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Article

Optimization of Producing Zein Clove Leaf Essential Oil Nanoparticles for Their Antioxidant Activities

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Abstract

Essential oils are often also referred to as flying oils produced by plants and usually volatile essential oils at room temperature, one of the essential oils with many uses is clove leaf essential oil (Syzygium aromaticum L). Zein-essential oil nanoparticles were prepared by varying the concentration of clove oil (0, 2, 4, 6, 8, and 20 mg/mL) added to 1.5 mg/mL zein and 1 ml tween 20 and sonicated for 20 minutes. The antioxidant activity of the zein-essential oil nanoparticles formed was tested using the DPPH method. The best antioxidant activity was found in the addition of clove leaf essential oil 8 mg/mL with a percentage of inhibition of 71.13%. Characterization test with a laser beam where the amount of light scattered by the solution. Chemical component analysis using Gas Chromatography Mass Spectrophotometry (GC-MS) showed the presence of 32 compounds with three main compounds, namely trans-β-caryophyllene (46.69%), chavibetol (25.80%) and - humulene (10.84%).

INTRODUCTION

Essential oils are also called essential oils or flying oils (essential oil, volatile oil) produced by plants, the characteristics of these oils are volatile at room temperature, smell good according to the smell of the producing plants, generally soluble in organic solvents and insoluble in water. The chemical components of essential oils are generally hydrocarbons and oxygenated hydrocarbons. The types of hydrocarbons contained in essential oils consist of terpenes, paraffins, olefins, and aromatic hydrocarbons [1].

One of the potential plant to produce the largest essential oil in Indonesia is namely the clove plant, clove essential oil is needed in various industries such as raw materials for food flavoring and fragrances, the cosmetic industry, the pharmaceutical industry [2]. Clove leaves themselves have not been used much by farmers compared to clove flowers or stalks which are often used in the cigarette and food industries [3]. The main components contained in clove leaf essential oil are eugenol (80%-90%) and caryophyllene [4]. Based on research by Nurhadianty, the results of isolating clove leaf essential oil by steam distillation method for 6 hours were analyzed using Gas Chromatography Mass Spectrophotometry (GC- MS) and found that the main compounds were total eugenol (71.34%) and total caryophyllene (25.52%) [5]. The eugenol compound in clove oil is one of the phenolic compounds which acts as an antioxidant, this compound is important in maintaining a healthy body because it functions as a scavenger of free radicals in the body [6][7]. Free radicals are atoms or molecules that have high activity, because these compounds have unpaired free electrons [8]. One of the mechanisms of action of antioxidant compounds is by donating hydrogen atoms or protons to radical compounds, this causes the radical compounds to be more stable [9]. One method for determining antioxidant activity is the DPPH (2,2-diphenyl-1-picrylhidrazil) method, this method is used to test a component as a free radical scavenger in a substance or extract.

One way to improve the function of clove leaf essential oil is by adding a natural polymer matrix that is used to bind various compounds and provide stability to clove leaf essential oil. Zein is a natural polymer matrix derived from corn seed prolamin protein powder [10][11]. Zein functions as a natural and biocompatible carrier substance, which has advantages compared to animal protein because it does not have zoonotic disease transmission (diseases transmitted from animals to humans caused by parasitic microorganisms), but the disadvantage of zein is that it has low aggregation stability when suspended in water. , to increase the stability of zein by combining with polysaccharides as a second polymer matrix [12]. Tween 20 (polysorbate 20) is a polysaccharide widely used as an emulsifier or emulsifying agent and nonionic surfactant [13]. According to Murtiningrum's research (2013) tween 20 can be used in the formation of red fruit oil emulsions because it is hydrophilic but fat soluble, hydrophilic properties come from hydroxyl and oxyethylene groups while it can dissolve in fat due to the presence of long hydrocarbon chains [14]. In the study by Da Rosa et al., zein was used as a carrier besides that zein had an absorption value between 88-99%. A high absorption efficiency value indicates that zein protein has the ability to absorb active compounds [15]. This is the background for adding zein to clove leaf essential oil. According to Murtiningrum's research (2013) tween 20 can be used in the formation of red fruit oil emulsions because it is hydrophilic but fat soluble, hydrophilic properties come from hydroxyl and oxyethylene groups while it can dissolve in fat due to the presence of long hydrocarbon chains [16]. In the study by Da Rosa et al., zein was used as a carrier besides that zein had an absorption value between 88-99%. A high absorption efficiency value indicates that zein protein has the ability to absorb active compounds [17]. This is the background for adding zein to clove leaf essential oil. According to Murtiningrum's research (2013) tween 20 can be used in the formation of red fruit oil emulsions because it is hydrophilic but fat soluble, hydrophilic properties come from hydroxyl and oxyethylene groups while it can dissolve in fat due to the presence of long hydrocarbon chains [16].

Based on the above, Zein essential oil (Syzygium aromaticum L) was prepared, using variations in the concentration of essential oil (Syzygium aromaticum L) with zein and tween 20 as emulsifiers, then the value of antioxidant activity was determined using the DPPH method. Formula that provides optimal results and value of antioxidant activity in the analysis of chemical components with GC-MS.

EXPERIMENTAL SECTION

Materials

The materials used in this study included clove leaf essential oil (commercial), zein(sigma), ethanol (C2H5OH)(Sigma Alderich), distilled water (H2O), tween 20 (C26H50O10)(merck), ascorbic acid (C6H8O6) (merck), and DPPH (2,2-diphenyl-1-picryhidrazil)(Merck). The equipment used in this study were glassware, measuring pipette, suction ball, spatula, dark bottles, vials, analytical balance, 96-well plate, red laser pointer, sonicator (Bendelin electronic Type RK 510),microplate reader(iMarkTM),UV-Vis spectrophotometer (UV-1800 spectrophotometer, Shimadzu, Japan) and GC-MS-QP2010 (Shimadzu Tokyo, Japan).

Methods

Preparation of clove leaf essential oil

The sample used was clove leaf essential oil (Syzygium aromaticum L) sent from an essential oil company in Medan city.

Preparation of zein nanoparticles containing essential oils

The manufacture of zein-essential oil nanoparticles follows the procedure of Wulandari (2016). A total of 150 mg of zein protein powder was dissolved using 45 mL of 70% ethanol. Furthermore, clove leaf essential oil with several variations (0, 200, 400, 600, 800 and 2000) mg was dissolved in 45 mL of 70% ethanol, the two solutions were mixed then added 1 mL of tween 20. The solution was added with 70% ethanol until the volume of the solution was 100 mL, after which the solution was homogenized using a sonicator with a frequency of 35 kHz for 20 minutes. NPZSa was prepared with the composition shown in Table 1

Table 1. Composition of NPZSa

Sample Code	Zein (mg/mL)	*Syzygium aromaticum* Essential Oil (mg/mL)	Tweens 20 (%)
F1	1.5	0	1
F2	1.5	2	1
F3	1.5	4	1
F4	1.5	6	1
F5	1.5	8	1
F6	1.5	20	1

Determination of antioxidant capacity

Preparation of a standard solution of ascorbic acid calibration curve

The standards used in determining the antioxidant capacity were ascorbic acid standards (0.2; 0.4; 0.6; 0.8; 1; 1.2 mg/mL). Pipette 50 µL of each standard solution and put it into a 96 well microplate and add 75 µL of 0.4 mM DPPH solution, then incubate for 30 minutes in the dark room. The absorbance was measured using a microplate reader at a wavelength of 517 nm.

Determination of the antioxidant activity of NPZSa and Syzygium aromaticum leaf essential oil

Determination of antioxidant activity was carried out using the DPPH (2,2- diphenyl-1-picrylhidrazyl) method. The sample solution (F1 to F6) was diluted 10 times. Each of the samples was then taken 50 µL and put into a 96 well microplate and added with 75 µL of 0.4 mM DPPH solution. Then incubated for 30 minutes in a dark room. The absorbance of the sample was measured using a microplate reader at a wavelength of 517 nm.

Antioxidant activity can be expressed in units of percent inhibition. Percent inhibition formula:

Information :

$$\% \text{ inhibition} = (AB\text{-}AS/AB)\times 100\%$$

AB = absorbance blank

AS = Sample absorbance

Characterization of Nanoparticles

Characterization of essential oil-based nanoparticles was carried out using laser light, UV-Vis spectrophotomer, and GC-MS to determine the nature and character of the formed nanoparticles. For the characterization used nanoparticles in optimum conditions.

Characterization with laser light

Observation of the presence or absence of particles was carried out using the tindal effect method with a black background. The red laser pointer light source is focused by shooting a beam at the sample to be tested horizontally to see the particles.

Characterization with UV-Vis

Clove oil (Syzygium aroaticum) zein nanoparticles, confirmed by UV-Vis spectrophotometry (UV-1800 spectrophotometer, Shimadzu, Japan). Samples were diluted with 70% ethanol and UV-Vis spectra were recorded using a quartz cuvette with 70% ethanol as a blank. Spectrophotometer readings are recorded at a scan of the 250–400 nm range.

Characterization with Gas Chromatography Mass Spectrometry(GC-MS)

Characterization of NPZSA and clove leaf essential oil using GC-MS (Shimadzu QP2010) equipped with an Rtx-5MS column (5% diphenyl 95% dimethyl polysiloxane) with a length of 30.0 m, an inner diameter of 0.25 mm and a thickness of 0.25 µm. The mobile phase used in the GC-MS characterization is helium gas combined with a selective mass detector (mass spectrometer) and the stationary phase, namely polycycline. Samples were injected in split mode using an injector operated at 250°C. The oven temperature used is 120°C and then increased 5°C per minute until the temperature is 250°C. In conducting the qualitative analysis of the samples, QP2010 mass spectrophotometry was used. The chemical composition and levels of the compounds obtained were analyzed through the GC chromatogram peaks and then compared with the MS spectral data base of the characterization results.

RESULT AND DISCUSSION

The NPZSa which has composition of the hight amount addition of *Syzygium aromaticum* leaf essential oil which produces a cloudy color while the addition of a small amount of essential oil produces a clear color. When giving a red laser light to the formula, where F1 to F3 indicates the absence of particles in the solution, while F4 to F6 there are particles in the solution. The results of the antioxidant activity test with the DPPH method for each formula have the potential as antioxidants, the high antioxidant activity in formula 5 is 71.13%. The high antioxidant activity in the analysis of the chemical components contained in ZMDC was analyzed using GC-MS there were 32 compounds with 3 main compounds Chavibetol,The levels of Trans-β- Caryophyllene and α-Humulene, the 3 main compounds, changed after 2 months of storage. The percentage of Chavibetol and Trans-β- Caryophyllene increased by 3% while that of α-Humulene decreased by about 2%.

Zein nanoparticles of clove leaf essential oil

The ZMDC composition was prepared by adding 1.5 mg of zein concentration with several additions of clove leaf essential oil concentrations (0, 2, 4, 6, 8, 20) mg/mL. Each NPZSa formula that has the addition of essential oils that have more colors produces turbidity, while the addition of a little essential oil produces a clear color, which can be seen in Figure 1.

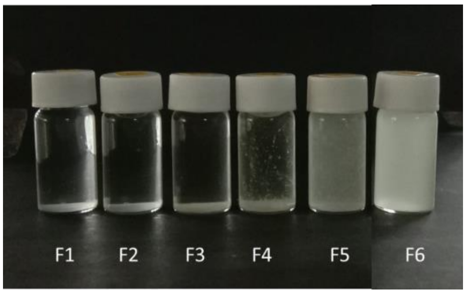

Figure 1. NPZSa Composition of zein nanoparticles in clove leaf essentialoil

Zein can absorb essential oils because zein has a hollow surface thatacts as hots, while clove leaf essential oil will stick and adjust to the surface of zein, this happens due to pore interactions that are ownedby zein, to stabilize the interfacial bond between zein and essential oil clove leaves then add tween 20.

Observation using a laser beam

NPZSa is a nano-sized colloidal solution, the presence of colloidal particles is indicated by the presence of a red laser beam on the formula, by irradiating the sample horizontally or by irradiating it from the side of the tube. According to Hidayat and Rachmadiyanto, the larger the particle size in a substance is indicated by the more scattering and the clearer the color change in the sample17. F1 to F3 indicates the absence of particles in the solution, while F4 to F6 indicates the presence of particles in the solution, as can be seen in Figure 2.

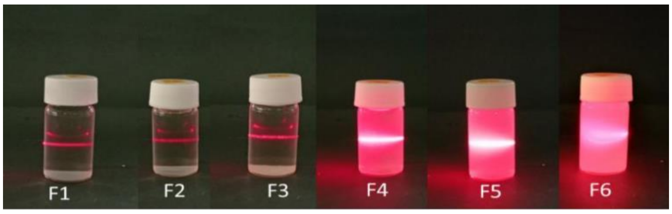

Figure 2. The effect of red laser light on the NPZSa formula

Antioxidant Activity Testing

Antioxidant activity was tested using the DPPH method (2,2- Diphenyl-1-pikrihidrazil). The color absorption of DPPH can bemeasured using a spectrophotometer at a wavelength of 515-520. Measurements at these wavelengths are caused by the purple color that is owned by DPPH. The decrease in absorbance in the DPPH solution indicates that the tested sample has activity in inhibiting free radicals. Inhibition of free radicals in samples can be determined using IC50, which is the minimum concentration to inhibit 50% of DPPH free radicals [18].

The results of the antioxidant activity test, each formula has the potential to be an antioxidant because it has a percentage of inhibition which can be seen in Table 2. The high or low antioxidant activity in the samples can be seen from the percentage of inhibition produced. The greater the percentage inhibition value, the higher the value of antioxidant activity [19] .

Table 2. Antioxidant inhibition

Formulas	inhibition (%)
F1	22.54
F2	63,71
F3	67.08
F4	69,83
F5	71,13
F6	70,79

Effect of Storage Time on ZMDC Components

ZMDC analysis using QP2010 SHIMADZU was carried out to determine the content and composition of the compounds contained in ZMDC

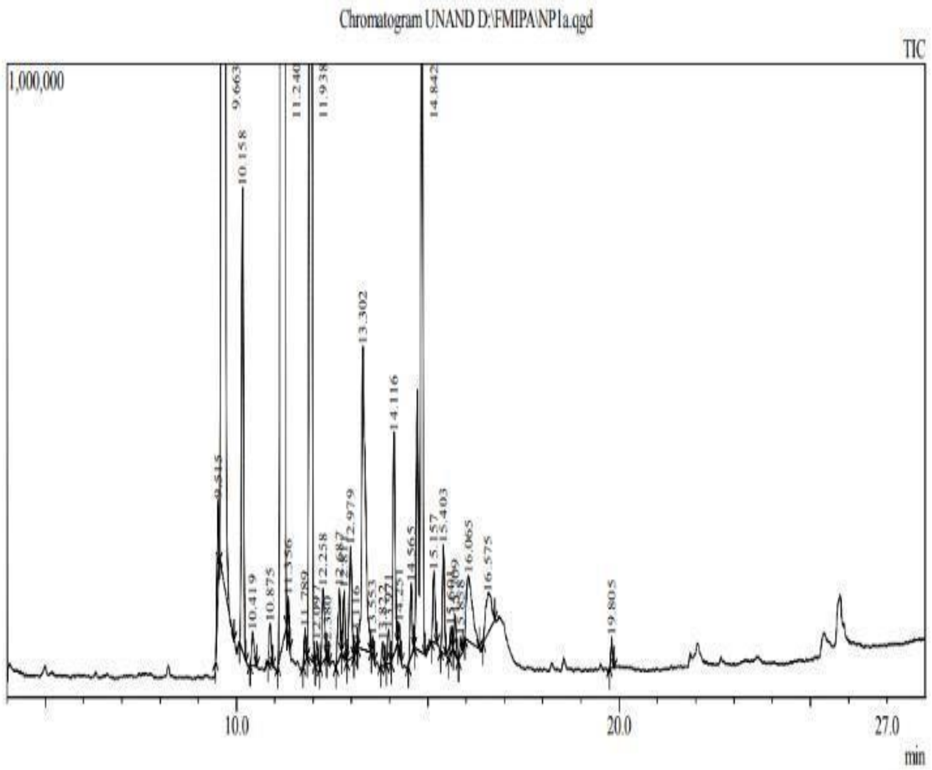

Figure 3. ZMDC chromatogram of day 0 storage

Table 3. ZMDC components of 0th day of storage

No	Compound Name	Molecular Formula	Retention Time (min)	Areas (%)	SI (%)
1	α-Cubebene	C15H24	9,515	0.34	93
2	Chavibetol	C10H12O2	9,665	25.80	96
3	Copaene	C15H24	10,160	2,10	95
4	Bicyclo[4.3.0]nonane,7-methylene-2,4,4-trimethyl-2-vinyl	C15H24	10,420	0.20	92
5	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	C15H24	10,875	0.17	95
6	Trans-β-caryophyllene	C15H24	11,240	46,69	93
7	Bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6;bis(methylene)	C15H24	11.355	0.12	91
8	Humulen-(v1)	C15H24	11,790	0.13	92
9	α-Humulene	C15H24	11,940	10.84	97
10	Aromadendrene	C15H24	12,095	0.09	93
11	Δ-Cadiene	C15H24	12,260	0.44	90
12	α-Bergamotene	C15H24	12,380	0.01	79
13	1,3,6,10-Dodecatetraene,3,7,11-trimethyl	C15H24	12,685	0.36	92
14	4.β.H,5.α.-Eremophila-1(10),11-diene	C15H24	12,810	0.29	88
15	Diethyl phthalate	C12H14O4	12,980	0.65	95
16	Bicycloelemene	C15H24	13.115	0.03	80
17	Δ-Cadiene	C15H24	13,300	2.43	92
18	Δ-Selinene	C15H24	13,550	0.06	94
19	α-Calacorene	C15H20	13,820	0.10	79
20	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	C15H26O	13,970	0.14	88

21	Caryophyllene epoxide	$C_{15}H_{24}O$	14.115	1.11	86
22	Caryophyllene epoxide	$C_{15}H_{24}O$	14,250	0.09	80
23	Palustrol	$C_{15}H_{26}O$	14,565	0.35	82
24	Caryophyllene epoxide	$C_{15}H_{24}O$	14,840	4.70	95
25	α- Bisabolene	$C_{15}H_{24}$	15.155	0.35	85
26	Humulene oxide	$C_{15}H_{24}O$	15.405	0.50	87
27	1H-Benzocyclohepten-7-ol,2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-,cis-	$C_{15}H_{26}O$	15,600	0.10	83
28	α-Cedrene	$C_{15}H_{24}$	15,710	0.16	84
29	7,11-Hexadecadien-1-ol	$C_{16}H_{30}O$	15,860	0.07	83
30	Caryophyllene epoxide	$C_{15}H_{24}O$	16,065	0.91	83
31	Isoaromadendrene epoxide	$C_{15}H_{24}O$	16,575	0.53	84
32	3-Ethyl-3-hydroxy-androstan-17-one	$C_{21}H_{34}O_2$	19,805	0.12	84

Analysis results with GC-MS ZMDC storage time of 0 days can beseen in Figure 3 and Table 3. ZMDC GC-MS analysis found 32 components with the largest levels beingTrans-β-karyophyllene 46.69%, Chavibetol 25.80% and α-Humulene 10.84%.

Figure 4. ZMDC chromatogram after 2 months of storage

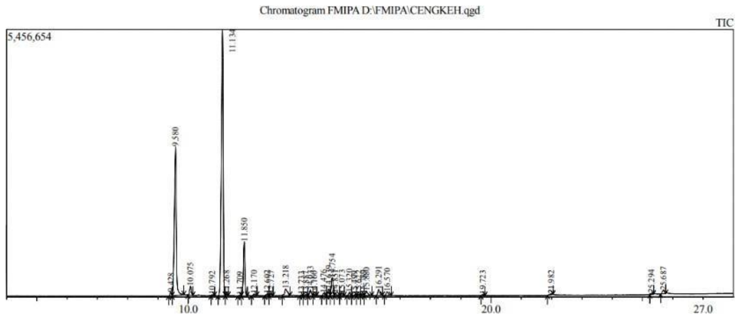

Table 4. ZMDC components after 2 months of storage

No	Nama Senyawa	Rumus Molekul	Waktu Retensi (min)	Area (%)	SI (%)
1	β -Cubebene	$C_{15}H_{24}$	9,428	0,54	90
2	Chavibetol	$C_{10}H_{12}O_2$	9,470	28,00	95
3	Copaene	$C_{15}H_{24}$	9,995	1,31	93
4	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	$C_{15}H_{24}$	10,755	0,14	90
5	Trans-β-kariofilen	$C_{15}H_{24}$	10,985	47,03	93

6	Bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6;bis(methylene)	C15H24	11,220	0,19	86
7	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	C15H24	11,640	0,14	87
8	α-Humulene	C15H24	11,755	8,03	97
9	Δ-Cadinene	C15H24	12,090	0,28	89
10	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	C15H24	12,520	0,30	90
11	Aristol-9-Ene	C15H24	12,665	0,28	89
12	Δ-Cadinene	C15H24	13,105	1,79	89
13	1-Isopropyl-4,7-dimethyl-1,2-dihydronaphthalene	C15H20	13,675	0,12	69
14	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	C15H26O	13,790	0,18	87
15	1,3,6,10-Dodecatetraene,3,7,11-trimethyl	C15H24O	13,945	1,05	87
16	1,3,6,10-Dodecatetraene,3,7,11-trimethyl	C15H24O	14,120	0,21	77
17	5,5,8a-Trimethyldecalin-1-one	C13H22O	14,405	0,41	81
18	Caryophyllene epoxide	C15H24O	14,560	1,05	87
19	Caryophyllene epoxide	C15H24O	14,690	2,93	95
20	1-ISOPROPYL-4-METHYL-7-METHYLENE	C15H24	14,825	0,43	72
21	Cyclohexene	C15H24	14,995	0,32	85
22	Humulene Oxide		15,240	0,45	86
23	Benzocyclohepten	C15H26O	15,390	0,16	82
24	α-Cedrene	C15H24	15,555	0,22	83
25	Oxatricyclo[8.2.0.0(4,6)]dodecane	C15H24O	15,690	0,29	83

26	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl	C15H24O	15,810	1,10	81
27	Isoaromadendrene epoxide	C15H24O	16,205	1,05	85
28	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)	C21H34O2	16,475	0,75	84
29	2-Pentadecanone, 6,10,14-trimethyl	C18H36O	19,670	0,15	82
30	Pentadecanoic acid	C15H30O2	21,865	0,18	85
31	2-HYDROXYCYCLO PENTADECANONE	C15H28O2	25,240	0,20	83
32	9-Octadecenoic acid	C18H34O2	25,600	0,70	85

Results of analysis with ZMDC GC-MS after 2 months of storage canbe seen in Figure 4 and Table 4. PercentageTrans-β-caryophyllene and chavibetol increased by about 3% and 1% while α-Humulene decreased by about 2%.Based on the GC-MS results obtainedβ-Caryophyllene and α-Humulene belong to the terpenoid group, whilethe chavibetol compound is a group of phenolic compounds that can act as antioxidants, because phenolic compounds have hydroxy groups attached to aromatic rings so that they are easily oxidized by donating hydrogen atoms to free radicals. The ability to form stable phenoxy radicals in oxidation reactions makes phenolic compounds very potential as antioxidants [5][6]

CONCLUSION

Based on research that has been carried out on zein nanoparticles - clove leaf essential oil, antioxidant activity was tested by the DPPH method, high antioxidant activity values were shown in formula 5 with a composition of 1.5 mg/mL zein, clove leaf essential oil 8 mg/mL and 1 mL tween 20 showed the best antioxidant activity with a percent inhibition value of 71.13%. The results from the ZMDC laser beam show that formula 5 can scatter light which proves the formation of particles. Analysis of the chemical components contained in ZMDC from Gas Chromatography Mass Spectrometry (GC-MS) analysis showed that there were three main compounds contained in ZMDC, namely trans-β-caryophyllene, chavibetol and α-humulene. , the percentage of Trans-β-caryophyllene and chavibetol increased by about 3% and 1% while α-Humulene decreased by about 2%.

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