Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.15, 2013

THE EFFECT OF PULSED ELECTRIC FIELD (PEF) ON GLANDULAR TRICHOME AND COMPOUNDS OF PATCHOULI OIL (Pogostemon cablin, Benth)

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Abstract
Patchouli oil could be obtained by extraction of patchouli leaves, where the leaves and patchouli is the main ingredient in the pharmaceutical industries such as cosmetics, perfumes, and recently for medical as antiseptic ingredient, because it is a fixative for other essential oils. Patchouli oil stored in glandular trichome (GT) cells and glandular mesophila (GM) cells within the leaves of patchouli plant. The research aimed to determine the effect of PEF (pulsed electric field) treatment on opening or damages on the cell wall of glandular trichome of fresh patchouli leaves and the quality of patchouli oil. Patchouli plant varieties of Sidikalang (Aceh) resulted by tissue culture in the Laboratory of Biology-Brawijaya University, after acclimatization planted in field trials. After 7 months age of plant, the fresh leaves (more than one leaves) were taken to observe the GT cell structure using light microscopy and SEM. Subsequently another leafe was treated with PEF at field strengths (E) between 50-150 V/Cm with a time of 2-3 seconds, and observed using SEM. Before distillation, 150 g of patchouli dry leaves was treated with PEF at 150 V/Cm and 15 seconds. The results showed that the PEF with field strength (E=100 V/Cm) and 2 seconds treatment, the GT cell walls were broken down (rupture). PEF treatment also increases 35% of the oil yield and better the proportion compound of patchouli oil.
Keywords: PEF, glandular trichome, cell wall changes, yield and oil compound

1. Introduction

Patchouli (Pogostemon cablin, Benth) is a native plant of tropical Asia (Kumaraswamy and Anuradha, 2010; Santos et al., 2010) and the essential oil is an export potential commodity for some countries in Asia (Sugimura et al., 1995; Santos, 2011). Patchouli oil is very important for the pharmaceutical industries such as perfumes, cosmetics and the medical world in the last decade is also using patchouli oil as an antiseptic ingredient (Lubbe and Verporte, 2011). As a result of the plant metabolism, essential oils are stored in special cells called glandular trichome (GT) and it is located on the abaxial and the adaxial of the leave and most are in the glandular mesophila cells (Amalia et al., 2011, Maeda and Miyake, 1997).
There are eight trichome types of patchouli leaves found, two of which were non-glandular and six is glandular (Rusydi et al., 2013). Glandular trichome is the greatest number and acts as a storage place and synthesis of essential oils and it is between the epidermal cell's position. Glandular trichome cells genus Lamiacaea as an organ function of essential oil production and there are two type glandular namely capitate and peltate glandular trichome (Lange and Turner, 2013). The form of glandular is oval and will be fully loaded with essential oil at the optimum age (Bouraui, et al., 2009). Essential oils are the result of secondary metabolites, stored in the glandular cells of leaves, twigs and stems of plants (Hopkins and Huner, 2009; Taiz and Zeiger, 2003).
Plant cell wall as the GT cell is composed of cellulose as major components (Cosgrove, 2005; Cooper and Hausman, 2007). In the extraction process so that the optimum oil carried out by damaging the cell wall (cellulose) which protects the oil, such as distillation using pressurized steam that had usually been done. Janositz and Knorr (2010) and Angersbach, Heinz and Knorr (2000) have reported the influence of Pulsed Electric Field (PEF) to rupture the plant cell walls. The rupture of the cell walls of cellulose cause the porous walls structure, thus facilitates the release of essential oils.
PEF treatment has been widely used in liquid extraction plant (Donsi, Ferrari and Pataro, 2010; Loginova, et al., 2010; Sack, et al., 2010; Vorobief and Lebovka, 2010), extraction of oil from peanut seeds (Zeng , Han and Zi, 2010) and seed corn oil extraction (Guderjan, et al., 2005). PEF treatment for extraction polyphenols from orange peel have also been reported by Luengo, Alvarez and Raso (2013). Besides, it has been widely used also

for preservation of food and drinks as well as fruit juice extraction (Praporsic, Muravetchi and Vorobiev, 2004; Grimi et al., 2010).
Pretreatment prior to the extraction process of plant bioactive materials using PEF has been reported (Belaya, et al., 2006; Fortuny et al, 2009). Extraction of colorants from red beet is reported by Loginova, Lebovka and Vorobiev (2011). However, the use of PEF treatment for extraction of essential oils of patchouli plant is still very limited, so it is very interesting to study. The aims of this research are to study the influence of PEF treatment on the change of the cell walls structure, the yields and quality of patchouli oil produced after the extraction of patchouli leaves. It is very important in the industrial world, as it deals with the time, cost and quality of the production, which are usually done without preliminary treatment in traditional industries of patchouli oil in Indonesia.

2. Materials and Methods

2.1. Materials

Patchouli plant varieties Sidikalang (Aceh) results in tissue culture propagation on the Laboratory of Biology Brawijaya University, after acclimatization then planted in field trials in district of Kesamben-Blitar, East Java was used in this research. After the age of 5 months the plant was transferred to a pot and brought to the Laboratorium and wait for 2 months for recovery before the plant is used for the research. Future observation was done to observe the leave cell morphology and changes that occur after PEF treatment, especially the cell trichome glands.

2.2. Sample Preparation

After picked from the pot, the fresh cut patchouli leaves with gold coating then performed using a sputter coater SC-7620 (Emitech). Most of the other leaves, before treated with PEF cut and carried coated.

2.3. PEF System and Treatments

PEF treatment of patchouli leaves is illustrated on Figure-1. Patchouli leaves picked at the bottom of the stalk, and placed into the chamber. PEF generator ignited for 2 and 3 seconds with the electric field (E) 50-150 V/Cm and leave the position (sample) is in the middle between the cathode-anode.

2.4. Microscopic Analisys

Microscopic observations performed on fresh leaves before and after treating with PEF using a Scanning Electron Microscope (SEM) FEI-Inspect S25-EDAX, light microscopy Olympus CX-31.

2.5. Extraction and Analysis Quality of Patchouli Oil

Leaves which are dried by dry air until 15% of water content. Patchouli oil is extracted from 150 g leaves which are dried and treated by PEF (E=150 V/Cm and 15 seconds) prior to steam distillation during 4 hours and patchouli oil was an analysis by GC-MS.

3. Resulsts

3.1. Observations of Fresh Leaves Cells Structure

Observations of fresh patchouli leaves cell structure with SEM (Figures-2A, B, C, D) seems very clear that there are glandular trichome cells (GT), and feathers (Tector). There are differences in cell wall structure of the abaxial and adaxial GT. GT cell walls tend to smooth for the abaxial, while the adaxial GT cell wall surface looks rough, it appears there was some sort of lines that are spread evenly across the surface. The results of the SEM do not describe the position of the oil in the cell GT, so it needs to be seen with a light microscope so that action can be taken further to PEF treatment. Position of essential oils in GT cells is very important to know, because it will determine how to and optimal PEF treatment to break up or make a porous cell wall.
The GT cell number on the mean leaf area of 1.96 mm2 were filled with essential oils are ± 13 cells, meaning that for an area of 1 cm2 the cell number is ± 65 GT, with cell diameter ranged from 53-63 µm. There are ± 6 cell GT were found not contained oil, it is probably already broken or damaged natural and essential oils have evaporated because the age of the leaves. The calculations show that the density of cells in the leaves GT is relatively high, so the amount of oil in the leaves is also great. GT cell structure observes by light microscopy as shown in Figures-3A, B.

Observations with the light microscope more visible presence of essential oils in GT cells, and the cell structure is also more obvious. Structure and the position of glandular mesophila (GM) cells that juts into the cuticular layer illustrates that the essential oils sheltered position. This is in accordance with the statement of Taiz and Zeiger (2003); Dunkic et al. (2007) and Bouroui et al. (2009) that the essential oils produced in the secretory cells and accumulated in the cavity between the secretory cells and the cuticula. Observation by polarimetre microscope indicated that in this cell presents of the rainbow and it is indicated by the oil of patchouli.

3.2. Observation of Cell Structure of Leaves After PEF Treatment

After PEF treatment with several variations of frequency, voltage and times it is shown the following results (Table 1). The structure of the GT cells after PEF treatment is change (rupture) and it is difference between abaxial and adaxial cell shape (Figures 4 and Figures 5)
Table-1 shown that the microscopic observation on the frequency 578 Hz and voltage 500 volts and 2 seconds PEF treatment, most of the GT cells was damaged (83%), whereas for 3 seconds the damage as much as 89%. To break up all the GT cells required frequencies above 1000 Hz, voltages above 1000 volts (E> 100 V / cm) with a time of ± 2 seconds. GT cell shape after PEF treatment in conditions such as a ball erupted. GT cells after PEF treatment contract and diameter change.

3.3. Quality of Patchouli Oil

Extraction of patchouli oil by distillation resulted in the volume and compound of patchouli oil was significantly different. Volume of oil (yield) up to 35% (compare with the yield of without PEF) and compound of oil change from 21 to 27 (Figures-6) and percentage of the compound of the oil is shown in Table-2. The chemical components of patchouli oil are very good for health because it is an antioxidant, anti-stress, anti inflammatory and diuritic (Manglani, Deshmukh and Kashyap, 2011). Patchoulol and α-patchoulene are important compounds of patchouli oil (Donelian, et al., 2009).

4. Discussion

GT cell morphological are differences between abaxial and adaxial leaf. The abaxial GT cells a smooth surface (Figures-2B), while the adaxial GT cells looks the fibers (Figures-2D). A GT cell wall at the abaxial look smooth because of the wax coating.
The morphology of the leaves (Figures-2) presented of the GT cells but not yet visible presence of patchouli oil. The results of SEM indicate that tector and GT much on the leaf surface (abaxial) with a high density. This suggests that the patchouli oil is relatively high amounts in the leaves. Glandular trichome is a place of production oil and storage potential of the oils without being affected by light (Huang, Kirchoff and Liao, 2008). There are two glandulars: the first, non-trichoma (Tector) of the plume and did not contain oils and the second is peltate trichoma and oval form with a bit position into the epidermis and contains the oil molecules (Figures-3). Both Glandulars are found in relatively high amounts on the leaf surface (abaxial). As noted by Siebert (2004) on the plant genus Lamiaceae found many trichoma peltate glands on the upper leaf surface (abaxial).
Glandular trichoma of patchouli leaves are peltate type and located on the abaxial and adaxial. Peltate glandular trichoma very large and full of oils (Figures-3). In addition the abaxial and adaxial present of the leaves hair is called tectore, as in the genus Lamiaceae (Zuzarte, et al., 2010; Bouraoui et al., 2009). The presence of glandular trichoma include the number and density (Gersbach, 2001) is very important for patchouli plant because it is a producing and storing essential oils. Glandular trichoma contain enzymes that synthesize distinctive and serves as the mono-and sesquiterpene volatile oil component (Zaks, et al., 2008).
Trichoma peltate glandular is a major secondary metabolite formation as a class of terpenes and storage as well as the lavender flowers (Guitton et al., 2010). Each type has a characteristic morphology of glandular trichoma, ontogeny, histochemistry and secretion independently (Kolb and Muller, 2004). Peltate glandular trichoma issued in the form of terpene compound and accumulates in the subcuticular space (Siebert, 2004). Subcuticular space can be enlarged by the essential oils so filled oval (Figures-2 and Figures-3). Essential oil consists of mono and sesquiterpene and present of its affected by specific amino acids called enzyme plant producing. The corelation between specific amino acids and essential oil production are reported by Degenhardt, Kollner and Gershenzon (2009).
PEF treatment of 1,0 kV (E=100 V/Cm), frequency 1,0 kHz, and cathode-anode distance of 10 Cm for 2 seconds to give a major influence on the structure of glandular trichoma cells patchouli leaves. Permeabilization of the cells membrane with direct current moderate electric field (DC-MEF) frequency is very important (Kulshrestha and Sastry, 2003). There is appear rupture and essential oils out (Figures-4 and Figures-5). Treatment of the subject seems too great so that to allow the process of cells not rupture but only increased porosity is required further study. Lebovka, Bazhal and Vorobief (2002) estimated that PEF 400 V/Cm is the optimum condition for

investigation cells disintegration. The optimal of electric field strength (E_{opt}) on the vegetables depends on the type of tissue and is higher for cells having developed secondary cell wall (Bazhal; Lebovka and Vorobiev, 2003). Electroplasmolysis with PEF affects not only plasmalemma membranes but also cell wall integrity of apple tissue (Bazhal; Ngadi and Raghavan, 2003).

The impact of PEF treatment at the combination voltages, frequencies and times on the structure of leaf cells (GT) is so transparent and patchouli oil is not a good look at glandular trichome and glandular mesophilla. Cell structure seems fragile and torn apart and are visible only chlorophyls. This suggests that to know the porosity of GT cells of patchouli leaves, treatment time should be under 2 seconds. Besides, the amount of voltage or energy needed to make glandular trichoma cells should be minimized become more porous. Rupture and cell damage is largely determined by the magnitude of the voltage, frequency, number of pulses and timeworn materials. As performed by Praporscic, Muravetchi and Vorobiev (2004) on sugar beet crops. Cell damage can be caused by voltage differences inside and outside the cell, and called the trans membrane potential (Jiahui et al., 2009). Evidenced by the high voltage (100 V/Cm) imposed on patchouli leaves for 2 seconds relative damaged cells. Patchouli leaf including the type of soft tissue, so treatment needs to be reviewed, to get the magnitude of the voltage, frequency, number of pulses and the optimal time.

Plant cell walls as like the glandular trichome cells is consisting of cellulose (Cosgrove, 2005). Pulsed electric field technology (PEF) is caused stress of membrane cell and a accouring of pore (Wijngaard et al., 2012). It is condition depends on field strength, frequency and times of PEF. In addition, that combination step of PEF and other extraction technology can increasing of the extraction efficiency. Cell shape of the glandular trichome (GT) of patchouli leaves is >50 µm (Figures 2), and breakdown of the cell membrane by the electric field strength with cell shape 50-120 µm is 400-800 V/Cm (Angersbach, Heinz and Knorr, 2000). Increasing of the oil distillation yield is 35% with an electric field 150 V/Cm and 15 seconds and change existing oil components also occur than without PEF (Figures 2 and Table 2). This suggests that PEF should be the improvement of the efficiency of the extraction process and also improve the quality of patchouli oil. Critical strength of plasma membrane treated with PEF are depend on characteristic of plant cells. Plasma membrane breakdown of onion tissue critical field strength is 67 V/Cm (Asavasanti et al., 2010). Diffusion of cells compound as an indicated of the electroporation of the cell membrane by PEF affected by frequency. The higher in frequency also higher of the threshold potential for permeabilization (Kulshrestha and Sastry, 2003). Ersus and Barrett (2010) reported that cell onion tissue is irreversible rupture with PEF at 333 V/Cm.

The percentage of macro components of the patchouli oil are decreasing, but a micro component is increased except palustrol, α-guaiene and 2,3,3-Trimethyl-2-(3'-Methyl-1 '3'-butadiene-1'-6-Methylidencyclohexanone. The number of components in the oil increased due to a number of 8 kinds of PEF despite missing 2 components (Table-2). This suggests that an increase in the quality and quantity of the PEF treatment on extraction of patchouli oil.

5. Conclussions

Patchouli plants have two types, namely of non-glandular trichoma and peltate glandular trichoma. Tectore shaped like feathers, and found on the abaxial and adaxial, but not containing patchouli oil. Peltate glandular trichoma round oval shape, located on the leaf surface (abaxial and adaxial) and filled the position of patchouli oil and protrudes into the inside subcuticula. Patchouli oil is also found on the inside of the leaf tissue patchouli plant is in glandular mesophila cells with numerous scattered positions.

PEF treatment with E >100 V/Cm for 2 seconds already showing damage of more than 95% of glandular trichoma cells of patchouli leaves. Patchouli oil yield was increased 35% (compare to without PEF) and the compound was changed (percentage and number of patchouli oil compound). Voltage, duration, frequency and the number of pulse optimal treatment to the porosity of the glandular trichoma cells wall of patchouli leaves need to be investigated further.

Acknowledgments

Thanks a lot to my wife and my son who has encouraged my studies. Thank you also to Nunung Harijati, Ph.D., who helped create the photographs.

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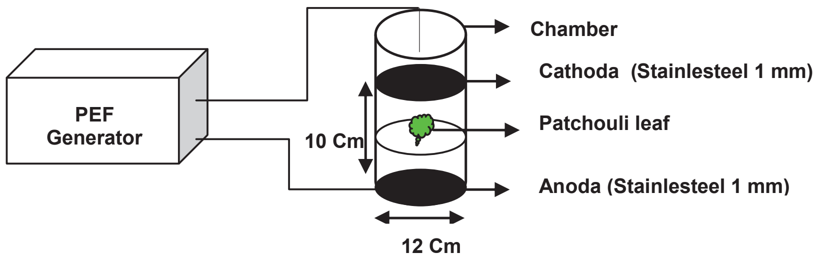
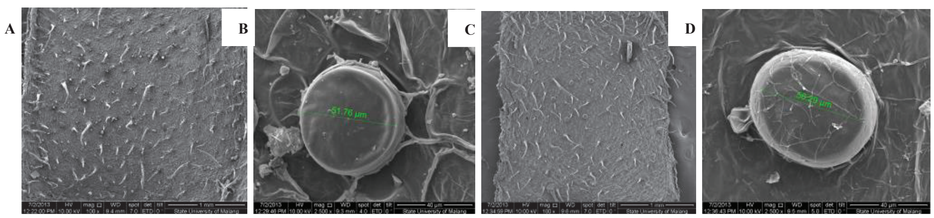
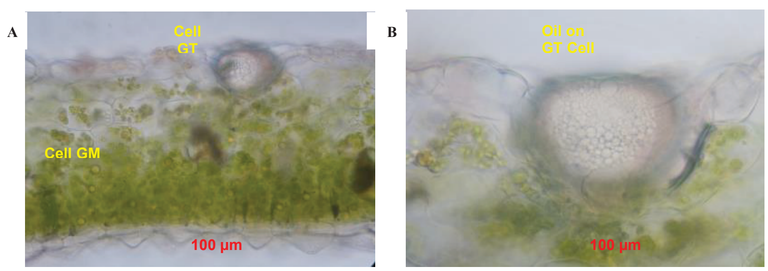


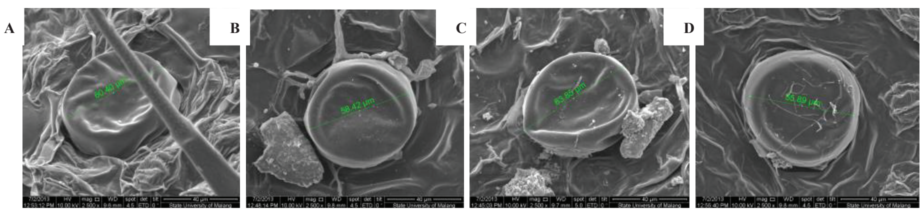
Figur 1. Scheme of PEF Treatment



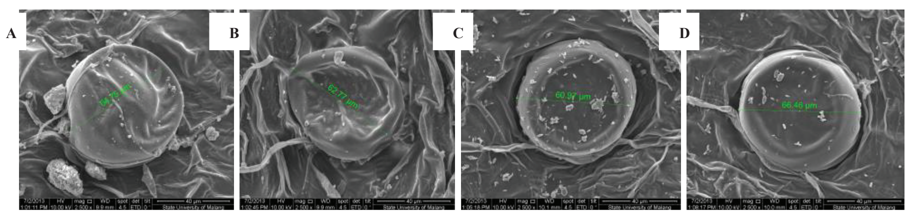
Figures 2. Abaxial and GT Cell (A, B) Adaxial and GT Cell (C, D)



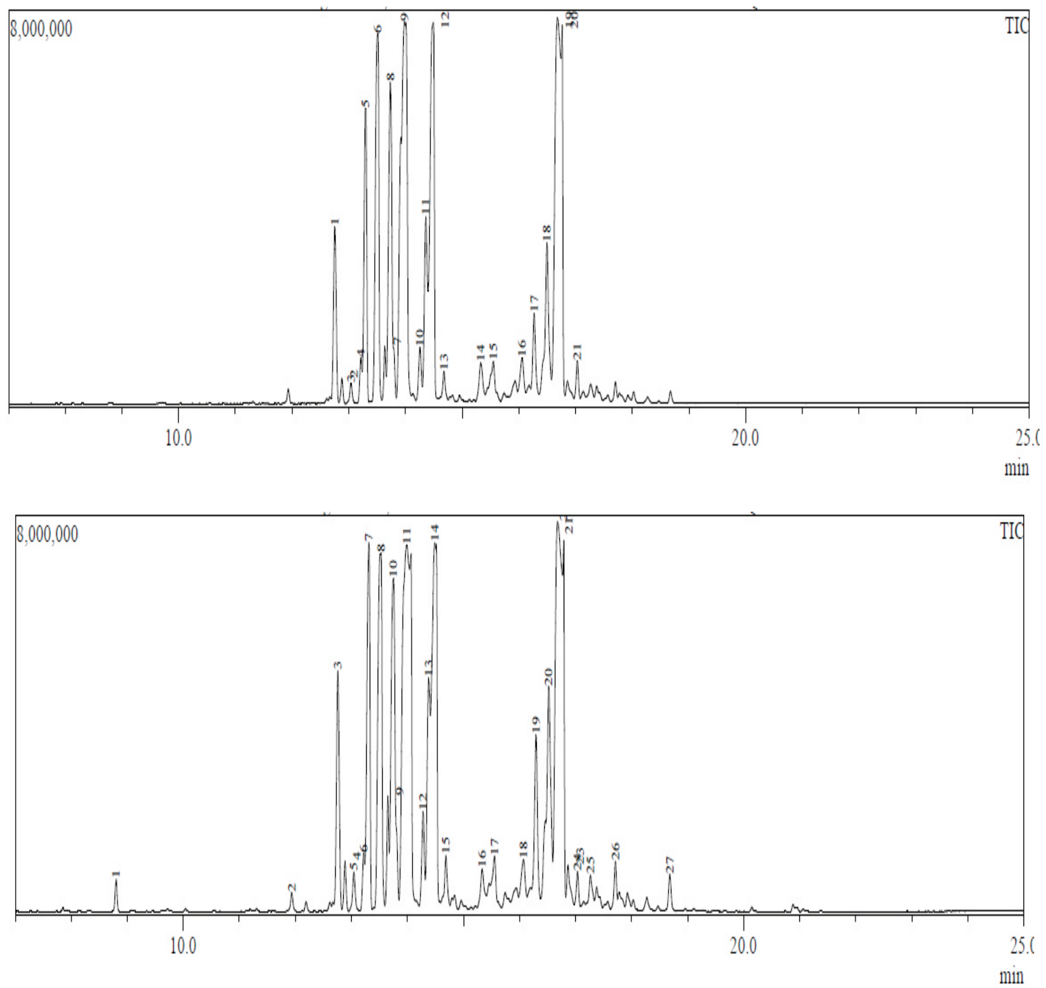
Figures 3. Light Microscopic of Fresh Patchouli Leaves.
A). Cross section, B). Oil on GT



Figures 4. Abaxial (A, B) and Adaxial (C, D) GT after PEF 578 Hz; 1100 Volt, 2 Secconds



Figures 5. Abaxial (A, B) and Adaxial (C, D) GT after PEF 578 Hz; 1100 Volt, 3 Secconds



Figures 6. Patchouli Oil Compound Changes Before and After PEF

Table 1. Percentage of the GT cells rupture in various treatment PEF Frekuency (Hz)	Voltage (Volt)	Pulsed with (%)	Times (Second)	Cells rupture (%)			Everage (%)
				I	II	III	
1108	1000	75	2	100	100	100	100
1002	500	75	2	96	100	97	98
578	1100	75	2	89	88	100	92,3
578	500	75	2	84	87	79	83,3
578	500	75	3	91	86	89	89
578	1000	75	3	97	98	98	97,7

*) Cathode-anode distance = 10 Cm

Table 2. Compounds of Patchouli Oil

Patchouli Oil Compounds without PEF	%	Patchouli Oil Compounds (PEF 150 V/Cm, 15 seconds)	%
Macro Compounds		**Macro Compounds**	
Patchouli alkohol	21,60	Patchouli alkohol	18,48
∂-guaiene	15,41	∂-guaiene	9,09
Seychellelene	9,08	Seychellelene	8,83
β-patchoulene	3,75	β-patchoulene	3,39
Micro Compounds		**Micro Compounds**	
Zingiberene	0,42	Zingiberene	0,69
β-Himachalene	0,41	β-Himachalene	0,67
Trans β-Caryophyllene	5,87	Trans β-Caryophyllene	6,49
β-Sesquiphellandrene	0,89	β-Sesquiphellandrene	1,37
Diepi-α-Cedren	19,81	Diepi-α-Cedren	20,81
Palustrol	0,93	Palustrol	0,83
∂-Guaiene	12,53	∂-Guaiene	11,29
Cyclohepten	0,57	Cyclohepten	0,79
Cyclohexanone	0,62	Cyclohexanone	0,90
Caryophyllene Oxide	0,82	Caryophyllene Oxide	0,89
γ-Gurjunenepoxid	1,59	γ-Gurjunenepoxid	2,65
2,3,3-Trimethyl-2-(3'-Methyl-1'.3'-Butadien-1'-6-Methylidencyclohexanone	0,62	2,3,3-Trimethyl-2-(3'-Methyl-1'.3'-Butadien-1'-6-Methylidencyclohexanone	0,50
Cis-Caryophyllene	1,20	-	
Veridiflorol	4,36	-	
-		∂-Elemene	0,24
-		β-Elemene	0,72
-		Citronella	0,36
-		Aciphyllene	4,34
-		Epiglobulol	4,35
-		γ-Gurjunene	0,73
-		Valerenyl Acetate	0,49
-		Cembrene	0,52
Total	**100**	**Total**	**100**