

The Potential at Phytochemical Compounds from the Indonesian Kalimantan Plant as Covid-19 Immunomodulators

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Abstract: Herbal plants are alternative medicine and immunity boosters related to the Covid-19 pandemic. Typical Kalimantan plants that have the potential are dayak onion (*Eleutherine americana* Merr.), karamunting (*Rhodomyrtus tematosa* (Aiton) Hassk), pasak bumi (*Eurycoma longifolia* Jack), and tigaron (*Crataeva adansonii* DC). This study aims to determine the potential of phytochemical compounds from Indonesian Kalimantan plants as Covid-19 immunomodulators. Descriptive research with cross-sectional study design by testing the typical plants of Kalimantan, which were extracted first through the maceration method and then carried out qualitative and quantitative phytochemical tests. Qualitative and quantitative results showed that the four positive plants contained phytochemical alkaloids, flavonoids, saponins, and tannins in certain levels. Quantitative results on dayak onion (*Eleutherine americana* Merr.) obtained alkaloid content; flavonoids; saponins; tannins were 74.540%; 22.125 mg/mL; 58.350%; 0.342 mg/mL. karamunting fruit (*Rhodomyrtus tematosa* (Aiton) Hassk) 72.815%; 20,500 mg/mL; 78.225%; 0.541 mg/mL. pasak bumi (*Eurycoma longifolia* Jack) 68.575%; 12,250 mg/mL; 53.930%; 0.383 mg/mL; and tigaron (*Crataeva adansonii* DC) 82.330%; 40,750 mg/mL; 75.395%; 0.609 mg/mL. The four phytochemical compounds have the potential as Covid-19 immunomodulators. This study concludes that the typical plant from Kalimantan that has been studied has the potential. This research can be continued by testing the effectiveness of phytochemicals as Covid-19 immunomodulators in experimental animals.

Keywords: Covid-19; immunomodulator; phytochemicals; plants typical of Kalimantan.

INTRODUCTION

During the Covid-19 pandemic, various efforts have been made, ranging from changes to a healthier lifestyle to implementing a vaccination program. However, there is an urgency to promote safe alternatives for handling Covid-19 by utilizing plant materials with immunomodulatory properties. According to Wulan and Agusni (2015), immunomodulators are materials that can modulate or stimulate the body's immune system, activating natural and adaptive defense mechanisms to restore immune system imbalances due to disease or pathogenic infection¹. The attack of SARS-Cov-2, which is the virus that causes Covid-19, will be inhibited through a modulation system by increasing the body's immune response.

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Phytochemical compounds (secondary metabolites such as flavonoids, alkaloids, saponins, tannins) from local plants have potential as immunomodulators. The results of Saputra's research stated that the dayak onion extract contains alkaloids, flavonoids, and phenolic compounds². In addition, Supriani's research stated that the phytochemical test results of the ethanolic extract of dayak onion contain flavonoid compounds, quinones, polyferols, steroids, and tannins³. The research results on flavonoid extract in karamunting fruit showed the highest flavonoid content in 70% ethanol extract⁷. The research results by Sari et al. stated that the flesh of karamunting fruit contains flavonoid and phenolic compounds, while the seeds of karamunting fruit contain flavonoid, phenolic, and saponin compounds⁴.

The research results of Supartini et al. stated that pasak bumi roots were extracted using ethanol as a solvent which contained tannins, triterpenoids, and coumarins but did not contain alkaloids flavonoids, and saponins⁵. Research results Rahmi et al. showed that tigar on contained total phenolics, flavonoids, and tannins⁶.

There has been researching on phytochemical compounds typical of Kalimantan plants, including dayak onion (*Eleutherine americana Merr.*), karamunting (*Rhodomyrtus tematosa (Aiton) Hassk*), pasak bumi (*Eurycoma longifolia Jack*), and tigar on (*Crataeva adansonii DC*). However, studies linking phytochemical content with plant potential as an immunomodulator have not been widely disclosed. This study aims to qualitatively and quantitatively determine the content of chemical compounds typical of Kalimantan plants which have the potential as Covid-19 immunomodulators.

MATERIALS AND METHODS

The type of research used is descriptive qualitative and quantitative test results of phytochemical tests typical of Kalimantan plants, including dayak onion (*Eleutherine americana Merr.*), karamunting (*Rhodomyrtus tematosa (Aiton) Hassk*), pasak bumi (*Eurycoma longifolia Jack*), and tigar on (*Crataeva adansonii DC*). The research was carried out in several stages: the determination test on each plant, making thick extracts using the maceration method, and qualitative and quantitative phytochemical tests. The qualitative and quantitative phytochemical test data for each plant typical of Kalimantan are described and presented tabularly.

Dayak onion (*Eleutherine americana Merr.*) comes from the city of Kapuas in Central Kalimantan province, karamunting (*Rhodomyrtus tematosa (Aiton) Hassk*) comes from Buntok city in Central Kalimantan province, pasak bumi (*Eurycoma longifolia Jack*) comes from Tamiang Layang village in Central Kalimantan, and tigar on (*Crataeva adansonii DC*) from Bati-bati village in South Kalimantan.

All selected parts of each fresh plant are pre-cleaned and dried; The next dry part is ground into a powder and sieved to make smaller powder particles. Then, each powder is mixed with each specific solvent and concentration for better results for a whole day. Each mixed liquid is then filtered with special paper so that the solution is evaporated with a water bath until the solution thickens to extract the working solution. This study used a solvent extract of dayak onions, namely 96% ethanol, karamunting, and pasak bumi roots used 70% ethanol, tigar on used methanol.

Quantitative tests of flavonoids and tannins used spectrophotometric and gravimetric methods to test alkaloids and saponins. For quantitative testing of alkaloids, several samples were first mixed with acetic acid in 10% ethanol, filtered until the solution

was evaporated on a water bath, added ammonium hydroxide little by little, and waited a while for precipitation to occur. The precipitation was then rewashed with ammonium hydroxide and evaporated again to obtain the residual weight, which was considered an alkaloid in the sample value. For the quantitative test of saponins, prepare a solution with several samples with 20% ethanol. Evaporation for the solution on a water bath for 55°C for 4 hours the residue must be filtered and re-evaporated for extraction. Then, the residue from the last process was evaporated at a temperature of 90°C to the last 40 ml. after that, put the last residue in a separatory funnel and take the liquid layer and add 60 ml of n-butanol, and mix. Finally washed with 10 mL of 5% NaCl, then evaporated again to get the residual weight in the form of saponins in the sample value.

The spectrophotometric method uses a standard curve. Measurement of total flavonoids made a solution with five successive variations in the value of the mixing control and aquadest. Also, mix with 5% NaNO₂, 10% AlCl₃, and add 4% NaOH to a total volume of 5 mL. Finally, measure in a UV-Vis spectrophotometer at 520 nm. The total flavonoid value is known by mixing several samples, 5% NaNO₂, 10% AlCl₃, adding 4% NaOH until the volume of the solution reaches 5 mL, and waiting for a while. Finally, measure in a UV-Vis spectrophotometer at 520 nm.

The standard tannin solution contained gallic acid with five successive concentrations of variation and was measured in a UV-Vis spectrophotometer with a wavelength of 700 nm. As for the total tannin value, a sample must be diluted with aquadest, then taken a little, and put some 0.8 M K₄Fe(CN)₆ and 0.1 M FeCl₃ into 0.1 M HCl distilled water until the volume reaches 10 ml. Finally, measure in a UV-Vis spectrophotometer at 700 nm.

RESULTS AND DISCUSSION

Plant determination was carried out at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru. The results showed that the samples used were dayak onion (*Eleutherine americana* Merr.), karamunting (*Rhodomyrtus tementosa* (Aiton) Hassk), pasak bumi (*Eurycoma longifolia* Jack), and tigarón (*Crataeva adansonii* DC).

Table 1. Qualitative Phytochemical Test Results

Typical Kalimantan Plants	Phytochemical Qualitative			
	Alkaloids	Flavonoids	Saponins	Tannins
Dayak onion (<i>Eleutherine americana</i> Merr.)	+	+	+	+
Karamunting (<i>Rhodomyrtus tementosa</i> (Aiton) Hassk)	+	+	+	+
Pasak Bumi (<i>Eurycoma longifolia</i> Jack)	+	+	+	+
Tigarón (<i>Crataeva adansonii</i> DC)	+	+	+	+

Table 2. Average Results of Quantitative Phytochemical Test

Typical Kalimantan Plants	Alkaloids (%)	Flavonoids (mg/mL QE)	Saponins (%)	Tannins (mg/mL GAE)
Dayak onion (<i>Eleutherine americana</i> Merr.)	74,540	22,125	58,350	0,342
Karamunting (<i>Rhodomyrtus tomentosa</i> (Aiton) Hassk)	72,815	20,500	78,225	0,541
Pasak Bumi (<i>Eurycoma longifolia</i> Jack)	68,575	12,250	53,930	0,383
Tigaron (<i>Crataeva adansonii</i> DC)	82,330	40,750	75,395	0,609

Test results of typical Kalimantan plant extracts showed that the four plants were positive for containing certain levels of phytochemical compounds of alkaloids, flavonoids, saponins, and tannins (table 1,2). The extraction of typical Kalimantan plants is carried out by the maceration method. The principle of the extraction process using the maceration method is to do it cold and not to heat it. Maceration extraction is intended for natural materials that contain chemical components that are not heat-resistant and have a soft texture². The extraction results were evaporated in a water bath until a thick extract was obtained to obtain the yield value. Yield is the ratio between the Extract obtained and the initial simplicia. The yield determines the number of secondary metabolites carried by the solvent, but the type of components contained cannot be known⁵.

Dayak onion extract using maceration method with 96% ethanol solvent. The use of ethanol is 96% because it is a universal solvent for the alcohol group that easily dissolves the appropriate compound fairly quickly. ethanol has a high polarity, has a low enough boiling point so that it can be evaporated quickly without using high temperatures, is inert, and has an affordable price. In addition, the toxicity of ethanol is much lower than that of other alcohol solvents⁷. Yuswi's research showed that using 96% ethanol solvent in the extraction of antioxidant dayak onions obtained the best extract results compared to other solvents⁸. The thick extract of dayak onion obtained by maceration extraction was brownish red with a yield of 10.12%. By using qualitative and quantitative tests (tables 1, 2), identification of phytochemical compounds in dayak onions dayak onions contains phytochemical compounds in the form of alkaloids, flavonoids, saponins, and tannins. This is in line with the results of Saputra's research, which states that the dayak onion extract contains alkaloids, flavonoids, and phenolic compounds².

Karamunting fruit extract in this study used the maceration method with 70% ethanol as a solvent. 70% ethanol is a universal solvent that can dissolve almost all organic compounds in the sample, both polar and non-polar compounds⁹. 70% ethanol concentration was used because it is more polar than 96% ethanol. The lower the concentration, the higher the polarity so that more polar compounds will be dissolved in 70% ethanol¹⁰. The thick extract of karamunting fruit obtained by maceration extraction is brown with a yield of 20.47%. Using qualitative and quantitative tests (tables 1, 2),

identifying phytochemical compounds in karamunting fruit contains phytochemical compounds in the form of alkaloids, flavonoids, saponins, and tannins. This is in line with the research results of Sari et al. stated that the flesh of karamunting fruit contains flavonoid and phenolic compounds. In contrast, the seeds of karamunting fruit contain flavonoid, phenolic, and saponin compounds⁴.

Pasak bumi root extract using maceration method with 70% ethanol solvent. Anwar's research shows that the optimal extraction process of pasak bumi roots is with 70% ethanol as solvent¹¹. The thick extract of pasak bumi root obtained by maceration extraction is brown with a yield of 7.34%. Using qualitative and quantitative tests (tables 1, 2), the identification of phytochemical compounds in pasak bumi roots contained phytochemical compounds in the form of alkaloids, flavonoids, saponins, and tannins. This is different from the research results of Supartini et al., which stated that pasak bumi roots were extracted using ethanol as a solvent that contained tannins, triterpenoids, and coumarins but did not contain alkaloids, flavonoids, and saponins⁵.

Tigaron flower extract in this study used the maceration method with methanol as a solvent. The use of methanol as a solvent because methanol is a solvent that quickly enters the cell through the cell wall of the extract. Secondary metabolites in the cytoplasm will dissolve in the solvent, and these compounds will be extracted entirely so that the maximum chemical compounds are obtained¹². Using qualitative and quantitative tests (tables 1, 2), the identification of phytochemical compounds in pasak bumi roots contained phytochemical compounds in the form of alkaloids, flavonoids, saponins, and tannins. This is in line with the research results of Rahmi et al. showed that tigaron and Jaruk tigaron flowers contained flavonoid compounds, total phenolics, and tannins⁶.

The body's defense system can be activated by providing an immunomodulator that can be used to increase a person's immune response. The body protects itself from attack by pathogenic microorganisms, including the virus that causes COVID-19 through the modulation system¹³. Phytochemical compounds in typical plants of Kalimantan include dayak onion (*Eleutherine americana* Merr.), karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk), pasak bumi (*Eurycoma longifolia* Jack), and tigaron (*Crataeva adansonii* DC), which have the potential as immunomodulators that can increase system activity. Immune system by activating natural and adaptive defense mechanisms and as an antiviral that inhibits the replication of the SARS-CoV-2 virus so that the virus cannot replicate in the host with the following mechanism:

Alkaloids are Indirubin compounds bisindole alkaloids from the *Isatis indigotica* plant, which can reduce the susceptibility to death from Influenza Virus (H1N1) in experimental mice (baby mice)¹⁴. Flavonoids are compounds that inhibit the reverse transcriptase enzyme in synthesizing RNA into cDNA so that the virus cannot reproduce (replication). Saponins are compounds that inhibit the formation of viral capsids and can increase host cell resistance to viral infections¹⁵. The SARS-CoV-2 virus has a nucleocapsid (N) protein type fused with a single-stranded RNA genome. Based on the mechanism of action of saponins on viruses, saponin compounds inhibit the formation of the nucleocapsid (N) of the SARS-CoV-2 virus. Tannins are compounds that prevent virus adsorption by inhibiting the interaction of host cell surface proteins and viral proteins, so that virus attachment and penetration into the host cell plasma membrane cannot occur¹⁵. The SARS-CoV-2 virus has a spike glycoprotein (S) surface protein that

recognizes and binds to receptors. Based on the mechanism of action of tannins on the virus, the surface glycoprotein spike (S) of the SARS-CoV-2 virus was inhibited by tannin compounds. Apart from being antiviral, each plant typical of Kalimantan has the following benefits:

Dayak onion (*Eleutherine americana* Merr.), with Flavonoid derivative compounds that have potential as immunostimulators are the flavonol group, namely quercetin and rutin¹⁶, then Karamunting fruit (*Rhodomyrtus tementosa* (Aiton) Hassk) with flavonoid compound that has the potential as an antiviral is a kaempferol. Karamunting also acts as an immunostimulator by increasing the activity of leukocytes in the form of neutrophils because it has the main compound in the form of Rhodomyrtone (Rom)¹⁷, and then Pasak Bumi root (*Eurycoma longifolia* Jack) with Flavonoid derivative compounds that have potential as immunostimulators are the flavonol group, namely quercetin and rutin¹⁶, Last is the tigarong flower (*Crataeva adansonii* DC) contains a flavonoid compound that has the potential as an antiviral is kaempferol, with the mechanism of inhibiting the neuraminidase enzyme and inhibiting the 3a channel so that the coronavirus progeny can be blocked¹⁸.

The limitation of this study was that it only tested the phytochemical content of plants without directly testing the immunomodulatory effects of plants against Covid-19. So that further research suggestions can conduct a more in-depth study with in vitro and in vivo tests on the immunomodulatory potential of this typical plant from Kalimantan Indonesia.

CONCLUSION

Typical plants of Kalimantan, including the dayak onion (*Eleutherine americana* Merr.), karamunting (*Rhodomyrtus tementosa* (Aiton) Hassk), pasak bumi (*Eurycoma longifolia* Jack), and tigarong (*Crataeva adansonii* DC), contains chemical compounds that have the potential as immunomodulators for Covid 19. This can be continued by testing the effectiveness of phytochemicals as Covid-19 immunomodulators in experimental animals.

ACKNOWLEDGMENT

The writing of this scientific paper could be completed thanks to the help of various parties. The author would like to express his gratitude to Politeknik Kesehatan Kementerian Kesehatan Banjarmasin, who has provided beneficial facilities and finances for compiling this scientific paper.

CONFLICT OF INTEREST

The author has declared no conflict of interest.

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