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
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 cacodylate 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 cacodylate buffer for 10 minutes each, 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 Harborne (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 and ion source temperature 200 °C. The carrier gas was helium. The spectrogram mass resulting from those processes was calculated then automatically 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) 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. baicaalensis*, *S. galleriulata* 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;
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 capitate 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* (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 capitate glandular trichomes in *H. capitata* had a short stalk with one cell and one head consisted of two cells. Type II of capitate 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 capitate 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 capitate 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

<table>
<thead>
<tr>
<th>Trichome types</th>
<th>Length of trichome stalk (µm)</th>
<th>Trichome head (µm)</th>
<th>Density (mm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adaxial</td>
<td>Abaxial</td>
<td>Adaxial</td>
</tr>
<tr>
<td>Peltate</td>
<td>7.5±0.5</td>
<td>7.8±0.9</td>
<td>27.7±0.3</td>
</tr>
<tr>
<td>Capitate type I</td>
<td>351±14.7</td>
<td>354.7±8.1</td>
<td>12.8±0.5</td>
</tr>
<tr>
<td>Capitate type II</td>
<td>343.7±32.6</td>
<td>342.7±44.2</td>
<td>27.5±0.7</td>
</tr>
<tr>
<td>Uniseriate</td>
<td>5.9±0.4</td>
<td>4.2±0.3</td>
<td>261.2±15.4</td>
</tr>
</tbody>
</table>

Note: s = significant; ns = non-significant (based on t-test at α = 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 forestii*, 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.
compounds like diterpene and monoterpenes in the 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.

![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)](image)

![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)](image)
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 rhiginum*) (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

<table>
<thead>
<tr>
<th>No</th>
<th>Ret Time</th>
<th>Metabolite Group</th>
<th>RI</th>
<th>Relative area (%)</th>
<th>Constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.950</td>
<td>Alcohol</td>
<td>85249549</td>
<td>10.02</td>
<td>(O-D)phenol</td>
</tr>
<tr>
<td>2</td>
<td>3.185</td>
<td>Amide</td>
<td>11316810</td>
<td>13.30</td>
<td>Acetamide, 2,2-dichloro- (CAS) Dichloroacetamide</td>
</tr>
<tr>
<td>3</td>
<td>4.501</td>
<td>Acetic acid</td>
<td>37932716</td>
<td>4.46</td>
<td>Acetic acid (CAS) Ethylic acid</td>
</tr>
<tr>
<td>4</td>
<td>5.228</td>
<td>Acetal</td>
<td>38862858</td>
<td>4.57</td>
<td>2-Propanone, 1-hydroxy- (CAS) Acetol</td>
</tr>
<tr>
<td>5</td>
<td>13.313</td>
<td>Ketone</td>
<td>17521339</td>
<td>2.06</td>
<td>1,2-CYCLOPENTANEDIONe</td>
</tr>
<tr>
<td>6</td>
<td>15.039</td>
<td>Phenol</td>
<td>13350624</td>
<td>1.59</td>
<td>Phenol (CAS) Izal</td>
</tr>
<tr>
<td>7</td>
<td>15.633</td>
<td>Terpenoid (Monoterpen)</td>
<td>15903086</td>
<td>18.69</td>
<td>L-Limonene</td>
</tr>
<tr>
<td>8</td>
<td>15.881</td>
<td>Ketone</td>
<td>16614244</td>
<td>1.95</td>
<td>2- Cyclopenten-1-one, 2-hydroxy-3-methyl- (CAS) Corylon</td>
</tr>
<tr>
<td>9</td>
<td>17.108</td>
<td>Phenol</td>
<td>37470245</td>
<td>4.40</td>
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<td>10</td>
<td>17.448</td>
<td>Aldheida alkyl</td>
<td>17828416</td>
<td>2.10</td>
<td>Pentanal (CAS) n-Pentanal</td>
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<tr>
<td>11</td>
<td>18.631</td>
<td>Aromatic aldehyde</td>
<td>9097365</td>
<td>1.07</td>
<td>Benzaldehyde, 2-methyl- (CAS) o-Tolualdehyde</td>
</tr>
<tr>
<td>12</td>
<td>19.688</td>
<td>Ester</td>
<td>13182646</td>
<td>1.55</td>
<td>2-Propenoic acid, 2-methyl, ethyl ester (CAS) Ethyl methacrylate</td>
</tr>
<tr>
<td>13</td>
<td>19.849</td>
<td>Acetic acid</td>
<td>15133588</td>
<td>1.78</td>
<td>2,3-dihydro-benzofuran</td>
</tr>
<tr>
<td>14</td>
<td>21.122</td>
<td>Aromatic heterocyclic</td>
<td>31783131</td>
<td>3.74</td>
<td>1H-Indole (CAS) Indole</td>
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<td>15</td>
<td>21.198</td>
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<td>29786120</td>
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<tr>
<td>16</td>
<td>21.813</td>
<td>Terpenoid</td>
<td>8964401</td>
<td>1.05</td>
<td>Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol</td>
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<tr>
<td>17</td>
<td>22.552</td>
<td>Alkaid</td>
<td>29383206</td>
<td>3.45</td>
<td>1H-Indole, 3-methyl- (CAS) 3-Methylindole</td>
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<tr>
<td>18</td>
<td>22.705</td>
<td>Terpenoid (Sesquiterpen)</td>
<td>8814772</td>
<td>1.04</td>
<td>Farnesol isomer A</td>
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<tr>
<td>19</td>
<td>22.915</td>
<td>Terpenoid (Sesquiterpen)</td>
<td>10811896</td>
<td>1.27</td>
<td>d-Nerolidol</td>
</tr>
<tr>
<td>20</td>
<td>23.496</td>
<td>Polycyclic aromatic</td>
<td>12729349</td>
<td>1.50</td>
<td>2H-1-Benzopyran-2-one (CAS) Coumarin</td>
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<tr>
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<td>26.417</td>
<td>Terpenoid (Sesquiterpen)</td>
<td>8734907</td>
<td>1.03</td>
<td>1-Dodecanol, 3,7,11-trimethyl- (CAS) Hexahydrofarnesol</td>
</tr>
<tr>
<td>22</td>
<td>27.717</td>
<td>Terpenoid (diterpen)</td>
<td>24454424</td>
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<td>Neophytadiene</td>
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<tr>
<td>23</td>
<td>27.999</td>
<td>Terpenoid</td>
<td>17043897</td>
<td>2.00</td>
<td>dodeca-1,6-dien-12-ol, 6,10-dimethyl-</td>
</tr>
<tr>
<td>24</td>
<td>28.207</td>
<td>Terpenoid (diterpen)</td>
<td>11252199</td>
<td>1.32</td>
<td>Neophytadiene</td>
</tr>
<tr>
<td>25</td>
<td>28.671</td>
<td>Fatty acid</td>
<td>13380605</td>
<td>1.57</td>
<td>Tetradecanoic acid, 12-methyl-1, methyl ester (CAS) Methyl 12- methylheptadecane</td>
</tr>
<tr>
<td>26</td>
<td>29.191</td>
<td>Fatty acid</td>
<td>1212786</td>
<td>1.42</td>
<td>9-Octadecenoic acid (Z)- (CAS) Oleic acid</td>
</tr>
<tr>
<td>27</td>
<td>29.537</td>
<td>Vitamin</td>
<td>10213731</td>
<td>1.20</td>
<td>Vitamin d2-7,8-diol</td>
</tr>
<tr>
<td>28</td>
<td>30.656</td>
<td>Fatty acid</td>
<td>9972296</td>
<td>1.17</td>
<td>Tricyclo[3.2.2.02,2]non-8-en-exo-6-exo-7-dicarboximid, 3,3-dic</td>
</tr>
<tr>
<td>29</td>
<td>31.789</td>
<td>Fatty acid</td>
<td>19635591</td>
<td>2.31</td>
<td>Hexadecanamide (CAS) Amide 16</td>
</tr>
<tr>
<td>30</td>
<td>43.507</td>
<td>Fatty acid</td>
<td>17180948</td>
<td>2.02</td>
<td>2,6,10,14,18,22-Tetracosahexeane, 2,6,10,15,19,23-hexamethyl- (CAS) Squalene</td>
</tr>
</tbody>
</table>

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 clausenianum (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-ellemene, beta-pynene, (+)-3-Carene, trans-beta-carophyllene 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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