Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 1, 2023, pp. 173-187.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

STANDARDIZATION AND GC-MS ANALYSIS OF KERSEN (MUNTINGIA CALABURA L.) FRUIT ETHANOL EXTRACT AS AN HERBAL RAW MATERIAL

S. Nur[1*], A. N.Aisyah[2], Nursamsiar[1], F. J. Sami[1], A. Fadri[1], N. Khairi[2], and A. Sapra[2]

[1]*Department Pharmacetical Chemistry, Sekolah Tinggi Ilmu Farmasi Makassar, 90242, Makassar, Indonesia*
[2]*Depratment of Parmaceutical Technology, Sekolah Tinggi Ilmu Farmasi Makassar, 90242, Makassar, Indonesia*

Kersen (Muntingia calabura) is a plant widely reported to have pharmacological effects so it can be developed as a traditional medicine. This study aims to standardize the ethanol extract of kersen fruit from various locations where it grows in South Sulawesi Province, Indonesia. Standardization-specific parameters were carried out, including organoleptic analysis, phytochemical profiles, water, insoluble ethanol extracts, phenolic content, and total flavonoids. Non-specific parameters include total ash content, acid insoluble ash content, water content, drying shrinkage, specific gravity, and heavy metal contamination content of Pb, Cd, and Cu. The ethanol extract of the kersen fruit was also followed by GC-MS analysis. Specific parameters in the chemical content analysis showed the presence of groups of phenolic compounds, tannins, saponins, alkaloids, steroids, and terpenoids. The water-soluble extract content yielded <2%, and the ethanol-soluble extract content was 14-15.12%; The total phenolic content obtained was 11-12.20% EAG; total flavonoids obtained levels of 3-6.34% EQ. Non-specific parameters indicate that the total ash content is 0.19-0.31%; acid-insoluble ash content of 1.09-1.33%; water content <10%; Drying shrinkage <4%; specific gravity of 1.01-1.07 g/mL; total plate number and mold and yeast numbers obtained the number of colonies <20 colonies/g; and the content of Pb, Cd, and Cu respectively obtained levels of 0 mg/Kg. The results of the GC-MS analysis obtained 36 compounds belonging to the benzoate, phenolic, alkaloid, terpenoid, steroid, and fatty acid ester groups. It can be concluded that the ethanol extract of kersen fruit can be considered a raw material for developing traditional medicine in Indonesia.

Key word : *GC-MS analysis, Geographical location, Muntingia calabura, standardization, specific and non-specific parameters*

INTRODUCTION

The development and advancement of technology are currently attracting the attention of several researchers, especially in the field of natural product development, to develop several plants as medicine to support public health[1]. The community has long used natural materials as an empirical medicine to treat several diseases[2]. Indonesian traditional medicinal plants are increasingly used as standardized traditional medicines and phytopharmacology. Various research and development of natural products that take advantage of technological advances are also carried out to improve the quality, quality, and safety of products which are expected to increase further confidence in the benefits of these natural medicines[3]. However, in the utilization of natural materials, it is necessary to have control over natural product products that have been regulated by the drug and food control agency[4].

Kersen fruit (*Muntingia calabura* L) is a plant from the *Muntiangiaceae* family which has some scientific evidence regarding the

Received in 2/1/2023 & Accepted in 22/2/2023

*Corresponding author: S. Nur, E-mail: syamsunur19@gmail.com

activity possessed by kersen fruit, namely antioxidant[5&6], Has a protective effect against UV exposure[7], anti-aging[8], antibacterial[9&10], anti-inflammatory[11]. Besides having good activity, Kersen fruit also has a variety of nutritional content[12] and compounds[7,9,13–15] which can support the activity of kersen fruit.

In supporting the efficacy of using kersen fruit as a natural raw material, it is necessary to supervise and guarantee quality standards for raw materials from kersen fruit extract. Therefore, the researchers wanted to standardize the ethanol extract of the kersen fruit based on the geographical growth. Standardization of natural medicinal raw materials can be standardized with two standard parameters, specific and non-specific standards. Specific parameters include organoleptic tests, water and ethanol extract insoluble, and identification of compound content. At the same time, non-specific parameters can be determined based on water content, acid-insoluble ash content, total ash content, and microbial contamination. This research is also supported by the chemical content of kersen fruit extract, which is determined through the GC/MS approach.

MATERIALS AND METHOD

Material

The tools used include, aluminum foil, MIxer (Cosmos®), stir bar, erlenmeyer (Iwaki®), beaker (Iwaki®), measuring cup (Iwaki®), filter paper, Thin Layer Chromatogram (TLC) Merck (Germany), micropipette (DragonLab®), oven (Memmert®), spatula, analytical balance (Mettler Toledo®), rotary evaporator (Buchi®), drip plates, erlenmeyer (Iwaki®) and vials.

The chemicals used include, aquadest OneMed (Indonesia), $AlCl_3$ Sigma-Alderich (Gemarny), dragendorf, ethanol pro analysis Merck (Germany), $FeCl_3$ Merck (Germany), folin ciocalteus Merck (Germany), H_2SO_4 Merck (Germany), HCl 2 N Merck (Germany), Mayer reagent, n-Hexan Merck (Germany), sodium carbonate Sigma-Alderich (Gemarny), Wagner reagent, Mg (Merck, Germany), NaCl.

Plant Collection

Half-ripe kersen (*Muntingia calabura* L) fruit samples were obtained from eight growing locations in South Sulawesi Province, Indonesia. The eight locations are Telkomas (M1, -5º12’71”S, 119º 51’01”E), Tamalanrea (M2, -5º13’92”S, 119º 51’38”E), Biringkanaya (M3, -5º 08’59”S, 119º53’28”E) which were located in Makassar City, Gowa (M3, -5º33’12”S, 119º87’24”E) Takalar (T, -5º31’94”S, 119º34’69”E), Soppeng (S, -4º33’93”S, 119º96’08”E), Barru (B, -4º48’81”S, 119º61’15”E), Tana Toraja (TT, -3º11’02”S, 119º84’59”E) regency. The kersen fruit obtained was then washed and dried using an oven at 40º C for 2 times 24 hours.

Extraction

Kersen fruit simplicia was obtained from eight areas and was then extracted using the ultrasonic method. Each sample of 150 grams was put into a beaker glass container and then sonicated at 42 kHz for 30 minutes. After the extraction process, the sample was filtered, and the residue was re-sonicated until the material was completely extracted, indicated by a clear filtrate. The filtrate obtained from each sample was then evaporated using a rotary evaporator to obtain a thick extract from each sample. The viscous extract that has been obtained is then weighed to calculate the percent yield of the extract obtained using the following formula:

% Extract Yield = (Extract weight/Simplesia weight) x 100

Standardization Of Spesific Parameters

Screening Phytochemical

Identification of the ethanol extract of kersen fruit compounds was analyzed qualitatively using specific chemical reagents to identify alkaloids, flavanoids, saponins, steroids, phenolics and tannins. Identification was carried out following the procedure[16].

Flavonoid Content

Determination of flavonoid levels from ethanol extracts of kersen fruit according to the procedure[17&18]. Each kersen fruit sample was weighed as much as 10 mg dissolved in 1 mL of ethanol pro analysis and then homogenized using a vortex. After vortexing, 20 µL of the sample was put into the 99 well plates, and 20 µL of $AlCl_3$ (10% w/v) and sodium acetate were added to 1 M (20 µL). The volume was made up with ethanol pro analysis up to 200 µL, which was then incubated for 30 minutes. After the incubation period was over, the sample of the test solution was then measured

for its absorbance using a microplate reader at a wavelength of 435 nm.

Phenolic Content

Determination of phenolic content of kersen fruit ethanol extract according to the procedure[17,19] with a few modifications. Each kersen fruit sample was weighed as much as 10 mg dissolved in 1 mL of ethanol pro analysis and then homogenized using a vortex. After vortexing, 20 µL of the sample was put into the 99 well plates, then added 20 µL of 7.5% Na_2CO_3 and 20 µL of Folin Ciocalteu reagent. Then the volume was made up of distilled water up to 200 µL, which was then incubated for 30 minutes. After the incubation period, the sample of the test solution is then measured for its absorbance using a microplate reader at a wavelength of 640 nm.

Determination Of Water-Soluble Compound Levels

Five-gram (W1) extract was macerated for 24 hours with 100 mL of water chloroform using a plugged flask while shaking for the first 6 hours and then left for 18 hours and filtered. The chloroform and water layers were separated. Evaporate 20 mL of the aqueous layer filtrate to dryness in a porcelain cup with a flat bottom (W0). The residue was heated at 105ºC to constant weight (W2). Calculate the concentration in percent of the water-soluble compound to the initial extract weight. Three replications were carried out [20].

$$\%\ \text{Content of water soluble compounds} = \frac{(W2 - W0)}{W1} \text{X } 100\%$$

Determination of Levels of Ethanol Soluble Compound

Five-gram (W1) extract was macerated for 24 hours with 100 mL ethanol (96%) using a plugged flask while repeatedly shaking for the first 6 hours and then left for 18 hours. Filtered quickly by avoiding the evaporation of ethanol, then 20 mL of the filtrate was evaporated to dryness in an evaporating cup (W0), and the residue was heated at 105°C to a constant weight (W2). The initial extract weight calculated the concentration in percent (%) of the compound dissolved in ethanol. Three replications were carried out[20].

$$\%\ \text{Ethanol Soluble Compound Content} = \frac{(\text{W2} - \text{W0})}{\text{W1}} \text{X } 100\%$$

Determination of Metal Content

Analysis of the content of heavy metals leads (Pb), copper (Cu), and cadmium (Cd) was carried out by the destruction method and followed by analysis using an atomic absorption spectrophotometer[1]

Standardization of Non-Specific Parameters
Determination of Drying Shrinkage

Weigh 1 gram of the extract and put it in a covered porcelain crucible which has been previously heated at 105°C for 30 minutes and has been calibrated. Before weighing, the extract was leveled in a porcelain crucible by shaking the crucible to form a layer 5 mm-10 mm thick. Put in the oven, open the lid, and dry at 105°C until the weight remains. Cool in a desiccator. Repeat three times and then calculate the percentage[20].

$$\%\ \text{Drying Shrinkage} = \frac{\text{W1}(\text{W2} - \text{W0})}{\text{W1}} \text{X } 100\%$$

Determination of Water Content

Determination of the water content of the ethanol extract of kersen fruit according to the procedure[21] with a few modifications. A sample of 2 grams was weighed accurately (W1), put into a porcelain exchanger, and weighed empty (W0). Then dry in the oven at 105ºC for 5 hours and weigh then continue drying and weigh at intervals of 1 hour until the difference between successive weighing is not more than 0.25% (W2).

$$\%\ \text{Water content} = \frac{\text{W1} - \text{W0}}{\text{W1} - \text{W2}} \text{X } 100\%$$

Total Ash Content

A total of 2 grams of the extract was weighed carefully (W1) into a crucible that had been tarred and weighed (W0). After that, the extract was fired using a furnace, slowly increasing the temperature to 600 ± 25°C until the charcoal disappeared. After that, it was cooled in a desiccator, and the weight of the ash (W2) was weighed. Then calculate the percent total ash content. The work was carried out three times replication[20].

$$\% \text{ Total Ash Content} = \frac{(W2 - W0)}{W1} X\ 100\%$$

Acid Insoluble Ash Content

The ash obtained from the determination of the ash content was then boiled with 25 mL of dilute hydrochloric acid for 5 minutes. The acid-insoluble portions were collected and filtered through ash-free filter paper, and the crucible was rinsed with hot water. The filtered ash and filter paper was put back into the same crucible and ignited in the furnace slowly at 600 ± 25°C until the charcoal disappeared. Then weighed until the weight remained (W3). Determined the acid-insoluble ash content in percent of the initial sample weight. Replication was carried out three times[20].

$$\% \text{ Acid Insoluble Ash Content} = \frac{(W3 - W0)}{W1} X\ 100\%$$

Microbial Contamination

One gram of extract was dissolved in 10 mL of 0.9% NaCl solution and then shaken using a vortex until homogeneous to obtain a 10^{-1} dilution.

Total Plate Number (ALT)

The assay was prepared in 3 tubes filled with 9 mL of 0.9% NaCl solution. From the 10^{-1} dilution, 1 mL was pipetted into the first test tube until a 10^{-2} dilution was obtained, then shaken until homogeneous. Subsequent dilutions were made up to 10^{-5}, and then 15 mL of NA (Nutrient Agar) medium was poured at 45°C into each petri dish. The petri dish was shaken carefully until it became homogeneous with the seed. After the media solidified, the Petri dishes were incubated at 35°C for 24 hours in an inverted position. The number of colonies growing in each petri dish was counted. Replication was carried out three times[20].

Determination of Total Mold

Prepared 3 test tubes, each of which was filled with 9 mL of 0.9% NaCl solution. From the 10-1 dilution, 1 mL was pipetted into the first test tube until a 10-2 dilution was obtained, then shaken until homogeneous. Further dilutions were made up to 10-5 dilutions. Pipette 1 mL of extract from each dilution using a sterile pipette, then pour into a petri dish containing 15 mL of PDA medium (Potato Dextrose Agar) and shake until homogeneous, then incubate at 25°C for 5 days. Then observed and counted the number of colonies that grew and multiplied them by the dilution factor. Replication was carried out three times, and a blank test was carried out (WHO, 2005).

RESULT AND DISSCUSION

Result

In supporting the efficacy and quality assurance of kersen fruit as a raw material for traditional herbs, various parameters have been developed to ensure the quality of kersen fruit extract. In this study, samples of kersen fruit were obtained from several places in the city of Makassar and various areas in the province of South Sulawesi, Indonesia. The samples that have been obtained are then made dry simplicia using an oven at a temperature of 40°C to reduce water content.

After the process of making drying samples, the samples were then extracted using the sonicator method with the aim of avoiding damage to the compounds and maximizing the extraction results contained in the kersen fruit with the help of ultrasonic sound waves with ultrasonic wave propagation of 42 kHz[22&23]. Based on the extraction results using the sonicator method, it was found that the kersen fruit samples from the Tamalanrea location had a higher yield (%), namely 27.39%. However, in general, the yield percentage generated from each region is not significantly different. The resulting yield percentage is in the range of 21-27% b/b (Table 1). The yield results show that the extraction process was carried out perfectly with a %rendement value > 10%. The yield of sample extracts can be influenced by several factors, including the use of solvents, sound wave emission, and temperature during the extraction process which is carried out using the sonicator method[23&24]. The yield of the sample extract can be affected by several factors, including the use of solvents, emission of sound waves, and temperature during the extraction process which is carried out using the sonicator method[23–25].

In an effort to control the quality standards of medicinal raw materials (simplicia and extracts) it is intended to guarantee the quality, safety, and efficacy of drugs to be made from kersen fruit extract [26]. in this study used several specific parameters (organoleptic, identification of compound content, determination of total

flavonoid content and determination of total phenolic content, water insoluble extract and ethanol insoluble extract content) and non-specific parameters (total ash content, insoluble ash content acid, water content, drying shrinkage, specific gravity, microbial contamination) in accordance with extract standardization procedures [20&21].

Table 1: % yield of kersen fruit extract (*Muntingia calabura* L.) from each region.

Sample	Extract Yield (%b/b)
Telkomas (M1)	26.17
Toraja	21.09
Takalar	26.62
Gowa	25.91
Barru	24.39
Soppeng	27.02
Tamalanrea (M2)	27.39
Paccerakkang (M3)	24.47

Organoleptic analysis of the ethanol extract of kersen fruit was carried out with several parameters, namely physical form, color, odor, and taste. The results of the organoleptic test showed that the ethanol extract of kersen fruit from each place where it grew showed the same results, namely in the form of a viscous extract that was dark brown, had an atypical odor, and had a bitter taste. The presence of chemical content influences the organoleptic characteristics of kersen fruit extract. Standardization of the specific parameters of the ethanol extract of kersen fruit, in the identification test for the content of compounds present in the ethanol extract of kersen fruit (Table 2) using the colorimetric method showed that the ethanol extract of kersen fruit contains alkaloids, flavonoids, steroids, saponins, phenolics and tannins. Determination of the water soluble content of each ethanol extract of kersen fruit obtained a level average of <2% while in determining the level of ethanol extract of kersen fruit which is soluble in ethanol with an average level of 14-15%.

Table 2: Evaluation results of specific standardization of kersen fruit ethanol extract.

Parameter	**Results**							
	M1	**Toraja**	**Takalar**	**Gowa**	**Barru**	**Soppeng**	**M2**	**M3**
Specific Standardization								
• **Organoleptic type** a. Physical form b. Color c. Odor d. Taste	Thick extract Dark-brown Not typical Bitter	Thick extract Dark-brown Not typical Bitter	Thick extract Dark-brown Not typical Bitter	Thick extract Dark-brown Not typical Bitter	Thick extract Dark-brown Not typical Bitter	Thick extract Dark-brown Not typical Bitter	Thick extract Dark-brown Not typical Bitter	Thick extract Dark-brown Not typical Bitter
• **Phytochemical Screening**								
a. Alkaloids								
– Mayer's reagent	+	+	+	+	+	+	+	+
– Dragendorf reagent	+	+	+	+	-	+	+	+
– Wagner reagent	+	-	+	+	+	+	+	+
b. Flavonoid	+	+	+	+	+	+	+	+
c. Steroids	+	+	+	+	+	+	+	+
d. Saponins	+	+	+	+	+	+	+	+
e. Phenolic	+	+	+	+	+	+	+	+
f. Tannins	+	+	+	+	+	+	+	+
• **Water Soluble Extract Content (%)**	1.88 ± 0.27	1.62 ± 0.98	1.82 ± 1.06	2.05 ± 0.29	1.92 ± 1.82	1.85 ± 0.34	1.98 ± 0.23	1.70 ± 0.52
• **Ethanol Soluble Extract Content (%)**	14.96 ± 0.37	14.03 ± 0.52	15.05 ± 0.88	14.42 ± 0.49	14.92 ± 0.55	14.77 ± 1.52	15.12 ± 0.11	14.81 ± 1.28

In the parameters for determining the levels of compounds, in this case determining the levels of flavonoids and phenolics, there are differences in the content found in various regions. Tests for flavonoids and phenolics from kersen fruit extract were carried out based on the colorimetric method using specific reagents which were then analyzed quantitatively using a UV-Vis spectrophotometers[19,27]. In Table 3 there are differences in flavonoid and phenolic content in kersen fruit extract based on the profile of where the plants grow. The compound contained in the kersen fruit sample can depend on the region where the plant grows which will affect the compound content of a sample [28]. Based on the results obtained from the determination of the content of flavonoid and phenolic compounds, the Takalar area has a lower compound content of 3.01% QE (Flavonoid) and 9.95% GAE (Phenolic) compared to some other areas (Table 3). This can happen because the Takalar area has environmental conditions close to the coast so that the temperature in the area is high so that it can affect the production of secondary metabolites which will increase Reactive Oxygen Species (ROS) in plants which can trigger cell damage in plants [29]. However, in general, the flavonoid and phenolic content of each extract based on the geographic location where it grows tends to be uniform, with average levels of flavonoids and phenolic ranges from 4-5% QE and 11-12% GAE, respectively.

Table 3: Specific Parameters based on Total Phenolic and Flavonoid Content of Kersen Fruit Ethanol Extract.

Sample	Flavonoid (%QE)	Phenolic (%GAE)
Telkomas (M1)	4.64±0.63	11.26±0.77
Toraja	5.25±0.71	11.47±0.47
Takalar	3.01±0.59	9.95±1.67
Gowa	4.17±0.11	11.37±0.61
Barru	4.67±0.92	11.74±0.59
Soppeng	6.34±1.01	11.90±0.68
Tamalanrea (M2)	3.44±0.77	11.87±0.30
Paccerakkang (M3)	3.66±0.29	12.20±1.02

For non-specific standardization parameters, the ethanol extract of kersen fruit (Table 4) is determined for the total ash content, which aims to identify the presence of mineral content in each extract that is formed during the heating process to a temperature of 600^{o} C. The ash content in raw materials is very important to determine the feasibility of the material for further processing. Based on the results of determining the total ash content obtained of 0.2-0.3%. The ash content indicates that the extract contains minerals with limits that still follow the raw material standards. The results of the total ash obtained then tested the acid-insoluble ash content. The results obtained from determining acid insoluble ash content were 1-1.3 %. The acid-insoluble ash indicates that the extract still contains contaminants or impurity compounds.

In determining the water content of the ethanol extract of kersen fruit using the gravimetric method, the water content contained in the ethanol extract of kersen fruit was average 8%, this exceeds the standard [30] with the water content contained in the extract sample ≤10%. In the drying shrinkage test to determine the levels of compounds that evaporate during the heating process at 105^{o}C. Based on the results (Table 4) it shows that the average number of compounds that evaporation is 3.8%. In testing the specific gravity which aims to determine the density of a substance to the density of water with a mass/volume value and determine the chemical content dissolved in an extract[20]. Based on the results obtained from determining the specific gravity, it was obtained at ±1 g/mL.

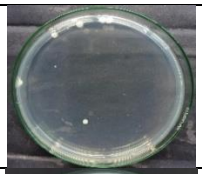
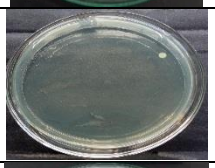
One of the requirements for the standardization of natural product extracts is that they are free or not polluted by the presence of heavy metals. Metal contaminants in extracts of natural materials must be limited to heavy metal contaminants because the heavy metals presence can cause complex reactions in the body that will have toxic consequences. Heavy metals do not exceed the limits set by the Indonesian national standard with number SNI 7387:2009. Based on SNI 7387:2009 states that heavy metal contamination of Pb does not exceed 2 mg/Kg, Cd does not exceed 0.2 mg/Kg, and Cu does not exceed 5 mg/Kg. Based on the analysis of heavy metal contamination from the ethanol extract of kersen fruit (Table 4), it was found that there was no heavy metal contamination of Pb, Cd, and Cu (0 mg/Kg) in the ethanol extract of kersen fruit.

Table 4: Non-specific parameters of kersen fruit ethanol extract.

Parameter	Results							
	M1	**Toraja**	**Takalar**	**Gowa**	**Barru**	**Soppeng**	**M2**	**M3**
Total Ash Content (%)	0.22± 0.006	0.27± 0.012	0.27± 0.08	0.19± 0.091	0.30± 0.000	0.26± 0.000	0.241± 0.010	0.309± 0.000
Acid Insoluble Ash Content (%)	1.32± 0.19	1.09± 0.08	1.29± 0.121	1.3± 0.01	1.29± 0.059	1.31± 0.002	1.33± 0.000	1.31± 0.37
Water content (%)	8.49± 0.37	8.43± 0.28	8.32± 0.23	8.61± 0.22	7.94± 0.17	7.99± 0.31	8.3± 0.77	8.40± 0.29
Drying Shrinkage (%)	3.82 ± 0.23	3.81 ± 0.43	3.82 ± 0.19	3.80 ± 0.45	3.79 ± 0.41	3.85 ± 0.4	3.77 % ± 0.42	3.80± 0.39
Specific Gravity (g/mL)	1.02 ± 0.32	1.07 ± 0.37	1.05 ± 0.31	1.03 ± 0.36	1.02 ± 0.38	1.02 ± 0.36	1.02 ± 0.30	1.01 ± 0.29
Pb content (mg/Kg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cd Content (mg/Kg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cu Content (mg/Kg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

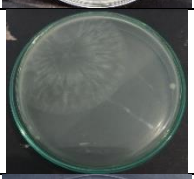
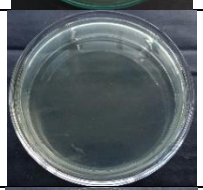
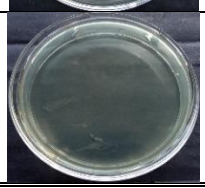
Testing for bacterial and yeast fungus contamination is one of the tests for extract purity. This test includes determining the allowable number of microorganisms and to indicate the absence of certain bacteria or yeast-fungus contaminant in the extract. The amount of microbial contaminants from the extract is expressed as colonies/gram. According to the Indonesian Food and Drug Supervisory Agency (2014), the limit for microbial contamination, both bacteria and fungi, in herbal medicines is a maximum of $1x10^5$ colonies/gram for bacterial contamination and $1x10^3$ for fungal contamination. Based on the results obtained (Table 5 and 6), it shows that the ethanol extract of kersen fruit has bacterial and yeast contamination according to standard (31), namely ≤ 100 colonies/g for total plate number (TPN) and yeast fungus number (YFN). The total plate numbers and mold and yeast numbers produced by the extracts were very small and followed the herbal raw materials standards. This can be influenced by the presence of compounds in the extract, which act as antimicrobials, so the resulting microbial contamination is very small.

Table 5: Test Results for Total Plate Number (ALT) of Kersen Extract Samples Nutrient Agar (NA) Media.

Sample Name	Dilution	Replication (TPN)			Profile in media	Results	Reference Value
		I	II	III			
Ethanol Extract	10^{-1}	7	6	4		17 Colony/g	≤100 Colony/g
	10^{-2}	2	1	1		4 Colony/g	
	10^{-3}	1	-	-		1 Colony/g	
	10^{-4}	-	-	-		-	

Note: - indicated the absence of colony.

Table 6: Test Results for Yeast Fungus Number (YFN) of Kersen Extract Samples on Potato Dextrose Agar (PDA) Media.

Sample Name	Dilution	Replication (YFN)			Profile in media	Results	Reference Value
		I	II	III			
Ethanol Extract	10^{-1}	5	4	4		13 Colony/g	≤100 Colony/g
	10^{-2}	2	2	1		5 Colony/g	
	10^{-3}	-	-	-		-	
	10^{-4}	-	-	-		-	

Note: - indicated the absence of colony.

GC-MS analysis performed on the ethanol extract of kersen fruit identified 36 compounds consisting of several compounds based on their group (Figure 1). The analysis showed that kersen fruit extract contained compounds belonging to the benzoat, phenolic, alkaloid, terpenoid, steroid, and fatty acid ester groups (Table 7). The results of the GC-MS analysis are in line with the analysis of the phytochemical screening using specific reagents in Table 2. The benzoate group in the form of Benzyl O-Nitro Benzoate is a benzoate derivative compound that has been reported as an anti-bacterial[31&32] Phenolic compounds in the form of 2,6-di-test-Butyl-4-Methylphenol have been widely reported as antioxidants[33&34]. Compound 1-Methyl-Cyclohexanacarboxylic Acid-(2H-[1,2,4]Triazol-3-YL)-Amide; 1,4-Diazabicyclo[4.3.0]Nonan-2,5-Dione, 3-Methyl; Pyrrolo[1,2-A]Pyrazine-1,4-Dione, Hexahydro; N, N'-Bis-3-Oxapentamethyleneformamidinum Dithiocarboxylate; and Propanoic Acid, 2,2-Dimethyl-, (2,3,3a,9a-Tetrahydro-3-Hydroxy-6-Oxo-6H-Furo [2',3':4,5] is an alkaloid compound and was reported pharmacological activities such as antibacterial[35&36], antibiotics and antioxidants[37]. Terpene compounds in the form of (2R,3R,4ar,5S,8as) -2-Hydroxy-4a,5-Dimethyl-3- (Prop-1-En-2-Yl)Octahydronaphthalen-1 was also reported to have pharmacological activity[38] and steroid compounds in the form of Cholesta-9(11),20(22)-Dien-23-One, 3,6-Bis(Acetyloxy)-, (3.Beta.,5.Alpha.,6.Alpha.)- has been reported to have effects as an antifungal[39] and a cholesterol inhibitor[40]. Information on the chemical content and bioactivity of the ethanol extract of kersen fruit reported in this study and previous studies can be used as a reference so that the ethanol extract of kersen fruit can be developed as a natural raw material for producing traditional medicinal products.

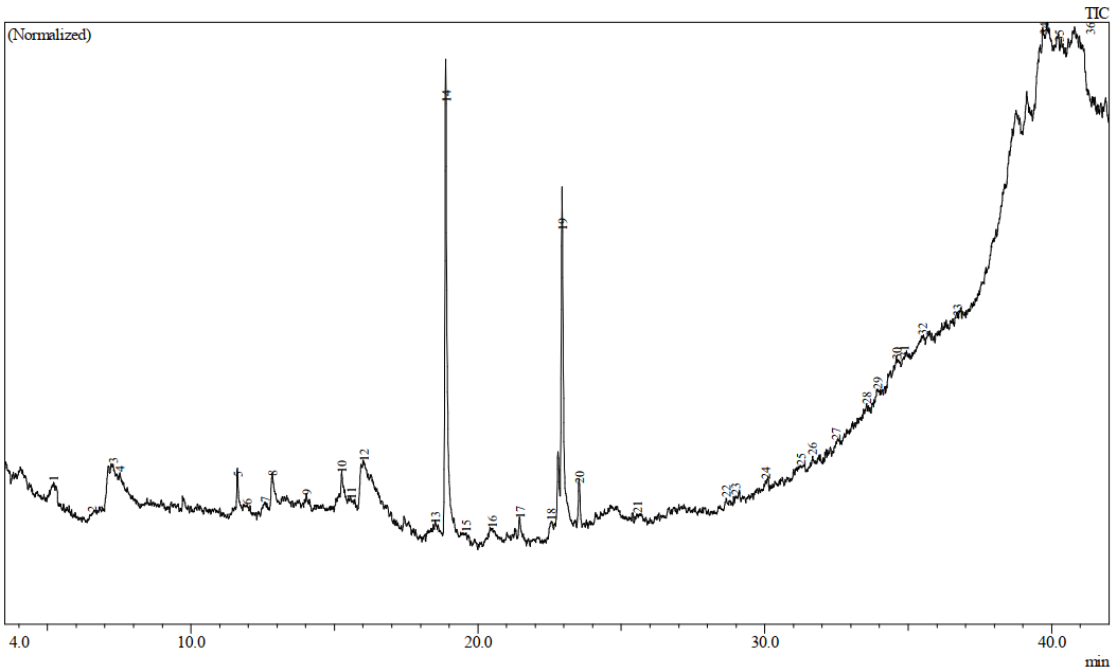


Fig. 1: GC-MS Spectrum of Kersen Fruit Extract.

Table 7: GC-MS analysis of Kersen Fruit extract.

ID Sampel	Retetion Time	Name Of The Compound	Molecular Weight	Molecular Formula	Class of Compound	Conc. (%)
1	5.220	Benzyl O-Nitro Benzoate	257	$C_{14}H_{11}NO_4$	Benzoate	0.69
2	6.558	1-Methyl-Cyclohexanacarboxylic Acid-(2H-[1,2,4]Triazol-3-YL)-Amide	208	$C_{10}H_{16}N_4O$	Alkaloid	0.68
3	7.281	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-	144	$C_6H_8O_4$	Heterocyclic alcohol	3.07
4	7.525	Pentanoic Acid, 2-(Methoxymethyl)-4-Oxo-	160	$C_7H_{12}O_4$	Fatty acid	1.93
5	11.630	2,6-Di-Tert-Butyl-4-Methylphenol	220	C15H24O	Phenolic	0.89
6	11.958	Benzo[B]Thiophene, 2,7-Diethyl-2,7-Diethyl-1-Benzothiophene	190	$C_{12}H_{14}S$	Aromatic	0.39
7	12.592	(R)-(+)-Arachidonyl-1'-Hydroxy-2'-Propylamide	361	$C_{23}H_{39}NO_2$	Fatty acid	0.25
8	12.847	Dodecanoic Acid, Ethyl Ester	228	$C_{14}H_{28}O_2$	Fatty acid	8.80
9	14.031	2-Propenoic Acid, Tridecyl Ester	254	$C_{16}H_{30}O_2$	Fatty acid	0.30
10	15.267	Ethyl Nonadecanoate	326	$C_{21}H_{42}O_2$	Fatty acid	1.20
11	15.592	1,4-Diazabicyclo[4.3.0]Nonan-2,5-Dione, 3-Methyl	168	$C_8H_{12}N_2O_2$	Alkaloid	0.61
12	16.031	Pyrrolo[1,2-A]Pyrazine-1,4-Dione, Hexahydro-	154	$C_7H_{10}N_2O_2$	Alkaloid	5.17
13	18.531	2-Aminoethanethiol Hydrogen Sulfate (Ester)	157	$C_2H_7NO_3S_2$	Ester	1.11
14	18.892	Ethyl Pentadecanoate	270	$C_{17}H_{34}O_2$	Fatty acid	7.52
15	19.592	2-((E)-[((E)-2-([(E)-(2-Hydroxyphenyl)Methylidene] Amino)Propyl)Im	282	$C_{17}H_{18}N_2O_2$	Phenolic	0.47
16	20.491	N,N'-Bis-3-Oxapentamethyleneformamidinum Dithiocarboxylate	260	$C_{10}H_{16}N_2O_2S_2$	Alkaloid	1.00
17	21.465	7-Hexadecenoic Acid, Methyl Ester, (Z)-	268	$C_{17}H_{32}O_2$	Fatty acid	1.02
18	22.558	13-Propoxy-13-Borabicyclo[7.3.1]Tridecane	236	$C_{15}H_{29}BO$	Heterocyclic	0.44
19	22.951	(E)-9-Octadecenoic Acid Ethyl Ester	310	$C_{20}H_{38}O_2$	Fatty acid	6.51
20	23.542	Octadecanoic Acid, Ethyl Ester	312	$C_{20}H_{40}O_2$	Fatty acid	0.66
21	25.558	2-Undecenoic Acid	184	$C_{11}H_{20}O_2$	Fatty acid	0.37
22	28.658	9,12,15-Octadecatrienoic Acid, 2-[(Trimethylsilyl)Oxy]-1-[[(Trimet	496	$C_{27}H_{52}O_4Si_2$	Fatty acid	0.30

Table 7: Continued.

23	28.992	Propanoic Acid, 2,2-Dimethyl-, (2,3,3a,9a-Tetrahydro-3-Hydroxy-6-Oxo-6H-Furo[2',3':4,5]	310	$C_{14}H_{18}N_2O_6$	Alkaloid	0.51
24	30.025	Nonanoyl Chloride	176	$C_9H_{17}ClO$	Fatty acid	0.52
25	31.258	Cyclopropanecarboxylic Acid,-2-(1-Trimethylsilylpropyn-3-Yl), Methyl Ester, Trans	210	$C_{11}H_{18}O_2Si$	Fatty acid	0.50
26	31.658	9-Octadecenoic Acid (Z)-, Methyl Ester	296	$C_{19}H_{36}O_2$	Fatty acid	0.42
27	32.492	3,5-Cyclohexadiene-1,2-Dione, 3,5-Bis(1,1-Dimethylethyl)-	220	$C_{14}H_{20}O_2$	Heterocyclic ketone	0.21
28	33.558	3-Isopropyl-6a,10b-Dimethyl-8-(2-Oxo-2-Phenyl-Ethyl)-Dodecahydro-Benzo[F]Chromen-7-	396	$C_{26}H_{36}O_3$	Terpene	0.93
29	33.925	9-Octadecenoic Acid (Z)-, Oxiranylmethyl Ester	338	$C_{21}H_{38}O_3$	Fatty acid	0.71
30	34.601	1,6,10,14,18,22-Tetracosahexaen-3-Ol, 2,6,10,15,19,23-Hexamethyl-, (All-E)-(.+/-.)-	426	$C_{30}H_{50}O$	Fatty acid	1.55
31	34.858	(+)-Nepetalactone	166	$C_{10}H_{14}O_2$	Fatty acid	1.53
32	35.492	(2R,3R,4ar,5S,8as)-2-Hydroxy-4a,5-Dimethyl-3-(Prop-1-En-2-Yl)Octahydronaphthalen-1	236	$C_{15}H_{24}O_2$	Terpene	2.06
33	36.725	Cholesta-9(11),20(22)-Dien-23-One, 3,6-Bis(Acetyloxy)-, (3.Beta.,5.Alpha.,6.Alpha.)-	498	$C_{31}H_{46}O_5$	Steroid	1.23
34	39.745	Dodecanoic Acid, 1,2,3-Propanetriyl Ester	638	$C_{39}H_{74}O_6$	Fatty acid	35.99
35	40.258	1-Dodecanoyl-3-Myristoylglycerol	484	$C_{29}H_{56}O_5$	Fatty acid	3.92
36	40.816	Octadecanoic Acid, 3-[(1-Oxohexadecyl)Oxy]-2-[(1-Oxotetradecyl)Oxy]Propyl Ester	806	$C_{51}H_{98}O_6$	Fatty acid	14.64

Conclusion

The ethanol extract of kersen fruit obtained from various locations where it grows has been standardized specifically and non-specifically. The test results showed that all the ethanol extracts of kersen fruit analyzed met the criteria based on the Indonesian National Standard (SNI). This shows that geographical differences do not significantly affect the extract's specific and non-specific characteristics. The results of GC-MS analysis of the ethanol extract of kersen fruit identified as containing compounds from the benzoate, phenolic, alkaloid, terpenoid, and steroid groups, as well as the ester fatty acid group. Based on the standardization that has been carried out, it indicates that the ethanol extract of kersen fruit can be used as a raw material in the development of traditional medicine.

Formatting of funding sources

This research received funding from the Indonesian Ministry of Education, Culture, Research and Technology through a Hibah Terapan Vokasi with contract numbers 131/SPK/D4/PPK.01.APTV/VI/2022 and 476/LL9/PK.00/PPM.PTV/2022.

Acknowledgment

The author would like to thank the STIFA Makassar Research and Community Service Institute and the research laboratory for the provided facilities and the students who collaborated in this research.

REFERENCE

1. Y. Syukri, R. Purwati, N. Hazami, H.T. Anshory, and A. Fitria, "Standardization of Specific and Non–Specific Parameters of Propolis Extract as Raw Material for Herbal Product", ***EKSAKTA: Int J Data Sci Anal,*** 1(1), 36–43 (2020).
2. N.I.E. Jayani, A.H. Krisnawan, N.D. Oktaviyanti, and K. Kartini, "Standardization of Phyllanthus niruri and Sonchus arvensis as Components of Scientific Jamu", ***Majalah Obat Tradisional***, 25(7), 7–14 (2020).
3. S. Anam, M. Yusran, A. Trisakti, N. Ibrahim, A. Khumaidi, R. Ramdanil and M.S. Zubair, "Standarisasi Ekstrak Etil Asetat Katu Sanrego (Lunasia amara Blanco)", ***Online J Nat Sci,*** 2(3)**,** 1–8 (2013).
4. ***Indonesia Food Drugs Authorization (BPOM)***, "Kriteria Tata Laksana Pendaftaran Obat Tradisional, Obat Herbal Terstandar dan Fitokimia". (2005).
5. S. Nur, M. Aswad, R. Yulianti, F.J. Sami, A.Burhan, A.Fadri, N. Khairi, and Nursamsiar, "Antioxidant activity profile of extract and fraction of kersen (Muntingia calabura L.) fruits prepared by different methods", ***IOP Conf Ser Earth Environ Sci***, 976**,** 012066 (2022).
6. S. Nur, M. Aswad, R. Yulianti, A. Burhan, W.J.D. Patabang, A. Fadri, and Nursamsiar, "Antioxidant Activity Profile of Kersen Fruit Extract and Fraction (Muntingia calabura L.) Using TAC and CUPRAC Methods", ***JPSCR***, 7**,** 79–88 (2022).
7. S. Nur, M. Aswad, R. Yulianti, Nursamsiar, A.E.E. Tumigolung and A. Burhan, "Screening Bioactivity of Kersen Fruits (Muntingia calabura L.) as a Sunscreens Candidate", ***Galenika Journal of Pharmacy***, 7(1), 29–38 (2021).
8. S. Nur, A.A. Angelina, M. Aswad, R. Yulianti, A. Burhan, and Nursamsiar, "In vitro anti–aging activity of Muntingia calabura L. fruit extract and its fractions", ***J Pharm Pharmacogn Res,*** 9, 409–421 (2021).
9. M. Krishnaveni, and R. Dhanalakshmi, "Qualitative And Quantitative Study Of Phytochemicals In Muntingia Calabura L. Leaf And Fruit", ***World J Pharm Res***, 3(6)**,** 1687-1696 (2014).
10. O.F.C. Mogollón, R.E. Gonzalez–Cuello, and J.S.G. López, "In vitro Antibacterial and Antioxidant Activity of Muntingia calabura Fruits Extract", ***Contemp Eng Sci***, 11**,** 881–890 (2018).
11. K. Preethi, P. Premasudha, and K. Keerthana, "Anti–inflammatory Activity of Muntingia calabura Fruits", ***Pharmacogn J***, 4(30)**,** 51-56 (2012).
12. L. Muslimin, I, Hasyim, N.F. Yusuf, F. Mubarak and R. Yulianty, "Nutrient Content, Mineral Content and Antioxidant Activity of Muntingia calabura Linn", ***Pak J Nutr***, 18**,** 726–732 (2019).
13. R. Gomathi, N. Anusuya and S. Manian, "A Dietary Antioxidant Supplementation of Jamaican Cherries (Muntingia calabura L.) Attenuates Inflammatory Related Disorders", ***Food Sci Biotechnol***, 22**,** 787–794 (2013).
14. G.A. Pereiraa, H.S. Arrudaa, D.R. Moraisb, M.N. deEberlinb, and G.M. Pastorea, "Carbohydrates, volatile and phenolic compounds composition, and antioxidant activity of calabura (Muntingia calabura L.) fruit Marcos Nogueira Eberlinb", ***Food Res Int***, 108**,** 264–273 (2018).
15. M. Zahara, and Suryady, "Kajian Morfologi dan Review Fitokimia Tumbuhan Kersen (Muntingia calabura L)", ***J. Ilm Pendidikan Dan Ppem,*** 5**,** 7 (2018).
16. J.B. Harbone, "Phytochemical Methods A Guide To Modern Tecniques Of Plant

Analysis", ***Chapman & Hall,*** London, UK (1998).
17. B. John, C.T. Sulaiman, S. George, and V.R.K. Reddy, "Total Phenolic and Flavanoid in Selected Medicinal Plant Form Kerala", ***Int J Pharm Pharm Sci***, 6(1), 406-408 (2014).
18. N. Nursamsiar, K. Khairuddin, M. Megawati, A. Fadri, and S. Nur, "Penentuan Kadar Flavanoid Dari Ekstrak Etanol dan Etil Asetat Daun Kesambi (Schleichera oleosa L.)" ***Acta Pharmaceutica Indonesia***, 46(2)**,** 33—37(2021).
19. S. Nur, S. Mus, M. Marwati, N. Nursamsiar, F.J. Sami, and A. Fadri, "Determination of Total Phenolic and Flavonoid Levels of Mangkokan Leaf Extract (Polyscias scutellaria)", ***Journal of Pharmaceutical and Medicinal Sciences***, 5(1)**,** 24–27 (2020).
20. R. I. Depkes, "Parameter standar umum ekstrak tumbuhan obat", Jakarta, ***Ministry of Health Republic Indonesia***, 4th edition (2000).
21. ***The Indonesian Herbal Pharmacopea***, Direktorat Jenderal Kefarmasian dan Alat Kesehatan, Jakarta (2017).
22. A. Golmohamadi, G. Möller, J. Powers, and C. Nindo, "Effect of ultrasound frequency on antioxidant activity, total phenolic and anthocyanin content of red raspberry puree", ***Ultrason Sonochem,*** 20(5)**,** 1316–1323 (2013).
23. K. Hao, W. Hu, M, Hou, D. Cao, Y, Wang, Q, Guan, X. Zhang, A. Wang, J. Yu and B. Guo, "Optimization of Ultrasonic–Assisted Extraction of Total Phenolics from Citrus aurantium L. Blossoms and Evaluation of Free Radical Scavenging, Anti–HMG–CoA Reductase Activities", ***Molecules***, 24(13)**,** 2368 (2019).
24. M. Rahardjo, G. Mangalik, M. Sihombing and J.F. Da Costa, "Effect of the Extraction Solvent Polarity and the Ratio of Feed and Solvent on the Phytochemical Content and Antioxidant Activity of Red Betel Leaves (Piper crocatum)", ***Indonesian J Agric Res***, 1(1)**,** 71–77 (2018).
25. N.P.Y.C. Riskiana and R.L. Vifta, "Kajian Pengaruh Pelarut Terhadap Aktivitas Antioksidan Alga Coklat Genus Sargassum dengan Metode DPPH", ***J Holistic Health Sci***, 3(2), 201–213 (2021).
26. H.A. Cahyanto, "Standardisasi Simplisia Dan Ekstrak Etanol Jahe Merah (Zingiber officinale Rosch. var rubrum) Dari Lahan Gambut Kubu Raya, Kalimantan Barat", ***Jurnal Borneo Akcaya*** 7(2)**,** 49–55 (2021).
27. S. Nur, F. Mubarak, C. Jannah, D.A. Winarni, D.A. Rahman, L.A. Hamdayani and F.J. Sami, "Total phenolic and flavonoid compounds, antioxidant and toxicity profile of extract and fractions of paku atai tuber (Angiopteris ferox Copel)", ***Food Res,*** 3(6)**,** 734–740 (2019).
28. M. Marwati, B. Taebe, A. Tandilolo, A and S. Nur, "Pengaruh Tempat Tumbuh dan Profil Kandungan Kimia Minyak Atsiri dari Rimpang Jahe Merah (Zingiber officinalle Linn. Var rubrum)". ***J Sains Kes,*** 3**(2),** 248–254 (2021).
29. D. S. Utomo, E.B.E. Kristiani, and A. Mahardika, "The Effect of Growth Location on Flavonoid, Phenolic, Chlorophyll, Carotenoid and Antioxidant Activity Levels in Horse Whip (Stachytarpheta Jamaicensis)", ***Bioma***, 22(2)**,** 143–149 (2020).
30. ***Indonesia Food Drugs Authorization (BPOM)***, "Persyaratan Mutu Obat Tradisional" (2014).
31. J. Matsumoto, T. Miyamoto, A. Minamida, Y. Nishimura, H. Egawa, H. Nishimura, "Pyridonecarboxylic Acids as Antibacterial Agents. 2.' Synthesis and Structure–Activity Relationships of 1,6,7–Trisubstituted 1,4–Dihydro–4–oxo1,8–naphthyridine–3–carboxylic Acids, Including Enoxacin, a New Antibacterial Agen", ***J Med Chem,*** 27(3), 292–301 (1984).
32. H. Diastuti, M. Chasani, and Suwandri, "Antibacterial Activity of Benzyl Benzoate and Crotepoxide from Kaempferia rotunda L. Rhizome", ***Indones J Chem***, 20(1)**,** 9–15 (2020).
33. F. S. Zarudii, G. Z. Gil'mutdinov, R. F. Zarudii, M. A. Myshkin, F. B. Gershanov, and T. A. Novikov, "2,6–Di–Tert–Butyl–4–Methylphenol (Dibunol, Ionol,

Tonarol): A Classical Antioxidant (A Review)",***Pharm Chem J,*** 35**,** 162–168 (2001).

34. O. D. Sparkman, Z.E. Penton, and F.G. Kitsonin *Gas Chromatography and Mass Spectrometry (Second Edition)* (eds. O.D. Sparkman, Z.E. Penton, and F.G. Kitson, ***Academic Press, Amsterdam***, 383–388 (2011).
35. J. Tangjitjaroenkun, W. Pluempanupat, R. Tangchitcharoenkhul, W. Yahayo, and R. Supabphol, "Antibacterial, antioxidant, cytotoxic effects and GC–MS analysis of mangrove–derived Streptomyces achromogenes TCH4 extract", ***Arch Biol Sci,*** 73(2), 223–235 (2021).
36. P. Sharma and D. Thakur, "Antimicrobial biosynthetic potential and diversity of culturable soil actinobacteria from forest ecosystems of Northeast India", ***Sci Rep,*** 10**,** 4104 (2020).
37. G.S. Kiran, S. Priyadharsini, A. Sajayan, A. Ravindran, and J. Selvin, "An antibiotic agent pyrrolo[1,2–a]pyrazine–1,4dione,hexahydro isolated from a marine bacteria Bacillus tequilensis MSI45 effectively controls multi–drug resistant Staphylococcus aureus", ***RSC Advances***, 8(32)**,** 17837–17846 (2018).
38. M. Gao, X. Han, Y. Sun, H. Chen, Y. Yang, Y. Liu, H. Meng, Z. Gao, Y. Xu, and Z. Zhang, "Overview of sesquiterpenes and chromones of agarwood originating from four main species of the genus Aquilaria", ***RSC Advances,*** 9**(8),** 4113–4130 (2019).
39. A. Sayik, A.S. Yusufoglu, L. Acik, G. Turker, and B. Aydin, "DNA– Binding, Biological Activities, and Chemical Composition of Wild Growing Epilobium angustifolium L. Extracts from Canakkale, Turkey", ***JOTCSA,*** 4(3)**,** 811–840 (2017).
40. G.T. Emmons, J.S. Pyrek, R. Dam, M. Martin, K. Kudo, and G.J. Schroepfer, "5 alpha–cholest–8(14)–en–3 beta–ol–15–one, a potent regulator of cholesterol metabolism: occurrence in rat skin", ***J Lipid Res,*** 29(8)**,** 1039–1054 (1988).

Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 1, 2023, pp. 173-187.

نشرة العلوم الصيدليـــــة
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

التوحيد القياسي وتحليل كروماتوغرافيا الغاز – مطياف الكتلة لمستخلص الإيثانول لثمار الكرز (مونتينجيا كالابورا ل.) كمادة عشبية خام

س. نور[1*] – أ.ن.عيسى[2] – نورسامسيار[1] – ف.ج. فادري[1] – ن.خيري[2] – أ.سابرا[2]

[1] قسم الكيمياء الصيدلية ، كلية ماكاسار للصيدلة ،٩٠٢٤٢ ، ماكاسار ، إندونيسيا

[2] قسم التكنولوجيا الصيدلانية ، كلية ماكاسار للصيدلة ،٩٠٢٤٢ ، ماكاسار ، إندونيسيا

الكرز (مونتينجيا كالابورا ل.) هو نبات معروف على نطاق واسع أن له تأثيرات فارماكولوجية لذا يمكن تطويره كدواء تقليدي. تهدف هذه الدراسة إلى توحيد قياس مستخلص الإيثانول لثمار الكرز من مواقع مختلفة حيث تنمو في مقاطعة سولاويزي الجنوبية بإندونيسيا. تم إجراء معايير خاصة بالتوحيد القياسي ، بما في ذلك التحليل الحسي ، والتحليل الفيتوكيميائي ، والمياه ، ومستخلصات الإيثانول غير القابلة للذوبان ، والمحتوى الفينولي ، وإجمالي مركبات الفلافونويد. تتضمن المعلمات غير المحددة محتوى الرماد الكلي ، ومحتوى الرماد غير القابل للذوبان في الأحماض ، ومحتوى الماء ، وانكماش التجفيف ، والجاذبية النوعية ، ومحتوى تلوث المعادن الثقيلة من الرصاص ، والكادميوم ، والنحاس. كما تبع مستخلص الإيثانول لثمار الكرز وتحليل كروماتوغرافيا الغاز – مطياف الكتلة. أظهر تحليل المحتوى الكيميائي وجود مجموعات من المركبات الفينولية والعفص والصابونين والقلويدات والمنشطات والتربينويدات. نتج محتوى المستخلص القابل للذوبان في الماء <٢٪ ، ومحتوى مستخلص الإيثانول القابل للذوبان كان ١٤–١٥,١٢٪ ؛ كان إجمالي المحتوى الفينولي الذي تم الحصول عليه ١١–١٢,٢٠٪ EAG ؛ مجموع الفلافونويد حصل على مستويات ٣–٦,٣٤٪ مكافئ. تشير المحددات الغير محددة إلى أن محتوى الرماد الكلي هو ٠,١٩–٠,٣١٪ ؛ محتوى الرماد غير القابل للذوبان في الحمض ١,٠٩–١,٣٣٪ ؛ محتوى الماء <١٠٪؛ انكماش التجفيف <٤٪ ؛ الجاذبية النوعية ١,٠١–١,٠٧ جم / مل ؛ إجمالي عدد الاطباق وأرقام العفن والخميرة التي تم الحصول عليها عدد المستعمرات <٢٠ مستعمرة / جم ؛ وحصل محتوى الرصاص والكادميوم والنحاس على مستويات ٠ مجم / كجم على التوالي. حصلت نتائج تحليل كروماتوغرافيا الغاز – مطياف الكتلة على ٣٦ مركبًا تنتمي إلى مجموعات إستر البنزوات والفينول والقلويد والتربينويد والستيرويد والأحماض الدهنية. يمكن الاستنتاج أن مستخلص الإيثانول لثمار الكرز يمكن اعتباره مادة خام لتطوير الطب التقليدي في إندونيسيا.