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The Spectrometic Quantification of Total Content of Flavonoid, Phenol, and Alkaloid in Kesum Leaf (*Polygonum minus Huds*) From West Borneo With Methanol and Ethanol Solvents

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ABSTRACT

Kesum (*Polygonum minus* Huds) is one of the typical plants that grow in West Borneo. The Kesum leaves are the part which is mostly used. Kesum leaves contain a lot of phytochemical compounds. This study quantitatively determined the total phenolic, flavonoid, and alkaloid contents in methanol and ethanolic extract of *Polygonum minus* Huds leaves using a spectrophotometric method. The samples were extracted by maceration. The content was determined by using a UV-Vis spectrophotometer. Based on the assay result, the methanol extract contained total of phenolic 49.351±0.430 ppmEAG (equivalent to gallic acid); total of flavonoids 44.128±0.116ppmEQ (quercetin equivalent); total of alkaloids 356,798±1,004ppmEP (piperine equivalent). The 96% ethanol extract contained total of phenol 46,196±0.329ppmEAG; total of flavonoids 34,969±0.116ppmEQ; total of alkaloids 183.525±0.766ppmEP. The 70% ethanol extract contained total of phenol 64.189+0.215ppmEAG; total of flavonoids 27.834±0.158ppmEQ; total of alkaloids 432,947±1,533ppmEP. The ethanol extract 50% contained total of phenol 67,343±0.164ppmEAG; total of flavonoids 26.063±0.158ppmEQ; total of alkaloids 679,142±2,525ppmEP. All of the results are preliminary data for further research, especially for the isolation of compounds with specific targets or for utilization as main ingredients for traditional medicine.
Keywords: Polygonum minus Huds; phenols; flavonoids; alkaloids; different solvents

INTRODUCTION

Traditional medicine plays still an important role in many diseases and there are some reasons including simple preparation and not be expensive. The utilization of plants as medicine is a traditional way to cure diseases without the addition of synthetic chemical compounds. In this modern era, the use of traditional medicine still has a place in people's hearts. This is because traditional medicine is easy to be founded and process, the price is cheaper than synthetic medicine, and the ingredients for traditional medicine can be planted at home (Suliannti *et al*., 2006), in terms of treatment. The use of plants as traditional medicinal ingredients needs to be researched to find out the truth about the benefits. The use of plants as medicine can be guaranteed with the data obtained scientifically. In recent times, however, there has been a growing concern about their safety. Therefore, this study was conducted to quantify the three common active compound groups. Of many various types of plants that can be used as medicinal ingredients, Kesum (*Polygonum minus* Huds) is one of the plants which has many chemical compounds that are efficacious for curing diseases. *Polygonum minus* Huds is one of the typical plants that grow in West Borneo, especially in Pontianak city. Local people use the leaves a lot as a cooking spice. Based on the research results of Kartikasari *et al.*, (2021). Extract of *Polygonum minus* Huds leaf contains chemical compounds, namely the phenol group, terpenoids, alkaloids, flavonoids, saponins, and tannins. Traditionally, the decoction of the leaves of *Polygonum minus* Huds is used to treat digestive problems, eliminate dandruff, and as a tonic after childbirth (Globinmed, 2010). Based on a phytopharmaceutical study, Polygonum minus Huds has activities as anthelmetic (Maulidya, 2017), antiviral, antibacterial, antifungal (Dewi *et al*., 2019), antioxidant (Purwantiningsih *et al*., 2018), anticancer and antiulcer.

Extraction is the process of separating solid and liquid materials with the help of a solvent. The solvent used must be able to extract the desired substance without dissolving the other material. Extraction using solvents is based on solubility (Ansel, 1989). The type of solvent in the extraction can affect the acquisition of active substances from plants. Therefore, the use of the best solvent will further enhance the optimization in sample extraction. This research uses methanol, 96% ethanol, 70% ethanol, and 50% ethanol as solvents because these four solvents are polar and

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Table I. Yield of Kesum Leaf Extract (*Polygonum minus* Huds) and Colour

Sample	Yield (%)	Colour
Metanol Extract	10,42	Blackish green
Etanol Extract 96%	21,85	Blackish green
Etanol Extract 70%	14,31	Blackish green
Etanol Extract 50%	9,68	Blackish green

soluble in water. Research related to the levels of total alkaloids, phenols, and flavonoids from *Polygonum minus* Huds leaves extracted using methanol, 50% ethanol, 70% ethanol, and 96% ethanol obtained from West Borneo, especially Pontianak City, has never been reported.

METHODOLOGY

Materials

The instruments used in this study included an evaporator (Buchi, Germany), pH meter (Eutech Instruments, France), centrifugator (Labsco, Germany), 1001000 L and 1-100 L micropipettes (Socorex, Switzerland), UV-spectrophotometer Vis (Thermoscientific, USA). The chemicals used are ethanol, methanol, ammonium hydroxide, and aquades (Brataco, Indonesia). Potassium iodide, potassium dihydrogen phosphate, sodium chloride, sodium acetate, sodium hydroxide, bismuth nitrate, nitric acid, bromkesol green solution, and FolinCiocalteu reagent (Sigma-Aldrich, USA). Glacial acetic acid, hydrochloric acid, sodium carbonate, disodium sulfide, (Merck, Germany) quercetin standard, gallic acid standard, reserpine standard. Kesum leaves (*Polygonum minus* Huds) originate from Pontianak City, West Borneo.

Methods

Process of extraction

Simplicia powder was macerated with methanol, 96% ethanol, 70% ethanol, and 50% ethanol as a solvent for 3 x 24 hours. Furthermore, it was evaporated to obtain a thick extract (Ahmad *et al*., 2015, 2016).

Determination of Total Phenol Level

A total of 0.25 mL of the test solution was added with 3.75 mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent, homogenized for 3 minutes. Then 1.25 mL of 20% sodium bicarbonate was added, homogenized, and incubated for 40 minutes at 40°C. Absorption was measured by spectrophotometry at a wavelength of 761 nm (Bobo-Garcia, 2014; Sari *et al.*, 2018).

Determination of Total Flavonoid Level

A total of 5 ml of the test solution was added with 2 ml of 10% w/v aluminum chloride solution and 0.1 ml of 0.1 mM sodium acetate solution, it has allowed to stand for 30 minutes and the absorption was measured by UV-Vis spectrophotometry at a wavelength of 430 nm, it was measured by using a standard quercetin (Ghosh *et al*., 2014; Do *et al*., 2014; Baba *et al*., 2015; Marinova *et al*., 2005).

Determination of Total Alkaloid Levels

As much as 50.0 mg of extract, dissolved in 5.0 mL of 2.0N HCl. stirred with a magnetic stirrer for 5 minutes, then filtered with filter paper. The filtrate was extracted with 25.0 mL of ethanol (extraction was repeated 3 times). Collect the ethanolic extract, and evaporate with an evaporator to a volume of 5.0 mL. add 5.0 mL of 2.0 N NaOH. centrifugation for 10 minutes, take the clear part. Then add 5 mL of phosphate-citrate buffer pH 4.7 and 5.0 mL of bromkesol green solution. Dilute with ethanol to a volume of 25.0 mL. read the absorbance at the maximum 424,1 (Shamsa *et al*., 2008). The total alkaloid content was calculated as the total equivalent of reserpine (RE) (Shamsa *et al*., 2008).

RESULT AND DISCUSSION

Polygonum minus Huds leaf extract was prepared by using the maceration method, dry simplicia, and soaked for three days with several solvents, namely, 50% ethanol, 70% ethanol, 96% ethanol, and methanol. The purpose of extracting *Polygonum minus* Huds leaves in several types of solvents was to see the content of chemical compounds present in the leaves of kesum with several types of solvents. The yield of *Polygonum minus* Huds leaf extract can be seen in Table I. It can be seen that the 96% ethanol extract produced the highest yield and color.

This research was conducted to quantify the total phenolic, flavonoid, and alkaloid content of methanol extract, 50% ethanol extract, 70% ethanol extract, and 96% ethanol extract of *Polygonum minus* Huds. The content of phenol compounds (ppm) was determined from the regression equation of the calibration curve (0.00934x-0.00621, R2 = 0.99981) and expressed in gallic acid equivalent was 49.351±0.430 in methanol extract, 46,196±0.329 in 96% ethanol extract, 64.189+0.215 at 70% ethanol extract, 67.343±0.164 at 50% ethanol extract.

The concentration of flavonoid (ppm) in quercetin equivalent was determined from the

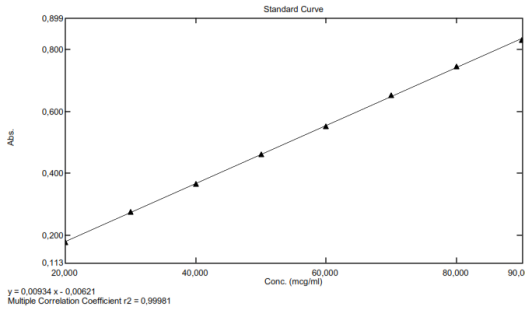

Figure 1. Standard calibration curve for total phenol content for gallic acid standard

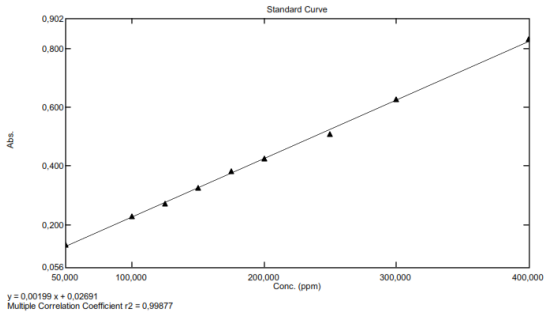

Figure 2. Standard calibration curve for total alkaloid content for reserpin standard

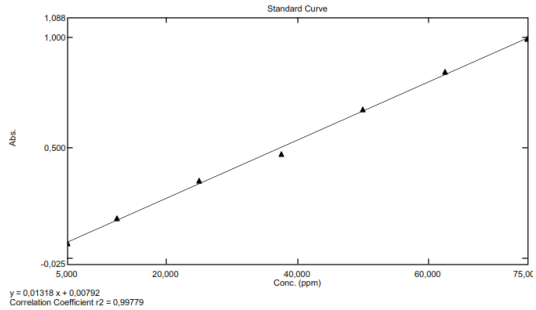

Figure 3. Standard calibration curve for total flavonoid content for quercetin standard

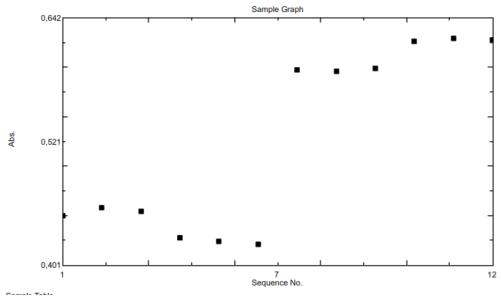

Figure 4. absorbance curve of polygonum leaf extract sample for total phenol content

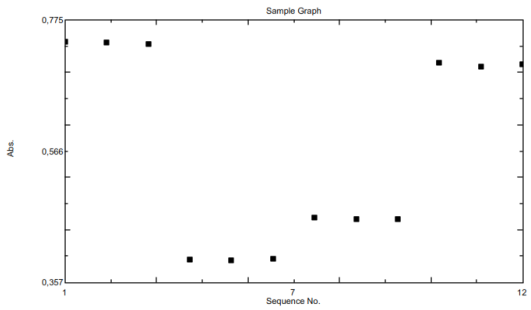

Figure 5. absorbance curve of polygonum leaf extract sample for total alkaloid content

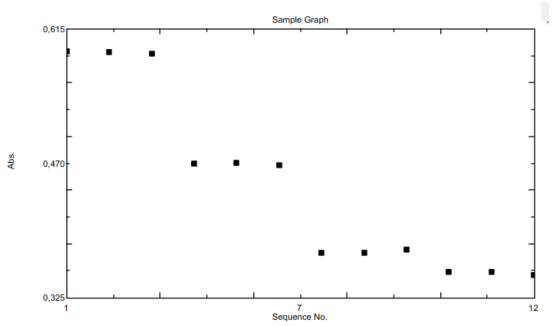

Figure 6. absorbance curve of polygonum leaf extract sample for total flavonoid content

regression equation of the calibration curve (y=0.01318x+0.00792, R2 =0.99779) was 44.128±0.116 in methanol extract, 34.969± 0.116 in 96% ethanol extract, 27.834±0.158 in 70% ethanol extract, 26.063±0.158 at 50% extract. Then, the total number of alkaloids determined using the calibration curve regression equation (y=0.00199x+0.02691, R2 = 0.99877) and expressed in reserpine equivalents was 356,798±1.004 in methanol extract, 183.525 ±0.766 in 96% ethanol, 432,947±1,533 on 70% ethanol, 679,142±2.525 on 50% ethanol extract. The standard calibration curves of gallic acid, quercetin, and reserpine can be seen in Figures 1 to 3. Table V was showed the total content of phenol, flavonoid, and alkaloid in the Polygonum leaf.

Figures 4 to 6 show the absorbance curve of polygonum leaf samples on extract methanol, 50% ethanol, 70% ethanol, 96% ethanol. Each sample was repeat for 3 times using spectrofotometri UV-Vis. Absorbance value can be seen in table II to IV.

It was revealed that the methanol extract, 96% ethanol extract, 70% ethanol extract, and 50% ethanol extract contained large amounts of phenolic compounds, flavonoids and alkaloids (Table V). The number of phytochemical compounds can support the use of Polygonum minus Huds leaves as staple for traditional

Table II. Absorbance of polygonum leaf extract sample for total phenol content at 761 nm

	Simple ID	Type	Ex	Conc	WL761,0	Comments
1	EKSTRAK METANOL REP	Unknown		48,708	0,449	
2	EKSTRAK METANOL REP	Unknown		49,551	0,457	
3	EKSTRAK METANOL REP	Unknown		49,135	0,453	
4	EKSTRAK METANOL 96 REP	Unknown		46,389	0,427	
5	EKSTRAK METANOL 96 REP	Unknown		45,984	0,423	
6	EKSTRAK METANOL 96 REP	Unknown		45,706	0,421	
7	EKSTRAK METANOL 70 REP	Unknown		63,909	0,591	
8	EKSTRAK METANOL 70 REP	Unknown		63,756	0,589	
9	EKSTRAK METANOL 70 REP	Unknown		64,109	0,593	
10	EKSTRAK METANOL 50 REP	Unknown		66,890	0,619	
11	EKSTRAK METANOL 50 REP	Unknown		67,220	0,622	
12	EKSTRAK METANOL 50 REP	Unknown		67,072	0,620	
13						

Table III. Absorbance of polygonum leaf extract sample for total alkaloid content at 424,1 nm

	Simple ID	Type	Ex	Conc	WL761,0	Comments
1	EKSTRAK METANOL REP 1	Unknown		357,731	0,740	
2	EKSTRAK METANOL REP 2	Unknown		356,873	0,738	
3	EKSTRAK METANOL REP 3	Unknown		355,932	0,736	
4	EKSTRAK METANOL 96 REP	Unknown		183,683	0,393	
5	EKSTRAK METANOL 96 REP	Unknown		182,932	0,391	
6	EKSTRAK METANOL 96 REP	Unknown		183,958	0,394	
7	EKSTRAK METANOL 70 REP	Unknown		217,226	0,460	Pengenceran 2x
8	EKSTRAK METANOL 70 REP	Unknown		216,085	0,458	Pengenceran 2x
9	EKSTRAK METANOL 70 REP	Unknown		216,032	0,457	Pengenceran 2x
10	EKSTRAK METANOL 50 REP	Unknown		340,864	0,706	Pengenceran 2x
11	EKSTRAK METANOL 50 REP	Unknown		338,153	0,701	Pengenceran 2x
12	EKSTRAK METANOL 50 REP	Unknown		339,922	0,704	Pengenceran 2x
13						

Table IV. Absorbance of polygonum leaf extract sample for total flavonoid content at 430 nm

	Simple ID	Type	Ex	Conc	WL761,0	Comments
1	EKSTRAK METANOL REP 1	Unknown		44,206	0,591	
2	EKSTRAK METANOL REP 2	Unknown		44,118	0,589	
3	EKSTRAK METANOL REP 3	Unknown		44,023	0,588	
4	EKSTRAK METANOL 96 REP 1	Unknown		34,991	0,469	
5	EKSTRAK METANOL 96 REP 2	Unknown		35,071	0,470	
6	EKSTRAK METANOL 96 REP 3	Unknown		34,866	0,467	
7	EKSTRAK METANOL 70 REP 1	Unknown		27,747	0,374	
8	EKSTRAK METANOL 70 REP 2	Unknown		27,706	0,373	
9	EKSTRAK METANOL 70 REP 3	Unknown		27,971	0,377	
10	EKSTRAK METANOL 50 REP 1	Unknown		26,121	0,352	
11	EKSTRAK METANOL 50 REP 2	Unknown		26,169	0,353	
12	EKSTRAK METANOL 50 REP 3	Unknown		25,898	0,349	
13						

medicine. This is a reinforcement to support the use of Polygonum minus Huds as anthelmintic, antiviral, antibacterial, antifungal, antioxidant free radical scavengers, anticancer and antiulcer etc. Furthermore, it could be conducted in more detailed examinations, especially the isolation of bioactive chemical compounds in Polygonum minus Huds plant extracts needed for treatment.

Table V. The Total Content of fenol, flavonoid, alkaloid in Polygonum Leaf

Plant Name	Family	The used part	Extract Investigation	Total of phenol (ppm) Plant Extract GAE	Total of flavonoid (ppm) Plant Extract QE	Total of alkaloid (ppm) Plant Extract RE
Polygonum minus Huds	Polygonaceae	The leaf	methanol	49,35±0,43	44,13±0,12	356,80±1,01
			ethanol 96%	46,20±0,33	34,97±0,12	183,53±0,77
			ethanol 70%	64,19±0,22	27,83±0,16	432,95±1,53
			ethanol 50%	67,34±0,16	26,06±0,16	679,14±2,53

Values are presented in the form of mean ± SD (n=3); GAE = gallic acid equivalent; QE = quercetin equivalent; RE = reserpin equivalent

CONCLUSION

The highest total phenolic content of the 50% ethanol *Polygonum* leaf extract is 67.343 ± 0.164 ppm GAE and the highest flavonoids content of the methanol extract is 44.128 ± 0.116 ppm QE. Furthermore, the highest total alkaloids content of the 50% ethanol extract is 79.142 ± 2.52 ppm RE. Therefore, it has provided a biochemical basis for the ethnomedicinal use of Polygonum leaf extract as a promising source of bioactive compounds and a very good source for useful medicine.

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REFERENCES

Ahmad, A., Husain, A., Mujeeb, M.,Khan, S. A., Alhadrami, H. A.A., & Bhandari, A. (2015). Quantification of Total Phenol, Flavonoid Content and Pharmacognostical Evaluation Including HPTLC Fingerprinting for The Standardization of Piper nigrum Linn Fruits. Asian Pasific Journal of Tropical Biomedicine, 5(2), 101–107.

Ahmad, I., Arifuddin, M., & Rijai, L. (2016). The effect of extraction methods of Bawang Dayak (Eleutherine palmifolia L. MERR) against TLC profiles and sunscreen activities. International Journal of PharmTech Research, 9(9), 428–436.

Ansel, H.C., 1989, Pengantar Bentuk Sediaan Farmasi, diterjemahkan oleh Farida Ibrahim, Asmanizar, Iis Aisyah, Edisi keempat, 255-271, 607-608, 700, Jakarta, UI Press.

Baba, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. Journal of Taibah University for Science, 9(4), 449–454. https://doi.org/10.1016/j.jtusci.2014.11.001

Bobo-García, G., Davidov-Pardo, G.,Arroqui, C., Vírseda, P., MarínArroyo,M., & Navarro, M.(2014). Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. Journal of the Science of Food and Agriculture, 95(1), 204–209. https://doi.org/10.1002/jsfa.6706

Dewi S, Asseggaf SNYRS, Natalia D, Mahyarudin, 2019, Efek Ekstrak Etanol Daun Kesum (Polygonum minus Huds.) sebagai Antifungi terhadap Trichophyton rubrum, Jurnal Kesehatan Andalas. 8(2), 199-204

Do, Q. D., Angkawijaya, A. E.,Tran-Nguyen, P. L., Huynh, L.H., Soetaredjo, F. E., Ismadji,S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of Food and Drug Analysis, 22(3), 296–302.https://doi.org/10.1016/j.jfda.2013.11.001

Ghosh, M., Sinha, B. N., Seijas, J.A., Vázquez-Tato, M. P., & Feás, X. (2014). Flavonoids and Phenolic Compounds from Litsea polyantha Juss. bark. Proceedings of The 18thInternational Electronic Conference on Synthetic Organic Chemistry, b016. https://doi.org/10.3390/ecsoc18-b016

Globinmed. (2010). Retrieved Januari 5 2021, from Globinmed: http://www.globinmed.com/index.php?o

ption=com_content&view=article&id=86112:hibiscus

Kartikasari D., Ika Ristia Rahman IR, Ridha A., 2021, Uji Fitokimia Dengan Beberapa Pelarut Pada Daun Kesum (Polygonum Minus Huds.) Dari Kalimantan Barat, Laporan Penelitian, Akademi Farmasi Yarsi Pontianak

Marinova, D., Ribarova, F., & Atanassova, M. (2005). Total phenolics and total flavonoids in bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy, 40(3), 255–260.

Maulidya DA., 2017, Daya Antelmintik Ekstrak Etanol Daun Kesum (Polygonum minus) terhadap Ascaridia galli secara in vitro, Jurnal Mahasiswa PSPD FK Universitas Tanjungpura, 3(1), 731-740

Purwaningsih I, Sapriani R, Indrawati R., 2018, Antioxidant Activity Of Methanol Extract Of Kesum Leaves (Polygonum Minus Huds.) Dpph Methodaktivitas Antioksidan Ekstrak Metanol Daun Kesum (Polygonum Minus Huds.) Metode Dpph, Jurnal Laboratorium Khatulistiwa, 1(2), 161-165

Sari, A. K., Alfian, R., Musiam, S.,Prasdianto, & Renny. (2018).Penetapan Kadar Fenolik dan Flavonoid Total Ekstrak Metanol Kayu Kuning (Arcangelisia flava Merr) dengan Metode Spektrofotometri UV-Visibel. Jurnal Insan Farmasi Indonesia, 1(2), 210–217.

Shamsa, F., Monsef, H., Ghamooshi, R., dan Verdian-rizi, M., 2008. Spectrofotometric Determination of Total Alkaloid in Some Iranian Medical Plants. Thai J.Pharm Sci, 32: 17-20

Sulianti S, Kuncari ES, Chairul SM. 2006, Pemeriksaan Farmakognosi dan Penapisan Fitokimia dari Daun dan Kulit Batang Calophyllum inophyllum dan Calophyllum soulatri. 7:25–9.