Phytochemical screening of *kaempferia galanga* l. Rhizome in Banguntapan, Bantul, yogyakarta

Muhammad Fadhiel Ihsan[1*], Reza Anindita[2], Intan Kurnia Putri[3], Noor Andryan Ilsan[4]
[1,2,3] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
[4]Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : muhammadfadhielihsan@gmail.com

Abstract

The rhizome of *Kaempferia galanga* L. is a medicinal plant with many benefits for human health. *Kaempferia galanga* L. rhizome extract has long been used in traditional medicine because it contains active compounds that have potential as medicinal ingredients. The place where *Kaempferia galanga* L. rhizomes are taken can affect the compounds contained therein. This research aimed to determine the secondary metabolites in *Kaempferia galanga* L. rhizomes harvested in Banguntapan, Bantul Regency, Yogyakarta. This type of research is qualitative and quantitative. The research design is experimental. The method used for extraction is maceration using 96% ethanol solvent. Phytochemical screening for alkaloids, phenols, flavonoids, essential oils, and tannins is carried out by adding color reagents and observing color changes in the ethanol extract of *Kaempferia galanga* L rhizomes. The data analysis used in this research is descriptive by interpreting the results of the tables presented in this research. The percentage yield from ethanol extraction of *Kaempferia galanga* L. rhizomes was 14.89%. The phytochemical screening test of the ethanol extract of *Kaempferia galanga* L. rhizomes showed that the test results with Mayer's reagent were positive for alkaloids (white precipitate), positive for FeCl3 for phenols and tannins (blackish green), concentrated HCl and Mg positive for flavonoids (orange), cheerful for anisaldehyde-sulphuric acid solution for essential oil (red). Screening results using Dragendorff and Wagner reagents produced negative alkaloids (no orange and brown precipitates). Based on the results of this research conclude that the ethanol extract of *Kaempferia galanga* L. from the Banguntapan area, Bantul Regency, Yogyakarta, positively had phytochemical contents such as alkaloids, phenols, flavonoids, essential oils, and tannins.

Keywords: banguntapan, ethanol, *Kaempferia galanga* L. rhizome, maceration, phytochemical screening.

Introduction

Indonesia is a country with a tropical climate that is rich in various types of plants, many of which are currently used as natural medicinal ingredients. One of the plants used as a natural medicine today is the rhizome of *Kaempferia galanga* L. The rhizome of *Kaempferia galanga* L. is an aromatic plant originating from India and cultivated, one of which is in Java (Subaryanti *et al*., 2020). Many benefits of the rhizome of *Kaempferia galanga* L. are often widely used by the public as herbal medicine and it is known that *Kaempferia galanga* L. rhizomes have analgesic, antibacterial, antidiarrheal, antioxidant, and anti-inflammatory effects (Cahyawati, 2020). The rhizome of *Kaempferia galanga* L. has 49 secondary metabolite components such as esters, terpenoids, steroids, tannins, flavonoids, polyphenols, sesquiterpenes, and monoterpenes which are mostly contained in its essential oil (Adianingsih *et al*., 2021).

The biochemical content of the rhizome of *Kaempferia galanga* L. depends on the cultivation method, the growing environment, and the variety so there may be differences in the biochemical content of *Kaempferia galanga* L. rhizomes from different regions (Utami *et al*., 2020). Central Java is the center of *Kaempferia galanga* L. rhizome cultivation, with production data obtained in 2018 by the Indonesian Central Bureau of Statistics of 8,759 tons per year (Adianingsih *et al*., 2021). The purpose of this study was to analyze the secondary metabolites contained in the ethanol extract of *Kaempferia galanga* L., which comes from the Banguntapan area, Bantul, Yogyakarta.

Methods

Sample preparation

This type of research is qualitative and quantitative, with the research design being experimental. The sample used was the rhizome of *Kaempferia galanga* L. 8-9 months old, harvested during the dry season in the Banguntapan area, Bantul Regency, Yogyakarta. The research was conducted at the STIKes Mitra Keluarga Phytochemical Laboratory.

Kaempferia galanga L. rhizome samples of as much as 5 kg of wet sorting were carried out by separating the rhizomes of *Kaempferia galanga* L. good with the rhizome of *Kaempferia galanga* L., which is rotten or damaged. Leaves, roots, and stems of *Kaempferia galanga* L. were cut with a knife and then washed (Hapsoh *et al*., 2010). The samples were chopped on *Kaempferia galanga* L. with a thickness of 3-4 mm. Then, the samples were dried in the hot sun for 4 days and covered with black cloth to avoid direct contact with sunlight. After that, drying is continued in the

oven for 2 days at 45˚C (Anggraini & Saputri, 2021). Dry sorting results were performed to select damaged or defective *Kaempferia galanga* L. rhizomes, such as charred, damaged rhizomes, or rhizomes that were not yet dry. The dry sorting results were pollinated using a blender, after which they were sifted using a mesh number 20 sieve (Azkiya *et al*., 2017).

Extraction

Simplisia rhizome of *Kaempferia galanga* L. as much as 200 grams dissolved in 1000 ml of 96% ethanol in a suitable airtight bottle, protected from sunlight. Maceration was carried out for 5 days and shaken every day, then continued with remaceration for 3 days. The results of maceration and remaceration are filtered using filter paper and stored in a brown bottle to avoid sunlight (Amelinda *et al*., 2018; Fajeriyati & Andika, 2017). The extraction results were then evaporated using a rotary evaporator to obtain a thick ethanol extract of *Kaempferia galanga* L. with a temperature setting of 48˚C, a pressure of 300 mbar, and a rotational speed of 90 rpm.

Phytochemical Screening

Phytochemical screening in this study was carried out by adding coloring reagents. In the phenolic compounds, the thick extract of *Kaempferia galanga* L. (0.5 gram) was added with FeCl3 (3-4 drops). A positive result for phenolic compounds will show a color change from bluish-black to dark-black. In the tannin compound, the extract (0.5 gram) is added to 10 mL of hot water and dripped with $FeCl_3$. A positive result will show a color change to black-green (Ningsih *et al*., 2020). Flavonoid compounds were screened by placing the extract (0.5 gram) in a test tube, adding 5 ml of ethanol, heating for 5 minutes, then adding 10 drops of concentrated HCl and 0.2 g of Magnesium powder. A positive result for flavonoids will show a color change to red-brown. In the alkaloid compound, the extract (0.5 gram) was put into a test tube along with 2 ml of chloroform and 10 ml of ammonia. The solution was then added 10 drops of $H2SO_4$ and shaken, then allowed to stand. The solution will be separated into 2 layers, then the $H2SO_4$ layer is transferred into 3 test tubes of 2.5 ml each and tested with Mayer, Dragendorff, and Wagner reagents. Positive results are indicated by a white precipitate in the Mayer reagent, a red-orange precipitate in the Dragendorff reagent, and a brown precipitate in the Wagner reagent (Rante *et al*., 2020). Screening of essential oil compounds was carried out by adding anisaldehyde-sulfuric acid to the extract (0.5 gram). Positive results of essential oils can be seen in visible light by producing blue, green, red, or brown colors, and some fluoresce in ultraviolet light with a wavelength of 366 nm (Hanani, 2016).

Data analysis

The data collected in this study is primary data from observations of the results of phytochemical screening tests. The data obtained is qualitative in the form of changes in sample color with positive or negative results. The resulting data from this study were processed and presented systematically in tables and figures and then described to determine the secondary metabolites in the ethanol extract of *Kaempferia galanga* L. rhizomes.

Results and Discussions

The extract yield calculation was carried out to determine the amount obtained by comparing the weight of the thick extract produced with the weight of the initial powder. The results of calculating the yield of *Kaempferia galanga* L. rhizome extract with a powder weight of 929 g and an extract weight of 138.32 g found that the extract yield percentage was 14.89%, which met the requirements set out in the Farmakope Herbal Indonesia, namely > 8.3 %. Phytochemical screening in this study was conducted to provide an overview of the secondary metabolite compounds contained in *Kaempferia galanga* L. rhizome extract. In this study, phytochemical screening was carried out qualitatively.

Table 1. Phytochemical Screening

No.	Compound	Reagent	Results	Information
1.	Alkaloids	Mayer	+	There is a white precipitate
		Dragendorf	-	The solution is not orange
		Wagner	-	The solution is not brown
2.	Phenol	$FeCl_3$	+	The solution is blackish-green
3.	Flavonoid	Concentrated HCl + Mg	+	Orange colored solution

	s			
4.	Essential oil	Anisaldehyde-sulfuric acid solution	+	Red in visible light
5.	Tannin	$FeCl_3$	+	The solution is blackish-green

Based on the results of the table above, the results of screening the ethanol extract of the rhizome of *Kaempferia galanga* L. with Mayer's reagent were positive for alkaloids (white precipitate), positive for $FeCl_3$ for phenol and tannin (blackish green), concentrated HCl and Mg positive for flavonoids (orange), anisaldehyde- positive sulfuric acid containing essential oil (red). Meanwhile, screening with Dragendorff and Wagner reagents produced negative alkaloids (no orange and brown precipitates).

Phytochemical screening is a qualitative preliminary test by observing the color reaction in *Kaempferia galanga* L. extract by adding specific reagents. The color change in the extract is caused by a chemical reaction between the compound detected and the reagent used (*Saputri*, 2021). Phytochemical screening in this study aims to identify or provide an overview of secondary metabolites in *Kaempferia galanga* L. rhizome extract with 96% ethanol solvent. The secondary metabolites identified include alkaloids, phenols, flavonoids, essential oils, and tannins.

Phytochemical screening in this study found that the ethanol extract of *Kaempferia galanga* L. rhizome positively contained alkaloids, phenols, flavonoids, essential oils, and tannins. Phytochemical screening of alkaloid compounds in this study used n-ammonia-chloroform to extract the sample which was then filtered and added H2SO4 acid to salt the alkaloids because alkaline alkaloids can dissolve in acidic solvents (Indarto, 2015; Puspa *et al*., 2017). After that, shake it so that two layers form. The top layer, the water phase, was tested with Dragendorff, Meyer, and Wagner reagents. These three tests showed positive results in the Meyer test with the formation of a white precipitate and negative results for the Dragendorff and Wagner tests. Based on Seniwaty *et al.* (2009) and Soniman *et al.* (2022), the reagent used is adjusted to the alkaloid's ability to combine with metals that have a high atomic weight, such as mercury, bismuth, tungsten, or iodine, in this case, mercury.

This phytochemical screening test for alkaloid compounds is based on the principle of a precipitation reaction that occurs by replacing a ligand. Alkaloids having a nitrogen atom with a lone pair of electrons can replace the iodo ion in the reagent. Dragendorff's reagent contains bismuth nitrate and potassium iodide, a solution of glacial acetic acid (potassium tetraiodobismutat (III)). Mayer's reagent contains potassium iodide and mercuric chloride (tetraiodomercurate (II)). Wagner's reagent contains potassium iodide (KI) and iodine (I_2), both of which react to produce brown I_3-. This precipitate is formed from a potassium-alkaloid complex, in which K+ ions form coordinate covalent bonds with nitrogen in the alkaloids (Julianto, 2016; Reiza *et al*., 2019).

Phytochemical screening for phenol and tannin compounds in this study was carried out using FeCl3 reagent, and positive results were found to be blackish green. FeCl3 reagent is widely used to identify polyphenolic compounds, including tannins. Therefore, other phenolic compounds in the extract can also give positive results. The color change is caused by FeC13 reacting with one of the hydroxyl groups in polyphenolic compounds (Sangi *et al*., 2008).

Phytochemical screening for flavonoid compounds in this study used the Wilstatter test method by adding magnesium powder and concentrated HCl to reduce the flavonoid compounds contained in the extract. The results of this test found a change in color to orange, which means it is positive for containing flavonoids. The cyanidin Wilstater test commonly detect compounds with an α-benzopyron nucleus (Julianto, 2016). The results of the phytochemical screening were also strengthened by research conducted by Hayati *et al*. (2015), which found that the phytochemical screening results were orange in color, and further identification using UV-Vis spectrophotometry suggested that the flavonoid compounds belonged to the flavonone or dihydro flavonol group.

Phytochemical screening of essential oil compounds in this study used anisaldehyde-sulfuric acid solution as a reagent and found positive results with a color change to red. *Sulfuric acid anisaldehyde* is a universal reagent used in identifying chemical compound classes of essential oil components. Sulfuric acid anisaldehyde reagent will change the color of the monoterpene alcohol and its ester compounds, cineol, citral, aldehyde, and citronellal. The color change to red is thought to be caused by the reaction of steroid/terpenoid compounds with anisaldehyde-sulfate reagent (Christiana & Soegianto, 2020; Syarifuddin & Sulistyani, 2019)

Conclusions

In summary, this study found that the presence of phytochemical contents such as alkaloids, phenols, flavonoids, essential oils, and tannins was detected in the ethanolic extract of *Kaempferia galanga* L by using the reagent test for each type of metabolites. According to the results, *Kaempferia galanga* L. has an antioxidant and antibacterial activity that helps to detect the bioactive principles and then develop products and medicines made from herbs, especially by using *Kaempferia galanga* L. Further studies should be conducted by doing extensive research and more detailed such as antibacterial and antioxidant tests using the ethanolic extract of *Kaempferia galanga* L.

References

Adianingsih, O. R., Widaryanto, E., Saitama, A., & Zaini, A. H. (2021). Analysis of bioactive compounds present in Kaempferia galanga rhizome collected from different regions of East Java, Indonesia. *IOP Conference Series: Earth and Environmental Science*, *913*(1). https://doi.org/10.1088/1755-1315/913/1/012074

Amelinda, E., Widarta, I. W. R., & Darmayanti, L. P. T. (2018). Pengaruh Waktu Maserasi Terhadap Aktivitas Antioksidan Ekstrak Rimpang Temulawak (Curcuma xanthorriza Roxb.). *Jurnal Ilmu Dan Teknologi Pangan (ITEPA)*, *7*(4), 165. https://doi.org/10.24843/itepa.2018.v07.i04.p03

Anggraini, M., & Saputri, A. R. G. (2021). Comparison of Moisture Content and Condition of Essensial Oil on Rhizome Kencur (Kaempferia galangal L.) With Different Drying Treatment. *Jurnal Analis Farmasi*, *6*(2), 79–83.

Azkiya, Z., Ariyani, H., & Nugraha, T. S. (2017). Evaluasi Sifat Fisik Krim Ekstrak Jahe Merah (Zingiber officinale Rosc. var. rubrum) Sebagai Anti Nyeri. *Journal of Current Pharmaceutica Sciences*, *1*(1), 2598–2095.

Cahyawati, P. N. (2020). Efek Analgetik dan Antiinflamasi Kaempferia Galanga (Kencur). *WICAKSANA: Jurnal Lingkungan Dan Pembangunan*, *4*(1), 15–19. https://doi.org/10.22225/wicaksana.4.1.1811.15-19

Christiana, I., & Soegianto, L. (2020). Skrining senyawa antibakteri dari minyak atsiri rimpang temu kunci (Boesenbergia pandurata) terhadap Staphylococcus aureus dengan metode bioautografi kontak. *Jurnal Farmasi Sains Dan Terapan*, *7*(1), 15–19. http://journal.wima.ac.id/index.php/JFST/article/view/2391

Fajeriyati, N., & Andika. (2017). Uji Aktivitas Antibakteri Ekstrak Etanol Rimpang Kencur (Kaempferia galanga L.) Pada Bakteri Bacillus subtilis dan Escherichia coli. *Journal of Current Pharmaceutical Sciences*, *1*(1), 36–41. journal.umbjm.ac.id/index.php/jcps

Hanani, E. (2016). *Analisis Fitokimia* (T. V. D. Hadinata (ed.)). Penerbit Buku Kedokteran EGC.

Hapsoh, Hasanah, Y., & Julianti, E. (2010). Budidaya dan Teknologi Pasca Panen Jahe. In *USU Press medan* (Vol. 3).

Hayati, E. K., Ningsih, R., & Latifah, L. (2015). Antioxidant Activity of Flavonoid from Rhizome Kaemferia galanga L. Extract. *Jurnal of Chemistry*, *4*(2), 127–137. https://doi.org/10.18860/al.v4i2.3203

Indarto. (2015). Uji Kualitatif Dan Kuantitatif Golongan Senyawa Organik Dari Kulit Dan Kayu Batang Tumbuhan Artocarpus Dadah Miq. *Jurnal Ilmiah Pendidikan Fisika Al-Biruni*, *4*(1), 75–84. https://doi.org/10.24042/jpifalbiruni.v4i1.82

Julianto, T. S. (2016). Analisis Fitokimia. *Jakarta Penerbit Buku Kedokteran EGC*, 58.

Puspa, O. E., Syahbanu, I., & Wibowo, M. A. (2017). Uji Fitokimia dan Toksisitas Minyak Atsiri Daun Pala (Myristica fragans Houtt) Dari Pulau Lemukutan. *Jurnal Kimia Khatulistiwa*, *6*(2), 1–6.

Rante, T. R. K., Simbala, H. E. I., & Mansauda, K. L. R. (2020). Skrining Fitokimia dan Potensi Antioksidan Dari Ekstrak Daun Tumbuhan Ekor Tikus (Stachytarpheta jamaicensis L) Dengan Metode 1.1 diphenyl-2-picrylhydracyl (Dpph). *Jurnal MIPA*, *9*(2), 91. https://doi.org/10.35799/jmuo.9.2.2020.29000

Reiza, I. A., Rijai, L., & Mahmudah, F. (2019). Skrining Fitokimia Ekstrak Etanol Kulit Nanas (Ananas comosus (L.) Merr). *Proceeding of Mulawarman Pharmaceuticals Conferences*, *10*, 104–108. https://doi.org/10.25026/mpc.v10i1.371

Sangi, M., Runtuwene, M. R. J., & Simbala, H. E. I. (2008). Analisa Fitokimia Obat di Kabupaten Minahasa Utara. *Chemistry Progres*, *1*(1), 47–53.

Saputri, F. L. (2021). *Uji Fitokimia Ekstrak Kencur (Kaempferia Galanga L.) Efektif Menurunkan Jumlah Koloni Candida Albicans Pada Basis Resin Akrilik Polimerisasi Panas: Literature Review*. Universitas Muhammadiyah Semarang.

Septia Ningsih, D., Henri, H., Roanisca, O., & Gus Mahardika, R. (2020). Skrining Fitokimia dan Penetapan Kandungan Total Fenolik Ekstrak Daun Tumbuhan Sapu-Sapu (Baeckea frutescens L.). *Biotropika: Journal of Tropical Biology*, *8*(3), 178–185. https://doi.org/10.21776/ub.biotropika.2020.008.03.06

Soniman, M., Syaputra, D., & Kurniawan, A. (2022). Efektivitas Senyawa Aktif Kombinasi Kencur Kaempera galanga dan Ilalang Imperata cylindrica Secara In Vitro terhadap Bakteri Gram Positif dan Bakteri Gram Negatif. *Journal of Aquatropica Asia*, *7*(1), 19–33.

Subaryanti, Sulistyaningsih, Y. C., Iswantini, D., & Triadiati, T. (2020). The Growth and Production of Galanga (Kaempferia galanga L.) in Different Altitudes. *Jurnal Ilmu Pertanian Indonesia*, *25*(2), 167–177. https://doi.org/10.18343/jipi.25.2.167

Syarifuddin, A., & Sulistyani, N. (2019). Karakterisasi Fraksi Teraktif Senyawa Antibiotik Isolat KP 13 dengan Metode Densitometri dan KLT-Semprot. *Jurnal Ilmiah Ibnu Sina*, *4*, 156–166.

Utami, L. P., Tandean, P. G., & Liliawanti, L. (2020). Pengaruh Pemberian Ekstrak Kencur (Kaempferia galanga L.) terhadap Peningkatan Zona Hambat Pertumbuhan Bakteri Staphylococcus aureus. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, *9*(2), 145. https://doi.org/10.30742/jikw.v9i2.883