Characterization of herbal oil with variation of spices ratio (brotowali, clove, cinnamon, kencur, sambiloto) and VCO

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Abstract. Herbal oil is a traditional oil used by people as medicine because the addition of some spices can increase the number of secondary metabolites and bioactivity. The study evaluates how different spice variation ratios and VCO affect phytochemical properties, physicochemical properties, and antioxidant activity in herbal oil. Herbal oil was made at 100ºC for 60 minutes using variations in the ratio of five total spices and VCO with weight per volume of 3:30 (formula A); 4:30 (formula B); 5:30 (formula C); 6:30 (formula D); and 7:30 (formula E). Phytochemical tests on VCO and herbal oil showed the addition of phenolic compounds in herbal oil. Physicochemical results showed a decrease in moisture content, free fatty acids, and peroxide value as the amount of spices added increased. Antioxidant activity test showed an increase in %inhibition with values of 15.774% (VCO); 30.009% (formula A); 30.054% (formula B); 30.099% (formula C); 30.863% (formula D); and 31.671% (formula E). The results showed that herbal oil with a formula E is the best with physicochemical properties: water content 0.009%; density 1.605 g/mL; pH 5.33; free fatty acids 0.005%; peroxide value 0.012 mEq/kg; and antioxidant activity with an IC50 value of 167.277 ppm.

1 Introduction

Herbal oils in Indonesia are widely used by the community in curing internal and external diseases, such as massage therapy to reduce body pain such as aches and pains [1, 2]. The use of herbal oils by the community because they are cheap, easy to make, and have low side effects compared to synthetic drugs [2]. In the Indonesian tradition, herbal oil is usually made with coconut oil (often referred to as VCO) mixed with several spices [3, 4]. The use of spices is intended to enrich VCO oil with secondary metabolites from spices and increase the bioactivity of herbal oils [4]. The addition of spices to VCO will also improve the herbal oil quality by reducing the levels of peroxides and free weak acids, making it safe for the skin [5–7]. Spices such as brotowali (*Tinospora crispa*), clove (*Syzygium Aromaticum*), cinnamon (*Cinnamomum burmanni*), kencur (*Kaempferia galangal*), and sambiloto (*Andrographis*

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paniculata) have the potential to be used as substituents in the manufacture of herbal oils [8–12]. The potential for use is due to the content of secondary metabolites and their bioactivity [6].

Brotowali is a spice that is widely used as a traditional medicine ingredient and has antioxidant, anti-inflammatory, immunomodulatory, anticholinesterase, antibacterial, antiviral, antiparasitic, cytotoxic, cardio-protective, antidiabetic, and antinociceptive bioactivity [12]. The bioactivity of brotowali is due to the content of secondary metabolite compounds in it, namely terpenoids, flavonoids, alkaloids, lignans, nucleosides, and sterols [12]. Cinnamon contains secondary metabolite compounds, namely flavonoids, tannins, alkaloids, saponins, glycosides, and triterpenoids/steroids [13]. Cinnamon is often used in traditional medicine because it has antibacterial, antioxidant, antitumor, anti-inflammatory, analgesic, antirheumatic, and antidiabetic bioactivity [8,14,15]. Galangal has biological activity as an anti-inflammatory, antioxidant, antitumor, antimicrobial, antiangiogenic, and antiinsecticide [9, 16]. The bioactivity of kencur is due the content of flavonoids, terpenoids, alkaloids, steroids, phenolics, ethyl-p-methoxycinnamate, gamma-muurolene, cyperene, pentadecane heptadecane,, 8-heptadecane, (2,2) 3,6-nonadienal, 3-Phenyl-2-Propenoate, and alpha-gurjunene [9, 16, 17]. Cloves are herbs that are rich in eugenol, β-caryophyllene, and eugenyl acetate compounds and are bioactive as antioxidants, antibacterials, antivirals, and anti-inflammatories [18]. Kencur is a traditional medicinal plant that has bioactivity as an anti-inflammatory, antioxidant, anticancer, anti-virus, antidiabetic, anti-diuretic, analgesic, and anti-infection, and this plant contains many diterpenoid, lactone, and flavonoid compounds [10, 11, 19].

In the manufacture of herbal oils, the bioactivity of herbal oils is often influenced by variations in VCO oil as a base with the spices to be added [20]. Emu et al., in their study, stated that increasing the concentration of bay leaf extract added to VCO in the manufacture of herbal oil can improve the quality of VCO oil in terms of its physicochemical properties [21]. The addition of medicinal plant herbs also increases the acceptability of the product in organoleptic tests [22]. Based on the literature review, this study will be conducted to make herbal oil from VCO with a combination of concentrations of brotowali, cloves, cinnamon, kencur, and sambiloto spices. The purpose of this study is to ascertain how the spices comparison and the VCO variations affect the physicochemical, phytochemical, and antioxidant properties of herbal oil preparations using variations in the comparison of total spices (brotowali, cloves, cinnamon, kencur, and sambiloto) and VCO.

2 Materials and Methods

2.1 Materials

The following ingredients were utilized in this study: distilled water (H_2O), starch (Merck), sulfuric acid (Merck), hydrochloric acid (Merck), glacial acetic acid (Merck), iron(III) chloride ($FeCl_3$), DPPH (TCI), ethanol (Merck), phenolphthalein (Merck), potassium hydroxide (Merck), potassium iodide (Sigmaaldrich), potassium iodate (Merck), chloroform (Smart-Lab), sodium thiosulfate (Smart-Lab), Dragendorff's reagent, Mayer's reagent, wagner, brotowali (*Tinospora crispa*), cloves (*Syzygium aromaticum*), cinnamon (*Cinnamomum burmanni*), kencur (*Kaempferia galangal*), sambiloto (*Andrographis paniculata*), and Virgin Coconut Oil (VCO).

2.2 Herbal oil preparation

All herbs used in this research were ground, dried, and sieved through a 40-mesh screen.. Making herbal oil based on research by Wahyuzan et al. has been modified [3]. This research was carried out by making five variations of herbal oil using five spices, namely brotowali: clove: cinnamon: kencur: bitter (ratio 1:1:1:1:1). The formulation of the herbal oil preparation uses 300 mL of VCO which is then added to each spice with a total weight of 30 g; 40 g; 50 g; 60 g; and 70 g. The five variations were heated for 60 minutes at a temperature of 100˚C. The heated oil is filtered using a vacuum pump.

Table 1. The formulation of herbal oil preparations.

Material	Variations in Herbal Oil Preparations				
	A	**B**	**C**	**D**	**E**
VCO (mL)	300	300	300	300	300
Brotowali (g)	6	8	10	12	14
Cloves (g)	6	8	10	12	14
Cinnamon (g)	6	8	10	12	14
Kencur (g)	6	8	10	12	14
Sambiloto (g)	6	8	10	12	14
Total weight (g)	30	40	50	60	70

2.3 Phytochemical test

2.3.1 Alkaloid test

The alkaloid test involved one millilitre of herbal oil placed inside the three tubes. The first and second tubes were dissolved with 1 to 2 mL of concentrated HCl. In the first tube, add two to three drops of Mayer's reagent, and in the second tube, add two to three drops of Dragendroff's reagent. In Mayer's test, the presence of alkaloids is indicated by the formation of a white-to-yellow precipitate, and in Dragendroff's reagent, there was a change in the precipitate to orange. One to two millilitres of concentrated sulfuric acid (H2SO4) was introduced in the third test tube. The solution was added back with Wagner's ragen around 1-2 mL. The results show positive alkaloids in Wagner's reagent if the solution contains a brown precipitate [23].

2.3.2 Phenol

The phenol test begins with 1 mL of herbal oil in a test tube, followed by three drops of 5% $FeCl_3$ added. The results show positive phenol is indicated by a change in the color of the solution to a strong green or blue [24].

2.3.3 Flavonoids

The flavonoid test begins with a pipette of 3 mL of herbal oil and is inserted into a reaction tube. Herbal oil was reacted with two millilitres of H_2SO_4 p.a solution. The results show positive for flavonoids if the solution changes color, namely yellow, red, or brown [24].

2.3.4 Saponin

The saponin test begins with about 1 mL of herbal oil being placed in a reaction tube. Herbal oil was mixed with three milliliters of aquadest and shaken. The results show positive for saponin if foam forms in the sample [24].

2.3.5 Steroids and terpenoids

The terpenoid and steroid test begins with a few drops of herbal oil put into a test tube. In herbal oil, one to two drops of concentrated sulfuric acid are added and one to two drops of concentrated glacial acetic acid are added. The results show positive for terpenoids if the solution turns red, pink, or violet, and positive for steroids if the sample turns blue or green [24].

2.4 Physicochemical Test

2.4.1 Density measurement

The density test begins with a 5 mL empty picno weighed empty without any water content. Empty picnos that have been weighed are added to the sample [25].

2.4.2 Water content

The water content test begins with 5 grams of oil being weighed in a cup whose constant weight is known. The oil was heated for 1 hour at 105°C in the oven. The oil is cooled in a desiccator for 15-30 minutes and weighed again to obtain a constant weight [26].

2.4.3 Measurement of pH

The pH test begins with 10 mL of herbal oil placed in a beaker. The pH meter is dipped in oil until there is a constant pH value on the pH meter.

2.4.4 Free fatty acid levels

The FFA content test based on the research of Noor et al. (2021) was modified [27]. First, 5 mL of herbal oil was measured and put into an Erlenmeyer flask (250 mL), 50 mL of ethanol (96%) was added, and two drops of phenolphthalein indicator. The sample was subsequently titrated with a KOH solution (0.1N) until a constant pink color formed for 10 seconds [27]. The following formula is used to calculate the levels of free fatty acids:

$$\%FFA = \frac{\text{a x n x m}}{\text{Sample weight (g)}} \quad (1)$$

Information:
a = number of mL KOH
n = KOH concentration
m = molecular weight of dominant fatty acid (lauric acid = 200.3 g/mL)
g = sample weight (g)

2.4.5 Peroxide number

The measurement of peroxide value is based on research by Bouta et al., which has been modified[7]. 2.5 grams of herbal oil were placed into a 250 mL Erlenmeyer flask, and 7.5 mL of a glacial acetic acid and chloroform mixture, in a 3:2 ratio, was added. The solution was shaken, and 0.25 grams of potassium iodide was added. After shaking the mixture, 0.5 mL of 1% starch and 7.5 mL of distilled water were added. Sodium thiosulfate 0.1 N solution was used to titrate the sample until the blue hue of the solution diminished or vanished [7]. The peroxide number is determined using the formula below:

$$Peroxide\ number = \frac{\text{A x N x 1000}}{g} \quad (2)$$

Information:
A = Number of mL of Na2S2O3 solution
N = Normality of Na2S2O3
G = Sample weight (grams)

2.5 Antioxidant activity test

2.5.1 Free radical reduction

The herbal oil was dissolved using chloroform with a concentration of 100 ppm. Two millilitres of herbal oil were placed in a test tube, and 2 millilitres of 0.1 mM DPPH solution were added. The solution was incubated at 37ºC for 30 minutes. The solution is then measured at the maximum wavelength using UV-Vis spectrophotometry [28]. The percentage value of DPPH radical inhibition is measured using the following formula:

$$\%\ Inhibition\ of\ DPPH\ radicals = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} x100\% \quad (3)$$

2.5.2 IC50 measurement

An herbal oil sample solution was made with a concentration of 100; 150; 200; and 250 ppm using chloroform solvent. Take 2 mL of each sample concentration and put it in a different test tube. Place 2 mL of DPPH solution (0.1 nM) into each test tube with the sample and incubate at 37ºC for 30 minutes. Afterwards, measure the solution at 528 nm using UV-Vis [28]. The results of the obtained inhibitory power are then drawn as a graph of the relationship between concentration (x-axis) and inhibitory power (y-axis) so that a linear regression equation is obtained. The equation is used to calculate IC50 which shows the compound can inhibit 50% of DPPH free radical antioxidants.

3 Results and discussions

3.1 Herbal oil preparation

In this research, five herbal oil formulations were made as can be seen in Tab. 1. In this research, five herbal oil formulations were made, as in Tab. 1. The results of herbal oil preparation obtained herbal oil, which was yellowish green in color, as can be seen in Fig. 2. There is a color change in all herbal oil products indicating that secondary metabolites from the plant have been dissolved into the VCO. The increase in secondary metabolites dissolved in VCO is confirmed by the qualitative increase in secondary metabolite content in the

phytochemical test, Tab. 2. Phytochemical test results showed that all herbal oil samples contained flavonoid, terpenoid, saponin, phenolic and alkaloid phytochemical compounds, while VCO contained flavonoid, terpene, saponin and alkaloid compounds. These results show that there is an additional compound content from spices, namely phenol, added to VCO.

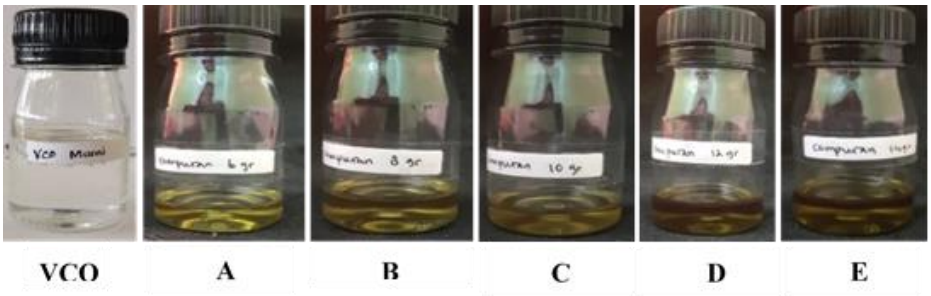

Fig. 1. The herbal oil formulated with a variety of spices and VCO.

Table 2. The phytochemical test results. (++) = many; (+) = little; (-) = none.

Phytochemical test	Test result					
	VCO	A	B	C	D	E
Alkaloids						
Dragendroff	+	++	++	++	++	++
Mayer	-	-	-	-	-	-
Waegner	+	+	+	+	+	+
Phenol	-	+	+	+	+	+
Flavonoids	+	++	++	++	++	++
Saponin	+	++	++	++	++	++
Steroids/ Terpenoids	+	++	++	++	++	++

3.2 Physicochemical test

Physicochemical tests on herbal oils are carried out by measuring the density, water content, pH, FFA content, and peroxide value. From physicochemical tests, data was obtained that compared to VCO, herbal oil had a higher density than VCO. This indicates that there has been an increase in dissolved secondary metabolites. Physicochemical tests also show that the overall water content of herbal oils decreases compared to the water content of VCO. The decrease in the water content of herbal oil is due to the heating process in making herbal oil which results in evaporation of the water contained in the oil.

Table 3. The Physicochemical test results of herbal oil.

Parameters	VCO	Formula				
		A	B	C	D	E
Density [g/mL]	0.920	1.059	1.098	1.167	1.204	1.605
Water content [%]	0.017	0.017	0.016	0.016	0.012	0.009
pH	4.98	5.07	5.13	5.17	5.26	5.33
Free Fatty Acids [%]	0.0080	0.0078	0.0073	0.0056	0.0054	0.0047
Peroxide Number [mEq/kg]	0.0190	0.0186	0.0186	0.0173	0.0173	0.0120

Determining FFA levels from herbal oils shows that overall, herbal oils have lower FFA levels than VCO. Table 3 also shows that the increasing spices included in VCO will further reduce the FFA content of the oil. This is because the presence of secondary metabolite compounds from spices will increase, which plays a role in inhibiting water from hydrolyzing ester bonds from oil [29]. This data is in accordance with research by Perera et al. (2020) that increasing the spices added to VCO will reduce the FFA levels of herbal oil [20]. This data is also reinforced by the pH value, which increases as the amount of spices added increases.

Measuring the peroxide value of herbal oils shows that all herbal oils have lower peroxide numbers compared to VCO. The data in Table 3 also shows that the increasing weight of herbs added will further reduce the oil's peroxide number. The decrease in the peroxide value of herbal oils is because secondary metabolite compounds from herbs are antioxidants that prevent the oxidation of fatty acids. The increased antioxidant activity of this herbal oil is confirmed by the research data in Table 4.

3.3 Antioxidant activity test

The antioxidant activity test of herbal oils is carried out in two ways, namely: (a) determining the ability of each herbal oil to reduce DPPH radicals, and (b) determining the IC_{50} value of the herbal oil that has the best ability to reduce DPPH radicals. From the research data, it was found that the addition of spices will increase the ability of herbal oils to reduce DPPH radicals. The data in Table 4 shows that formula E is the best herbal oil in reducing DPPH radicals. The increased ability of herbal oil to reduce DPPH radicals is due to the increased content of secondary metabolite compounds from spices which have antioxidant properties [8–12]. Next, the best formula determines the IC_{50} value.

Table 4. The ability of herbal oils to reduce DPPH radicals.

Sample	% Inhibition
VCO	15.774
Formula A	30.009
Formula B	30.054
Formula C	30.099
Formula D	30.863
Formula E	31.671

Determining the IC50 value for herbal oil formula E, the value was 167.277 ppm, as shown in Tab. 5. This value shows that the herbal oil has relatively weak antioxidant activity, with a value of $151 < IC_{50} < 200$ ppm. When compared with the antioxidant power of VCO, formula E has 1.44 times greater antioxidant power than VCO. The data in Tab. 5 gives the IC_{50} values of VCO, formula E oil, and tocopherol, respectively, at 240.936 ppm; 167.277 ppm; and 10.860 ppm.

Table 5. The IC50 value of the best herbal samples was compared with VCO and tocopherol

Sample	Regression equation	R^2	IC_{50}
VCO	y= 0,0171x + 45,88	0.9951	240.936
Formula E	y= 0,0595x + 40,047	0.9924	167.277
Tocopherol	y= 3,8069x + 17,736	0.9956	10.860

4 Conclusion

The mixed sample herbal oil preparation contains flavonoids, terpenoids, saponin, phenolic and alkaloid phytochemical compounds, and the VCO sample contains saponins, flavonoids,

alkaloids, and terpenes. Increasing the composition of herbal substituents will improve the quality of the oil by improving its physicochemical properties. Herbal oil with a composition formula E is the best with physicochemical properties: water content 0.009%; density 1.605 g/mL; pH 5.33; free fatty acids 0.005%; peroxide value 0.012 mEq/kg; and antioxidant activity with an IC50 value of 167.277 ppm.

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