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Phytochemical screening and compound purification of n-hexane fraction of sulatri leaves (*Calophyllum soulattri* Burm F.)

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ABSTRACT

Background: *Calophyllum soulattri* Burm F. (sulatri) is widely utilized in traditional medicine. It is necessary to identify secondary metabolites from *C. soulattri* leaves to determine the pharmacologically active chemicals.

Objective: This study aimed to screen the phytochemical content and purify the n-hexane fraction of *C. soulattri* leaves from Banyumas, Indonesia.

Methods: The n-hexane fraction was macerated with methanol, followed by liquid-liquid fractionation with n-hexane. The n-hexane fraction was tested for flavonoids, triterpenoids/steroids, saponins, and phenols using the test tube method. In addition, the compounds were purified using column chromatography. The purified compound was identified by the Liebermann-Burchard reagent, which was compared with commercially available steroid drugs as reference.

Results: Phytochemical analysis revealed that the n-hexane fraction of *C. soulattri* leaves contained secondary metabolites such as flavonoid, steroid, and phenol compounds. Analyses with the Liebermann-Burchard reagent indicated that the purified compound was potentially a steroid.

Conclusion: The compound extracted from the n-hexane fraction of *C. soulattri* leaves was expected as a steroid.

Keywords: phytochemical screening, *Calophyllum soulattri*, n-hexane fraction

Introduction

Indonesia is a tropical country with a large variety of medicinal plants. The sulatri (*Calophyllum soulattri* Burm F.) grows in Indonesia and is generally practiced in daily life. Leaves, bark, seeds, and flowers have been utilized in traditional medicine. For instance, the leaf infusion is used to wash inflamed eyes. A decoction of the bark is employed to treat vaginal discharge and rheumatism. The seeds are often used to treat scabies,

ulcers, and hair growth [1]. A pharmacological effect in this plant is attributable to the presence of an active ingredient in the plant.

A study has shown that the South Kalimantan sulatri leaf contains flavonoids, triterpenoids, steroids, phenols, tannins, and saponins [2]. Another study discovered that an ethanolic extract of sulatri leaf from West Kalimantan lacked triterpenoid but contained the same secondary metabolite compounds [3]. In the leaves of sulatri from East Nusa Tenggara, additional secondary metabolites, including essential oils, lipids, steroids/triterpenoids, carotenoids, and tannins have been identified [1]. The ethanol extract, ethyl acetate, and n-hexane fraction of sulatri have also shown antifungal activity [4]. The sulatri leaf n-hexane extract from Banyumas was the most effective at inhibiting the growth of *Candida albicans*.

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The n-hexane fraction extracted from Banyumas sulatri leaves must be further purified to identify the components responsible for the activity. Consequently, it may be necessary to explore the phytochemical screening and purification of compounds contained in the n-hexane fraction of sulatri leaves from Banyumas, Central Java.

Methods

Preparation of leaves simplicia

C. soulattri Burm F. leaf samples were collected at Cilongok, Banyumas District, Central Java. After removing any remaining soil and debris, the samples were thoroughly cleaned under running water. The sample was then sun-dried using a black fabric cover in order to obtain simplicia. After drying, the simplicia was then ground into a powder.

Extraction and fractionation

As much as 8 kg of sulatri leaf simplicia powder (*C. soulattri* Burm F.) was immersed in 20 L of methanol solvent (ratio of powder and solvent was 1:5) during the extraction process. After immersing the sample for 24 hours at room temperature, the sample was remacerated five times using the same solvent, methanol. Using a vacuum rotary evaporator, the resulting extract was then concentrated until the thick extract was obtained [5]. The sulatri leaf methanolic extract with the weight of 1.3 kg and yielding 16.25 percentages, was obtained. Through a liquid-liquid extraction technique using n-hexane and aquadest as a solvent, the thick methanolic extract of sulatri leaves was subsequently fractionated to obtain the n-hexane fraction. A thick n-hexane fraction with weight of 176.28 grams with a yield value of 13.56 percentages were produced by partitioning a total of 1.3 kg of methanol thick extract using a ratio of n-hexane and aquadest of 1:1.

Phytochemical screening

Several phytochemical screening tests were carried out to determine the class of compounds contained in the sample including tests for flavonoids, terpenoids and steroids, saponins, and phenols [3]. The thick fraction of n-hexane from sulatri leaves was dissolved in methanol for the flavonoid compound group test prior to getting placed in a test tube. After adding concentrated hydrochloric acid solution and magnesium powder to the tube, the color changes were

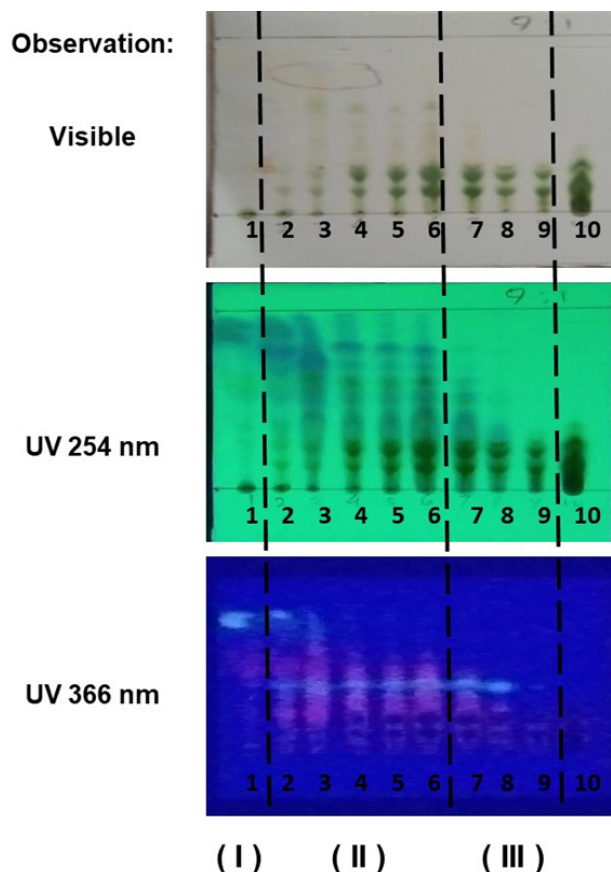


Figure 1. TLC profile from the first VLC with silica gel GF254 as a stationary phase with n-hexane: ethyl acetate (9:1) as a mobile phase

observed. By transferring the thick n-hexane fraction of sulatri leaves into a test tube and dissolving it with n-hexane, the terpenoid and steroid compounds were detected. The color change was observed after adding concentrated sulfuric acid and glacial acetic acid to the test tube. The procedure used to evaluate the saponin component class involved preparing a thick fraction of n-hexane from sulatri leaves that was then further dissolved in warm water before being loaded in a test tube. Ten ml of water was then added, cooled, and forcefully agitated until the foam was formed in the test tube. Afterward, the test for phenolic chemicals involved dissolving the thick fraction of n-hexane from sulatri leaves in methanol, pouring it in a test tube, and adding three drops of a 1% solution of iron (III) chloride reagent. Subsequent color changes were seen.

Purification and identification of isolates

Using vacuum liquid chromatography (VLC) and column gravity chromatography with n-hexane: ethyl

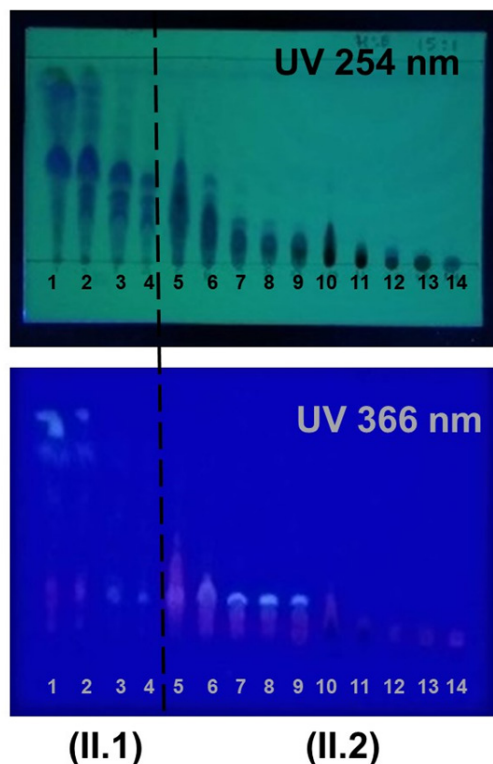


Figure 2. TLC profile from the second VLC with silica gel GF254 as a stationary phase and n-hexane: ethyl acetate (15:1) as a mobile phase

acetate as an eluent in a gradient mode (90 to 100 percent ethyl acetate), the compounds were separated from the thick n-hexane fraction of sulatri leaves. Up to 11 fractions were obtained as a result of the initial VLC's separation. Using n-hexane:ethyl acetate as an eluent in a ratio of 9:1, the fractions obtained from the VLC were investigated by thin layer chromatography (TLC) to achieve four primary subfractions, namely subfraction I (subfraction 1), II (mixture of subfraction 2-6), III (mixture of subfraction 7-9), and IV (subfraction 10), which are shown in Figure 1.

Subfraction II was separated again by VLC with silica gel G 60 F254 as the stationary phase, and gradient elution using mobile phase as follows n-hexane three times, n-hexane: ethyl acetate in a ratio of 15:1 (five times) and 9:2 (two times), ethyl acetate (2 times), and methanol as the final eluent to obtain 14 fractions. Following a second TLC analysis, the re-separated fraction was evaluated under 254 nm and 366 nm UV light. Based on the TLC analysis data from 14 obtained fractions, the fractions with the same chromatogram were combined to obtain two combined subfractions, namely combined subfraction II.1 (subfraction number 1-4) and combined subfraction II.2 (subfraction number 5-14) that were shown in Figure 2. In addition

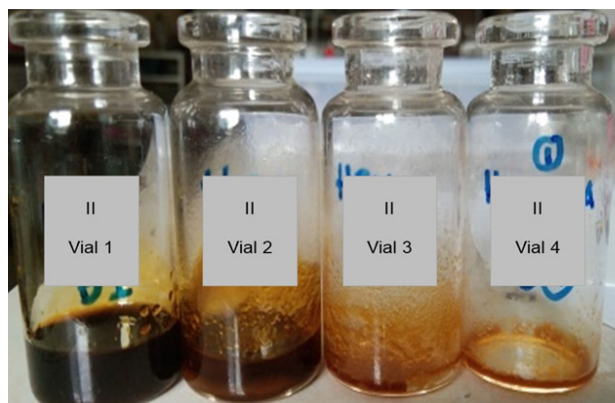


Figure 3. Crystals in the vials 1, 2, 3, and 4 from subfraction II after re-separated processes

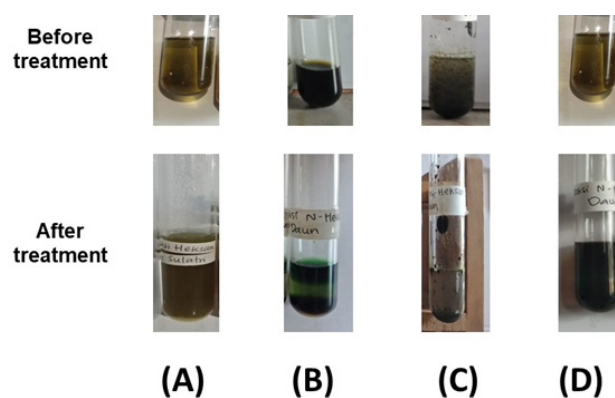


Figure 4. The results of the test of compound groups (A) flavonoids, (B) terpenoids/steroids, (C) saponins, (D) phenols

to the data from the TLC analysis, the combination of the obtained subfractions was also carried out based on the characteristics of the obtained fractions, namely the presence of crystals formed in the vial of combined subfraction II.1 (subfraction numbers 1, 2, 3 and 4) as shown in Figure 3. Subfraction II.1 was further separated using column chromatography with n-hexane as a mobile phase to obtain white crystalline isolates.

Results

A small sample of the n-hexane fraction of the sulatri leaf was taken, and the appropriate reagent was added in accordance with the compound to be identified. The flavonoid, steroid, and phenolic compounds were identified to be present in significant amounts in the phytochemical screening results from the n-hexane fraction of sulatri leaves (Figure 4).

The n-hexane fraction of sulatri leaf was purified by vacuum liquid chromatography and column gravity chromatography using the appropriate eluent. The

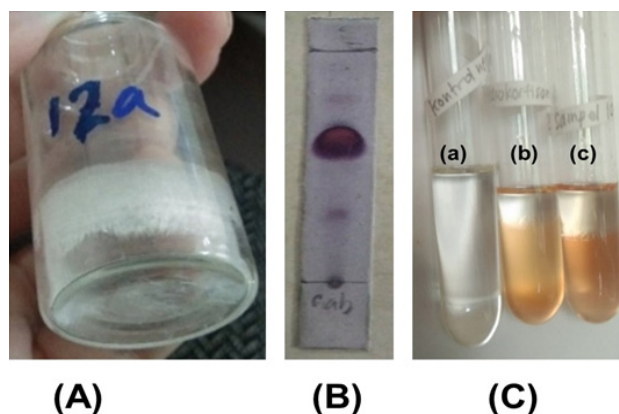


Figure 5. The results of the identification of purified compounds from fraction II.1. (A) Crystals of purified compounds, (B) TLC profile after adding anisaldehyde-hydrochloric acid spray reagent, (C) tube test results with Liebermann-Burchard reagent with (a) control solution, (b) sample solution, (c) standard solution

fractions were collected in a separate vial and then spotted on a TLC plate with the appropriate mobile phase under UV lamps at 254 nm and 366 nm. The purifying process yielded an isolate consisting of white crystals. Both TLC and test tubes were used to identify the isolates, which were compared to standards (Figure 5).

Discussion

In the flavonoid test, magnesium and concentrated hydrochloric acid were added to reduce the benzopyran in the flavonoid structure and produce red, yellow, and orange flavilium salts. The flavonoid test tube yielded positive results, as evidenced by a prominent yellow change (Figure 4A). It is consistent with prior research indicating the sulatri leaf extract contains flavonoids [3,6].

The Liebermann-Burchard method was used to test for steroids and triterpenoids. In a test tube, the thick part of n-hexane was mixed with n-hexane, and glacial acetic acid and concentrated sulfuric acid were added. The presence of a bluish-green ring suggested positive results on the steroid test. Some steroids, including b-sitosterol and stigmasterol, were identified in the sulatri leaf [3], which were also found in the root bark ethyl acetate extract and the bark n-hexane extract.

The presence of saponin was confirmed by a foam test, in which adding water and shaking the sample produced foam. The saponin test on the n-hexane fraction of sulatri leaf was negative because no foam was formed. Another study revealed that the ethanol extract of sulatri leaf contained saponin, indicating that saponin was more extracted using ethanol extracts,

which were more polar than those obtained with n-hexane solvent [3].

The purpose of the separation is to obtain a fraction that is more pure or devoid of undesired components, such as lipid and chlorophyll [9]. TLC analysis of the obtained crystal isolate, three spots were observed, suggesting that the obtained isolates were not completely pure (Figure 5B). When the three spots were observed under UV light at 254 nm and 366 nm, they did not fluoresce, indicating that the isolated compound's structure lacked a conjugated double bond. Followed by the addition of the anisaldehyde-hydrochloric acid sprayed reagent, TLC results revealed a purple.

The isolates were then compared with one of the commercially available steroid-derived substances, hydrocortisone cream. The addition of Liebermann-Burchard reagent changed the color of both sample solutions, making both a similar color of reddish-brown (Figure 5C). Steroid compounds contain hydroxyl and methyl groups and no conjugated double bonds as their main structural features [10]. In addition, steroid molecules have several functions, including antifungal properties [11].

Conclusion

Secondary metabolites of flavonoids, steroids, and phenols were found in the n-hexane fraction of the sulatri leaf. This study's also purified substance that was expected as a steroid compound.

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Author contributions

S and MSF developed the research method; AY assisted with data collecting; AY and S wrote the first script; S, MSF, AY, HW, and THW contributed to data analysis and data visualization; and all authors contributed to data interpretation, manuscript editing, and manuscript approval.

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References

1. Sulianti SB, Kuncari ES, Chairul SM. Phytopharmaceuticals and phytochemicals evaluation on leaves and bark of *Calophyllum inophyllum* and *Calophyllum soulatri*. *Biodiversitas*. 2006;7. <https://doi.org/10.13057/biodiv/d070108>
2. Violet V. Identifikasi pemanfaatan tradisional dan penapisan senyawa fitokimia ekstrak daun bintangur (*Calophyllum soulatri* Burm F.). *es*. 2018;14: 70. <https://doi.org/10.20527/es.v14i1.4896>
3. Fajriaty I, Hariyanto IH, Andres A, Setyaningrum R. Skrining fitokimia dan analisis kromatografi lapis tipis dari ekstrak etanol daun bintangur (*Calophyllum soulatri* Burm. F.). *Jurnal Pendidikan Informatika dan Sains*. 2018.
4. Gilchrist RE. Profil Kromatografi Lapis Tipis Ekstrak Etanol, Etil Asetat, dan N-Heksan Daun Slatratri (*Calophyllum Soulatri* Burm. F.) dan Aktivitas pada *Candida albicans* - Repository Universitas Jenderal Soedirman [Internet]. 2017 [cited 19 May 2022]. Available: <http://repository.unsoed.ac.id/6542/>
5. Jang DS, Han A-R, Park G, Jhon G-J, Seo E-K. Flavonoids and aromatic compounds from the rhizomes of *Zingiber zerumbet*. *Arch Pharm Res*. 2004;27: 386-389. <https://doi.org/10.1007/BF02980078>
6. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of *Calophyllum soulatri*. *Fitoterapia*. 2002;73: 741-743. [https://doi.org/10.1016/S0367-326X\(02\)00242-3](https://doi.org/10.1016/S0367-326X(02)00242-3)
7. Mah SH, Ee GCL, Teh SS, Rahmani M, Lim YM, Go R. Phylatrin, a new cytotoxic xanthone from *Calophyllum soulatri*. *Molecules*. 2012;17: 8303-8311. <https://doi.org/10.3390/molecules17078303>
8. Saputra DE, Handayani N, Wartono MW. Isolation and identification of beta-sitosterol and stigmasterol mixture from root bark of sulatri (*Calophyllum soulatri* Burm. F.). *ALCHEMY JPenKim*. 2018;10: 87. <https://doi.org/10.20961/alchemy.10.1.14.87-93>
9. Malik A, Ahmad AR, Najib A. Pengujian aktivitas antioksidan ekstrak terpurifikasi daun teh hijau dan jati belanda. *IJPF*. 2017;4: 238-240. <https://doi.org/10.33096/jffi.v4i2.267>
10. Suryelita S, Etika SB, Kurnia NS. Isolasi dan karakterisasi senyawa steroid dari daun cemara natal (*Cupressus funebris* Endl.). *eksakta*. 2017;18: 86-94. <https://doi.org/10.24036/eksakta/vol18-iss01/23>
11. Masendra M, Purba BAV, Lukmandaru G. Antifungal Activity of Triterpenoids and Steroids Isolated from *Pinus merkusii* Bark Against *Phanerochaete chrysosporium*. *Wood Res J*. 2021;11: 65-71. <https://doi.org/10.51850/wrj.2020.11.2.65-71>