomes were tested positive for terpenoids and alkaloids indicated by yellow to brownish-yellow color. Uniseriate-type trichomes were tested positive for terpenoids as indicated by brown color as well as for alkaloids indicated by yellow to brownish-green color (Fig. 3).

Various studies reported on the metabolites produced by the glandular trichomes in Lamiaceae plants. Nazzar *et al.* (2010) reported that peltate and capitate glandular trichomes in Lamiaceae generally secrete essential oils in the form of terpenoid compounds. According to Gang *et al.* (2002), terpenoids in *Ocimum basilicum* (Lamiaceae) were produced by the peltate glandular trichomes. Glandular trichomes in *Mentha piperita* and *Mentha spicata* produced terpenoids in form of monoterpenes (Fahn 1979). In *Salvia sclarea* L., capitate glandular trichomes play a role in producing essential oil

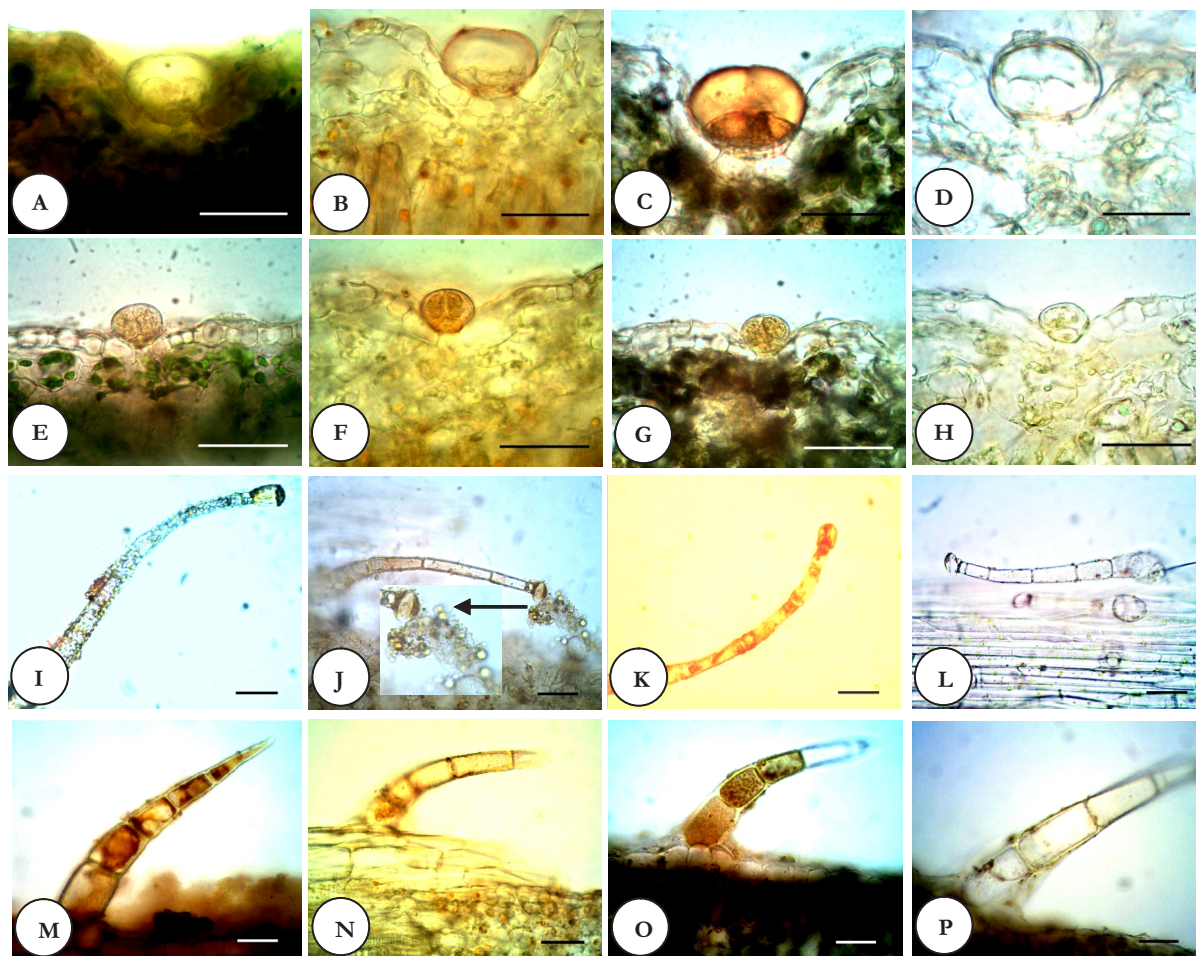


Figure 3 Glandular trichomes in *H. capitata*. Histochemical tests using cupric acetate for terpenoid (A, E, I and M); Sudan IV test for lipophilic substance (B, F, J and N); glandular trichome in water (control) (L); Wagner test for alkaloid (C, G, K and O); negative control for alkaloid test using tartaric acid reagent (D, H and P) (Note: Bar = 50 μm)

compounds like diterpene and monoterpenes in form of linalool and linalyl acetate compounds. Peltate glandular trichomes of *Salvia sclarea* L. produce sesquiterpene compounds (Schmiderer *et al.* 2008). Liu and Liu (2012) reported that

the glandular trichomes in the leaves of *Isodon rubescens* (Lamiaceae) contain a combination of phytochemical compounds, consisting of phenolic compounds, terpenoids, flavonoids, carbohydrates and alkaloids.

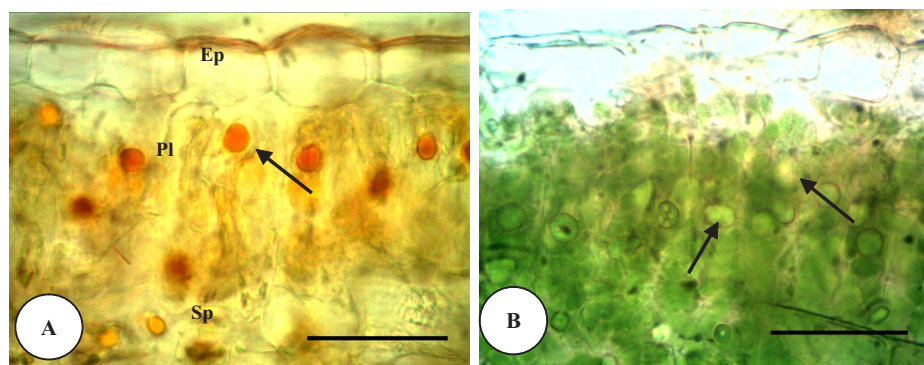


Figure 4 Histochemical results for idioblast cells in leaf transversal section; using sudan IV for lipophilic compounds (A) and water as a control (B) Ep = Epidermis, Pl = Palisade parenchyma and Sp = Spongy parenchyma (Note: Bar = 50 μm)

Histochemical tests conducted on the idioblast cells in the leaves of *H. capitata* did not detect terpenoids and alkaloids in these structures. However, these cells demonstrated the presence of lipophilic compounds, as indicated by yellow to orange colors (Fig. 4). Such cell types were found in *Physostegia virginiana* (L.) (Lamiaceae) (Lersten & Curtis 1998), but the idioblast cells containing such compounds are not specific to this family. Similar cell types are also present in various families, such as Lauraceae, Asteraceae, Caprifoliaceae, Rosaceae, Leguminosae (*Caesalpinia echinata*) and Bixaceae (*Cochlospermum rbegium*) (Lersten *et al.* 2006; Teixeira *et al.* 2007; Filho *et al.* 2014).

Gas Chromatograph-Mass Spectrometer (GC-MS)

GC-MS analysis revealed the presence of alkaloids, terpenoids, fatty acids and phenolic compounds (Table 2). L-limonene, eugenol, farnesol isomers A, d-nerolidol, hexahydrofarnesol, neophytadiene and dodeca-1,6-dien-12-ol, 6,10-dimethyl were the predominant terpenoids found in the leaf tissue. L-limonene was terpenoid detected in the largest quantity (18.69%). The alkaloids were detected in only one substance i.e. 1H-Indole, 3-methyl-(3-Methylindole). Those compounds support the healing of wound or infection and also act as antibacterial agents. According to Pei *et al.* (2009),

Table 2 Phytochemical compounds of *H. capitata* leaves using GC/MS

No	R Time	Metabolite Group	RI	Relative area (%)	Constituent
1	2.950	Alcohol	85249549	10.02	(O-D)ethenol
2	3.185	Amide	113186810	13.30	Acetamide, 2,2-dichloro- (CAS) Dichloroacetamide
3	4.501	Acetic acid	37932716	4.46	Acetic acid (CAS) Ethylic acid
4	5.228	Acetal	38862858	4.57	2-Propanone, 1-hydroxy- (CAS) Acetol
5	13.313	Ketone	17521339	2.06	1,2-CYCLOPENTANEDIONE
6	15.039	Phenol	13550624	1.59	Phenol (CAS) Izal
7	15.633	Terpenoid (Monoterpene)	159030886	18.69	L-Limonene
8	15.881	Ketone	16614244	1.95	2- Cyclopenten-1-one, 2-hydroxy-3-methyl- (CAS) Corylon
9	17.108	Phenol	37470245	4.40	Phenol, 4-methoxy- (CAS) Hqmmme
10	17.448	Aldheida alkyl	17828416	2.10	Pentanal (CAS) n-Pentanal
11	18.631	Aromatic aldehyde	9097365	1.07	Benzaldehyde, 2-methyl- (CAS) o-Tolualdehyde
12	19.688	Ester	13182646	1.55	2-Propenoic acid, 2-methyl-, ethyl ester (CAS) Ethyl methacrylate
13	19.849	Acetic acid	15135388	1.78	2,3-dihydro-benzofuran
14	21.122	Aromatic heterocyclic	31783131	3.74	1H-Indole (CAS) Indole
15	21.198	Phenol	29786120	3.50	Phenol, 4-ethenyl-2-methoxy-
16	21.813	Terpenoid	8964401	1.05	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol
17	22.552	Alkaloid	29383206	3.45	1H-Indole, 3-methyl- (CAS) 3-Methylindole
18	22.705	Terpenoid (Sesquiterpene)	8814772	1.04	Farnesol isomer A
19	22.915	Terpenoid (Sesquiterpene)	10811896	1.27	d-Nerolidol
20	23.496	Polycyclic aromatic	12729349	1.50	2H-1-Benzopyran-2-one (CAS) Coumarin
21	26.417	Terpenoid (Sesquiterpene)	8734907	1.03	1-Dodecanol, 3,7,11-trimethyl- (CAS) Hexahydrofarnesol
22	27.717	Terpenoid (diterpene)	24454424	2.87	Neophytadiene
23	27.999	Terpenoid	17043839	2.00	dodeca-1,6-dien-12-ol, 6,10-dimethyl-
24	28.207	Terpenoid (diterpene)	11252199	1.32	Neophytadiene
25	28.671	Fatty acid	13380605	1.57	Tetradecanoic acid, 12-methyl-, methyl ester (CAS) Methyl 12-methyltetradecanoic acid
26	29.191	Fatty acid	12112786	1.42	9-Octadecenoic acid (Z)- (CAS) Oleic acid
27	29.537	Vitamin	10213731	1.20	Vitamin d2-7,8-diol
28	30.656	Fatty acid	9972296	1.17	Tricyclo[3.2.2.0e2,4]non-8-en-exo-6,exo-7-dicarboximid, 3,3-dic
29	31.789	Fatty acid	19635391	2.31	Hexadecanamide (CAS) Amide 16
30	43.507	Fatty acid	17189048	2.02	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (CAS) Squ
Total			850925187	100	

eugenol (a member of terpenoids) also acts as antibacterial agent. Eugenol is able to inhibit the growth of gram-negative and gram-positive bacteria (Catherine *et al.* 2012). Antibacterial tests of eugenol proved that this substance can inhibit the growth of *Salmonella typhi*, a gram-negative bacteria (Devi *et al.* 2010). D'Alessio *et al.* (2014) reported that d-limonene acts as an anti-inflammatory that can significantly heal skin inflammation caused by murine 12-O-Tetradecanoylphorbol-13-acetate (TPA). This substance can also heal wounds by reducing neovascularization in mice. Kusma *et al.* (2009) reported that essential oil of *Salvia sclarea* leaves is used as analgesic, anti-inflammatory, antioxidant, antifungal and antibacterial agents. This oil is toxic to various pathogenic microbes, including *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* fungus. Curvelo *et al.* (2014) reported that nerolidol extracted from *Piper claussonianum* (Miq.) C. DC. is able to inhibit the development of *C. albicans*, a fungus which causes candidiasis. According to Ragasa *et al.* (2009), neophytadiene can inhibit the growth of some fungi and bacteria, including *C. albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes*, *E. coli* and *Pseudomonas aeruginosa*. Neophytadiene compounds serve as antipyretic, analgesic, anti-inflammatory, antimicrobial and antioxidant agents (Raman *et al.* 2012).

The leaf extract of *H. suaveolens* is used as traditional medicine in Brazil to treat inflammation, peptic ulcers, infections and can reduce gastric lesions in oral application in mice (Jesus *et al.* 2013). According to Shaikat *et al.* (2012), the leaf extract can cure diarrhea in mice previously treated with castor oil. *H. suaveolens* contains a combination of terpenoids, consisting of eucaliptol, gamma-ellene, beta-pyrene, (+)-3-Carene, trans-beta-cariophyllene and germacrene (Moreira *et al.* 2010). According to Malar *et al.* (2012) *H. suaveolens* also contains alkaloids, glycosides, saponins, tannins and flavonoids; the leaf extract of *H. suaveolens* can inhibit the growth of various bacteria such as *Aeromonas formicans*, *Aeromonas hydrophila*, *Bacillus subtilis*, *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Malar *et al.* 2012), as well as the growth of pathogenic fungi *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, *A. fumigatus* and *A. niger* (Moreira *et al.* 2010).

H. verticillata Jacq. is used as traditional medicine in Central America. This plant contains lignans, triterpenes, diterpenes, sesquiterpenes, monoterpenes, flavonoids, polyphenols and alkaloids. The crude plant extracts and isolated phytochemicals play a role in antibacterial and anti-fungal treatment of infectious diseases (Picking *et al.* 2013).

CONCLUSIONS

H. capitata leaves have secretory structures in the form of glandular trichomes and idioblast cells. The secretory structures produce secondary metabolite compounds. Glandular trichomes generally contain terpenoids and alkaloids. Idioblast cells contain lipophilic compounds. The terpenoid compounds, such as limonene, eugenol, farnesol isomers A, d-nerolidol, koumarin and neophytadiene, play roles in the healing process of wound or infection and act as antibacterial agents.

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