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GC-MS and Antioxidant Capacity Analysis in Propanol Extract of *Carthamus Tinctorious L*

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***Abstract*— Safflower, were extracted using propanol solvent at different time intervals: 10, 20, and 30 min at a constant temperature of 40°C. The extracts were analyzed by GC/MS technique. The major compounds identified were tetrapentacontane, tetracontane, triacontanol, gamma sitosterol, myristic acid, linoleic acid, stearic acid, palmitic acid, oleic acid, and lauric acid. However, some levels of palmidrol, beta-amyrin, cubenol, and tocopherol were also found in safflower extracts. Most of the volatile compounds were detected between 10–30 min time of extraction. The 30 min time of extraction also showed the maximum content of polyphenols and antioxidants in safflower extracts. Thus, 30 min was suggested as the most suitable time for maximum extraction of bioactive volatiles, antioxidants, and polyphenols from Safflower using propanol solvent.**

Keywords: Antioxidants; Gas chromatography-mass spectrometry; Polyphenols; Propanol; Safflower.

I. Introduction

Safflower *(Carthamus tinctorius L)* is known by the Bugis-Makassar community as '*Kasumba turate'* or '*Kasumba ogi'*. Safflower is used as a dye on foodstuffs and traditional medicines empirically used by the people of South Sulawesi to treat measles. Natural antioxidants can be found in plants that are high in these compounds. Safflower has long been used as a source of edible fat, food colorants, and Chinese medicine. There are some reports on antioxidative compounds derived from Safflower that describe their activity in scavenging free radical species such as superoxide anion (O_2) [1] and a,a-diphenyl-h-picrylhydrazyl (DPPH) radical [2], [3], indicating the importance of safflower as antioxidant source material. Bioactive compounds of yellow and red Safflower are flavonoids, kaempferol, glycosides, serotonin, and sterols. Phenolic compounds, flavonoids, and carotenoids are secondary metabolites with antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergy, and anti-cancer activity [4].

Some studies explain that antioxidant compounds can inhibit free radicals in the body, which are obtained endogenously (superoxide dismutase enzymes) and exogenous through food or supplements [5], [6]. Some antioxidant compounds can be found in plants derived from polyphenols, vitamin C, vitamin E, β-carotene, and flavonoids [6], [7]. Kasumba turate potential to be developed as a functional food, as it contains polyphenols and antioxidants that are useful to ward off free scavenging activity. Research [8], examined the potential of Safflower is subjected to natural pollination by *Apis mellifera honeybees* in Tacheng City, China. Safflower is extracted by maceration using ethanol solvent 95% for 24 h, obtained 14 polyphenol components such as protocatechuic acid, gallic acid,

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caffeic acid, and ferulic acid. The flavonoids produced are about 83.4%, only about 43.4% are classified as having a high antioxidant activity (DPPH IC_{50}<50). Then, research on Safflower using a combination of ultrasonic-assisted extraction methods and direct solvents in ethanol solvents, showed the maximum potential of Safflower as an antioxidant and antibacterial with an extraction time of 45 min and a temperature of 37^{o}C [9]. In addition, the potential of safflower type yellow A originating from Terra e Vita, Italy. Results showed that this plant is classified as a high antioxidant because it contains polyphenols 3.5 (GAE)/100 g and flavonoids 330 (CE)/100 [10].

Antioxidants from plants are obtained through extraction with solvents based on the level of solubility of these compounds. Alcoholic compounds such as ethanol, methanol, and propanol are solvents for extracting all groups of flavonoids. The most commonly used extraction method is maceration, immersing the sample using a solvent with or without stirring. Maceration is generally slow, requires a lot of solvents, and produces low yields. Sufficiently high temperature is used to increase the extracted compounds' solubility to accelerate the antioxidant oxidation process. Ultrasonic-assisted extraction (UAE) is one of the ultrasonic-assisted extraction methods. Ultrasonic waves have a sound frequency above human hearing (≥ 20 kHz) [11], [12]. The process of extracting organic compounds in plants and grains using organic solvents can take place more quickly with the help of ultrasonics. Ultrasonic vibrations break down the cell wall of the material, and the contents come out easily [13], [14]. The main advantages of ultrasonic-assisted extraction over conventional extraction using maceration are greater efficiency and shorter operating time.

Identify the bioactive compounds of Safflower to gain a better understanding of the physiological and pharmacological properties of Safflower. The effect of extraction time with ultrasonic-assisted extraction methods on the bioactive compounds of Safflower has not yet been reported, to the best of the authors' knowledge. In general, gas chromatography and mass spectrometry (GC–MS) are used to analyze bioactive compounds. This study aimed to comprehensively investigate the effect of extraction time with ultrasonic-assisted extraction methods using propanol solvents on safflower bioactive compounds, antioxidants activity, and polyphenolic content.

II. Research Methodology

2.1. Materials

Dried Safflower (Fig. 1) were purchased from the local market and stored at room temperature until use. Gallic acid monohydrate, sodium carbonate, quercetin, Folin Ciocalteau's phenol reagent, potassium acetate, and 1,1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) were all purchased from Sigma-Aldrich (Singapore). Propanol were purchased from Merck (Singapore). Most of the chemicals are used analytical grade and were used and without purification.

Figure 1. Safflower *(Carthamus tinctorius L)*

2.2. Extraction of Safflower

In the early extraction stages, 2 g of dried Safflower were extracted in 20 ml propanol solvent (1:20) at a constant temperature of 40°C for 10, 20, and 30 min using ultrasonic-assisted extraction methods (Elma Easy Ultrasonic 10 H) with a frequency of 37 kHz. The extracts were concentrated in a rotary evaporator under reduced pressure (Buchi Rotavapor R-100).

2.3. Conditions for the instrument and chromatography

The filtrate was pipetted using PVDF Syringe Filter (SFPVDF013022NA) pore size 0.22 µm, diameter 13 mm (membrane solutions), and collected in vial glass. GC-MS Shimadzu QP-2010 Plus was used to analyze the extracts. GC temperature was set at 100^{o}C for 2 min and then increased to 240^{o}C for 18 min. Capillary

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column RTX-5 (30 mm x 0.25 mm x 0.25 µm), Split ratio 40:1, heating rate 10°C min^{-1}, up to 300°C, maintained for 5 min with a total analysis time of 25 min. Helium was used as a carrier gas flowing at a constant 1.0 ml/min; pre-column pressure was 80 kPa, Ionization voltages 70 eV.

2.4. Free Radical Scavenging activity of Safflower

Method of [6] with some modifications used for analysis antioxidant activity of Safflower. The stock solution of propanol extracts were diluted 1:10 (v/v) with methanol to produce sample solutions, then diluted with a concentration of 300, 350, 400, 450 ppm. Aliquots 4 ml of different concentrations of the safflower extract in methanol (300–450 µL/mL) were mixed with 1 mL of 1 mM DPPH solution; for 30 min, the thoroughly mixed solution was left at room temperature and in the dark. A Shimadzu UV Mini 1240 visible spectrometer was used to measure the mixture's absorbance at 515 nm. The inhibition activity of the samples was calculated according to Eq. (1):

$$Inhibition\ (\%) = \left[\frac{A_{control} - A_{Sample}}{A_{control}}\right] x\ 100 \qquad (1)$$

2.5. Determination of phenolic content of Safflower

Phenolic content of propanol extract safflower was determined using the Folin–Ciocalteu colorimetric method [15] and expressed as mg gallic acid equivalents (GAE) per 100 g dry weight (mg GAE/100 g dw). Safflower extract (1 ml) was mixed with Folin-Ciocalteu reagents (2 ml), Na_2CO_3 (20 ml), and added aquades to the limit mark on the volumetric flask 50 ml. The mixture was shaken for 2 min, and 2 ml of the saturated sodium carbonate and kept in the dark for 60 min at room temperature (28–30°C). A spectrophotometer measures sample uptake with a wavelength of 740 nm.

III. Results and Discussion

3.1. Identifying and analyzing major components

GC–MS chromatograms of propanol extract of 10, 20, and 30 min different time extraction. Safflower samples for different periods of extraction are given in Figure 2. Figure 2 shows that peak samples are identified components totally in 10, 20, and 30 min time of extraction, about 145, 179, and 174 compounds in sequence identified from propanol extract in Safflower. The number of volatile safflower sample constituents as described by the peak varies to a certain extent accordingly due to varying processing and extraction time. Different compounds were identified and characterized by comparing the mass spectra of the constituents to the NIST 2.7 and Willey 8 libraries. Table 1 shows the relative percentages of some major compounds present in almost all of the samples. The representative compound is Tetrapentacontane which accounts for 28.38, 27.45, and 30.79%. They were identified as myristic acid, gamma sitosterol, palmitic acid, lauric acid, stearic acid, oleic acid, linoleic acid, phenols, ascorbic acid, hexatriacontane, tetracontane, tetracosane, triacontanol, and tocopherol were more abundantly present in the safflower extract. However, there are some compounds that are only found at different extraction time treatments, such as palmidrol and Santolina triene (10 min), cubenol and norolean-12-ene (20 min), and beta.-amyrin was identified at the extraction time of 30 min. As the extraction duration is increased, the relative percentage of most physiologically relevant volatile chemicals decreases. As seen in Table 1, most of the chemicals found were beneficial to health and physiologically relevant.

3.2. Total phenolic content of Safflower in different extraction time

The effect of extraction time on the polyphenolic content of safflower extracts is depicted in Table 2. The content of polyphenols in tea is one of the parameters in the quality of Safflower. This is because polyphenols are one of the chemical compounds that have an essential role in maintaining health. Phenol or polyphenols are compounds with hydroxyl groups (OH) that are bound to aromatic rings. Testing the levels of these polyphenols using the reagent Follin ciocalteu and sodium carbonate will change the color of the extract to blue. This concentration of blue color will be measured and compared to gallic acid as a reference solution for calculating polyphenol levels contained in the extract. Total phenolics are presented in Table 2 and expressed as mg of gallic acid equivalents/100 g FW. Total

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phenolics ranged from 11.89 to 96.64 GAE/g. The results revealed that the time of extraction 30 min in Safflower has a high polyphenols 96.64 GAE/g. As a result, the most significant amount of total phenols was detected after 30 min of extraction, implying that this is the most efficient extraction duration for full phenolic component release in Safflower.

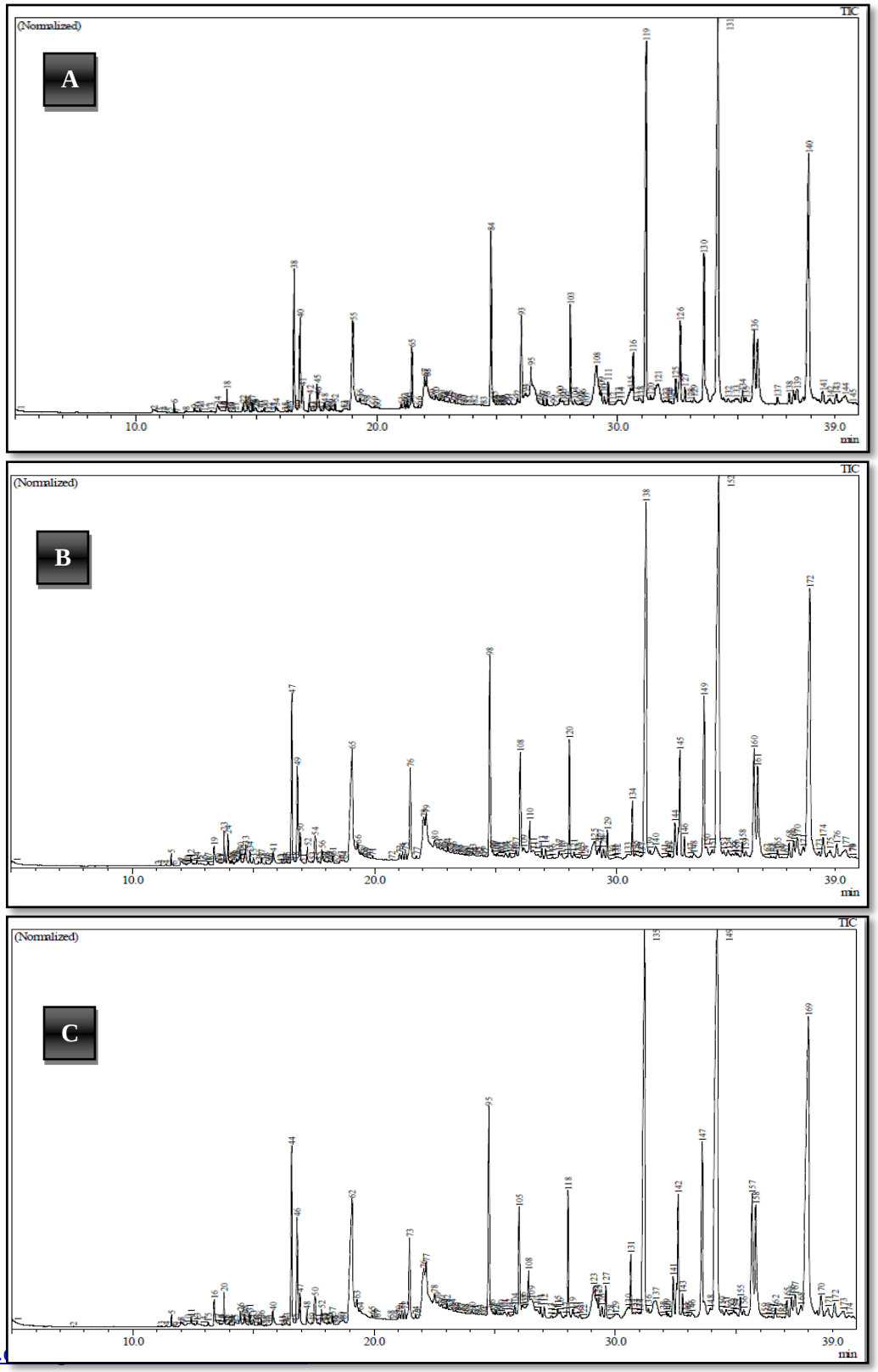

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Figure 2. GC-MS chromatograph propanol extract of Safflower. (A) extraction time 10 min; (B) extraction time 20 min; (C) extraction time 30 min.

Table 1. Relative percentages of some major compounds present in Safflower with different time extraction

Bioactive compounds	Area (%)			Bioactivity	Reference
	10 min	20 min	30 min		
Myristic acid	0.17	1.06	0.68	Antioxidant, nematicidal, anti-carcinogenic, antibacterial activity	[12]
Palmitic acid	0.24	0.61	1.94	Antioxidant, anti-carcinogenic antibacterial activity, nematicide	[12]
Stearic acid	1.12	1.43	1.32	Antibacterial activity	[12]
Oleic acid	0.19	0.19	0.36	Anti-tumor, anti-cancer agents, and antioxidant	[12]
Linoleic acid	3.34	4.25	4.61	Anti-obesity effects, hypoglycaemic, Alzheimer's dementia	[17]
Phenolics	0.22	0.08	0.04	Antioxidant	[6]
Gamma sitosterol	1.64	0.04	0.56	Anti-fungi, antibacterial activity, anti-inflammatory	[12]
Tetracontane	2.4	11.22	10.51	Anti-tumor activities, antidiabetic and antibacterial activity	[18]
Palmidrol	0.01	-	-	Antioxidative	[19]
Hexatriacontane	6.30	12.05	8.92	Blood glucose lowering potency	[20]
Santolina triene	0.03	-	-	Antibacterial activity	[21]
Lauric acid	0.31	0.39	1.1	Antidiabetic and antioxidant activities	[22]
Ascorbic acid	3.89	4.35	4.35	Antioxidant	[23]
Phytol	0.08	1.71	1.34	Precursor of synthetic vitamin E and vitamin K was cytotoxic to breast cancer cell lines. (MCF7)	[18]
Dotriacontane	2.85	0.12	0.51	Antimicrobial agent	[24]
Beta.-amyrin	-	-	1.58	Anti-inflammatory, anticonvulsant, antidepressive, analgesic, antipancreatitic, anticholytic, antihyperglycemic, gastroprotective, hepatoprotective, and hypolipidemic effects	[25]
Tetracosane	10.06	0.31	0.39	Anti-cancer activity against MDA-MB-231, HT-2918, AGS, and NIH 3T3 cell lines	[26][18][27]
Norolean-12-Ene	-	1.10	-	Potential for cytotoxic activity against tumor	[28]
Triacontanol	1.66	5.78	5.63	anti-tumor activity.	[29]
Tetrapentacontane	28.38	27.45	30.79	Hydroxylation of liver enzymes during phase I metabolism, hair growth promoter, uric acid production, and arachidonic acid inhibitor in the human body are therapeutic and pharmaceutical benefits.	[30]
Cubenol	-	0.14	-	Antimicrobial activity	[31]
Tocopherol	0.38	0.56	0.67	Alzheimer's and cardiovascular disease, anti-aging, antioxidant properties	[32]

The decrease in total phenolic content could be due to changes in phenolic compounds' molecular structure, which could reduce extractability due to the degree of polymerization [16].

3.3. Free Radical Scavenging activity of Safflower (DPPH assay)

Analysis of the antioxidant activity of safflower extract using radical DPPH (2,2 diphenyl-1-pikrilhydrazil). Antioxidant compounds will react with DPPH radicals through the hydrogen atom donation mechanism and cause color decay. DPPH from purple to yellow is measured at a wavelength of 517 nm. The parameter of

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this DPPH method is the inhibition concentration value of 50% (IC_{50}) or a concentration that can reduce free radical activity by 50%. Compounds have very strong antioxidant activity if IC_{50} values are less than 50 ppm, strong IC_{50} between 50-100 ppm, medium if the IC_{50} value is 101-150 ppm, and weak if the IC_{50} value is between 150-200 ppm [33]. Safflower extract using propanol solvents with various extraction times is classified as a weak antioxidant activity with a range of 1577.33 to 366.79. It is well known that plant extracts containing polyphenolic components have antioxidant activity because of their ability to donate hydrogen atoms or electrons and capture free radicals [34]. Free radicals are well-known to play a role in biological damage. In summary, the antioxidant reduces DPPH reduction capacity by lowering its absorbance at 517 nm. The extent of the reaction is determined by the antioxidant's hydrogen-donating ability [16].

Table 2. Effects of extraction time on total phenolic content and antioxidant activity of safflower extracts

Time (min)	Propanol solvents		Antioxidant properties
	Total Phenolic Content (GAE/g)	IC_{50} (µg/ml)	
10	49.01	181.25	Weak
20	11.89	366.79	no activity
30	96.64	157.33	Weak
Quercetin (control)	121.38	7.26	Very strong

IV. Conclusion

This study confirms the presence of various health-beneficial compounds using GC-MS. Safflower extract using propanol solvents was found to be richer in myristic acid, lauric acid, palmitic acid, linoleic acid, stearic acid, and oleic acid, as well as the dominant Tetrapentacontane content of this plant, serving as an inhibitor in the human body, are some of the therapeutic and anti-cancer activity. The antioxidant and phenolic content of Safflower was found to be higher at 30 min of extraction time. It was concluded that the extraction time of 30 min is best suited for maximum retention of bioactive compounds in Safflower.

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