The_Extraction_of_Gambier.pdf

by

Submission date: 22-Jul-2022 04:42PM (UTC+0800)
Submission ID: 1873720871
File name: The_Extraction_of_Gambier.pdf (543.87K)
Word count: 3229
Character count: 17288

The Extraction of Gambier by Using Ultrasonic Assisted at Various Temperature

The Extraction of Gambier by Using Ultrasonic Assisted at Various Temperature

T Anggraini[1], Rini[1], L Aini[1]

[1] Faculty of Agricultural Technology, Andalas University. Limau Manis-Padang, West Sumatera, Indonesia 25163

Corresponding author's email address: tuty@ae.unand.ac.id

Abstract. Gambier is an extract from the leaves *Uncaria gambier* Roxb plant that contain polyphenolic compounds. One of polyphenol compounds in gambier is catechin, that can act as antioxidant and antimicrobial. This study aimed to determine the effect of heat on the catechin extraction from gambier by using ultrasonic assisted. The experimental design used a completely randomized design with four temperature treatments, i.e. 50°C, 60°C, 70°C, and 80°C with 3 replications. The variables measured were IC50, polyphenol content and catechin content. This study showed that the heating process during the extraction with ultrasonic waves has an impact on the content of antioxidants, polyphenols and catechins of the extract produced. The extraction process using ultrasonic assisted at 50°C showed the best result for antioxidant activity, polyphenol content, and catechin content. The value of IC50 was 1.3538 mg/L, polyphenol content was 6.6724 mg/L, and catechin content was 957.01µg/mg
Keywords: Gambier; Ultrasonic Assisted Extraction; Heating Process; Catechin; Antioxidant

1. Introduction

Gambier is an extract from the leaves of *Uncaria gambier* Roxb plant. Gambier contains polyphenolic compounds such as tannins and catechin which are utilized in pharmaceutical, textile and food industries [1]. In food industries, catechin can act as antioxidant and antimicrobial component. Catechins have proven useful because of their high potency for preventing lipid peroxidation of oil-containing foods. They have ability to scavenge free radicals and stop the auto-oxidative degradation of lipids [2].

Catechin gambier purification can be conducted using an extraction process with organic solvents and water which utilized the differences in the solubility between catechins and tannins in water. In the pure state, catechins dissolve in hot water, alcohol and ethyl acetate [3]. Yeni (2017) found that crude gambier extracted using water at 70°C produced a product with 65-74% cathecin content. Catechins obtained by solvent extraction using methanol and ethyl acetate increase the purity of catechins gambier to 95% and 98%, respectively [4].

Although methanol and ethyl acetate give a much higher yield than water solvents, chemical solvents such as methanol, dioxane, acetonitrile, acids, formaldehyde, and tetrahydrofuran are toxic, not environmentally friendly and also will increase production costs [5, 6]. For this reason, an optimization of extraction using water could be both practical and environmentally friendly.

To optimize the process of extracting organic compounds in gambier using water as the solvent we can apply ultrasound waves and it is known as Ultrasonic-assisted extraction (UAE). Ultrasonic waves are acoustic waves with frequencies greater than 16-20 kHz [7]. Ultrasonic extraction is non-

destructive and non-invasive, so it can be easily adapted to various applications. Ultrasonic can also reduce the operating temperature so it is suitable for the extraction of bioactive compounds that are not heat-resistant [8]. Ultrasonic-assisted extraction of supercritical extraction ginger has been found to increase yield 30% and reduce the extraction time [9].

In previous study, it has been shown that the extraction process of catechin gambir with water solvent and the use of ultrasonic waves for 90 minutes can help increase the percentage of antioxidant and polyphenol levels of catechin gambier extracted with catechin levels 933.45µg/mg. This study aims to determine the effect of ultrasonic assisted in the extraction of catechin compounds from gambier with water of various temperatures.

2. Materials and Methods

2.1. Raw Materials

Dry Gambier (*Uncaria gambier* Roxb.) from Tarusan, South Pesisir, West Sumatra was used.

2.2. Chemicals and Equipment

The chemicals used were Aquabides, Sigma brand catechin standard, DPPH, methanol, Na2CO3, Folin-Ciocalteu reagent, HPLC methanol, PA methanol, formic acid and water. The equipment used included a blender to break down dry gambier and Universal 320 R centrifuges, HPLC equipment, spectrophotometer for analysis, and oven.

2.3. Gambier Re-extraction with water

A modification of Yeni et al.,(2017) was used for extraction. Crude gambier was reduced to powder and mixed with distilled water (1: 5), mixed in a vortex mixer, then ultrasonicated (ultrasonic water bath (280 W, 50/60 Hz, S 10H Elmasonic) supplied by Elma (Singem, Germany) for 90 minutes at various temperature (50, 60,70,and 80^{o}C). The mixture was filtered through a 100 mesh filter. The filtrate was left to stand for 24 hours. The precipitate obtained was repeatedly washed with water until a yellowish suspension was obtained and then centrifuged to re-settle out the precipitate which was dried in an oven at 40^{o}C for 15 hours.

2.4. IC 50 with DPPH (Diphenyl Pycryl Hydrazyl) Radical Scavenging Activity

The antioxidant activity test was carried out by determining the levels of the radical compound DPPH (Diphenyl Pycryl Hydrazyl) added to the material. This test was carried out using a modification of the method described by Zhang et al (2016), 1 gram of sample was added to 10 ml of methanol (10^5 mg/l) and homogenized. The resulting mixture was diluted with methanol down to 1, 2, 3, 4, and 5 mg/l. Next, 2 ml of each diluted sample was placed with 1 ml of DPPH solution in methanol in a test tube. After incubating in a dark room for 30 minutes, spectrometer readings were carried out at a wavelength of 517 nm. DPPH radical scavenging activity was obtained by the equation below:

DPPH radical scavenging activity = [1- - A517 (sample) / A517 (blank)] x 100%

2.5. Polyphenol Content

The polyphenol content of the sample was determined using the FolinCiocalteu colorimetric method modified by Tumbarski et al (2019). 1 gram of sample was added to 10 ml of methanol and homogenized (10^5 mg/l). Then the mixture was diluted further with methanol to 5 mg/1. 1 ml of this was homogenized with 2 ml of distilled water and 1 ml Follin-Ciocalteu reagent for 5 minutes. After that, 1 ml of Na2CO3 was added and the suspension incubated in a dark room for 2 hours at room temperature. Absorbance was measured at 725 nm. Phenol content was calculated using gallic acid as a standard (3,4,5-trihydroxy benzoic acid ($C_6H_2(OH_3)CO_2H$)) and expressed as mg of Gallate Acid Equivalent (GAE) / 100 g dry weight.

2.6. HPLC for catechin content

2.6.1. Sample Preparation

The sample was dissolved with methanol (HPLC grade) solvent containing 1% formic acid to a concentration of 200 ppm and homogenized by ultrasonication for 5 minutes

2.6.2. Standard preparation
The standard was prepared by dissolving the standard in methanol HPLC: aquabidest (1: 1) and then adding 0.1% formic acid to the standard concentrations of catechins at 500 ppm.

2.7. Sample Analysis
Dried extract from mashed raw dried gambier that had been obtained after 90 minutes with various temperature (50, 60, 70, and 80°C) ultrasonication which had been dried was tested. The tests included antioxidant levels calculated as IC_{50} and polyphenols by spectrophotometer. Subsequently, an analysis of catechin content was carried out on samples of gambier extracted by HPLC. The HPLC method used for the analysis of catechin was using Shimadzu Technical Note L373A. The catechins were determined by a high-pressure pump HPLC system, an autosampler, a column (Prominence HPLC 20A Shimadzu), and a reverse phase chromatography column (agilent Zorbax SB-C18 column) paired with a visible UV detector. The mobile phase A was aquabidest with a mixture of 0.2% phosphoric acid and 1% tetrahydrofuran. The mobile phase B was acetonitrile with 1% tetrahydrofuran. Separation was developed under a polarity gradient system of 5-25% B for 20 minutes with a flow rate of 0.8 ml / min with a wavelength of 230 nm.

3. Results and Discussion

3.1. Raw Crude Gambier
IC_{50} analysis of 1, 2, 3, 4 and 5 ppm concentration samples showed the antioxidant activity of the original gambier was 1.939 mg/mL and the crude gambier polyphenol content in the 5 ppm concentration solution was 6.004 mg /ml solution.

The DPPH method was chosen to test for antioxidant compounds because it is simple, easy, fast, sensitive and does not require a large sample. DPPH is a free radical compound that can react with compounds that can donate hydrogen atoms changing the color of DPPH from purple to yellow [10]. Antioxidant compounds in the crude gambier donate hydrogen atoms into DPPH reducing the intensity of the color purple. Antioxidant content in gambier comes from polyphenols, catechins, epicatechins, and caffeic acid [1]. The raw crude gambier used is shown in figure 1.

Figure 1. Raw Crude Gambier

Antioxidant activity can be considered very high because very low concentrations (1, 2, 3, 4 and 5 ppm) were used for IC_{50} testing. According to Molyneux (2004), if the IC_{50} value is less than 50 ppm, antioxidants are classified as strong antioxidants [11].

3.2. Antioxidant Activity
Antioxidants are electron donors for compounds that function as radicals or Reactive Oxygen Species (ROS) [1]. The antioxidant activity of phenol compounds is due to their ability to form phenoxide ions which can donate an electron to free radicals. The antioxidant phenol compounds (PhH) reacts with free radical (ROO •) to form ROOH and a radical phenol compound (Ph •) which is relatively unreactive but can react again with another free radical (ROO •) to form compounds that are not radicals [12]. DPPH is a free radical compound that is able to react with compounds that donate

hydrogen atoms such as the antioxidant compounds (polyphenols) in gambier. As DPPH binds to this hydrogen a reduction in the intensity of the color purple occurs which can be measured by spectrophotometer at 517 nm. The results can be seen in Table 1.

Table 1. % DDPH Scavenging Activity of Gambier Extracted using Ultrasonication with various temperature

Temperature (°C)	DPPH Scavenging Activity (%)				
	1 mg/L	2 mg/L	3 mg/Ml	4 mg/L	5 mg/L
50°C	36.23 ± 3.86	60.65 ± 1.65	87.66 ± 1.29	94.55 ± 0.37	94.81 ± 0.00
60°C	18.99 ± 1.69	34.53 ± 1.31	50.13 ± 4.98	61.42 ± 1.22	71.31 ± 1.13
70°C	20.07 ± 4,62	40.90 ± 0.29	50.56 ± 0.79	62.57 ± 1.28	77.50 ± 1.57
80°C	30.37 ± 0.34	39.15 ± 0.17	57.62 ± 0.26	73.11 ± 0.09	93.90 ± 0.00

IC_{50} testing was successfully carried out at extract concentrations of 1 to 5 mg /L indicating very strong antioxidant activity. As the extraction time increased, the DPPH Radical Scavenger activity also increased. The results of the antioxidant activity of gambier extracted by ultrasonication using water solvents with various temperature can be seen in Table 2.

Table 2. Antioxidant Activity

Temperature (°C)	IC 50 (mg/L) ± SD	
50°C	1.36 ± 0.19	a
80°C	2.45 ± 0.01	b
70°C	2.98 ± 0.05	c
60°C	3.21 ± 0.11	c

SD: Standard Deviation (α<0.05)

The highest antioxidant activity was obtained when the ultrasonication was at 50 °C (1.36 mg/ml). It is mean that it takes 1.36 mg/ml of the sample to inhibit 50% of the oxidation activity. This is stronger than the IC_{50} antioxidant activity of crude gambier which is 1.939 mg/L.

The Catechins can donate hydrogens from the hydroxyl groups in their structure, they have been found to have excellent antioxidant activities, expressed through their free radical scavenging ability being more powerful than vitamin C, vitamin E, or b-carotene. They have also been shown to chelate transition metal ions, modulate oxidant and antioxidant enzymes, and affect gene expression [2].

3.3. Polyphenol Content

Polyphenols are secondary metabolites of plants and include catechins and tannins. Tannins are complex phenolic compounds which have a molecular weight of 500-3000 and are colloidal in water and weakly acidic [1, 13]. The Polyphenol Content of the extracted gambier can be seen in Table 3.

Table 3. Polyphenol Content of The Gambier Extracted

Temperature (°C)	Polifenol content (mg/L) ± SD		
60°C	1.89 ± 0.50	a	
70°C	2.38 ± 0.26	a	b
80°C	2.92 ± 0.40		b
50°C	6.71 ± 2.60		c

SD: Standard Deviation (α<0.05)

In the 5 mg/L sample, the best polyphenol levels were obtained in the gambier extract which was processed for 90 minutes. The extraction process with water using ultrasonic at 50°C showed the best result (6.71 mg/L).

3.4. Catechin content

The catechins are receiving considerable interest for their potential benefits on human health. The recent in vivo and epidemiology studies have linked the green tea catechins with the prevention of some skin and liver cancers. Other studies have linked the catechins with a reduced development of lung, gastric, and breast cancers. In addition, catechins have been linked with reductions in cardiovascular disease, dental decay, obesity, diabetes, and an improvement in the immune system [2]. Catechin content in gambir sxtracted with water as solvent using ultrasonic at various temperature can be seen in Table 4.

Table 4. Catechin content

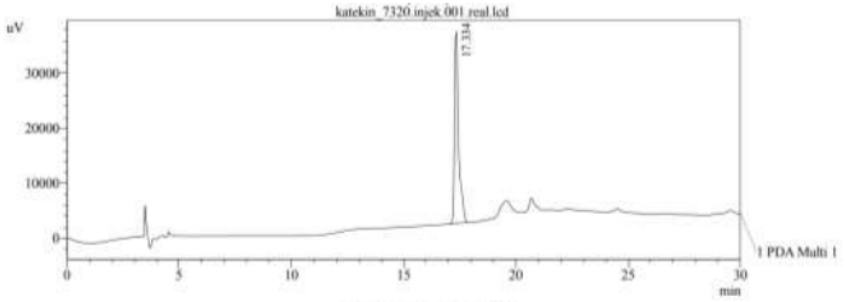
Extraction Time (Minutes)	Catechin Content (µg/mg) ± SD
80°C	818.31 ± 6.30 a
60°C	880.89 ±12.08 b
70°C	889.97 ± 4.08 b
50°C	957.01 ±10.46 c

SD: Standard Deviation (α<0.05)

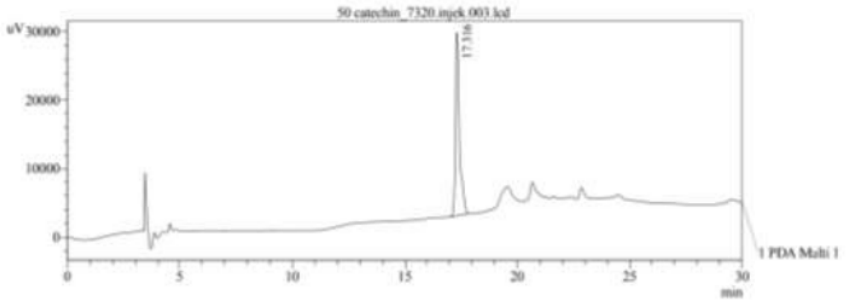
The highest levels of catechin content in gambier extracted were obtained at 50°C (957.01 µg / mg). The yields of 81.8 - 95.7% were far higher than that measured in West Sumatran raw gambier extract by Anggraini (2017) who found Cubadak Gambier catechin content was 26%, Gambier Udang 25 %, and Riau Mancik Gambier 27% [1]. This indicates that ultrasonication combine with heat treatment can improve the extraction of the components in gambier. The advantage of ultrasonic-assisted extraction is the direct contact between solvents and solids achieved with ultrasonic waves [14].

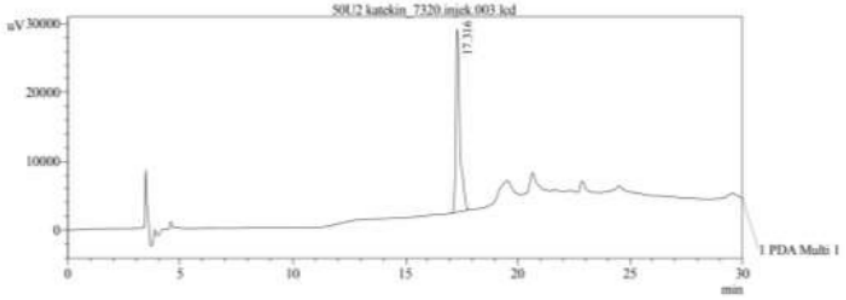
Damanik, Surbakti, and Hasibuan (2014) found up to 87.14% of catechins could be extracted using a 60 °C maceration temperature over 6 hours using ethyl acetate 95%, as the solvent [15]. This present study acheived comparable results using water which is safer and environmentally friendly solvent. HPLC result for Catechin content shown in figure 2.

A

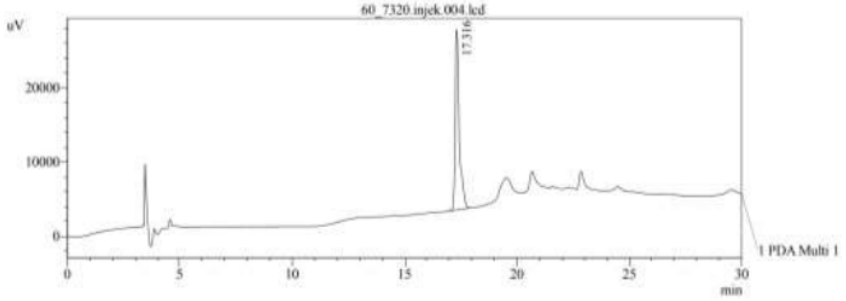


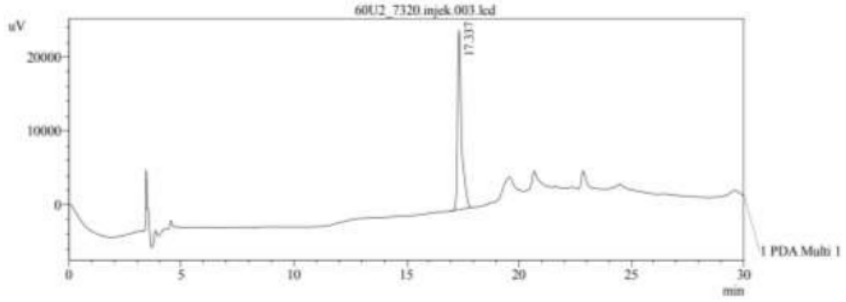
B



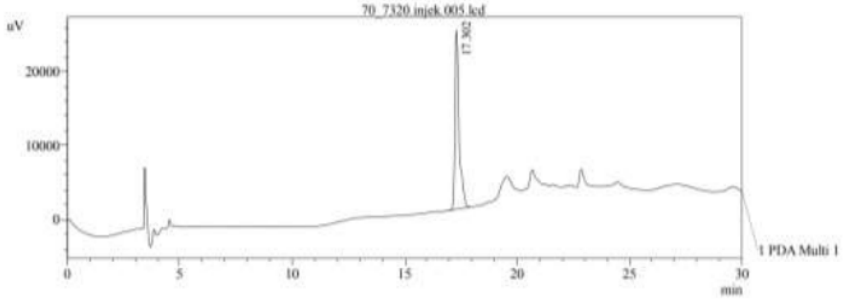

50U2 katekin_7320 injek 003.lcd
uV
30000
20000
10000
0
17.316
0
5
10
15
20
25
30
min
1 PDA Multi 1

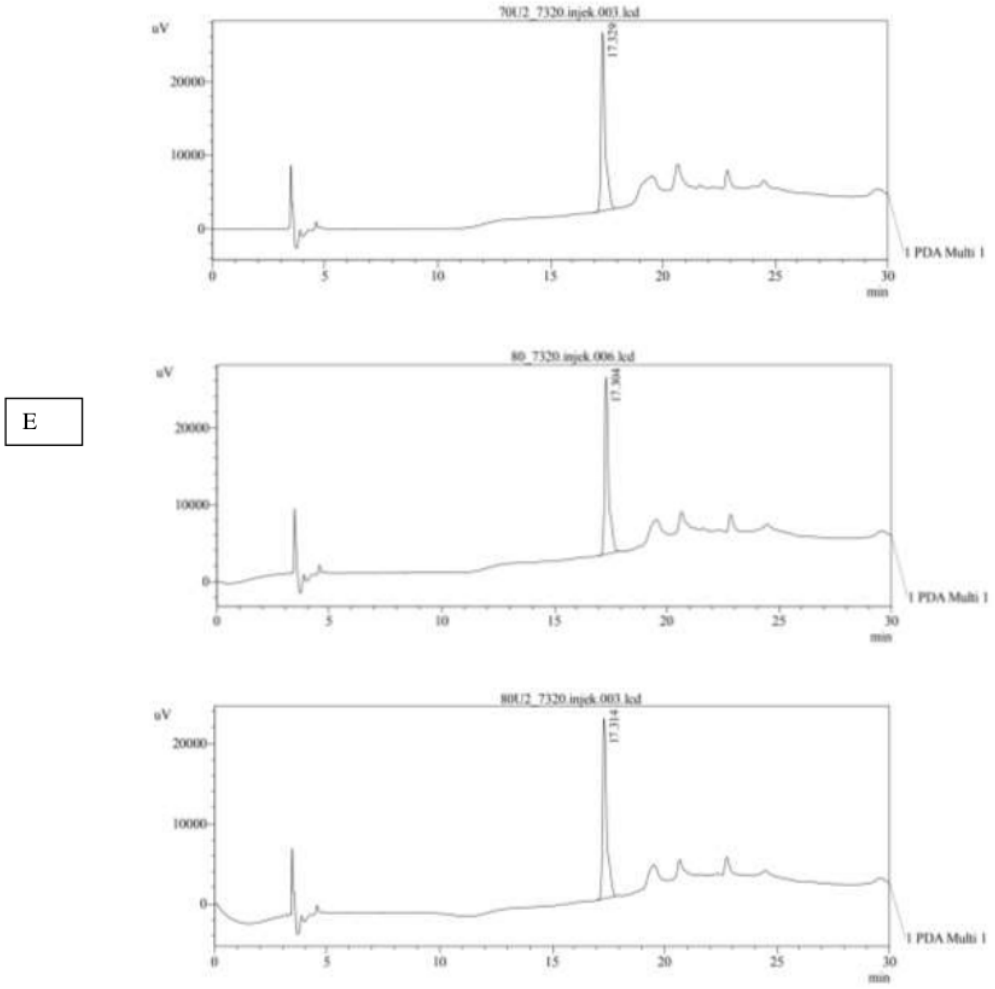
C


60_7320 injek 004.lcd
uV
20000
10000
0
17.316
0
5
10
15
20
25
30
min
1 PDA Multi 1


60U2_7320 injek 003.lcd
uV
20000
10000
0
17.337
0
5
10
15
20
25
30
min
1 PDA Multi 1

D


70_7320 injek 005.lcd
uV
20000
10000
0
17.302
0
5
10
15
20
25
30
min
1 PDA Multi 1



E

Figure 2. HPLC result for A.Catechin standard, B. Catechin extraction at 50°C, C. Catechin extraction at 60°C D. Catechin extraction at 70°C E. Catechin extraction 80°C

4. Conclusion

Crude gambier can be successfully purified in water using ultrasonic. The optimal sonication temperature for this was found to be 50°C. The resulting diluted extract had strong antioxidant properties IC_{50} 1.36 mg/L, catechin and polyphenol content are 957.01µg / mg, and 6.71 mg /L respectively. This method avoids the ongoing use of dangerous and expensive chemicals. It also removes the need to separate chemicals out of the final product so making the process more efficient in time and labor. However, it requires the use of an ultrasonic bath. Mass production of large ultrasonic baths, which could be powered by micropower generation (solar or hydro) for use by farmer cooperatives would allow the farmers to significantly increase the quality and prices they get for their product while avoiding environmental damage.

References

[1] Anggraini, T. 2017. Sumber Antioksidan Alami. Penerbit Rumah Kayu. Padang.
[2] Vuong, Q.V., Golding, J.B., Nguyen, M., and Roach, P.D. 2010. Review: Extraction and Isolation of Catechins from Tea. Journal of Separation Science. 33(21):3415-3428
[3] Muchtar, H., Yeni, G., Hermianti, W., Diza, Y.H., 2010. Pembuatan konsentrat polifenol gambir (Uncaria gambir Roxb) sebagai bahan antioksidan pangan. J. Ris. Ind. 4, 71–82.
[4] Yeni, G., Syamsu, K., Mardliyati, E., danMuchtar, H. 2017.PenentuanTeknologi Proses Pembuatan Gambir Murni dan Katekin Terstandar dari Gambir Asalan. Jurnal Litbang Industri.7(1): 1-10
[5] Capello, C., Fischer, U. dan Hungerbuhler, K. 2007. What is a green solvent? A comprehensive framework for the environmental assessment of solvents. Green Chemistry. Isuue
[6] Welton, T. 2017. Solvents and sustainable chemistry. sustainable chemistry. Proc. R. Soc. A471:20150502
[7] Liu, Y., Luo, X., Lan, Z., Tang, J., and Zhao, P.K.H. 2017. Ultrasonic-assisted extraction and antioxidantcapacities of flavonoids from Camellia fascicularis leaves. Journal of Food, 16(1): 105-112.
[8] Zou, T.B., En-Qin, X., Tai-Ping, H., Ming, Y.H., Qing, J., and Hua-Wen, L. 2014. Ultrasound-Assisted Extraction of Mangeferin from Mango Leaves Using Response Surface Methodology. Molecules. 19: 1411-1421
[9] Balachandran, S., Kentish, S.E., Mawson, R., and Ashokkumar, M. 2006. Ultrasonic enhancement of the supercritical extraction from ginger.UltrasonSonochem. 13:471-479.
[10] Ghosh, M.N. 1998. Fundamentals of Experimental Pharmacology, 2nd Edn., Scientific Book Agency: Calcutta. 174-179
[11] Molyneux, Philip. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity.Songklanakarin J. Sci. Techno. 26(2): 211-219
[12] Dhianawaty, D., danRuslin. 2015. Kandungan Total PolifenoldanAktivitasAntioksidandariEkstrakMetanolAkarImperatacylindrica (L) Beauv. (Alang-alang). MKB 47(1):60-64
[13] Irianty, R. S.,danYenti, S.R. 2014. PengaruhPerbandinganPelarutEtanol-Air Terhadap Kadar Tanin pada Sokletasi Daun Gambir (UncariagambirRoxb).Sagu 13 (1):1-7
[14] Wiyarno, B., Rosli, M.Y., and Maizzirwan, M. 2010. Ultrasound Extraction Assisted (UEA) of Oil From Microalgae (Nannochoropsis).International Journal of Science Engineering andTechnology. 3 (1), 55-59.
[15] Damanik, D.D.P., Surbakti, N., danHasibuan, R. 2014. EkstraksiKatekindariDaunGambir (Uncariagambirroxb) denganMetodeMaserasi.JurnalTeknik Kimia USU. 3(2)

The_Extraction_of_Gambier.pdf

ORIGINALITY REPORT

24%	21%	19%	10%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

10%

★ Ifmalinda, Andasuryani. "The Design of the Divider Door for the Orange Grading Machine Based on the Diameter and Color Uses Image Processing", IOP Conference Series: Earth and Environmental Science, 2021

Publication

Exclude quotes	On	Exclude matches	< 3%
Exclude bibliography	On		