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The Temperature Effect of Madeira Vine (Anredera Cordifolia) Leaf Oil Extraction and Its Characterization as An Additive in Health Supplement Product

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ABSTRACT

The immune system is one of the most important things that need to be preserved in the midst of the Corona Virus Disease 2019 (Covid-19) pandemic. Consuming the components of the compounds contained in Madeira vine (Binahong) leaf oil in the right and regular doses can be one way to enhance the immune system. This oil can be obtained by carrying out the extraction process with the reflux method on Madeira vine leaf. This study aimed to determine the optimal time and temperature of Madeira vine leaf oil extraction process. The extraction process was carried out at 40, 50, and 60 °C for 90, 100, and 110 minutes. Madeira vine leaf oil obtained was analyzed for its density, solubility in ethanol, and chemical compounds. The results showed that the highest yield (62.45% w/w) was achieved using optimal temperature of 60 °C and time of 110 minutes. The density of the oil obtained ranges from 0.786-0.812 gr/ml. Analysis using Gas Chromatography-Mass Spectrophotometry (GCMS) showed that the resulting extract contained several components namely Neophytadiene; 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (phytol); Hexadecanoic Acid, methyl ester (methyl palmitate); 9,12-Octadecadienoic acid (Z,Z), methyl ester (methyl linoleate); 9-Octadecenoic acid (Z), methyl ester (methyl oleate); 9-Octadecenoic acid, methyl ester;and Octadecanoic acid, methyl ester (Stearic acid).

1. Introduction

Free radicals are highly reactive chemical molecules and are often cited as one of the causes of premature aging and diseases such as cancer, atherosclerosis, rheumatism, and diabetes [1]. The formation of free radicals can occur through normal cell metabolism processes, inflammation, nutritional deficiencies, or as a result of responses to influences from outside the body, such as environmental pollution, ultraviolet light, and cigarette smoke [2]. Abnormal levels of free radicals that enter the body can attack vulnerable compounds, such as lipids and proteins and have

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implications for the emergence of various diseases [3]. Maintaining the immune system can help the body to prevent and inhibit foreign objects, such as viruses, that enter the body [4]. In biological systems, the body can usually produce its own antioxidants in the form of enzymes, namely superoxide dismutase, catalase, and glutathione peroxidase (endogenous antioxidant). However, the process of natural antioxidants formation in the body is not proportional to the number of free radical attacks that occur throughout life [5]. The high accumulation of free radicals that is not accompanied by the sustained ability of antioxidants in the body could causes oxidative stress [6]. Therefore, additional antioxidants must be obtained (exogenous antioxidants) [7].

Antioxidants are compounds that can inhibit reactive oxygen species/reactive nitrogen species (ROS/RNS) and free radicals [2]. If applied as a functional food ingredient, antioxidants will act as free radical scavengers, but if applied to prevent food damage, antioxidants could act as a peroxidation inhibitor [8]. Antioxidants are divided into two types, namely natural antioxidants and artificial antioxidants [9]. Artificial/synthetic antioxidants commonly used by the food industry, such as BHA or butylated hydroxy aniline and BHT or butylated hydroxy toluene could cause some side effects, including liver damage [10]. Meanwhile, the choice and availability of natural antioxidants are still limited.

Indonesia is one of countries that has a largest biodiversity in the world with more than 30 thousand species of medicinal plants through scientific research and only about 180 species have been used in traditional medicinal plants by the Indonesian traditional medicine industry [11]. One of the medicinal plants is the Madeira vine plant. Madeira vine is a medicinal plant that almost all parts of it can be used; starting from the roots, stems, leaves, and flowers. However, the part that is often used is the leaves [12]. Madeira vine leaves have the potential to treat several types of diseases because it contains active substances such as saponins, polyphenols, flavonoids and polysaccharides [13].

According to Susanty and Bachmid, the leaves of the Madeira vine plant contain oleanolic acid, a compound that can be an alternative source of antioxidant [14]. The technique that is often used to isolate the active antioxidant substance is solvent extraction [15]. Reflux extraction is widely used because it is efficient, easy to operate, and cost-effective [16]. Reflux extraction is a method of extraction using a solvent and operate at its boiling point temperature, for a certain time, with a limited amount of solvent that is relatively constant, and in the presence of reverse cooling. There are several factors that affect the extraction results such as extraction time and temperature [17].

The solvent used in this study is ethanol with concentration of 96% v/v. The concentration of the solvent is an important parameter in this experiment, because it could affect the extraction rate constant. According to Gong, et al. (2020), the yield of flavonoids would be increased along with the increasing concentration of ethanol [18]. The purpose of this study was to determine the optimum temperature and extraction time of the Madeira vine leaf extraction process, and to determine the components of the compounds contained in the Madeira vine leaf extract.

2. Research Method

2.1 Materials

The research materials in this study are Madeira vine leaves were obtained from Semarang City, Central Java. 96% v/v ethanol solvent was purchased from hepilab store, Semarang City, Central Java.

2.2 Methods

2.2.1 Extraction of Madeira Vine (Binahong) leaf oil

Dried Madeira vine leaves were crushed by a blender to form the powder. 29.5 grams of Madeira vine leaf powder were taken and put it in a three neck boiling flask. Into the boiling flask, 96% v/v ethanol was added as much as 200 ml. The solution was then mixed thoroughly using a stir bar for 90, 100, and 110 minutes at a speed of 200 rpm and temperatures of 40, 50, and 60°C. Then the obtained extract was filtered by using filter paper. Eq. (1) was used to determine the percentage of Madeira vine leaf oil extract produced from 29.5 grams of dried Madeira vine leaves and 200 ml of 96% v/v ethanol solvent.

$$\%Yield = \frac{Weight\ of\ extract\ (final)}{Weight\ of\ simplicia\ (initial)} x\ 100\% \quad (1)$$

2.2.2 Density measurement

An empty pycnometer was weighed. After that, the pycnometer was filled with Madeira vine (Binahong) leaf oil until full. Then the weight of the extract was measured. And empty pycnometers are washed using alcohol and dried.

2.2.3 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Analysis using GC-MS was carried out in order to determine the chemical compounds that contained in the obtained Madeira vine (Binahong) leaf oil. The GCMS used is Shimadzu GC 2010 Plus.

3. Results and Discussion

Ethanol was used as a solvent in this Madeira vine leaf extraction process. This compound can dissolve polar compounds and non-polar compounds [19]. In addition, using ethanol as a solvent because it is easy to obtain and it can extract almost all of natural compounds found in plants [20]. The Madeira vine leaf oil was obtained by filtering the filtrate using filter paper to obtain a thick extract of Madeira vine leaves. The results of Madeira vine leaf extraction with temperature variables of 40, 50, 60°C and time of 90, 100, 110 minutes is presented in Table 1.

Table 1
Extraction Observation Results

Temperature (˚C)	Time (minutes)	Extract Weight (gram)	Volume Extract (ml)	Density (gram/ml)	Yield (% w/w)
40	90	77.90	99.0	0.786	41.59
40	100	103.00	130.0	0.792	54.99
40	110	101.22	127.0	0.797	54.04
50	90	110.00	137.5	0.800	58.72
50	100	112.00	139.0	0.805	59.79
50	110	106.00	131.5	0.806	56.59
60	90	105.00	130.0	0.807	56.05
60	100	106.00	131.0	0.809	56.59
60	110	116.98	144.0	0.812	62.45

3.1 Effect of Temperature Towards Yield of Madeira Vine Leaf Extraction

The extraction temperature greatly affected the extraction results in terms of quantity as well as the photochemical content of the extract. Lower temperature could not maximize the extraction process. However high extraction temperature also has a drawback. It could cause some heat-resistant organic compounds to evaporate and disappear [21]. From Figure 1, It can be seen that the higher the extraction temperature, the more extracts will be produced. This is in line with research [22] which states that this is because the increasing temperature of the effective component can cause a gradual decomposition of the extracted chemical.

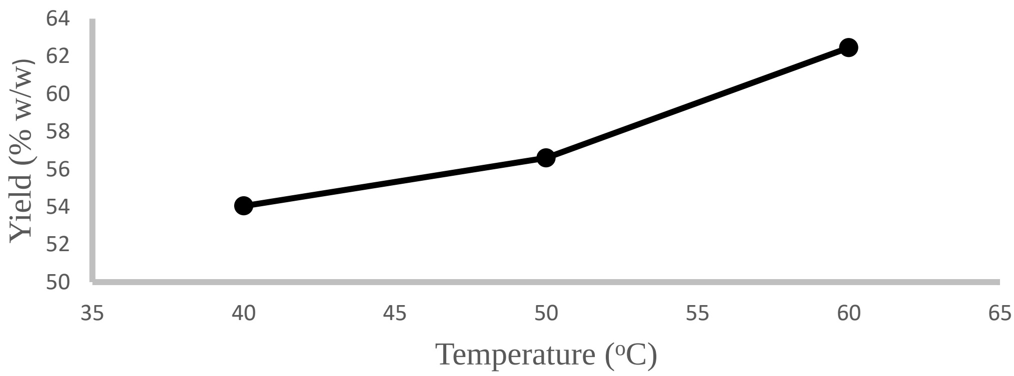

Fig. 1. The yield profile of Madeira vine leaf extract with extraction temperature variation

3.2 Effect of Time Towards Yield of Madeira Vine Leaf Extraction

Time greatly affects the extraction results. Short extraction time will lead to small amount of yield as not all of the targeted substance had been extracted, while longer time means requires more energy. The final equilibrium of extract will be reached between the solute concentration in the Madeira vine leaf matrix and in the bulk solution after a certain time. From Figure 2, it can be seen that the highest extraction yield was found at 110 minutes at temperature of 60 ^oC.

Based on Figure 2, it can be concluded that the longer the extraction time, the more extracts will be produced. This is in line with research conducted by [21] which stated that the longer the extraction time, the higher the extraction yield.

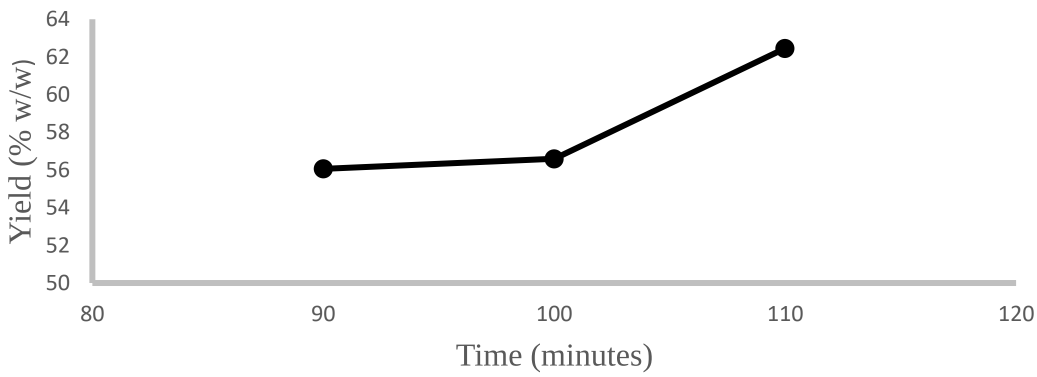

Fig. 2. Time effect on the yield profile of Madeira vine leaf extract

3.3 Density Character of Madeira Vine Leaf Extract

The results of the density of Madeira vine leaf oil can be seen in Table 1. The highest density was obtained when the extraction temperature was 60°C and the extraction time was 110 minutes, while the lowest density was at the extraction temperature of 40°C and the extraction time of 90 minutes. After being observed, it turned out that two layers were formed, which means the Madeira vine leaves oil was completely dissolved in the ethanol solvent.

3.4 Analysis Using Gas Chromatography-Mass Spectrophotometry (GCMS)

Analysis using Gas Chromatography-Mass Spectrophotometry (GCMS) showed that the resulting extract contained several components. From Figure 3 and Table 2, It can be seen that the main components of Madeira vine leaf extract in the form of terpenoids are *Neophytadiene* (1.61%) and *2-Hexadecen-1-ol, 3,7,11,15-tetramethyl* (6.09%). In addition, fatty acid groups are *Hexadecanoic Acid, methyl ester (methyl palmitate)* (25.05%); *9,12-Octadecadienoic acid (Z, Z), methyl ester (methyl linoleate)* (8.13%); *9-Octadecenoic acid (Z), methyl ester (methyl oleate)* (28.17%)*; 9-Octadecenoic acid, methyl ester* (6.71%); and *Octadecanoic acid, methyl ester (Stearic acid)* (4.97%).

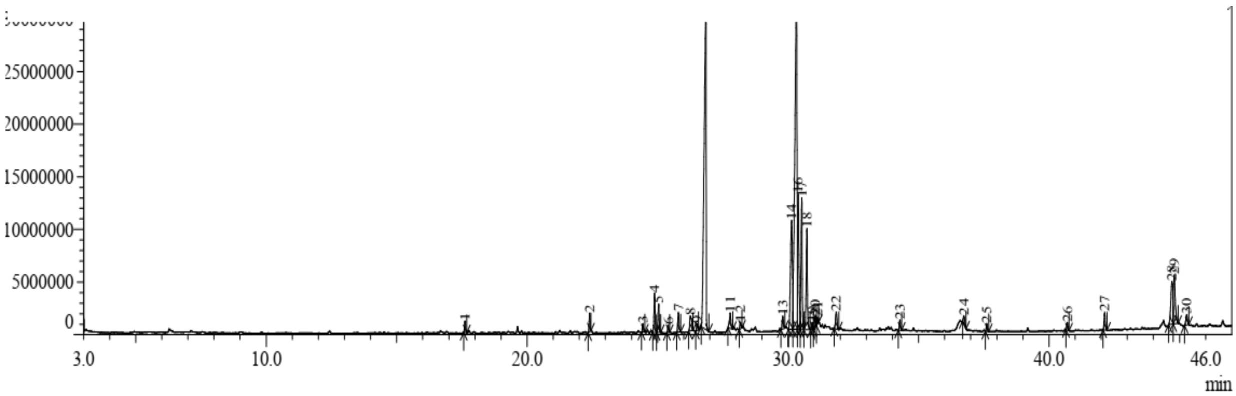

Fig. 3. Chromatogram analysis of GC-MS from Madeira vine leaf extract

Table 2
List of components contained in Madeira vine leaf extract

Retention Time	% Area	Component
24.884	1.61	*Neophytadiene*
26.848	25.05	*Hexadecanoic Acid, methyl ester (methyl palmitate)*
30.134	8.13	*9,12-Octadecadienoic acid (Z,Z), methyl ester (methyl linoleate)*
30.317	28.17	*9-Octadecenoic acid (Z), methyl ester (methyl oleate)*
30.387	6.71	*9-Octadecenoic acid, methyl ester*
30.525	6.09	*2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (phytol)*
30.721	4.97	*Octadecanoic acid, methyl ester (Stearic acid)*

This group of fatty acids and terpenoids can function as antioxidants, and antimicrobial compounds. This indicates that the compound structure of the *Madeira vine* leaves oil affects its antioxidant activity which can reduce the level of free radical reactivity. This is in line with research conducted by [23] [24] which stated that the fatty acid group such as *Hexadecanoic acid, 9-Octadecenoic acid methyl ester, 9-Octadecenoic acid, Octadecanoic acid* can inhibit microbial growth.

In addition, one of the Madeira vine leaves oil is antiglycemic because it contains phytol components that can stimulate insulin secretion by pancreatic β cells and lower blood glucose [25].

4. Conclusions

Based on the research that has been done, the most optimal condition for Madeira vine leaf extraction was found at a temperature of 60°C with an extraction time of 110 minutes. The effect of the increasing temperature on the extraction process is that the effective component could cause gradual decomposition of the extracted chemical, so that if the temperature is too high, it will damage the components in the Madeira vine leaves oil. The effect of extraction time on extract yields is that the longer the extraction time, the higher the extraction density. The compounds contained in the Madeira vine leaf extract include in the form of terpenoids are *Neophytadiene* and *2-Hexadecen-1-ol, 3,7,11,15-tetramethyl*. In addition, fatty acid groups are *Hexadecanoic Acid, methyl ester (methyl palmitate); 9,12-Octadecadienoic acid (Z, Z), methyl ester (methyl linoleate); 9-Octadecenoic acid (Z), methyl ester (methyl oleate); 9-Octadecenoic acid, methyl ester;* and *Octadecanoic acid, methyl ester (Stearic acid).*

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