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Essential Oil from Fresh and Dry Leaves of Rosemary (*Rosmarinus Officinalis* L.): Antioxidant Activity and Microscopic Structure

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Abstract: Rosemary (*Rosmarinus officinalis* L.) is a medicinal plant from the Lamiaceae family, originally from the Mediterranean region and can be found in most parts of the world. Rosemary essential oil is central to medication, personal health care, cosmetic ingredients, spices, and food preservation with considerable biological activities. This research aimed to measure the oil yield of fresh and dry rosemary leaf samples, identify the chemical constituents of the oil, measure the antioxidant activity of the oil, and analyze the secretory cells of the leaves. These are important to support the development and deployment of rosemary essential oil. The method of drying leaf samples is essential for preserving the oil compounds and it influences both essential oil yield and antioxidant activity. Rosemary leaves were harvested from the field of Dasong Village, Sub-District of Sukasada, and the District of Buleleng, Bali, Indonesia. One part (5 kg) of the leaves samples was naturally dried at room temperature at natural atmospheric pressure for seven days and this was referred to shade drying. Another part of the leaf samples (5 kg) was freshly processed. Both shade-dried and freshly harvested rosemary leaves were extracted using steam distillation with three replications. The composition of the essential oils was determined using Gas Chromatography-Mass Spectrometry. Cross-sections of the leaves were prepared by the paraffin embedding method and stained with safranin. Leaf microscopic structure was observed under a light microscope. This study confirmed that the yields of essential oils from dry leaf samples were higher than fresh samples (0.56% and 0.36%, respectively). The oil from fresh and dry leaves contained 183 and 164 compounds respectively. The main constituents of both oils were eucalyptol, α -pinene, geraniol, linalool, and caryophyllene. The novelty of this study was that both oils from fresh and dry leaf samples have the same strong antioxidant activity with IC_{50} of 83.08 ppm. This might be useful for the utilization of the oil for different purposes. The secretory structure of the essential oil was glandular trichomes of two types: peltate and capitate.

Keywords: capitates glandular trichomes, essential oil, peltate glandular trichomes, *Rosmarinus officinalis*, steam distillation.

来自迷迭香(迷迭香大号.)鲜叶和干叶的精油：抗氧化活性和微观结构

摘要：迷迭香(迷迭香.)是唇形科植物的一种药用植物，原产于地中海地区，在世界大部分地区都能找到。迷迭香精油是药物、个人保健、化妆品成分、香料和食品保鲜的核心，具有相当大的生物活性。本研究旨在测量新鲜和干燥的迷迭香叶样品的出油量，鉴定油的化学成分，测量油的抗氧化活性，并分析叶子的分泌细胞。这些对于支持迷迭香精油的开发和部

署非常重要。干燥叶子样品的方法对于保存油类化合物至关重要，它会影响到精油产量和抗氧化活性。迷迭香叶采自印度尼西亚巴厘岛须贺定分区 Dasong 村和布莱冷区。将一份 (5 公斤) 的叶子样品在室温和自然大气压下自然干燥 7 天，这被称为阴干。另一部分叶样品 (5 千克) 是新鲜加工的。阴干和新鲜收获的迷迭香叶均采用蒸汽蒸馏法提取，重复三次。使用气相色谱-质谱法测定精油的成分。通过石蜡包埋法制备叶子的横截面并用番红染色。在光学显微镜下观察叶片显微结构。该研究证实，干叶样品的精油产量高于新鲜样品 (分别为 0.56% 和 0.36%)。来自新鲜和干燥叶子的油分别含有 183 种和 164 种化合物。两种油的主要成分是桉叶油素、 α -蒎烯、香叶醇、芳樟醇和石竹烯。这项研究的新颖之处在于，来自新鲜和干燥叶子样品的两种油具有相同的强抗氧化活性，我测得了 50 为 83.08 ppm。这可能有助于将油用于不同目的。精油的分泌结构是两种类型的腺毛状体：盾状和头状。

关键词：头状腺毛，精油，盾状腺毛，迷迭香，蒸汽蒸馏。

1. Introduction

Rosemary (*Rosmarinus officinalis* L.) is known as a medicinal plant from the family of Lamiaceae, is native to the Mediterranean region, and can be found almost worldwide. The plant can grow in different soil types widely distributed between 0 and 1600 meters above sea level. The rosemary plant is a densely branched shrub with a height of up to 1.8 meters, evergreen, either erect or procumbent. The shape of the leaf is very narrow and pointed like needles, non-petiolate, small ovals with a size of 10-41 x 1-3 mm, and variable sizes among branches. The leaves' color is dark green above and grey underside with a very distinctive odor [1]. Numerous glandular and non-glandular trichomes are present on the under (abaxial) side of the leaf. Small purple-white flowers with bilabiate corolla are arranged in short clusters [2].

Rosemary essential oil (REO) is central to medication, personal health care, cosmetic ingredients, and food preservation with considerable biological activities [1, 3]; therefore, it is necessary to study the yields, chemical constituents, antioxidant activity, and microscopic structure of the leaf. For the medication, the antioxidant activity of rosemary essential oil (REO) protects against liver damage by reducing liver-degrading enzymes, such as aminotransferase and aspartate aminotransferase enzymes. This decrease is a physiological defense response that protects the liver from the effects of free radicals [4]. The antioxidant activity of REO has been confirmed via the reduction of the blood glucose level of alloxan-induced diabetic mice [5]. Rosemary essential oils are used for food and cosmetics preservation due to their antifungal and antibacterial properties [2]. Rosemary essential oil is a natural compound as an alternative to synthetic preservatives used for citrus fruit preservation that prevents post-harvest diseases such as fungus infecting

the fruits. The antifungal property of REO against *Penicillium digitatum* that causes economic loss post-harvest makes it suitable for safe and environmentally friendly citrus preservation [6].

The antioxidant activity of essential oils can be measured using an oxidant DPPH (2, 2-Diphenyl-1-picrylhydrazyl). Currently, consumers are very concerned about the negative impact of using synthetic materials in products, especially foods [7]. This encourages research on natural additives as an alternative to synthetic materials. Natural ingredients are not only safe for health but also beneficial due to the existence of special biological activities in natural ingredients such as antioxidants, anti-diabetes, anti-cancer, anti-bacterial, anti-bacterial fungi, anti-inflammatory, anti-mutagenic and others [8]. Rosemary essential oil has all these biological activities so it is a potential additive for health products and medicines, cosmetics, perfumes, cooking spices, and food preservation. Some bacteria and fungi are inhibited by REO during the processing and storage of stirred-like yogurt at 4°C, such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella marcescens*, *Aspergillus flavus*, and *Candida albicans* [9].

The yield and compounds that make up the REO are strongly influenced by environmental factors [10] such as climate, altitude, temperature, soil texture, soil type, and soil organic matter content and sample conditions [11]. The method of drying leaf samples also greatly influences essential oil yield and antioxidant activity. The recommended drying method for higher oil yield and lower IC₅₀ is shade drying, which is naturally dried at room temperature at natural atmospheric pressure [12]. Time of harvest influences oil yield and oil compounds where early (5 am) at late (5 pm) harvests result in higher oil yield and higher compounds than afternoon harvest [13]. However, the yield of REO from fresh and dry leaves shows mixed results.

According to [12, 13], higher yield of REO are obtained from dry leaves, while, according to [11], dry leaf samples has significantly lower oil yield than fresh leaf samples. Therefore, it is essential to cross-check the yield of REO using both fresh and dry leaf samples and this will enable to give a practical recommendation to farmers, researchers, and essential oil entrepreneurs.

Rosemary plants grow in Bali in the highlands but can also adapt to the lowlands. Dasong Village, Sukasada District, Buleleng Regency, is a center for planting rosemary in Bali, Indonesia. The aims of the present study were to measure the oil yield of fresh and dry rosemary leaf samples, identify the chemical constituents of the oils, measure the antioxidant activity of the oil, and analyze the secretory cells of the leaf. These are important to support the development of REO, both to be marketed directly or in the form of derivative products such as cosmetics, medicinal products, and food preservatives.

2. Methodology

The research began with harvesting rosemary leaves and then shade drying for 7 days. Both freshly harvested and dried leaf samples were extracted using steam distillation to obtain REO. The chemical constituents of REO were analyzed using gas chromatography-mass spectrometry (GCMS). The antioxidant activity of REO was measured using the DPPH assay. The microscopic structure of the leaves was prepared in slides using a tissue processor. The main steps of the research process are summarized in Fig. 1.

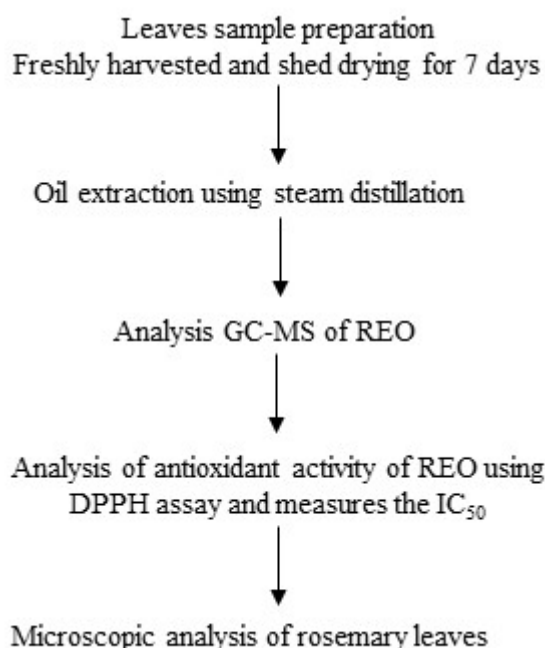


Fig. 1 The main step in the research process (Developed by the authors)

2.1. Sample Preparation and Oil Extraction

Freshly harvested rosemary leaves from Dasong

Village, Sub-District of Sukasada and District of Buleleng, Bali, Indonesia, were divided into two with 5 kg each. One part (5 kg) of the leaf samples was naturally dried at room temperature at natural atmospheric pressure for seven days. This is called shade drying [12]. Each day, the leaf samples were moved sideways so that all parts get equal drying. Both fresh and dry leaf samples were extracted for essential oil using steam distillation with three replications according to the method by [14] and [15]. Briefly, fresh or dry leaf samples were placed into the sample tank with a slightly loose pack so that the steam can penetrate the samples easily. The tank was closed with a tight lid to avoid steam leaking. The boiler was filled with $\frac{3}{4}$ volume of water and then the stove was ignited and this generated steam. The steam flowed through a pipe connected to the sample tank, where distillation of the leaf samples occurred. Steam-containing essential oils flowed into the condenser and dripped into a separator. Essential oils were separated from hydrosol using a cloth. Excess water was absorbed by adding Na_2SO_4 to the oil and then filtered using filter paper. The extraction was performed thrice. The yield of essential oils was measured using the following formula:

$$\text{Oil yield} = \frac{\text{weight of the oil (g)}}{\text{weight of sample (g)}} \times 100\%$$

2.2. Analysis of Chemical Constituents of the Essential Oils

Analysis of the chemical constituents of REO from both fresh and dry leaf samples was performed with GCMS using Shimadzu-QP 2010 [16]. The oil sample (20 μL) was diluted using 5 mL absolute ethanol by thoroughly mixing followed by filtering with Whatman # 1 filter paper. This was taken for injection (1 μL) to the column with a temperature program 50°C for 2 min to 280°C for 5 min. The injector temperature was 300°C with a split flow of 50 mL min⁻¹ and a split ratio of 50. The column HP-5MS UI had 30 meters' length, an inner diameter of 0.25 mm, and a film thickness of 0.22 μm diameter. The carrier gas used was helium with a purge flow rate of 3 mL min⁻¹. The identification of the chemical constituents of the essential oils was performed by comparing peaks with the library.

2.3. Antioxidant Activity of the Essential Oil

The antioxidant activity of REO was measured using DPPH according to the method by [17]. The oil was diluted in methanol analytical grade to make a stock solution of 1000 ppm. A series of concentrations of the test solution was made from the stock solution, namely 50, 60, 70, 80, and 90 ppm. A stock solution of DPPH was prepared in 1000 ppm using methanol, and then an aliquot was taken to make a concentration of 40 ppm. The absorbance of 40 ppm DPPH was measured to determine the maximum wavelength (from 400 to 600 nm) that can be absorbed. The antioxidant activity

of samples was measured by mixing samples, and DPPH with a ratio of 1:4 for all sample concentrations (0, 50, 60, 70, 80, and 90 ppm), and these were incubated for 30 minutes at room temperature. A blank (0 ppm) solution was prepared by mixing methanol and DPPH in the same ratio. The absorbance was measured using a UV-Vis spectrophotometer at 515.6 nm. The free radical-scavenging activity toward DPPH radical was calculated using the following formula:

$$\% \text{ SC} = \frac{A_0 - A_1}{A_0} \times 100$$

where % SC - percent scavenging, A0 - the absorbance of the blank, and A1 - the absorbance of the sample. A regression curve was created between the percentage of inhibition and concentrations of essential oil, and a regression equation was generated. The antioxidant activity is expressed as IC₅₀ which is the concentration of antioxidant (rosemary essential oil) needed to scavenge 50% of the oxidant DPPH. The IC₅₀ was calculated using the following regression equation:

$$y = (bx + a)/b$$

where y = value of IC₅₀, b = 50.

2.4. Microscopic Analysis of the Leaves

Both fresh and air-dried leaf samples were prepared for microscopic slides in a tissue processor (Tissue Tek II, Model RX-II B, Sakura Seiki Co, Lid) following a method by [14]. The process began with the fixation of the samples in 10% neutral-buffered formalin (NBF) solutions for 24 hours. Samples were dehydrated using NBF solution followed by 70% alcohol for 2 h each. The next step is dipping the tissue in a series of absolutes three times for 2 h each and toluol twice for 2 hours each. The final dehydration step was 3 h in liquid paraffin. The samples were embedded in liquid paraffin and then cooled to form blocks. Tissues were sliced into 4 μm thick pieces using a microtome and then placed into an object glass. Each object glass contained 4 tissue slices that were stained with 1% aqueous safranin and then enclosed with a cover glass. These were sealed with Canada balsam to keep the tissue in place. The excess Canada balsam was removed using absolute alcohol and xylol. The microscopic structure of the leaf was observed under a microscope and photographs were taken using an Optilab camera.

3. Results

3.1. Essential Oil Yields

The yields of REO from fresh rosemary leaf samples were significantly different from dry leaf samples (P > 0.05). The oil yields were 0.37 % and 0.56 % for fresh and dry leaf samples, respectively (Fig. 2).



Fig. 2 Essential oil yield of rosemary using fresh and dry leaves (Developed by the authors)

3.2. Chemical Constituents of REO

The compounds of REO from fresh and dry leaf samples were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). There were 183 and 164 peaks identified for fresh and dry leaf samples, respectively, that appeared at different retention times. The difference in peak number indicates the difference in chemical compounds that constitute essential oils. Compounds having an area of more than 0.5%, which made 87.49% of the oil from fresh leaf samples, are presented in Table 1. Among these, there were 8 major compounds, namely: eucalyptol/1,8-cineole (14,7%), α-pinene (11,82%), linalool (7,53%), geranial (4,06%), caryophyllene (4,02%), β-pinene (3,51%), α-terpineol (3,22%) and, camphene (3,16). The identification of REO compounds from dry leaf samples is presented in Table 2. There were 27 compounds with an area of more than 0.5% that made 90.33% of the oil. Of these, there were 7 major compounds, namely eucalyptol/1,8-cineole (15.17%), α-pinene (10.05%), pinocarvone (7.80%), geraniol (6.29%), linalool (6.10%), and caryophyllene (4.22%).

Table 1 Identification of compounds of rosemary essential oil from fresh leaf samples (Developed by the authors)

No.	Retention time (minutes)	Compound	Area (%)
1	6.96	α-Pinene	11.82
2	7.39	Camphene	3.16
3	8.09	β-Pinene	3.51
4	9.64	Eucalyptol	14.70
5	10.51	?-Terpinene	1.11
6	10.78	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	0.98
7	11.36	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	1.27
8	11.77	Linalool	7.53
9	12.44	3,5-Heptadien-2-ol, 2,6-dimethyl-	0.54
10	12.98	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	2.94
11	13.09	Trans-Verbenol	1.35
12	13.71	Endo-Borneol	2.09
13	13.85	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	1.50

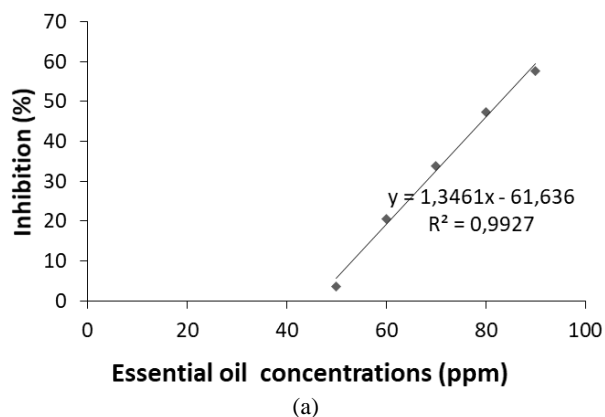
Continuation of Table 1			
14	13.89	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1a,2β,5a)-	1.47
15	14.04	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	2.61
16	14.57	α-Terpineol	3.22
17	14.91	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	7.47
18	15.79	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1a,2β,5a)]-	0.69
19	16.31	Geraniol	4.06
20	17.02	Bornyl acetate	2.15
21	17.09	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	1.93
22	19.59	Geranyl acetate	1.71
23	20.19	Benzene, 1,2-dimethoxy-4-propenyl-, (Z)-	0.54
24	20.54	Caryophyllene	4.02
25	21.42	Humulene	1.14
26	22.11	Germacrene D	1.24
27	24.56	Caryophyllene oxide	1.07
28	28.61	Benzyl Benzoate	1.11
29	34.63	Cyclohexanepropanol, 2,2-dimethyl-6-methylene-	0.57
Total			87.49

Table 2 Identification of compounds of rosemary essential oil from dry leaf samples (Developed by the authors)

No.	Retention time (minutes)	Compound	Area (%)
1	6.92	α-Pinene	10.05
2	7.37	Camphene	2.62
3	7.48	Bicyclo[3.1.0]hex-2-ene,4-methylene-1-(1-methylethyl)-	0.56
4	8.09	β-Pinene	2.50
5	8.54	β-Myrcene	2.24
6	9.65	Eucalyptol	15.17
7	11.34	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	0.71
8	11.77	Linalool	6.10
9	13.05	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	4.20
10	13.52	Pinocarvone	7.80
11	14.06	Terpinen-4-ol	2.25
12	14.59	α-Terpineol	1.47
13	14.70	Terpineol	2.47
14	14.90	3-Caren-10-al	2.09
15	15.00	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	7.65
16	15.59	6-Octen-1-ol, 3,7-dimethyl-, (R)-	0.59
17	16.32	Geraniol	6.29
18	16.70	2,6-Octadienal, 3,7-dimethyl-, (E)-	1.05
19	17.03	Bornyl acetate	2.11
20	17.09	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	1.63
21	19.62	Geranyl acetate	1.41
22	19.99	4,7,7-Trimethylbicyclo[4.1.0]hept-3-en-2-one	0.86
23	20.19	Benzene, 1,2-dimethoxy-4-propenyl-, (Z)-	0.54
24	20.53	Caryophyllene	4.22
25	21.42	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	0.96
26	24.55	Caryophyllene oxide	1.85
27	28.61	Benzyl Benzoate	0.52
Total			90.33

3.3. Antioxidant Activity of the Essential Oils

Inhibition of REO from fresh and dry leaves to DPPH is presented in Fig. 3a and 3b. The higher the essential oil concentrations the higher inhibition to DPPH with R^2 values of 0.9927 and 0.9855 for fresh and dry leaf samples respectively. These values indicate a highly positive correlation between essential oil concentrations and the percentage of inhibition of the radical of DPPH.



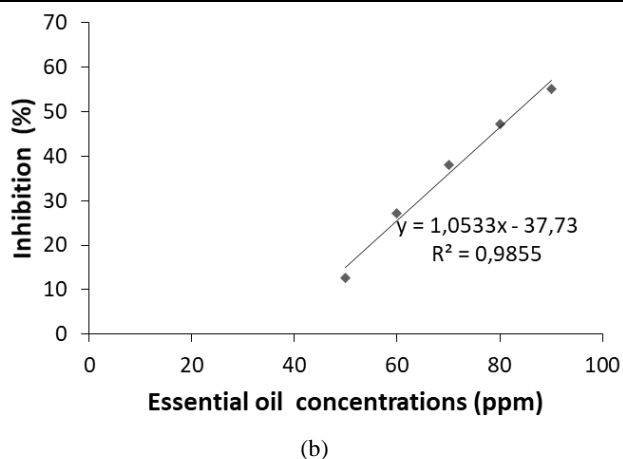


Fig. 3 a. Inhibition of rosemary essential oil from (a) fresh and (b) dry leaf samples to DPPH (Developed by the authors)

Based on the independent sample T-test, the antioxidant activity of REO as measured by IC_{50} is not different between fresh and dry leaf samples ($P > 0.05$) and presented in Fig. 4. The IC_{50} of REO from fresh and dry leaf samples were 82.76 and 83.40 ppm, respectively, with the mean of 83.08 ppm.

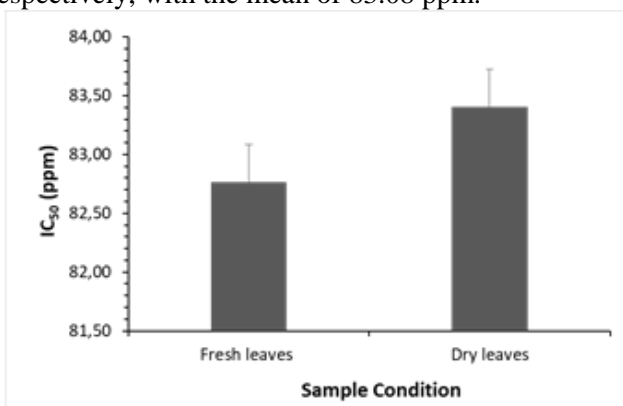


Fig. 4 The IC_{50} of rosemary essential oil from fresh and dry leaf samples (Developed by the authors)

3.4. Microscopic Structure of Leaves

The microscopic structure of a cross-sectioned fresh rosemary leaf is presented in Fig. 5. The adaxial surface of the leaf was covered by a transparent cuticle and very rare short non-glandular trichomes. The epidermis was located under the cuticle, which appears thin and flat. Beneath the epidermis, there were large cells called the hypoderm, which generally consisted of one layer, but in some parts, there were 2-3 layers. The palisade parenchyma was a slender cylindrical structure consisting of 2-3 layers. The spongy parenchyma was located under the palisade parenchyma and composed of loose cells. On the abaxial side, there was a layer of the epidermis, which was slightly rounded in shape, and many trichomes. There are two types of trichomes: glandular and non-glandular, which can be seen scattered around the abaxial epidermis because they were unattached during the process of making microscopic incisions. Non-glandular trichomes appeared long with the pointed end in a large number, while glandular trichomes appeared shorter with

rounded ends. There are two types of glandular trichomes (Fig. 6): capitate and peltate. The capitate glandular trichomes consisted of one head cell as a secretory cell, one to two supporting cells, and one basal cell. Peltate glandular trichomes consisted of one basal cell, one very short support cell, and a large head.

A cross-section of dried rosemary leaves is presented in Fig. 7 with almost the same structure as fresh leaves. Dried leaves absorb red from safranin, so the picture appeared to be dominated by red. The microscopic difference between dry and fresh leaves was that the cells are shorter and smaller in dry leaves because they lost water during seven days of drying at room temperature.

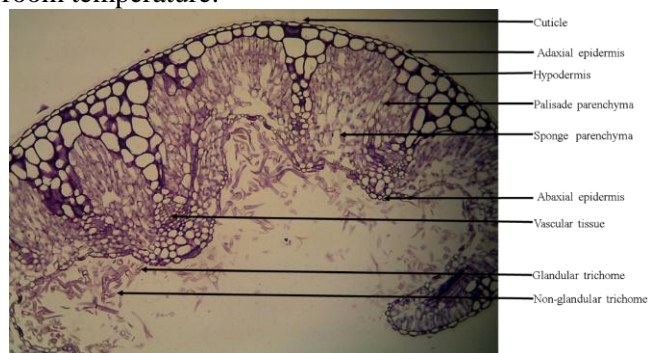


Fig. 5 Microscopic structure of a cross-sectioned fresh rosemary leaf (Developed by the authors)

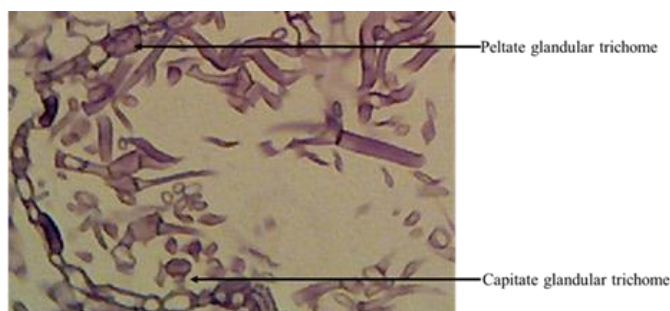


Fig. 6 Cross-section of a fresh rosemary leaf with an emphasis on trichomes (Developed by the authors)

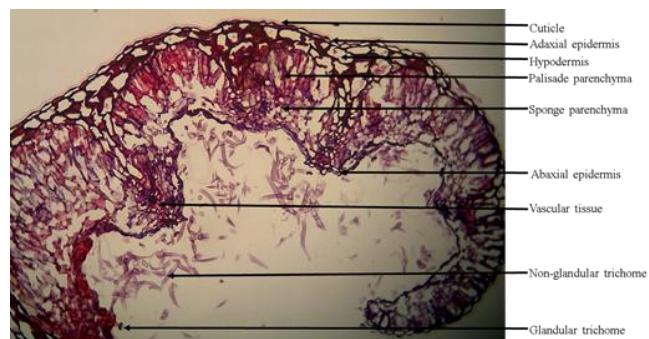


Fig. 7 Cross-section of a dry rosemary leaf (Developed by the authors)

4. Discussion

The yields of REO were 0.37% and 0.56% from fresh and dry leaf samples, respectively. The dry leaf sample contained 0.19% higher oil than the fresh leaf samples. The method of shed drying is recommended for higher oil yield. Drying leaf samples creates a

porous structure of the plant tissue and this facilitates the evaporation of soluble compounds, including essential oils. The porous structure also causes easy penetration access of steam during distillation [18] and resulted in higher oil yield of dry than fresh leaf samples. In other species, such as *Lippia citriodora*, shade drying preserves glandular trichomes, while higher drying temperatures using either an oven (40°C) or freeze-drying (60°C) are vulnerable to glandular trichomes. High temperatures deform and crack trichomes [19]. Shade drying in this study might also contribute to higher oil yield due to preservation of glandular trichomes. According to [20], preservation of trichomes integrity by the shed drying method ensures a high yield of essential oil. This is in contrast to another research [11] that higher oil yield is obtained from fresh leaf samples. In this study, the yield of REO from shade-dried samples was higher than that from fresh samples due to the aforementioned reasons. Therefore, shade drying of rosemary leaves for one week is recommended for higher oil yield.

The GC-MS analysis of the oils found 183 and 164 compounds in fresh and dry leaf samples, respectively. Major oil compounds on fresh leaf samples were eucalyptol (14.7%), α -pinene (11.82%), linalool (7.53%), geraniol (4.06%), caryophyllene (4.02%), β -pinene (3.51%), α -terpineol (3.22%), and camphene (3.16). Major oil compounds on dry leaf samples were eucalyptol (15.17%), α -pinene (10.05%), pinocarvone (7.80%), geraniol (6.29%), linalool (6.10%), and caryophyllene (4.22%). Essential oil from dry leaf samples contained fewer compounds probably due to the evaporation of some compounds during drying. [11] report some compounds lost during the drying of rosemary leaves, namely trans verbenol, levomenthol, and L-borneol. In this study, there were four compounds only found in the fresh leaf samples, namely endo-borneol, garmacerene D, trans-verbenol, and humulene. These compounds are lost during shade drying as indicated by their absence in dry leaf samples. According to [12], biotransformation occurs during drying; therefore, the compounds of essential oils from fresh and dry leaf samples are different. For example, endo-borneol was present in the fresh leaf sample, while it was absent in the dry leaf sample. Common and most abundant compounds in both samples were eucalyptol (1,8-cineole), α -pinene, linalool, geraniol, and caryophyllene. In another study, eucalyptol is one of the main constituents of essential oil from rosemary [12]. The material used (e.g., fresh or dry) influenced both the yield and composition of REO [21].

The IC_{50} values of REO from fresh and dry leaf samples were the same (mean of 83.08 ppm), indicating a strong antioxidant activity. The value of IC_{50} indicates that the concentration of the essential oil requires inhibiting 50% of the radical DPPH. The smaller value of IC_{50} the higher the antioxidant activity

and vice versa. Many factors can influence the antioxidant activity of REO, such as environmental factors, sampling techniques, extraction methods, plant organs [22], geographic origin, and seasonal variations [23]. For example, the IC_{50} of REO from Serbia is 77.6 ppm [4], while a much lower IC_{50} of 3.53 ppm is obtained from Hammam-Dalâa, Nigeria [6], and wider ranges of REO from five governorates in Palestine from 10.2 ppm to 158 ppm [25]. The antioxidant activity as a measure of IC_{50} is due to the presence of terpenes in the REO [26]. Particularly, eucalyptol (1,8-cineole) is one of the main antioxidants in REO [27]. In this study, REO contained major compounds of terpenes, such as eucalyptol (1,8-cineole), α -pinene, linalool, geraniol, caryophyllene, β -pinene, α -terpineol, camphene, pinocarvone, and geraniol. REO grown in Dasong Village, Sukasada District, Buleleng Regency, Bali, Indonesia, had a strong antioxidant activity. This study gives useful information on the usage of the oil for different purposes.

The microscopic structure of rosemary showed two types of glandular trichomes. Non-glandular trichomes on the abaxial side form grooves that coalesce to form a mechanical layer that maintains the integrity of the glandular trichomes and protects the leaves from herbivorous attacks. A large number of non-glandular trichomes also play a role in lowering leaf temperature and affecting light reflection. Rosemary leaves are adapted to dry areas characterized by xeromorphic characteristics; for example, small leaf size plays a role in water and nutrient resistance. According to [28] and [24], the head of the peltate glandular trichome consists of eight radial secretory cells with a larger size and more volume than the capitate. The cuticle layer on the head of the peltate trichomes detaches from the wall of the secretory cells to form a space to accommodate the secretions. The histochemical and phytochemical analysis of rosemary leaves showed that glandular trichomes play a role in the production of lipophilic components and secrete volatile terpenoids. Glandular trichomes were the major sites of biosynthesis and accumulation of volatile terpenoid compounds.

The recommendation for future research is to look more deeply at the microscopic structure of rosemary for both fresh and shed-dried leaf samples by using a scanning electron microscope (SEM) analysis. Comparing and counting the number of intact and broken trichomes in both leaf samples and relating this to the oil yield.

5. Conclusion

In conclusion, the yield of REO from dry leaves was higher than that from fresh leaf samples, which were 0.56% and 0.36%, respectively. Common oil compounds for both fresh and dry leaf samples were eucalyptol (1,8-cineole), α -pinene, linalool, geraniol, and caryophyllene. The IC_{50} of REO was 83.08 ppm, indicating a strong antioxidant activity. The secretory

structure of rosemary leaves was glandular trichomes both capitate and peltate types.

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