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Research Article

Analysis of Antioxidant Activity of Ethanol Extracts of Cocoa Fruit Peel (*Theobroma cacao* L.) and Arabica Coffee Fruit Peel (*Coffee arabica* L.) with Maceration and Reflux Extraction Method

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Abstract: Cocoa and coffee are well-known commodities in the world, including Indonesia. The seeds of cocoa and coffee are often utilized. Cocoa beans are usually processed into chocolate products, while coffee becomes a beverage product. Utilization of cocoa and coffee beans, and fruits produces waste, namely the skin. The more cocoa and coffee production, the more fruit skin waste. One of the efforts to utilize fruit skin waste is by extracting the antioxidant content from it. The choice of extraction method is important to produce extracts with the maximum amount of extract. Therefore, a study was conducted on the comparison of maceration and reflux extraction methods in determining the highest level of antioxidant activity. Cocoa and coffee fruit peels were extracted using ethanol, and the extracts were tested for antioxidant activity using DPPH with ascorbic acid as a positive control using UV-Vis spectrophotometry. The results of the antioxidant activity test conducted with the DPPH method showed that the IC_{50} value of macerated cocoa fruit peel extract was 5.440 ppm, and coffee was 62.99 ppm the reflux extract of cocoa fruit peel was 4.999 ppm, and coffee was 61.68 ppm. This shows that the maceration and reflux methods of cocoa can be categorized as very strong antioxidants and strong arabica coffee. from the two extraction methods of this study, it is concluded that there is no significant difference between maceration and reflux.

Keywords: Cocoa pods, Arabica coffee pods, antioxidant, maceration, reflux

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) and is one of the most recognized commodities in the world including Indonesia. The part of the cocoa fruit that is often utilized is the seeds. Cocoa beans are usually processed into chocolate products. Utilization of cocoa beans and fruits produces waste, namely skin [9] Based on statistical data on cocoa fruit production in Indonesia, South Sumatra is high in 2021 reaching 10.1 thousand tons and increasing in 2022, which is 10.2 thousand tons. One of the cities in South Sumatra producing cocoa fruit is in the city of Pagar Alam with a plant area of 1,209.00 hectares [3]. The increasing production of cocoa fruit, the more cocoa skin waste is produced.

Cocoa fruit skin is the mesocarp or wall part of the cocoa fruit, which includes the outer shell to the pulp. Cocoa fruit skin has not been optimally utilized and not many people know that cocoa fruit skin has secondary metabolite compounds [11]. Previous research states that cocoa fruit peel extract has secondary metabolite compounds of flavonoids alkaloids, terpenoids and saponins. The content of secondary metabolites is influenced by various internal factors such as genes and external factors such as light, temperature, humidity, Ph, nutrient content in the soil and altitude, and extraction method [10].

Indonesia is a developing country as the world's largest coffee exporter [15]. Coffee production in Indonesia is dominated by smallholder plantations which reached 636.7 thousand tons in 2017 and has been exported to five continents [5]. The types of coffee in Indonesia are Arabica and Robusta

with total production in 2017 of 173,765 tons and 463,775 tons respectively [6]. Geographical conditions, differences in soil properties, microlimates and farming patterns of coffee farmers affect the productivity of the coffee produced. Coffee skin is obtained from the processing of coffee fruit or coffee cherries which go through pulping stages either wet process, namely coffee cherries, washing, pulping [16]. Or dry process, namely washing, drying, and pulping [1]. In every ton of wet coffee cherry fruit, 200 kg of dry skin will be produced [16]. Coffea skin is a material that can be utilized as a caffeine, polyphenol, and bioethanol producing material [4] as well as antioxidants and antimicrobials [8].

Cocoa and coffee pods are extracted using cold and hot methods, namely by using maceration and reflux extraction methods. Testing of antioxidant activity in research [14] entitled comparison of maceration and reflux extraction methods on phenolic content of corn cob extract (Zea mays L) states that the results obtained from the comparison of antioxidant activity using reflux and maceration methods of greater antioxidant activity obtained from the reflux method which is 0.397 mg/g maceration 0.312 mg/g. While in research [17] with the title of the comparison of maceration extraction with reflux method of antioxidant activity and toxicity of mangkokan leaves *Mathopanax scutellarium* merr the results obtained are 21.63% maceration while reflux 12.55% so the maceration method produces extracts with greater antioxidant activity.

The use of maceration extraction and reflux extraction methods was carried out to determine whether there are differences in antioxidant activity in cocoa and coffee fruit peels. The maceration extraction method was chosen because the process is easy, the tools used are simple and reflux extraction was chosen because the process is easy, fast does not require a long time and the solution used is small. This research is expected to be used in determining which extraction method is better and optimal for cocoa and coffee fruit skin extracts as antioxidants.

2. MATERIALS AND METHODS

2.1. Tools and Materials

The tools used for research are beaker glas, aluminum poil, test tube, stirring rod, measuring cup, spatula, drop pipette, scale, filter paper, blender, jar, petr cup, hot platt, rotary evaporator, drop piprt, mesh 60 sieve, reflux, UV-Vis spectrophotometry.

While the research materials used are cocoa and arabica coffee fruit skin waste, 96% ethanol solvent, hydrochloric acid, anhydrous acetic acid, sulfuric acid, Fecl3, potassium persulfate, DPPH, ascorbic acid, distilled water, methanol.

2.2. Methods

This study uses an experimental method by comparing the antioxidant activity of cocoa pods (*Theobroma Cacao* L.) and arabica coffee (*Coffea arabica* L.) with maceration and reflux extraction methods, which were carried out at the Pharmacy Laboratory of the Kader Bangsa University in Palembang and the Agriculture Laboratory of Sriwijaya University in July 2023.

3. RESULTS AND DISCUSSION

The results of the determination carried out at the Bandung Institute of Technology Laboratory showed that the samples used were in accordance with the samples used, namely *Theobroma cacao* L. and *Coffea arabica* L. The yield of cocoa fruit peel extract obtained in the maceration extraction method was 3.459%, and the reflux method was 1.934%, while the arabica coffee skin in the maceration extraction method obtained a yield of 4.9581% and in the reflux extraction method of 2.0092%, using the following formula: % yield.

$$\%\ Yield = \frac{weight\ of\ extract\ (a)}{\text{powder weight}}\ x\ 100\%$$

Table 1. Results of Cocoa and Coffee Fruit Peel Yield Calculation

Extraction Method	Empty Container Weight	Container Weight & Extract	Result
Cocoa Pods			
Maceration	51.2150 gr	61.5933 gr	3.459 %
Reflux	51.0487 gr	56.8524 gr	1.934 %
Coffee Fruit Skin			
Maceration	51.1558 gr	66.0301 gr	4.9581%
Reflux	51.1485 gr	57.1761 gr	2.0092 %

In Table 1, it can be seen that in both samples of cocoa and coffee fruit peels between maceration and reflux extraction methods it can be seen that the use of the maceration method produces more extracts than the reflux method. In the phytochemical test to determine the secondary metabolites contained in cocoa and coffee fruit peels, the results showed that cocoa fruit peels in maceration and reflux extraction methods both contained flavonoids, saponins, and tannins, as shown in Table 2. For coffee fruit skin in the phytochemical test, the results showed that the coffee fruit skin in maceration and reflux extraction methods both contained alkaloids, flavonoids and tannins which are shown in Table 3.

Table 2. Phytochemical Test Results of Cocoa Fruit Peel

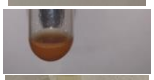
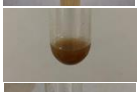
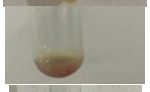
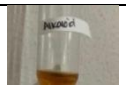
No	Test	Method Results		Method Result Image	
		Maceration	Reflux	Maceration	Reflux
1.	Alkaloid	-	-		
2.	Flavonoid	+	+		
3.	Saponin	+	+		
4.	Terpenoid	-	-		
5.	Tanin	+	+		

Table 3. Phytochemical Test Results

No.	Chemical Content	Method Results		Method Result Image	
		Maceration	Reflux	Maceration	Reflux
1.	Alkoloid	+	+		
2.	Flavonoid	+	+		
3.	Saponin	-	-		
4.	Terpenoid	-	-		
5.	Tanin	+	+		

Measurement of the maximum wavelength of DPPH is done to determine the maximum or highest absorbance of the sample and obtained at a wavelength of 517 ppm getting absorbance results

of 0.846 nm. In determining the operating time of ascorbic acid obtained at 30 minutes is the maximum or highest absorbance with an absorbance value of 0.682 nm which is then carried out absorbance of ascorbic acid solution at a predetermined wavelength and the inclusion time obtained is 30 minutes the results obtained in the ascorbic acid solution curve with a linear regression equation Y = 9.6065X-4.4143, the regression results show the value of R2 = 0.992. In measuring the antioxidant activity of ascorbic acid with concentrations of 1,2,3,4,5,6,7,8 and 9 ppm, the following results were obtained:

Table 4. Ascorbic Acid Antioxidant Activity Measurement Results

Concentration (ppm)	Absorbance	Blank	% inhibition
1	0.813	0.847	4.01
2	0.701	0.847	17.24
3	0.662	0.847	21.84
4	0.562	0.847	33.65
5	0.482	0.847	43.09
6	0.370	0.847	56.32
7	0.317	0.847	62.57
8	0.209	0.847	75.32
9	0.182	0.847	78.51

Ascorbic acid is used as a comparator because it functions as a secondary antioxidant by capturing free radicals and preventing chain reactions. Vitamin C belongs to a class of secondary antioxidants that can counteract various extracellular free radicals. This is because vitamin C has a free hydroxy group that acts as a free radical catcher and if it has a polyhydroxy group it will increase antioxidant activity [12].

The extracts of cocoa and coffee fruit peels are then tested for antioxidant activity by spectrophotometry Vis to determine how much antioxidant content and which extraction method is better the first step is wavelength measurement Based on the results of the wavelength obtained is 517 nm used for further measurements. The use of the maximum wavelength is because it has maximum sensitivity to the largest change in absorbance. The wavelength is sought to determine how much the highest light energy is absorbed by the solution [2].

Table 5. Measurement of Antioxidant Activity of Cacao Peel

Method	Concentration (ppm)	Repetition	Absorbance	Average	% inhibition	Average % inhibition
Maceration	2	1	0.681	0.647	18.44	22.51
		2	0.601		28.02	
		3	0.659		21.07	
	4	1	0.424	0.517	49.22	38.08
		2	0.546		34.61	
		3	0.583		30.17	
	6	1	0.318	0.376	61.92	54.97
		2	0.473		43.35	
		3	0.327		60.79	
	8	1	0.282	0.233	66.22	72.10
		2	0.194		76.76	
		3	0.223		73.29	
	10	1	0.179	0.130	78.56	83.84
		2	0.085		89.82	
		3	0.127		84.79	
Reflux	2	1	0.658	0.63	22.58	25.88
		2	0.663		22.00	
		3	0.569		33.05	
	4	1	0.438	0.471	48.47	44.59

continued Table 5…

	2	0.533		37.29	
	3	0.442		48.00	
6	1	0.357	0.371	58.00	56.35
	2	0.331		61.05	
	3	0.426		48.70	
8	1	0.278	0.225	67.29	73.53
	2	0.241		71.64	
	3	0.157		81.52	
10	1	0.109	0.103	87.17	87.88
	2	0.129		84.82	
	3	0.072		91.53	

Table 6. Measurement Result of Antioxidant Activity of Arabica Coffee Fruit Peels

Method	Concentration (ppm)	Repetition	Absorbance	Average	% inhibition	Average % inhibition
Maceration	20	1	0.551	0.605	35.18	28.78
		2	0.608		24.47	
		3	0.657		22.71	
	40	1	0.497	0.458	41.53	46.03
		2	0.441		48.12	
		3	0.438		48.47	
	60	1	0.326	0.382	61.65	55.06
		2	0.351		58.71	
		3	0.469		44.82	
	80	1	0.324	0.325	61.88	61.76
		2	0.309		63.65	
		3	0.342		59.76	
	100	1	0.212	0.232	75.06	72.62
		2	0.236		72.23	
		3	0.25		70.59	
Reflux	20	1	0.539	0.627	36.59	26.16
		2	0.661		22.24	
		3	0.683		19.65	
	40	1	0.458	0.484	46.12	43.06
		2	0.472		44.47	
		3	0.522		38.59	
	60	1	0.417	0.389	50.94	54.16
		2	0.368		56.71	
		3	0.384		54.82	
	80	1	0.321	0.256	62.24	69.81
		2	0.208		75.53	
		3	0.241		71.65	
	100	1	0.162	0.183	80.94	83.92
		2	0.107		87.41	
		3	0.141		83.41	

From the data in Table 5 and 6, it is known that the higher the concentration of the extract, the % inhibition value will also increase.

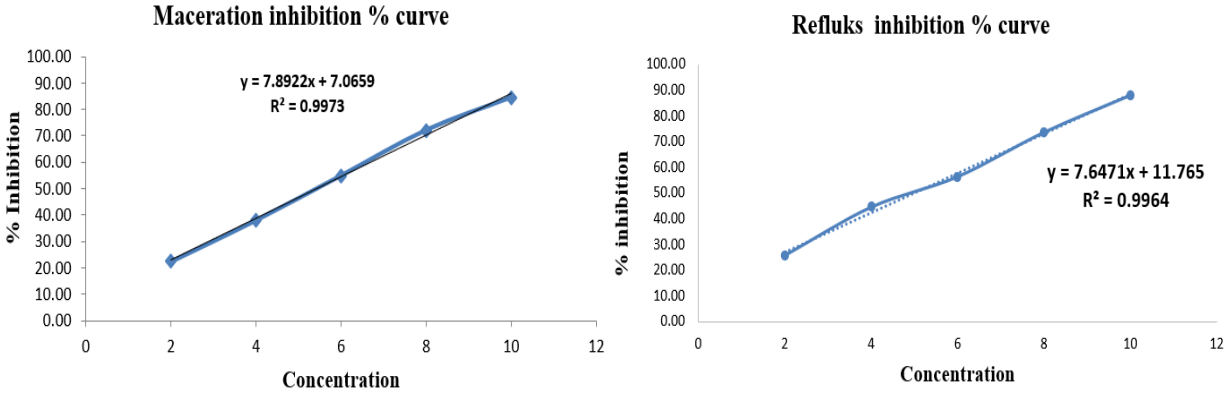


Figure 1. Antioxidant Activity Curve of Cocoa Fruit Peels by Maceration and Reflux Extraction Methods

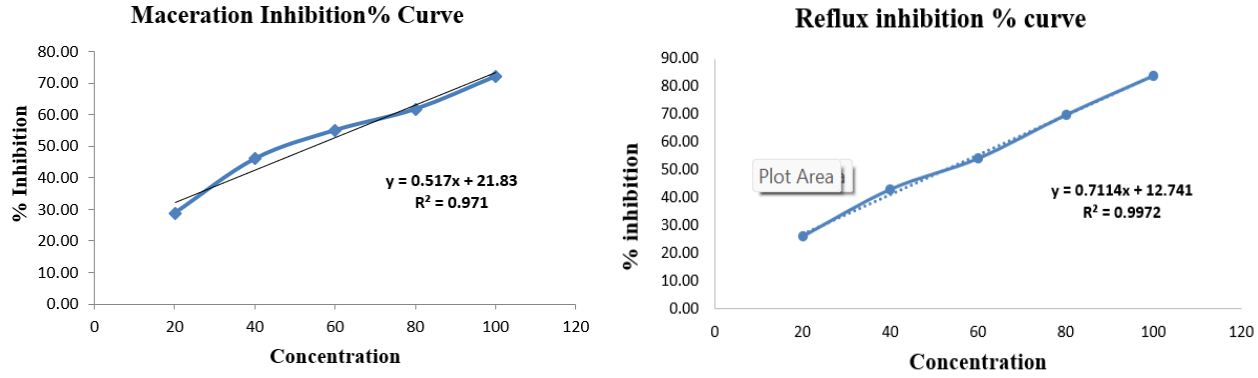


Figure 2. Antioxidant Activity Curve of Coffee Fruit Peels by Maceration and Reflux Extraction Methods

The IC_{50} value can be determined by analyzing the probit of the log concentration data with the % inhibition value by entering the % inhibition data into the probit table and making a graph between log concentration and probit to obtain a linear regression equation y = bc + a which is found in the % inhibition curve Figure 1 and 2.

Table 7. IC_{50} Value of Ethanol Extract Solution of Cocoa Fruit Peel, Arabica Coffee and Ascorbic Acid

No	Sample	Regression Equation	Value R_2	Value IC_{50}	Category
1	Ascorbic Acid	Y = 7.8922x + 7.0659	0.992	5.66 ppm	Very Strong
2	Cocoa Maceration Extract	Y = 7.6471x + 11.7647	0.9973	5.44 ppm	Very Strong
3	Cocoa Reflux Extract	Y = 7.6471x + 11.7647	0.9964	4.99 ppm	Very Strong
4	Coffee Maceration Extract	Y = 5.17 x + 21.21.83	0.971	54.47ppm	Powerful
5	Coffee Reflux Extract	Y = 0.711 x + 12.74	0.997	52.40ppm	Powerful

The IC_{50} value of the ethanol extract of cocoa fruit peel is known to have a very strong category where the IC_{50} value of the ascorbic acid sample is 5.66 ppm, macerated extract 5.44 ppm and reflux extract 4.99 ppm. The ethanol extract of cacao fruit peel is known to have a strong category where the IC50 value, macerated extract 54.47 ppm and reflux extract 52.40 ppm. The compound is said to have very strong antioxidant activity if the IC_{50} value is less than 50 ppm, strong if between 50-100 ppm, moderate if between 100-150 ppm, and weak if between 150-200 ppm. The IC_{50} value is obtained using a linear regression equation of maceration extraction method, namely, y = 7.8922x + 7.0659 with a correlation coefficient value of 0.9973, IC50 value of 5.440 ppm and reflux extraction 7.6471 x + 11.7647 correlation coefficient value of 0. 9964, IC50 value of 4,999 ppm while the coffee fruit skin extract

obtained the results of the strong IC50 value category with the regression equation on the extract of arabika coffee fruit leaves that is = 0.517 x + 21.83 so that the IC_{50} value is 54.47. Then the regression equation on the extract of arabican coffee fruit dehydration is y = 0.711 x + 12.74, resulting in an IC_{50} value of 52.40.

Inhibition data of maceration and reflux methods are known to have normally distributed and homogeneous data with a p-value> 0.05, using the Shapiro Wilk test (Normality) and Levene Test (Homogeneity) on SPSS software. Because the data is normally distributed and homogeneous, the test is continued with the one way anova test, so the results show that there is no significant difference in antioxidant levels between maceration and reflux extraction methods, this is evidenced by the p-value of 0.841>0.05.

In previous studies that also compared extraction methods entitled comparison of maceration extraction methods with reflux methods on antioxidant activity and toxicity of Nothopanax scutellarium Merr mangkokan leaves concluded that statistically the maceration method is better than reflux with the results of the IC_{50} value of maceration 37.1387 ppm and reflux 58.3349 ppm [17]. While in a study entitled comparison of extraction methods on total flavonoid content and antioxidant activity of Boehmmeria virganta stems obtained the results of reflux method better than maceration with IC50 results of reflux 30.58 mg/L and maceration 100.89 mg/L [7]. From the results of previous studies above, it can be concluded that each sample has a different extraction method to produce a good IC50 value.

4. CONCLUSION

The present study confirms that cocoa fruit peel extract contains secondary metabolites, namely flavonoids, saponins, and tannins. Similarly, Arabica coffee peel extract was found to contain alkaloids, flavonoids, and tannins. The antioxidant activity, evaluated using the DPPH assay, revealed that the IC_{50} value of cocoa peel extract obtained via maceration was 5,440 ppm, while the extract obtained via reflux yielded an IC_{50} value of 4,999 ppm. In comparison, the IC_{50} value of Arabica coffee peel extract was 54.47 ppm for maceration and 52.40 ppm for reflux extraction. Statistical analysis indicated no significant difference in antioxidant activity between the maceration and reflux extraction methods for both cocoa and Arabica coffee peel extracts. These findings suggest that both extraction methods are comparable in effectiveness for isolating antioxidant compounds from the respective plant materials.

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Conflicts of interest: The authors declare no conflict of interest

Reference

1. Aisyah, N. S. (2018). Analisis Nilai Tambah Industri Pengelolahan Buah Cherry Kopi (Kasus Di Mahkota Java Coffee Garut) Nur Syamsi Aisyah.Institut Pertanian Bogor.
2. Amelia, P. 2011, Isolasi, Elusidasi Struktur dan Uji Aktivitas Antioksidan Senyawa Kimia Dari Daun Garcinia benthami Pierre Tesis, Fakultas Matematika Dan Ilmu Pengetahuan Alam, Universitas Indonesia.
3. Badan Statistik Indonesia. (2023). Statistik Indonesia 2023 Data Statistik Kondisi Sosial Dan Kependudukan, Ekonomi Dan Perdagangan Serta Pertanian Dan Pertambangan Di Indonesia. In 2023.

4. Boniilla-Heirmosa, V. A. , D. W. F. , & S. R. F. (2014). Utilization Of Coffee By-Products Obtained From Semi-Washed Process For Production Of Value-Added Compounds. Bioresource Technology.
5. BPS. (2017). Statistik Kopi indonesia (Indonesian Coffee Statistic) 2017. (S. D. S. T. Perkebunan, Ed.). Badan Pusat Statistik.
6. Dirjein Perkebunan. (2017). Statistik Perkebunan Indonesia 2015-2017 Kopi. Kementrian Pertanian.
7. Hasan, T., Farmasi Fakultas Kedokteran Dan Ilmu Kesehatan Universitas Islam Negeri Alauddin Makassar, J., Yasin Limpo No, J. H., Sombaopu Kabupaten Gowa, K., Selatan, S., Matematika Dan Ilmu Pengetahuan Alam, F., Islam Makassar, U., Perintis Kemerdekaan, J. K., Indah, T., & Makassar, K. (2023). Perbandingan Metode Ekstraksi Terhadap Kadar Flavonoid Total Dan Aktivitas Antioksidan Batang Boehmeria Virgata Comparison Of Extraction Methods On Total Flavonoid Content And Antioxidant Activity Of Boehmeria Virgata Stem.
8. Jimeneiz-Zamora, A. , P. S. , & R.-H. J. A. (2015). Revalorization Of Coffee By-Products. Prebiotic, Antimicrobial And Antioxidant Properties. LWT - Food Sciense And Technology.
9. Kusuma, I. G. N. S., Putra, I. N. K., & Darmayanti, L. P. T. (2019). Pengaruh Suhu Pengeringan Terhadap Aktivitas Antioksidan Teh Herbal Kulit Kakao (Theobroma Cacao L.). Jurnal Ilmu Dan Teknologi Pangan (ITEPA), 8(1), 85. Https://Doi.Org/10.24843/Itepa.2019.V08.I01.P10
10. Mentari, D., Naima, M., Wulansari, R., Widada, J., Rini Nuringtyas, T., Wibawa, T., Wijayanti, N., Studi D-, P., Transfusi Darah STIKES Guna Bangsa Yogyakarta, T., & Studi Biologi UIN Walisongo, P. (2019). Pengaruh Perbedaan Metode Ekstraksi Metabolit Sekunder Streptomyces Sp. GMR22 Terhadap Toksisitas Pada Sel BHK-21 Effects Of Extraction Methods Of Secondary Metabolite Streptomyces Sp. GMR22 Towards BHK-21 Cells Toxicity. In Jurnal Farmasi Indonesia (Vol. 16, Issue 1). Http://Journals.Ums.Ac.Id/Index.Php/Pharmacon
11. Rusli, R., & Rijai, L. (2015). Aktivitas Antioksidan Kulit Buah Kakao Masak Dan Kulit Buah Kako Muda. In Jurnal Sains Dan Kesehatan.(Vol. 1, Issue 1).
12. Setiabudi, A., Pringgenies, D., & Ridlo, A. (2020). Aktivitas Penangkapan Radikal Bebas DPPH Dan Daya Reduksi Ekstrak Gracilaria Verrucosa. JRST (Jurnal Riset Sains Dan Teknologi), 4(2), 47. Https://Doi.Org/10.30595/Jrst.V4i2.5761
13. Sholekah, F. F. (2017). Prosiding Seminar Nasional Pendidikan Biologi Dan Biologi Jurusan Pendidikan Biologi, Fakultas MIPA, Universitas Negeri Yogyakarta.
14. Susanty, L., & Bachmid, F. (2016). Perbandingan Metode Ekstraksi Maserasi Dan Refluks Terhadap Kadar Fenolik Dari Ekstrak Tongkol Jagung (Zea Mays L.) (Susanty, Fairus Bachmid).
15. Valduga, A. T., & Goncalveis, Ii. L. , M. Ei. , & F. J. R. D. (2018). Cheimiistry, Pharmacology And Neiw Treinds Iin Tradiitiional Functiional And Meidiiciinal Beiveirageis. Food Reiseiarch Iinteirnatiional.
16. Widyotomo, S. (2013). Potency And Technology Of Coffee Trash Diversification Product To Increase Good Quality And Added Value. Review Penelitian Kopi Dan Kakao.
17. Yanti, D. (2013). Perbandingan Metode Ekstraksi Maserasi Dengan Metode Refluks Terhadap Aktivitas Antioksidan Dan Toksisitas Dari Daun Mangkokan Nothopanax Scutellarium Merr.