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The Effect of Extraction Method on Total Phenolic Content and Antioxidant Activity of Salam Leaves (*Syzygium polyanthum*) using DPPH (1,1-Diphenyl-2-Picrylhidrazil)

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ABSTRACT

Extraction is an important step in separating bioactive compounds from the plant. The selection of extraction technique is also important in the standardization of herbal products, for if not observed it can remove the desired soluble constituents. One of Indonesia's endemic plants which have been proven to have an antioxidant activity is Salam leaves (*Syzygium polyanthum*). This study aims to determine the effect of extraction method on total phenolic content and antioxidant activity of *S. polyanthum* leaves by counteracting free radicals mechanism using DPPH. The *S. polyanthum* leaves were extracted by maceration, soxletation, and infusa methods, and tested for the total phenolic content and the antioxidant activity. The results showed that the total phenolic content of each method extraction is different. The total of the phenolic content extraction by maceration, soxhlet, and infusa method respectively were 338.62±21.3; 227.72±21.6; and 144.48±8.2 mgGAE/g. The best antioxidant activity was maceration method with IC_{50} 17.53±0.11 µg/mL followed by soxhlet and infusa which were 18.73±0.31 and 40.26±0.18 µg/mL. The research conclusion is that the extraction method has an effect on the total phenol and antioxidant activity of *S. polyanthum* leaves.

Keywords: Extraction Method, *Syzygium polyanthum*, total phenol, Antioxidant Activity

INTRODUCTION

Extraction is an important step in the itinerary of phytochemical processing constituents from plant materials. Various extraction techniques such as maceration, percolation, soxhlet, infundation, supercritical extraction, ultrasonic extraction and microwave extraction had previously been developed for discovery of bioactive compounds from plants. Some compounds could be degraded in heat and light. Therefore, selection of a suitable extraction methods is also important for standardization of herbal products as it is utilized in the removal of desirable soluble compounds (Dhanani *et al.,* 2017).

Polyphenol, flavonoid, and phenolic acids compounds are most abundantly present in natural bioactive compounds that have antioxidant activity (Diem *et al.,* 2013; Upadhya *et al.,* 2015). Several kinds of research showed that the extraction methods and solvent influence total phenol and total flavonoid content, and the activity of the compounds. The study of the extraction of *Limnophila aromatica* using various solvent showed that the extract obtained by 100% ethanol highest phenolic and flavonoid content and also highest antioxidant activity from extract obtained by 50% aqueous acetone (Diem *et al.,* 2013).

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Another study showed that the extract of rhizome of *Zingiberis officinale* using maceration by ethanol solvent obtained the highest level of total phenol and total flavonoid content and higher antioxidant activity than using decocta method by water solvent (Andriyani *et al.,* 2015). A study conducted by Hasmida *et al*, the extract of *Quercus infectoria* Galls plant using Supercritical CO2 extraction obtained higher phenolic content and the antioxidant activity than soxhletasi method (Hasmida *et al.,* 2014).

Salam (*Syzygium polyanthum*) is a plant known as a seasoning and as also a traditional medicine in Indonesia. As a traditional medicine, *S. polyanthum* leaves has been proved has various pharmacological activities such as antioxidant, anti-inflammation, antidiabetic, antihypertension, antibacterial, immunomodulator, anticancer and antidiarrhoe (Malik & Ahmad, 2013; Sutrisna *et al.,* 2016; Rizki & Hariandja, 2016. Many kinds of research proved its antioxidant activity. The methanol extract of *S. polyanthum* leaves shown the antioxidant activity with the inhibitory concentration (IC_{50}) is 90.85 µg/ml (Har & Ismail, 2012). Another study showed the activity of its radical scavenging is not different significantly to *Ziziphus Mauritania* plant. The effectivity concentration (EC_{50}) is 20.90±0.26 µg/ml, with the data of each total phenol contents was 333.74±1.92 GAE/g and flavonoid contents 65.2±1.83 CE/g

(Perumal *et al.,* 2012). The study conducted by Hassan *et al.,* 2015 showed that aqueous extract of *Eugenia poliantha* leaves from Malaysia, a synonym of *S. polyanthum* leaves, has antioxidant activity with IC_{50} is 0.15±0.01 mg/ml and the phenolic and flavonoid contents were 213.15±1.10 GAE and 2.47±0.06 QE each gram of sample. Another study also to showed that the extracts of *S. polyanthum* bark by methanol-water solvent have inhibitory activity against free radical that it is higher than the extract obtained by methanol or water solvent (Lelono *et al.,* 2009). Base on description above, phytochemical processing of *S. polyanthum* is essentially required to optimize the concentration of phenolic and flavonoid contents and also to maintain their antioxidant activity.

METHODOLOGY

Instrument

This research used Spectrophotometer UV-Vis (*Shimadzu* type 2450), UV lamp lambda 254 and 366 nm (*Merck* type 1.13203.0001), Oven (Memmert), rotary evaporator (*Heldolph*®), *hot plate* (*Schott Instrument*), micropipette (*Rainin* E1019705K®), analytical balance (*Precise* TYP 320-9410-003), *waterbath* (*Memmert* tipe WNB-1314) and glassware (*Pyrex*).

Material

Sample of *S. polyanthum* leave from Mekar baru village, Kubu raya, Indonesia; chemical compound 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich)**,** gallat acid (Sigma Aldrich), *Folin Ciocalteu* (Sigma Aldrich) and chemical solvent grade pro analysis.

The way of Research

The determination of the plant

The sample was determined in the Biology Laboratory of Matematika dan Ilmu Pengetahuan Alam, (FMIPA) Universitas Tanjungpura.

The extraction of Salam (*S. polyanthum*) leaves

The sample is divided into three groups based on the extraction methods. Sample I was macerated by methanol solvent. Sample II was extracted with soxhlet methods by methanol solvent and sample III was infusion by water. Sample I and sample II were concentrated using rotary evaporator. Sample III was concentrated used *freeze dry*.

Phytochemical Screening of Sample

Phytochemical screening sample I, II and III is conducted with color tests to analyze the metabolite content of alkaloid, flavonoid, steroid/terphene, polyphenol, tannin, and saponin.

The Screening of Antioxidant Activity and Chromatography Profile of Sample

Sample I, II and III were analyzed using thin layer chromatography (TLC). Each sample was eluted using silica plat GF_{254} as stationary phase and Buthanol: Acetic acid: Water (4: 1: 5) as mobile phase. The plat was identified using DPPH to analyze the antioxidant activity, $FeCl_3$ to analyze phenol content and $AlCl_3$ to analyze flavonoid content.

The Determination of Total Phenolic Contents

Total phenolic contents in the extracts were determined spectrophotometry by Folin-Ciocalteau method. The solution extracts 1% added 0.2 ml solution of Folin Ciocalteu for 10 seconds and then let stand for 5 minutes. After that each solution was added to 2 ml of Na_2CO_3 7% b/v (in aquabides) for 30 seconds ad homogen and finally added aquabides ad 5 ml. The solution is then allowed to stand for 95 minutes and the absorbent of mixture as measured at 749,5 nm. Gallate acid is used as a positive control.

The antioxidant activity assay

The antioxidant assay was adapted from Kikuzaki *et al.,* 2002 and Molyneux, 2004. Antioxidant activity was determined using DPPH method with methanol as a blank. Extracts diluted by methanol and divided several concentration were 5, 10, 15, 20, 25 and 30 µg/mL Each solution added solution DPPH 28 µg/.mL with ratio 1: 5. After 30 minutes, the absorbance of mixture as measured at 517 nm. The inhibitory activity was determined by UV-vis Spectrophotometric method (Kikuzaki *et al.,* 2002; Molyneux, 2004).

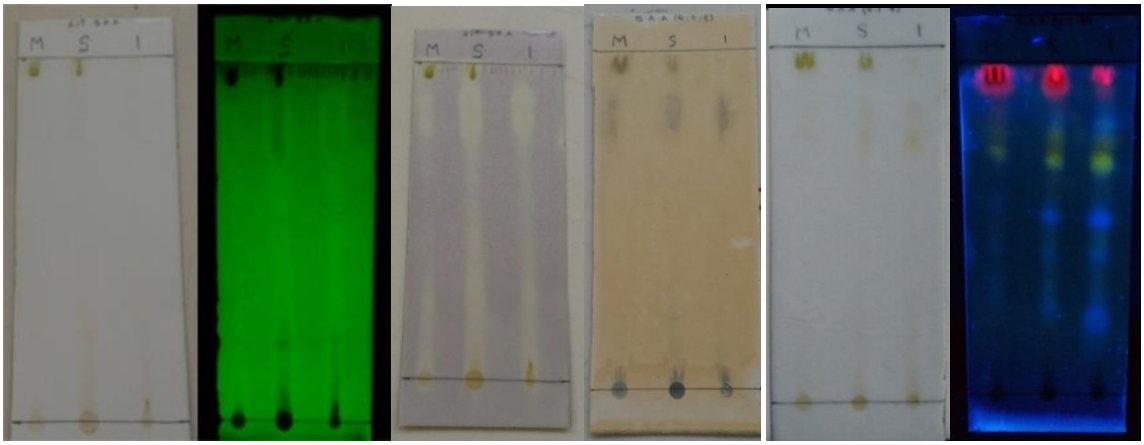
The data analysis

The data results were interpreted using *software* SPSS 21 to analyze the normal distribution and the Varian homogeneity of the result data (p>0,05), and *One-Way* ANOVA method and *Post Hoc* LSD with interval 95% to analyze the hypothesis.

RESULTS AND DISCUSSION

The result of plant determination and extract preparation

The determination result shows that the samples are Salam (*Syzygium polyanthum*.) which the family is *Myrtaceae* species is *Syzygium polyanthum*. Rendemen of the extract obtained



(a) (b) (c) (d) (e) (f)

Figure 1. Result of Chromatogram Profil TLC Exstracts used Maseration, Sohxlet and Infusion

Legends : Stationary phase Silica gel GF $_{254}$ and Mobile phase Buthanol : acetic acid : Water (4:1:5) (a) visible Lght; (b) UV 254 nm ligth; (c) after DPPH 0,2% spray; (d) after $FeCl_3$ spray; (e) After $AlCl_3$ spray and (f) After $AlCl_3$ spray-UV 366 nm

by maceration, soxhlet and infusion were 26.84; 25.28 and 10.64 % respectively.

The Result of Phytochemical Screening

The result shows that extract of *S. polyanthum* leaves obtained by maceration, soxhlet and infusion contains alkaloid, flavonoid, steroid/terpene, polyphenol, tannin, and saponin. This result is in accordance with the previous research conducted by Hassan (Hassan et al. 2015; Bahriul et al. 2014) and the research conducted by Nor that analyzed terpene such as nerodiol, α,β,γ-tocopherol, β-sitosterol, linalool, phytol in extract of *S. polyanthum* leaves using Gas Chromatography-Mass Spectroscopic (Nor *et al.,* 2018).

The Result of Antioxidant Activity Screening and Chromatography Profile

As shown in Figure 1, the chromatogram profile of extract using DPPH showed a change in color from violet to yellow. The chromatogram profile of extract using $FeCl_3$ showed a dark blue color that shown phenol content. The chromatogram profile of extract using $AlCl_3$ showed yellow color that shown flavonoid content.

The Result of Total Phenolic Content

The result showed the extract contains phenol that is confirmed by positive control, gallic acid. The result also showed the extract that extracted by maceration contains the highest of total phenol content. The total phenol level of maceration, Soxhlet, and infusion were 338.62±21.3; 227.72±21.6 and 144.48±8.2 mgGAE/g sample respectively. This result shows that infusion could not extract well the compound from the leaves. This is in accordance with the previous research obtained the total phenolic content of the methanol extract of *S. polyanthum* leaves by maceration is 11.125 mg GAE/100 g sample (Har & Ismail, 2012), but obtained the total phenolic content of extract obtained by water extraction is 213.15±1.10 mgGAE/g (Hassan *et al.,* 2015). The difference between both extraction results could be caused temperature and solvent (Andriyani *et al.,* 2015). Extraction to obtained phenolic compounds could use water, methanol, ethanol, and acetone. The extraction of *Limnophila aromatica* using, methanol, ethanol, acetone, and mix of water-methanol, ethanol-acetone in series concentration of 50, 75, and 100% showed that extract that extracted by ethanol 100% obtained the highest level of total phenol that is 40.50±0.08 mgGAE/g. The extract that extracted with acetone, methanol, and water obtained the total phenol level were 40.30±0.20; 3.,50±1.6 and 6.25±0.24 mgGAE/g respectively. The extract that extracted by mixing of water-methanol, ethanol and acetone obtained the total phenol contents lower than methanol, ethanol, and acetone 100% (Diem *et al.,* 2013). Phenolic compounds from *S. polyanthum* leaves has antioxidant activity that could scavenge the free radical (Bahriul *et al.,* 2014; Hassan *et al.,*

Table I. Result Yield, Total Phenol and Antioxidant Activity (IC_{50}) of *S. Polyanthum* Leave

Extraction Methods	Yield (%)	Total Phenol (mgGAE/g)	IC_{50} (µg/mL)
Maseration	26.84	338.62±21.3	17.53±0.11
Sohxlet	25.28	227.72±21.6	18.73±0.31
Infusion	10.64	144.48±8.20	40.26±0.18

2015; Perumal *et al.,* 2012). Therefore, the higher phenol content in extract and the higher contribution to the antioxidant activity of the extract. (Chaisawangwong & Gritsanapan, 2009; Har & Ismail, 2012; Diem *et al.,* 2013).

Antioxidant Activity

The result shows that all of the extracts have antioxidant activity. The IC_{50} for extract that was obtained by maceration, Soxhlet, and infusion were 17.53±0.11; 18.73±0.31 and 40.26±0.18 µg/mL extracts respectively. These data show that all of the extracts have IC_{50} less than 100 µg/mL. This means that all of extract are active as antioxidant because these IC_{50} are less than 50 ppm (Ruchiyat, 2013). Moreover, as shown in (Table I), the result is the higher rendemen of extract, the higher level of total phenol and antioxidant activity of the extract (Table I).

The result shows that the extraction method determines antioxidant activity. This is in accordance with previous researches. The study conducted by Andriyani shows that the ethanol extract that obtained by maceration has the level of antioxidant activity that is different with water extract that obtained by decocta (Andriyani *et al.,* 2015). But, Chaisawangwong obtained that the extract of *Azadirachta indica* flower obtained by decocta had higher antioxidant activity than extract obtained by maceration, percolation, soxhlet, ultrasonic extraction and distillation with EC_{50} 11.36 µg/mL (Chaisawangwong & Gritsanapan, 2009). A study conducted by Dhanani also shows that the different extraction method to obtain *Withnaia somnifera* L extract such as soxhlet, ultrasonic and microwave and mix of solvent soxhlet the total phenol level and antioxidant activity (Dhanani *et al.,* 2017). A study conducted by Sutrisna obtained IC_{50} 27.8 µg/mL for the antioxidant activity of 70% ethanol extract of Salam leaves (Sutrisna *et al.,* 2016). A study conducted by Ruchiyat obtained IC_{50} 9.10 µg/mL for the methanol extract of Salam leaves (Ruchiyat, 2013). Another study conducted by Perumal shows that the methanol extract of *S. polyanthum* leaves could scavenge the free radical with EC_{50} 20.90±20.26 µg/mL (Perumal *et al.,* 2012). In addition, the water extract of *S. polyanthum* leaves from Malaysia, *Eugenia polyantha* also showed IC_{50} 0.15±0.01 mg/mL of the extract (Hassan *et al.,* 2015). The result of this research shows that the extract that was obtained by water solvent has lower antioxidant activity.

CONCLUSION

P. niruri ethanol extract gel with added 1% menthol has shown better hair length and weight of the test animals compared to the other gel formulation without menthol, with an average hair length of 12,45±3,457 mm and an average hair weight of 28,53±7,681 mg, whereas the gel formulation without enhancer yielded 10,67±2,455 mm of average hair length and 19,87±9,552 mg of hair weight.

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