PHYTOCHEMICAL SCREENING TEST, SPECIFIC AND NONSPECIFIC PARAMETER TEST OF JOHAR LEAF EXTRACT

Adinda Tri Octaviani[1], Annysa Ellycornia Silvyana[2*], Yonathan Tri Atmodjo Reubun[3]
[1,2,3]Program Studi Sarjana Farmasi, Sekolah Tinggi Ilmu Kesehatan Medistra Indonesia

[*]Corresponding author: annysa150793@gmail.com

Abstract

The Johar leaf (Cassia siamea Lamk.) is a plant with potential antimicrobial chemical compounds. This study aims to identify the compounds present in Johar leaf extract using solvents with different polarity levels. The Johar leaf samples were collected from Karang Bahagia Village, Bekasi Regency, West Java. The extraction was performed using the maceration method with successive solvents: n-hexane, ethyl acetate, and 96% ethanol, followed by phytochemical screening, and specific and non-specific parameter tests. The results of the phytochemical screening showed that the 96% ethanol extract of Johar leaves contains tannins, saponins, terpenoids, alkaloids, phenols, and flavonoids, but does not contain steroids; while the ethyl acetate and n-hexane extracts contain terpenoids. Specific parameter tests revealed that the water-soluble extract content was 2% in n-hexane and ethyl acetate extracts, and 10% in the 96% ethanol extract. The ethanol-soluble extract content was recorded as 5% for n-hexane extract, 6% for ethyl acetate extract, and 16% for 96% ethanol extract. In the non-specific parameter tests, the drying loss results were 5% for the n-hexane extract, 10% for the ethyl acetate extract, and 38% for the ethanol extract, while the moisture content was 89% for the n-hexane extract, 98% for the ethyl acetate extract, and 66% for the ethanol extract. It was concluded that the Johar leaf extract contains various secondary metabolites according to its polarity level, and the results of the non-specific and specific parameter tests all meet the established criteria.

Keywords: *Johar leaf, Phytochemical screening, Specific parameters, Non-specific parameters, Polarity.*

1. INTRODUCTION

Johar (*Cassia siamea* Lamk.) is a large tree commonly used as a shade plant. It is also known by various local names, such as ciebrek in Aceh, juwar in Betawi, bujuk or dulang in Sumatra, and English, it is called black-wood cassia, Bombay blackwood, or kassod tree [1]. Johar contains many chemical compounds, and several studies have shown that this plant has compounds beneficial to health. Its fruit and leaves offer numerous benefits. Johar leaves, which belong to the Fabaceae family, are known for their various uses [2]. In Indonesia, Johar leaves are often used in traditional medicine by squeezing or boiling them to extract the juice [2]. The use of Johar leaves is based on traditional knowledge and practices passed down through generations in various cultures [3].

Johar leaves are also known to have hypoglycemic effects that can help lower blood sugar levels, making them beneficial for people with diabetes [4]. Several secondary metabolites found in Johar leaves include saponins, anthraquinones, flavonoids, and alkaloids, with flavonoid and alkaloid content allowing its extract to function as an antibacterial agent [5]. Additionally, some glycosides in Johar leaves can provide diuretic effects [6]. Johar leaf extract also has anti-inflammatory properties that can reduce inflammation in the body [6]. According to a study conducted by Fitriah and her team in 2017, Johar leaves are known to contain polar and semi-polar antibacterial compounds, but they do not contain non-polar antibacterial compounds. The Johar leaf extract showed the greatest inhibitory effect against gram-negative bacteria with a diameter of 12.9 mm in the ethanol extract and gram-positive bacteria with a diameter of 14.9 mm [5]. This study aims to identify the compounds in Johar leaf extract using extraction solvents with different polarity levels.

2. METHODOLOGY

2.1 Research Location and Time

This research was conducted from January to March 2024 in the laboratory of the Bachelor of Pharmacy Study Program, Medistra Indonesia College of Health Sciences.

2.2 Equipment and Materials

Glass laboratory equipment (Pyrex), dropper pipettes, analytical balances (CHANGSHU QINGHUA, Indonesia), analytical balance (Biobase, China), filter paper, hot plate stirrer (Thermo Scientific Cimarec+ Hotplate Stirrer, USA), spatula, stirring rods, tweezers, aluminum foil, dropper pipettes, glass jars, test tubes (Pyrex), test tube racks, vortex mixer (Smartcare, Taiwan). Johar leaf simplicia, 96% ethanol, ethyl acetate, n-hexane, distilled water, reagents (Dragendorff, Bouchard, Mager), magnesium, HCl, FeCl3, 10% NaCl, chloroform.

2.3 Phytochemical Screening

2.3.1 Flavanoid

The flavonoid test was conducted by weighing 5-10 mg of Johar (*Cassia siamea*) leaf extract and placing it into a test tube. Approximately 20 ml of ethanol was added using a dropper, followed by 10 drops of concentrated hydrochloric acid and 3-4 grains of magnesium. After shaking briefly, the color change was observed. A positive flavonoid reaction is indicated by a change in color to red, orange, or yellow [7].

2.3.2 Phenol

The phenol test was performed by adding 5-10 mg of Johar (*C. siamea*) leaf extract into a test tube along with 20 ml of hot water and 5 drops of 10% NaCl, then dividing the solution into two test tubes. The first tube served as a **control**, while the second tube was added with 3 drops of 1% FeCl3. A positive phenol reaction is indicated by a color change to blue or dark blue [7].

2.3.3 Saponin

For the saponin test, 0.5 mg of the extract was mixed with 10 ml of distilled water in a test tube, then the mixture was shaken for 30 seconds and observed. The formation of stable foam for 30 seconds indicates the presence of saponins [7].

2.3.4 Tannin

The tannin test was conducted by dissolving 5 mg of ethanol extract and adding FeCl3 reagent, where a color change to blue or greenish-black indicates the presence of tannins [7].

2.3.5 Steroids & Terpenoids

The Steroid & Terpenoid test involved dissolving 5 mg of extract in ethanol, then adding Lieberman-Bouchard reagent. The formation of a brownish or purple ring indicates the presence of terpenoids, while a bluish-green ring indicates the presence of steroids [7].

2.3.6 Alkaloid

The alkaloid test was conducted by dissolving 10 mg of extract in ethanol, then dividing the resulting filtrate into three parts and adding Mager, Lieberman, and Dragendorff reagents. A positive alkaloid reaction is indicated by the formation of a white or yellow precipitate with Mager reagent, a reddish-brown precipitate with Lieberman reagent, and an orange precipitate with Dragendorff reagent [7].

2.4 Determination of Water-Soluble Extract Content

1 gram of Johar leaf extract was macerated with 20 ml of chloroform and distilled water mixture for 24 hours in a volumetric flask, with shaking done during the first 6 hours. Then, 4 ml of the maceration product was filtered and evaporated in a previously weighed dish at 105°C until a stable weight was obtained [8]. The percentage of water-soluble extract content is calculated using the following formula:

$$\text{Water-Soluble Extract Content} = \frac{\text{Weight of Water Extract}}{Weight\ of\ Extract} \times 100\%$$

2.5 Determination of Ethanol-Soluble Extract Content

1 gram of Johar leaf extract was macerated for 24 hours with 20 ml of 96% ethanol, with shaking done during the first 6 hours. Then, 4 ml of the 18-hour soaking product was filtered and evaporated in a previously weighed dish, then heated at 105°C until a stable weight was obtained [8]. The percentage of ethanol-soluble extract content is calculated using the following formula:

$$\text{Ethanol-Soluble Extract Content} = \frac{\text{Weight of Water Extract}}{Weight\ of\ Extract} \times 100\%$$

2.6 Determination of Drying Loss

1 gram of Johar leaf extract was heated for 30 minutes at 105°C using a calibrated dish, then weighed again. The extract was leveled to form a layer 5 to 10 mm thick. The infusion was then dried until a stable weight was reached. The dish was left open and placed in a desiccator until it reached room temperature [8]. The measurement results were then recorded. The formula for calculating drying loss is as follows:

Drying Loss = $\frac{\text{Dry Weight}}{\text{Wet Weight}}$x 100%

2.7 Determination of Water Content

1 gram of Johar leaf extract was heated in a calibrated dish (A) for 3 hours until dry at 105°C. To maintain a stable temperature, the dish was then placed in a desiccator (B) [8]. The results obtained were recorded. The percentage of water content is calculated using the following formula:

Water Content = $\frac{A-B}{A}$ x 100%

3. RESULT

3.1 Phytochemical Screening

The results of the phytochemical screening tests for the three different extracts are shown in Table 1, with variations possibly due to differences in the polarity levels of each solvent. The solubility principle of "like dissolves like" applies, where polar solvents dissolve polar compounds, and non-polar solvents dissolve non-polar compounds [9]. Ethanol 96% was capable of dissolving various compounds such as basic alkaloids, essential oils, glycosides, coumarins, flavonoids, anthraquinones, steroids, and chlorophyll, although tannins, saponins, and fats were only slightly soluble. At concentrations above 20%, ethanol 96% is considered a more selective solvent as it does not allow the growth of mold and bacteria on the skin [10]. The n-hexane solvent effectively dissolved non-polar compounds such as terpenoids, essential oils, triterpenoids, fats, sterols, fatty acids, carotenoids, alkaloids, resins, and chlorophyll [10].

Ethyl acetate, which is semi-polar, was able to extract glycoside flavonoids and aglycones. Ethyl acetate can also dissolve compounds in the alkaloid, flavonoid, saponin, tannin, and polyphenol categories [11]. Compounds in plants or simplicia can be extracted using universal solvents such as ethanol 96% [5]. The type of solvent used is one of the factors that influence the chemical compounds contained in Johar leaf extract. The methoxy group, which can form hydrogen bonds, is present in the semi-polar solvent ethyl acetate [12]. Although ethyl acetate can form hydrogen bonds, these bonds are weaker compared to those formed in methanol, a polar solvent. Therefore, ethyl acetate is not strong enough to dissolve the alkaloid and flavonoid compounds found in Johar leaves [13].

Saponin compounds were detected in the ethanol 96% extract, indicated by foam formation. These saponins are known for their antimicrobial and antifungal activities [14]. Alkaloids tested with Dragendorff's reagent were detected in the n-hexane and ethanol 96% extracts, indicated by the formation of an orange precipitate. In contrast, with Lieberman's reagent, a reddish-brown precipitate was observed in the ethanol 96% and ethyl acetate extracts. For Mager's reagent, a white precipitate was visible only in the n-hexane extract [15]. Tannin compounds were detected in the ethanol 96% extract, indicated by a change in color to greenish-black. Tannins play a role in astringent and antimicrobial activities [16]. A change in color to yellow indicates the presence of flavonoids in the ethanol 96% and ethyl acetate extracts. These flavonoids are known for their high antioxidant activity [17].

Phenol compounds were detected only in ethanol 96%, indicated by a change in color to bluish-black. Phenols found in Johar leaves include flavonoids, tannins, and

phenolic acids [18]. Steroid compounds were not detected in any of the extracts, while terpenoids were detected in all extracts, indicated by the formation of a brown ring. Terpenoids have anti-inflammatory properties that help reduce inflammation in the body [19]. The results of the phytochemical screening can be seen in Table 1.

Table 1. *The results of the phytochemical screening tests*

No.	Compounds Class	Reagent	Results		
			Ethanol 96% Extract	**Ethyl Acetate Extract**	**n-Hexane Extract**
1.	Alkaloids	Dragendorf	+	-	+
		Bouchard	+	+	-
		Mager	-	-	+
2.	Flavonoids	Mg + HCl	-	+	-
3.	Saponins	S+ aquadest	+	-	-
4.	Tannins	$FeCl_3$	+	-	-
5.	Phenols	Nacl 10% + $FeCl_3$ 1%	+	-	-
6.	Steroids	Lieberman-Bouchard	-	-	-
7.	Terpenoids	Lieberman-Bouchard	+	+	+

3.2 Specific Parameters

The purpose of testing the water-soluble extract and ethanol-soluble extract is to identify the active compounds present in ethanol or water based on the polarity of each solvent. This determination is carried out by dissolving the extract using solvents such as water or ethanol [10]. The test results indicate that the water-soluble extract content is 2% for n-hexane and ethyl acetate extracts, and 10% for ethanol extract. Meanwhile, the ethanol-soluble extract content is 5% for n-hexane extract, 6% for ethyl acetate extract, and 10% for ethanol extract. These results meet pharmacopoeial standards, although the extraction method used may be less than optimal [20]. The details can be seen in the following table.

Table 2. *Results of Specific Parameter Tests*

No	Name Extract	Water-Soluble Extract Content	Ethanol-Soluble Extract Content
1.	N-hexane Extract	2%	5%
2.	Ethyl acetate Extract	2%	6%
3.	Ethanol 96% Extract	10%	16%

3.3 Non-Specific Parameters

The results of the drying loss test show 0.95% for n-hexane extract, 0.62% for ethyl acetate extract, and 0.9% for ethanol extract. The purpose of this test is to determine the amount of substance lost during the drying process. The principle involves measuring the

remaining substance after drying at 105°C for 30 minutes. This test aims to determine the water content in the sample after drying. The material content is highly dependent on water content, where a thick extract should have a water content between 5% and 30%, a dry extract less than 10%, and a liquid extract more than 30%. The higher the water content in the material, the more susceptible the sample is to decay and damage due to microbial growth. Therefore, water content significantly affects the stability and quality of the extract and the formation of the extract preparation [21].

Table 3. *Results of Non-Specific Parameter Tests*

No	Name Extract	Drying Loss Result	Water Content Result
1.	N-hexane Extract	0,95%	0,11%
2.	Ethyl acetate Extract	0,62%	0,02%
3.	Ethanol 96% Extract	0,9%	0,36%

4. CONCLUSIONS

This study concludes that Johar leaf extract contains various secondary metabolite compounds that are consistent with the polarity of the solvents used. The non-specific parameter tests show results that meet the criteria, as do the specific parameter tests. This indicates that Johar leaf extract has good quality and meets the criteria established for both types of parameters.

5. ACKNOWLEDGEMENTS

Thanks to BAZNAS (Badan Zakat Amil Nasional), Indonesia, for providing general research funding support for this study.

6. REFERENCES

[1] Hanum, S., Sulaiman, M. I., & Martunis, M. (2018). Potensi Antioksidan Daun Johar (Cassia Seamea Lamk.). *Jurnal Ilmiah Mahasiswa Pertanian*, *3*(1), 401-408.

[2] Aprianti, H. (2006). Uji Toksisitas Akut Daun Johar (Cassia Siamea L.) Dengan Metode Brine Shrimps Lethality Test (Bst) Beserta Profil Kromatografi Lapis Tipis Fraksi Paling Aktif.

[3] Sumanto, S. E., & Takandjandji, M. (2014). Identifikasi Pemanfaatan Hasil Hutan Oleh Masyarakat: Upaya Konservasi Sumber Daya Genetik Dan Sosial Budaya. *Buletin Plasma Nutfah*, *20*(1).

[4] Soewandi, A. (1981) Isolasi Serta Penentuan Beberapa Sifat Kimia Dan Biokimia Suatu Komponen Daun Johar {Cassia Siamea, Lamk) Yang Berkhasiat Hipoglikemik.

[5] Fitriah, F., Mappiratu, M., & Prismawiryanti, P. (2017). Uji Aktivitas Antibakteri Ekstrak Daun Tanaman Johar (Cassia Siamea Lamk.) Dari Beberapa Tingkat Kepolaran Pelarut. *Kovalen: Jurnal Riset Kimia*, *3*(3), 242-251.

[6] Amirah, S., & Herman, H. (2015). Uji Efek Antiinflamasi Fraksi Kloroform Daun Johar (Cassia Siamea Lamk.) Dengan Metode Rat Hind Paw Edema. *As-Syifaa Jurnal Farmasi*, *7*(2), 182-189.

[7] Haslindha, N. N. (2022). *Uji Aktivitas Antibakteri Kombinasi Ekstrak Daun Miana (Coleus Athropurpureus L. Benth) Dan Daun Salam (Syzygium Polyanthum)*

Terhadap Bakteri Staphylococcus Aureus Dan Escherichia Coli Secara In Vitro (Doctoral Dissertation, Stikes Karya Putra Bangsa Tulungagung).

[8] Maryam, F., Taebe, B., & Toding, D. P. (2020). Pengukuran Parameter Spesifik Dan Non Spesifik Ekstrak Etanol Daun Matoa (Pometia Pinnata Jr & G. Forst). *Jurnal Mandala Pharmacon Indonesia*, *6*(01), 1-12.

[9] Isma, M. L. (2017). *Uji Aktivitas Fraksi Aktif Ekstrak Kulit Batang Garcinia Dioica Blume Terhadap Enzim Malate: Quinone Oxidoreductase Dari Plasmodium Falciparum (Pfmqo)* (Bachelor's Thesis, Uin Syarif Hidayatullah Jakarta: Fakultas Kedokteran Dan Ilmu Kesehatan, 2017).

[10] Rolita, D. Uji Aktivitas Larvasida Ekstrak Etanolik, Fraksi N-Heksana, Fraksi Etil Asetat, Dan Fraksi Air Biji Srikaya (Annona Squamosa L.) Terhadap Larva Nyamuk Aedes Albopictus Instar Iii.

[11] Elviana, E., Koapaha, T., & Lalujan, L. E. (2022). Antibacterial Activity Test Of Kecombrang Fruit (Etlingera Elatior) Extracted Using Multiple Solvents. *Jurnal Agroekoteknologi Terapan*, *3*(2), 287-294.

[12] Febriani, Y., Riasari, H., Winingsih, W., Aulifa, D. L., & Permatasari, A. (2018). The Potential Use Of Red Ginger (Zingiber Officinale Roscoe) Dregs As Analgesic. *Indonesian Journal Of Pharmaceutical Science And Technology*, *1*(1), 57-64.

[13] Febriani, Y., Riasari, H., Winingsih, W., Aulifa, D. L., & Permatasari, A. (2018). The Potential Use Of Red Ginger (Zingiber Officinale Roscoe) Dregs As Analgesic. *Indonesian Journal Of Pharmaceutical Science And Technology*, *1*(1), 57-64.

[14] Hidayah, N., Sumandiarsa, I. K., & Alqadiri, W. M. (2024). Kandungan Senyawa Fitokimia Dan Aktivitas Antifungal Ekstrak Padina Sp. Menggunakan Ultrasound Assisted Extraction Terhadap Aspergillus Flavus. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *27*(4), 297-308.

[15] Sulistyarini, I., Sari, D. A., & Wicaksono, T. A. (2020). Skrining Fitokimia Senyawa Metabolit Sekunder Batang Buah Naga (Hylocereus Polyrhizus). *Cendekia Eksakta*, *5*(1).

[16] Susmayanti, W., & Rahmadani, A. (2023). Uji Aktivitas Antioksidan Fraksi Daun Melinjo (Gnetum Gnenom L.) Menggunakan Metode Cuprac (Cupric Ion Reducing Antioxidant Capacity): Antioxidant Activity Of Fraction From Gnetum Gnenom L. Leaves Using Cuprac (Cupric Ion Reduxing Antioxidant Capacity) Methods. *Indonesian Journal Of Pharmacy And Natural Product*, *6*(01), 97-106.

[17] Mabruroh, A. I. (2015). *Uji Aktivitas Antioksidan Ekstrak Tanin Dari Daun Rumput Bambu (Lopatherum Gracile Brongn) Dan Identifikasinya* (Doctoral Dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).

[18] Septiani, N. K. A., Parwata, I. M. O. A., & Putra, A. A. B. (2018). Penentuan Kadar Total Fenol, Kadar Total Flavonoid, Dan Skrining Fitokimia Ekstrak Etanol Daun Gaharu (Gyrinops Versteegii). *Wahana Matematika Dan Sains: Jurnal Matematika, Sains, Dan Pembelajarannya*, *12*(1), 78-89.

[19] Heliawati, L. (2018). Kimia Organik Bahan Alam. *Universitas Pakuan Bogor*.

[20] Febrianti, D. R., Mahrita, M., Ariani, N., Putra, A. M. P., & Noorcahyati, N. (2019). Uji Kadar Sari Larut Air Dan Kadar Sari Larut Etanol Daun Kumpai Mahung (Eupathorium Inulifolium Hb &K). *Jurnal Pharmascience*, *6*(2), 19-24.

[21] Mewar, D., & As' Ad, M. F. (2023). Standarisasi Parameter Spesifik Dan Non Spesifik Ekstrak Etanol Daun Gatal (Laportea Decumana (Roxb.) Wedd) Sebagai Bahan Baku Obat Herbal Terstandar. *Jurnal Penelitian Kesehatan" Suara Forikes"(Journal Of Health Research" Forikes Voice")*, *14*(2), 266-270.