Review: *Eucalyptus globulus* essential oil extraction method

N Z Immaroh, D E Kuliah Sari, and S D Nugraheni

Department of Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia

E-mail: nundiahzi@gmail.com

Abstract. *Eucalyptus globulus* (one of the species *Eucalyptus*) is an essential oil-producing plant that widely used in the medical sector. Generally, the useful part of *Eucalyptus* is leaves. Bioactive compounds contained in plant essential oils include gallic acid, cypellocarpin A, eucaglobulin, cuniloside and (1S, 2S, 4R)-trans-2-hydroxy-1,8-cineole-4-D-glucopyranoside. The presence of these bioactive compounds made of essential oils can be used as an antibacterial, antioxidant, and anti-inflammatory. The extraction method has a major effect on the production of essential oil because it can affect yield and bioactive compound. The extraction methods can be used to produce essential, such as maceration, Ultrasound-Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), water distillation, steam distillation, water-steam and distillation. This review discusses the extraction methods and the main factor can affect the extraction process to produce *E. globulus* essential oils.

1. Introduction

*Eucalyptus* comes from the *Myrtaceae* family, widely grown in the world and commonly used for medicinal plants. There are many species in the world, one of them is *Eucalyptus globulus*. The essential oil can be found in *Eucalyptus globulus*, especially in the leaves. The essential oil is used in many sectors, such as health, flavoring, perfume, cosmetics, and pharmaceuticals. The bioactive content in essential oil can be used for antioxidant, anti-bacterial, and anti-inflammatory. In the food sector, the function of essential oil is an additional ingredient for aroma and flavor, and a natural preservative [1].

*Eucalyptus* leaves have secondary metabolites, such as 1,8-cineole (eucalyptol), monoterpene, sesquiterpene, aldehyde, and ketone. The standard of cineole content in the essential oil must less than 70%. The chemical content in the essential oil depends on the species, geographic location, season, leafage, harvest time, and extraction method [2].

The extraction method can affect the yield and chemical content of the essential oil. The selection of effective extraction methods can produce high quality, maximum yield, and maintain the chemical content. Generally, the extraction methods used are maceration, Ultrasound-Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), distillation with water or steam, and others. The effective extraction method can extract essential oil with the best quality and not damage the existence of bioactive compounds. The review aims to inform the extraction methods of essential oil in *Eucalyptus globulus*. 
2. Discussion

2.1. The profile of Eucalyptus globulus

*Eucalyptus* is Myrtaceae's family, the genus of *Eucalyptus*, and belongs to the species of *Eucalyptus globulus* [3]. This plant has up to 100 meters in height, round-shaped and bluish color on the leaves' surface, which produces an aroma when crushed. *Eucalyptus* has a fragrant and refreshing aroma. *Eucalyptus globulus* attracts the researcher's interest because it has beneficial oil [4].

Chemical compound contained in the leaves of *Eucalyptus globulus* like α-pinene, camphene, β-pinene, β-sabinene, limonene 1, 8-eucalyptol, cis-β-ocimene, γ-terpinene, unidentified, unidentified, terpinolene, linalool,1 fenchol 1, 7, 7-trimethylbicyclo [2.2.1] hept-5-en-2ol, pinocarveol, 5, 5-dimethylene-3-hylenecyclo [2.2.1] heptan-2-one, 2, 6-dimethyl-1, 5 , 7-octatrien-3-ol, isobornyl formate, terpien-4-ol, α-terpineol, trans-carveol, 2-methylene-5-(1-methylethenyl), cyclohexan, 3, 7-dimethyl-2, 6-octadien-1-ol, exo-2-hydroxycineole, α-terpineol acetate, geranyl acetate, isolateden, isopulegol acetate, α-gurjunene, (-)-cis-carvyl acetate, β-panasinsene β-gurjunene, alloromadendrene, aromadendrene, 2-phenylethyl isovalerate, eudesma-4(14), 11-diene, α-guaiene, (+)-ledene, cubenol, ledol, spathulenol, (-)-gubinol, α-cadinol, Y-eudesmol, unidentified, α-eudesmol, β-eudesmol 1.2.3, 3a, 4, 5, 6. 7-octahyfo-e, 3. 8-1-5- azulenenthanol [5].

2.2. The profile of Eucalyptus globulus essential oil

Essential oil is colorless liquid material that contains a volatile and aromatic compound from part of plants such as seeds, flowers, bark, stems, and leaves. One of the plants which contain essential oil is the *Eucalyptus globulus*, especially in the leaves. Essential oil is a secondary metabolite that has bioactive compounds can be used for the medical sector [6].

Essential oil consists of oxygenated monoterpen, monoterpenese and oxygenated sesquiterpene. 1, 8-eucalyptol (72.71%), α-terpineol (2.54%), terpinen-4-ol (0.34%), and linalool (0.24%) are oxygenated monoterpenes, while α-pinene (9.22%), and β-pinene (0.4%) are monoterpenese and α-eudesmol (0.39%), (-) - globulol (2.27%), and epiglobulol (0.44%) are sesquiterpene.

The essential oil components of *Eucalyptus globulus*’s leaves are camphor (9.58%), borneol (7.63%), 1.8 cineole (51.25%), and camphene (3.77%). The 1,8 cineole is the most crucial compound due to its high concentration of 51.25% [7]. 1.8-cineole is a cyclic ether with the empiric formula of C10H18O and the systematic name of 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane, which belongs to the group of monoterpen oxygenated hydrocarbon component [8]. 1.8-cineol in the commercial world is referred to as “eucalyptol” [2]. 1.8-cineole compound’s characteristic has camphor aroma, spicy taste, and fresh.

2.3. Usefulness

The essential oil of Eucalyptus globulus’s leaves has several leading benefits that play a role in the medical world as an antibacterial [9], anti-oxidant, [10] and anti-inflammatory [11].

2.3.1. Anti-bacterial

The component of *Eucalyptus globulus*’s leaves essential oil can inhibit the bacteria’s growth are 1.8-cineole, linalool, and pinocarveol [9]. Research conducted showed that the essential oil of *Eucalyptus globulus* could be used as an anti-bacteria on *Staphylococcus aureus* and *Escherichia coli* at a dose of 20 µL. The higher the concentration has the higher the inhibition zone [12].

2.3.2. Anti-oxidant

Antioxidant is a molecule that can breaks down free radicals and protects against cell damage. Free radical affects energy production, biomolecule synthesis, and cell growth. Free radical can make an imbalance free radicals and anti-oxidants, that can cause oxidative stress—natural antioxidants obtained from the diet. Antioxidant of *Eucalyptus globulus*’s essential oil can break down 2,2-diphenyl-1-picrylhydrazyl (DPPH). Antioxidant activity on *E.globulus* was lower than ascorbic acid. Anti-oxidant activity of essential oil was affected by the concentration and absorption of the radical. The low activity
of antioxidants from *Eucalyptus* globulus’ essential oil may be caused by the absence of phenolic compounds such as thymol and carvacrol [10].

2.3.3. *Anti-inflammatory*

The aromatic compound of essential oil can be applied as an analgesic, anti-inflammatory, and antipyretic [11]. Eucalyptol can inhibit the production and synthesis of TNF-α, interleukin-1β (IL-1β), leukotriene B4, and thromboxane B2 in human blood monocytes. It showed that eucalyptol is a potent cytokine inhibitor, making it suitable for long-term treatment of inflammation of the airways in bronchial asthma and other steroid-sensitive disorders. Furthermore, in double-blind testing, the placebo became controlled. The anti-inflammation activity of eucalyptol was applied for acute asthma patients and it demonstrated success as a mucolytic agent in the upper and lower airways [13]. The above statement helped to explain the application of essential oil of *Eucalyptus* globulus as anti-inflammatory.

2.4. *Extraction method*

The extraction method of essential oil can be divided into two categories, conventional and modern methods. The conventional method includes hydro-distillation, steam distillation, and extraction using solvents. The modern method includes supercritical fluid extraction, microwave-assisted hydro-distillation, and ultrasound-assisted extraction.

2.4.1. *Hydro-distillation*

This method is a traditional extraction method used for essential oil. In this method, the essential oil is evaporated by heating a mixture of water and material or with other solvents, then the vapor is liquefied in the condenser. The result then flows into a separate room in which there will be separated into essential oil and water. This method is quite simple, it can be used in either a small or big scale, can avoid chemical content loss due to the long extraction process, and saves energy used [14,15,16]. In eucalyptus essential oil extraction, 15 grams of sample was immersed in 300 ml water and distilled for 5 hours. The oil extracted by hydro-distillation contained volatile compounds while the oil from SFE and soxhlet contained volatile and higher molecular weight compounds. The Eucalyptus oil yield from 3.1 at 1 h to 3.8% at 5 h of hydro-distillation extraction [17]

2.4.2. *Steam distillation*

This method is a standard method used for temperature-sensitive materials (such as oil, resin, hydrocarbon, and many others), insoluble in water, and can decompose at its boiling point. The mechanism of this method is separation compound or a mixture of the compound at the boiling point below the boiling point of the compound (close to the boiling point of water, 100°C at atmospheric pressure) so that the volatile components whose boiling point of 150 to 300 °C can be evaporated at the water temperature [18]. Water vapor is passed to the material that will be distilled without immersing the material in water. Furthermore, the compounds included in the water vapor enter the condenser and then are separated for its water and essential oil compound. The essential oil that has been cooled and returns to liquid comes down from the condenser and is collected in the container under it, called a separator. In this separator flask, the water and essential oil gather with the position of the essential oil is floating on the water. *Eucalyptus* globulus’ essential oil extraction, generally, uses this method. The part used in the process is a fresh or dry leaf with oil yields 1.0% - 2.4% (fresh weight) using fresh or dry leaf [14].

2.4.3. *Solvent extraction*

Solvent extraction, liquid-to-liquid separation, is a separation method based on solubility. Solvent extraction is commonly used in the processing of perfumes, vegetable oils, or biodiesel. Solvent extraction is used for soft texture or fragility plants, sensitive to heat, and large quantities of essential oils with low cost [19]. *E. Globulus* leaves were cleaned, air-dried for 4 days until the average humidity
is around 9%, then ground, sieved to pass 0.5mm, and packed in sealed plastic bags. Eucalyptus leaves were extracted in an orbital shaker with temperature control using aqueous ethanol as solvent. Two grams of leaf powder then put in 100ml erlenmeyer using a solid/liquid ratio of 1:20 g/mL then a shaking speed of 120 rpm. The extract was filtered using filter paper under vacuum, and the filtrate obtained will be analyzed. The results extraction ranged from 24.4 to 33.1% [20].

2.4.4. Supercritical fluid extraction
Supercritical Fluid Extraction (SFE) refers to a separation process of a component from the matrix using supercritical fluid as the extraction solvent. The extraction was usually from a solid matrix or liquid. In general, the supercritical fluid used CO₂, but it was modified by the addition of other solvents like ethanol or methanol [21]. This extraction method obtained a higher yield, higher diffusion coefficient, lower viscosity, and better extract quality (functional and biological activities) than the conventional method. However, the disadvantage of the SFE method was costly (the price of the equipment), and it was not easy to handle or operate [22]. E. Globulus leaves were air-dried for two days and by drying the samples in the oven set at 103°C for 5 h. Then, the samples were ground with a knife grinder and the ground sample was sieved using a sieve shaker. Five g of eucalyptus leaves were weighed and put in the SC CO₂ extraction vessel. Installing glass wool at both ends of the extractor to prevent the entry of the substrate. SFE can be started when the pressure and temperature are appropriate. The flow rate of gas CO₂ was set at 2 L/min in all runs. Results extract included in the amber bottle, the bottle was placed in an ice bath for dynamic extraction step, which can also function in minimizing the loss of volatile compounds due to the sublimation of CO₂. Deposition in all the pipes is washed using ethanol and then mixed with the extract collected in a bottle. Then put in a rotary evaporator and conducted weighing the extract. The resulting oil has increased along with the increasing pressure and temperature at 50 °C of 2.99% to 3.39% and at 70 °C of 2.51% to 4.66% at 70 °C [17]. The extraction E.globulus with this method at pressure (350 bar), temperature (80 °C) and flow rate of CO₂ (12 g min⁻¹) gave the highest percentage yield (3.6%) compare with hydro distillation, solvent extraction, and ultrasonic-assisted extraction method [23].

2.4.5. Microwave-assisted hydro-distillation
Microwave-Assisted Hydro-distillation is the hydro-distillation technique through a microwave oven during the extraction process. This method successfully reduced the required time and solvent volume of the extraction, minimized the environmental impact by releasing less CO₂ in the atmosphere, and required less energy [24]. On the other hand, this method was also able to foster the purify of essential oil [25]. Extraction of essential oil from E. globulus leaves with this method at the ratio of raw materials to water is 1:3 mL/g, with 60 min in extraction time, and 450W microwave power can give yield 2.65 mg/L (ground material) with the main ingredients of essential oils were Eucalyptol (38.771%) [26].

2.4.6. Ultrasound-assisted extraction
Ultrasound-assisted extraction was defined as the extraction method which producing high-value compounds. This method was beneficial for extracting the essential oils, especially from flowers, leaves, or seeds [27]. Although requires a high price, this method increases yield in a shorter time. The research was done by [23] revealed that the yield produced by this method on Eucalyptus globulus leaves was higher than the hydro-distillation and extraction method a (2.2%) with important compounds extracted are aliphatic saturated hydrocarbons, organic acids, and esters.

2.5. Factors affecting the extraction process
The effectiveness of the extraction process depends on the quality and quantity (yield) of the essential oil. Several factors that can be affecting are material, extraction method, time, temperature, and pressure.
2.5.1. Raw material
The content of essential oil in different part of the plant can be different too. In the Eucalyptus globulus plant, the highest content of essential oil was on leaves [28]. Furthermore, the variety of E. globulus and harvesting time also affected the yield and quality of essential oil [29, 30]. Other factors that can influence the quality and quantity of yield are the physical conditions (dry or wet) and the size of the materials being used [31].

2.5.2. The extraction method used
The extraction method used in the production of essential oils can affect the biological composition and yield. It also gave an effect on the properties and functions of the oil. The extraction of essential oils from the leaves of Eucalyptus globulus through different methods obtained different yields. The yields obtained were 2.0% (hydro-distillation), 2.2% (solvent extraction), 2.6% (the extraction by using ultrasound) and 3.6% v/w (supercritical carbon dioxide). In the supercritical method, carbon dioxide produced the monoterpene compounds, sesquiterpene, and oxygenated sesquiterpene hydrocarbons. Whereas hydrodistillation, solvent extraction, and ultrasound methods produced the saturated aliphatic hydrocarbons, organic acids, and esters compounds [24]. Moreover, maceration produced extracts with the greatest antioxidant, while microwave-assisted hydro-distillation was able to reduce the extraction time [32].

2.5.3. Time
Time for the extraction process affected the yield. In some extraction methods, the longer of extraction time applied, the higher the yield would be. Microwave-assisted hydro-distillation for 3 minutes produces the highest yield if compared to 1 and 5 minute-extraction time [33].

2.5.4. Temperature
The temperature used during the extraction affected the yield of essential oil is produced. The extraction on the leaves of Eucalyptus camendulis by using the water distillation method at 100°C was capable of producing the highest yield [34].

3. Conclusions
Eucalyptus globulus is a plant whose benefits as antibacterial, antioxidant, anti-inflammatory, and others. Bioactive compounds contained in Eucalyptus globulus have the potential to be further developed. Thus, the appropriate extraction method is needed in maximizing the extraction yield obtained from the Eucalyptus globulus. In general, the extraction methods used are hydro-distillation, steam distillation, distillation using solvents, microwave-assisted hydro-distillation, and ultrasound-assisted extraction.

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