

JURNAL BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejournal.brin.go.id/JBBI/index

MICROBIAL CONTAMINATION AND BIOACTIVE COMPOUNDS OF JAMU BERAS KENCUR

Wani Devita Gunardi1*, Virginia Marsella Teiseran¹ , Kris Herawan Timotius1,2

¹Department of Microbiology, ²Center for Jamu and Herbal Medicine, Faculty of Medicine and Health Sciences, Krida Wacana Christian University (UKRIDA), Jakarta, Indonesia [*Email:](mailto:penulis_pertama@address.com) wani.gunardi@ukrida.ac.id

ABSTRACT

Background: Jamu Beras Kencur (JBK) is recognized as an herbal medicine, containing two main components: the rhizome of *Kaempferia galanga* and rice. While previous research has identified bioactive compounds in galangal rhizomes, such as Ethyl p-Methoxycinnamate (EPMC), Ethyl-cinnamate, and Kaempferol, there are few reports on polar or aqueous compounds in JBK. LC-MS/MS and GC-MS enable comprehensive analysis of bioactive compounds, with LC-MS/MS detecting non-volatile, polar, and thermally sensitive compounds like flavonoids and glycosides, while GC-MS analyzes volatile and semi-volatile compounds, such as terpenoids, providing precise separation and identification. Therefore, this study were to know the amount and the growth of contaminant bacteria, yeast and mold; to determine the main bioactive compounds in JBK; and to determine the bioactive compound in aqueous and ethanolic extracts of rhizome that analysed with LC-MS/MS and GC-MS. **Methode**: JBK samples were sourced from local producers in West Jakarta, freshly prepared, and immediately analyzed for microbial contamination and bioactive compounds. **Result**: The analysis revealed microbial contamination in JBK, including *Escherichia coli*, *Staphylococcus aureus*, Coliform, yeast, and mold. Additionally, three novel flavonoid glycosides were identified: Chrysoeriol-4'-O-β-D-glucopyranoside, Patuletin-7-O-[6′′-(2-methylbutyryl)]-glucoside, and Acacetin-7-galactoside. **Conclusion**: Therefore, from the pharmacological perspective, JBK has the potentials as a healthy herbal drink. However, further preclinical and clinical studies are essential to validate its safety and efficacy for clinical use, which could pave the way for its integration into mainstream healthcare as a natural therapeutic option.

Keywords: Flavonoid, GC-MS, Jamu beras kencur, Kaempferia galangal, LC-MS/MS

ABSTRAK

Latar Belakang: Jamu Beras Kencur (JBK) adalah obat herbal yang terbuat dari rimpang *Kaempferia galanga* dan beras. Meskipun studi sebelumnya telah mengidentifikasi senyawa bioaktif dalam galanga, seperti Ethyl p-Methoxycinnamate (EPMC), Ethylcinnamate, dan Kaempferol, informasi mengenai senyawa polar atau akua dalam JBK masih terbatas. LC-MS/MS dan GC-MS memungkinkan analisis komprehensif senyawa bioaktif; LC-MS/MS mendeteksi senyawa non-volatil, polar, dan sensitif terhadap panas seperti flavonoid dan glikosida, sementara GC-MS menganalisis senyawa volatil dan semi-volatil, seperti terpenoid. Penelitian ini bertujuan untuk menilai tingkat dan pertumbuhan bakteri, ragi, dan jamur kontaminan dalam JBK, mengidentifikasi senyawa bioaktif utamanya, serta menganalisis ekstrak akua dan etanol dari rimpang menggunakan LC-MS/MS dan GC-MS. **Metode**: Sampel JBK diperoleh dari produsen lokal di Jakarta Barat, disiapkan segar, dan segera dianalisis untuk kontaminasi mikroba dan senyawa

bioaktif. **Hasil**: Analisis mengungkapkan kontaminasi mikroba dalam JBK, termasuk *Escherichia coli*, *Staphylococcus aureus*, koliform, ragi, dan jamur. Selain itu, tiga glikosida flavonoid baru diidentifikasi: Chrysoeriol-4'-O-β-D-glucopyranoside, Patuletin-7-O-[6′′-(2-methylbutyryl)]-glucoside, dan Acacetin-7-galactoside. **Kesimpulan**: Dari perspektif farmakologis, JBK memiliki potensi sebagai minuman herbal yang sehat, tetapi studi praklinis dan klinis lebih lanjut diperlukan untuk memvalidasi keamanan dan efektivitasnya untuk penggunaan klinis.

Kata kunci: *Flavonoid, GC-MS, Jamu beras kencur, Kaempferia galangal, LC-MS/MS*

INTRODUCTION

Jamu is a traditional herbal formula medicine that has been practised for many centuries in the Indonesian community to maintain good health and to treat diseases(Elfahmi et al., 2014). As one of the famous jamu, jamu beras kencur (JBK, galanga-rice jamu) is principally made from two main components, a rhizome of *Kaempferia galanga* and rice. Additional components such as brown sugar, cinnamon and ginger are often added. JBK is good for hypertension, pectoral and abdominal pains, headache, toothache, rheumatism, dyspepsia, coughs, and inflammatory tumour(Wang FangLin et al., 2013). JBK is well known for its antioxidant, antimicrobial, analgesic, anti-inflammatory, sedative, vasorelaxant, nematocidal, mosquito repellent, larvicidal, antiprotozoal and wound healing activities(Nag & Mandal, 2015). It is also good to help with restlessness, stress, anxiety, and depression(Wang FangLin et al., 2013). *K. galanga* is also known for its induction to the activity of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) as an index of thermogenesis(Nishidono et al., 2017).

Previous has research reported the bioactive compounds from the rhizome of galanga, such as Ethyl p-Methoxycinnamate (EPMC), Ethyl-cinnamate, Kaempferol(Nag & Mandal, 2015). Most of the reported compounds can be extracted with organic solvents, either non or semi-polar solvents. However there are few reports on the polar or aqueous compounds found in JBK.

The use of LC-MS/MS and GC-MS for analyzing bioactive compounds is based on their complementary strengths. LC-MS/MS is particularly effective for detecting non-volatile, polar, and thermally sensitive compounds, such as flavonoids and glycosides, with high sensitivity and precision(Pitt, 2009). On the other hand, GC-MS is ideal for analyzing volatile and semi-volatile compounds, such as terpenoids, providing excellent separation and identification, supported by extensive spectral libraries(Gomathi et al., 2015). These techniques together enable a comprehensive analysis of a wide range of bioactive compounds in complex mixtures.

The objectives of this study were to assess the amount and growth of contaminant bacteria, yeast, and mold; to identify the main bioactive compounds in jamu beras kencur; and to determine the bioactive compounds in aqueous and ethanolic extracts of the rhizome, analyzed using LC-MS/MS and GC-MS.

MATERIAL AND METHOD

Jamu Beras Kencur (JBK)

JBK was obtained directly from the local producers/sellers in West Jakarta (Figure 1). The samples were freshly prepared and directly counted for the contaminated microbe and for the GC-MS and LC-MS/MS analyses (Figure 2). After two days of storage in room temperature, the contaminated microbe in JBK was recounted. For the aqueous and ethanolic extracts, the rhizomes were obtained from the same producer/sellers.

Figure 1. Traditional carrier of jamu gendong (left) and a glass of "ready to drink" jamu beras kencur (right)

Microbial counting (CFU) with 3M Petrifilm

The JBK was first filtered using sterile cooking shovels strainers to eliminate the fibers and residues of the herbal drink. Then the JBK was diluted in Butterfield's Phosphat dilluent at various dilution factors. Each diluted sample was taken 1 mL and inoculated to Petrifilms-3M for total bacteria, Coliform, Staphylococcus, yeast and mold according to the instruction given by Petrifilm-3M. The incubation was at $37^\circ \pm 1^\circ$ C. The incubation period for *Staphylococcus aureus* was 24 ± 2 hours, for Coliform was 48 ± 3 hours, for *Escherichia coli* was 24 ± 2 hours, and for yeast and mold were 72-120 hours. After incubation, the CFU was counted. The before and after incubation, the CFU was counted. All measurements are triplicates**.**

LC-MS/MS analysis

Mariner Biospectrometry was used for the LC-MS/MS analysis. It was equipped with a binary pump (Hitachi L 6200). The equipment was interfaced with a Q-tof mass spectrometer fitted with an ESI (Electrospray Ionisation) source with positive and negative ion mode. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140 °C. Shimp-pack C8, 150 \times 6 mm i.d., was used as a column. The solvent was methanol with 0.3% formic acid and delivered at a total flow rate of 1 ml/min. A 5 ul sample was injected and eluted isocratically.

GC-MS analysis

GC-MS was carried out on a GCMS-QP20105 SHIMADZU for the ethanolic extract of rhizome of *K. galanga*. The electron ionisation energy was 70 Ev Ion source temperature was 250°C and the interface temperature 305^oC. An Abdel 5MS column (30 m x 0.25 mm i.d.and 0.25 μm film thickness) was used. The oven temperature was programmed from 60° C –300 $^{\circ}$ C at 10 $^{\circ}$ C C/min. Data acquisition was performed with software for the mass ranges 28 – 600 amu with a scan speed of 1250. Carrier gas helium was used at the rate of 0.51 mL/min. Injection temp 300^oC. Injection mode was splitless.

Figure 2. Research flowchart

Microbial Contamination and Bioactive Compounds… Gunardi et al.

RESULT AND DISCUSSION

Microbial contamination of jamu beras kencur

After two days incubation of JBK at room temperature, the CFU number of coliforms, *Escherichia coli*, Pseudomonas,

Staphylococcus aureus, yeasts and molds increased significantly more than 100% (Table 1) (Figure 3). Meanwhile, the microbial count varies among the three samples, possibly due to different home industries which influenced the hygiene.

Figure 3. Representative colonies of 3M Petrifilm. a) *Staphylococcus aureus with the red colonies till the violet-black colour; b)* Coliform colonies: pale red with gas bubbles*; c) Escherichia coli*: with blue colonies and gas bubbles*; and d)* Yeast and mold: small blue colonies for the yeast, large blue colonies for the mold

Glucosides content of jamu beras kencur (LC-MS/MS and GC-MS analysis)

Liquid chromatography (LC) is widely used to separate compounds in a sample prior to analysis, often in combination with mass spectrometry. The separation is based on interactions between the compounds and the mobile and stationary phases, with each compound's affinity for the mobile phase determining its separation. After elution from the column, the compounds are desolvated, ionized, and analyzed by a mass spectrometer(Ardrey, 2003; Pitt, 2009; Thermo Scientific, 2024). Similarly, in Gas Chromatography (GC), the sample is vaporized and separated into components by a capillary column, propelled by an inert gas such as helium, hydrogen, or nitrogen. The compounds elute at

different times based on their boiling points and polarity, allowing GC to resolve complex mixtures with hundreds of compounds.

LC-MS/MS is particularly effective for detecting non-volatile, polar, and thermally sensitive compounds, such as flavonoids and glycosides, with high sensitivity and precision(Pitt, 2009). On the other hand, GC-MS is ideal for analyzing volatile and semi-volatile compounds, such as terpenoids, providing excellent separation and identification, supported by extensive spectral libraries(Gomathi et al., 2015). The use of these two methods aims to enhance analysis because some bioactive compounds cannot be detected by LC-MS but are found in GC-MS, and vice versa(Table 1).

Note: LA: least abundance; POS: abundance; nd: not detected

LC-MS/MS analysis (negative mode) revealed the abundance presence of three glucosides in jamu beras kencur. They are Chrysoeriol-4'-O-β-D-glucopyranoside, Patuletin-7-O-[6′′-(2-methylbutyryl)]-glucoside, and Acacetin-7-galactoside (Figure 4). All

belong to the flavonoid compounds with the same flavone ring structure but different substitutions (Figure 5 and Table 2). The positive mode of LC-MS/MS analysis revealed only less abundance compounds (Figure 6).

2500 2000 Intensity [Counts]
1500
1000 Chrysoeriol-4'-O-β-D-glu... 7.77 Patuletin-7-O-[6'... Å, 500 1.37 1,2,6-Tri-O-galloyl-B-D-g $\overline{7}$ \overline{z} $\overline{3}$ $\overline{4}$ $\overline{5}$ 6 $\overline{8}$

Figure 4. Chromatogram of LC-MS/MS (negative mode) of jamu beras kencur

Retention time [min]

Figure 5. a.) Flavon aglycones and their glucosides in jamu beras kencur and b.) Flavone aglycones and their glucosides in jamu beras kencur

Figure 6. LC-MS/MS Chromatogram (positive mode) of the rhizome aqueous extract

LC-MS/MS (positive mode) revealed that the positive compounds in the aqueous extract of rhizome contains Isorhamnetin-3- O-β-gentiobioside and Quercetin-3',4',7-trimethylether (Figure 6). The ethanolic extract contains Quercetin-3',4',7-trimethyl ether, Feralolide, Skullcapflavone Ⅱ, and Yakuchinone B (Figure 7) with have closed retention times, 16.73, 16.64, 16.67, and 16.96

respectively. The negative mode of LC-MS/MS revealed less abundance compounds in ethanolic extract. GC MS analysis revealed that the ethanolic extract of rhizome contains Ethyl p-Methoxycinnamate as the sole dominant component (Table 2, Figure 8). These constituents were not found in the aqueous and ethanolic extracts of the rhizome.

Figure 7. LC-MS/MS Chromatogram (positive mode) rhizome etanolic extract

JBK is easily contaminated by many microbe that may become a source of foodborne disease. The contamination is happened during the preparation and keeping of the product, particularly when it is kept at room temperature. The presence of *E. coli* and other coliform bacteria (*Klebsiella and Enterobacter*) are reported already(Jilan Maulida, n.d.; Walther et al., 2016). But, so far, no reports on the presence of *Salmonella spp* and *Shigella spp*(Walther et al., 2016). The contamination source of JBK may come from the water used during the preparative process of JBK, the ineffectiveness of the heating process in killing the contaminant microbe, the cleanness of the container and equipment particularly for grinding the material/rhizome, and the material or rhizome itself. The presence of Coliform, *Escherichia coli*, *Staphylococcus aureus*, yeast, and mold are indicative enough for the problem of contamination and the growth of the microbe in JBK. The JBK is not able to inhibit or limit the growth of contaminant microbe. Therefore, the amount of contaminant increased during

the keeping of the JBK. From this information, it is strong enough to say that the maker and the seller do not have enough good knowledge and skill on sanitation. This report is the first report on the growth of contaminant microbe in JBK.

It is also the first report on the presence of Chrysoeriol-4'-O-β-D-glucopyranoside, Patuletin-7-O-[6′′-(2-methylbutyryl)] glucoside, and Acacetin-7-galactoside in JBK (Table 3). These flavonoid glycosides were not detected in the aqueous and ethanolic extract of the rhizome. Ethyl p-methoxy cinnamate is only detected by GC MS in ethanolic extract of the rhizome. This means that the preparative process of JBK is no guaranty that the essential bioactive compounds from the rhizome are available in JBK. But, a correlation between a microbial community in JBK and the quality of BJK is also subject to further research. The prospects and trends in evaluating natural glycosides in JBK can make it a new effective therapeutics(Bartnik & Facey, 2017).

Figure 8. GC-MS Chromatogram of the ethanolic extract of *Kaempferia galangal*

Many previous reports used extract obtained from the rhizome of K. galanga by the help of organic solvents, such as Ethyl p-Methoxycinnamate. This compound is detected clearly in ethanol extract but not in aqueous extract. Ethyl p–methoxycinnamate has antibacterial activity. The

number of bacteria in jamu beras kencur, a water extract of rhizome mixed with other ingredients, is increased significantly. This antibacterial capacity is not expressed in jamu beras kencur because ethyl p-methoxycinnmate is not soluble in water.

Aglycon of Acacetin, Chrysoeriol, Patuletin glucosides in JBK exerts a wide range of activities (Table 3). They are antioxidative, anti-inflammatory, and immunomodulators, and anticancer agents(Tanagornmeatar et al., 2014). Therefore, JBK is a promising herbal medicine that needs to be improved in its specificity and efficacy. Acacetin and Chrysoeriol may act as a potential therapeutic agent for brain diseases involving neuroinflammation such as Alzheimer's disease, Parkinson's disease,(Limboonreung et al., 2020) and ischemia.(Ha et al., 2012)

Table 4. Bioactive compound of aqueous and ethanolic of rhizome of *Kaempferia galangal*

Among bioactive compounds found in aqueous and ethanol compounds, ethyl pmethoxycinnamate has been reported in several reports.(Hasegawa et al., 2016; Kumar, 2020; Tritripmongkol et al., 2020; Yu et al., 2000). The other bioactive compounds are the first reported in this report (Table 4). They have wide biologically activities. Unfortunately, their presence is not detected in JBK. A combination between rice ('beras") and the rhizome of *Kaempferia galanga* (kencur) made jamu beras kencur unique in its composition. Not all bioactive compounds are extractable`e in JBK.

CONCLUSION

Jamu beras kencur is contaminated with a lot of Gram negative and Gram positive bacteria, such as Coliform, *Escherichia coli*, Pseudomonas, Staphylococcus. Yeast and mold contaminated also jamu beras kencur. All of them are able to grow in jamu beras kencur. Jamu beras kencur is a habitat and good nutrients for the growth or fermentation of the microbe. It is recommended that jamu beras kencur should be made more hygiene to minimalize the contamination and store or sold in cooled condition. But on the other side, there is a possibility that special fermentation is happening during the making process of jamu beras kencur.

This study reported for the first time, the results of LC-MS/MS analysis revealed the presence of three flavonoid glycosides, Chrysoeriol-4'-O-β-D-glucopyranoside, Patuletin-7-O- [6′′-(2-methylbutyryl)]-glucoside and Acacetin-7-galactoside in jamu beras kencur. The LC-MS/MS analysis confirmed that components of jamu beras kencur are not similar with the components of aqueous and ethanolic extracts of the rhizome of *Kaemferia galanga*. GC-MS revealed that Ethyl-p-methoxycinnamate is found in rhizome ethanolic extract.

From the pharmacological point of view, jamu beras kencur has the potentials as a healthy herbal beverage. But further preclinical and clinical studies are required to justify its clinical use.

CONFLICT OF INTEREST

Authors declare no conflict of interest

ACKNOWLEDGEMENT

The authors thank you for the technical assistance of Mr. Adit Widodo Santosa

REFERENCES

Abd-Alla, H. I., Abu-Gabal, N. S., Hassan, A. Z., El-Safty, M. M., & Shalaby, N. M. M.

(2012). Antiviral activity of Aloe hijazensis against some haemagglutinating viruses infection and its phytoconstituents. *Archives of Pharmacal Research*, *35*(8), 1347–1354. https://doi.org/10.1007/s12272-012-

0804-5 Amuamuta, A., Plengsuriyakarn, T., & Na-Bangchang, K. (2017). Anticholangiocarcinoma activity and toxicity of the Kaempferia galanga Linn. Rhizome ethanolic extract. *BMC Complementary and Alternative Medicine*, *17*, 1–11.

- Arambewela, L. S. R., Perera, A., & Wijesundera, R. L. C. (1999). Antibacterial activity of Kaempheria galanga. *Fitoterapia*, *70*(4), 425–427.
- Ardrey, R. E. (2003). *Liquid chromatographymass spectrometry: An introduction* (Vol. 2). John Wiley & Sons. https://books.google.com/books?hl=id& lr=&id=L8U5ZtLsI-

FUC&oi=fnd&pg=PR9&dq=Liquid+Chromatog-

raphy+%E2%80%93Mass+Spectrometry:+An+Introduction.+Robert+E.+Ardrey+Copy-

right+%C2%B6+2003+John+Wiley+%2 6+Sons,+Ltd&ots=tainTG3KuH&sig=P xEiyyj0dqX07ui7f5wKZSaKBrc

- Bartnik, M., & Facey, P. (2017). Glycosides. In *Pharmacognosy: Fundamentals, Applications and Strategy* (pp. 101–161). https://doi.org/10.1016/B978-0-12- 802104-0.00008-1
- Bayati, S., & Yazdanparast, R. (2011). Antioxidant and free radical scavenging potential of yakuchinone B derivatives in reduction of lipofuscin formation using H2O2-treated neuroblastoma cells. *Iranian Biomedical Journal*, *15*(4), 134.
- Bonham, M., Posakony, J., Coleman, I., Montgomery, B., Simon, J., & Nelson, P. S. (2005). Characterization of chemical constituents in Scutellaria baicalensis with antiandrogenic and growth-inhibitory activities toward prostate carcinoma. *Clinical Cancer Research*, *11*(10), 3905–3914.
- Boussouar, A., Barette, C., Nadon, R., Saint-Léger, A., Broucqsault, N., Ottaviani, A., Firozhoussen, A., Lu, Y., Lafanechère, L., & Gilson, E. (2013). Acacetin and chrysin, two polyphenolic compounds,

alleviate telomeric position effect in human cells. *Molecular Therapy-Nucleic Acids*, *2*. https://www.cell.com/molecular-therapy-family/nucleic-ac-

ids/fulltext/S2162-2531(16)30174-3

- Bui, T. T., Piao, C. H., Song, C. H., & Chai, O. H. (2017). Skullcapflavone II attenuates ovalbumin-induced allergic rhinitis through the blocking of Th2 cytokine production and mast cell histamine release. *International Immunopharmacology*, *52*, 77–84.
- Chandrasekaran, C. V., Thiyagarajan, P., Deepak, H. B., & Agarwal, A. (2011). In vitro modulation of LPS/calcimycin induced inflammatory and allergic mediators by pure compounds of Andrographis paniculata (King of bitters) extract. *International Immunopharmacology*, *11*(1), 79–84.
- Cheng, H.-L., Zhang, L.-J., Liang, Y.-H., Hsu, Y.-W., Lee, I.-J., Liaw, C.-C., Hwang, S.-Y., & Kuo, Y.-H. (2013). Antiinflammatory and antioxidant flavonoids and phenols from Cardiospermum halicacabum (倒地鈴 Dào Dì Líng). *Journal of Traditional and Complementary Medicine*, *3*(1), 33–40.
- Choi, D.-Y., Lee, J. Y., Kim, M.-R., Woo, E.- R., Kim, Y. G., & Kang, K. W. (2005). Chrysoeriol potently inhibits the induction of nitric oxide synthaseby blocking AP-1 activation. *Journal of Biomedical Science*, *12*(6), 949–959. https://doi.org/10.1007/s11373-005- 9028-8
- Chun, K.-S., Kang, J.-Y., Kim, O. H., Kang, H., & Surh, Y.-J. (2002). Effects of yakuchinone A and yakuchinone В on the Phorbol ester-induced expression of COX-2 and iNOS and activation of NFkB in mouse skin. *Journal of Environmental Pathology, Toxicology and Oncology*, *21*(2).
- Chun, K.-S., Sohn, Y., Kim, H.-S., Kim, O. H., Park, K.-K., Lee, J.-M., Lee, J., Lee, J.- Y., Moon, A., & Lee, S. S. (1999). Antitumor promoting potential of naturally occurring diarylheptanoids structurally related to curcumin. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *428*(1–2), 49–57.
- Corrêa, W. R., Serain, A. F., Aranha Netto, L., Marinho, J. V. N., Arena, A. C., Figueiredo De Santana Aquino, D., Kuraoka-Oliveira, Â. M., Júnior, A. J., Bernal, L. P. T., Kassuya, C. A. L., & Salvador, M. J. (2018). Anti-Inflammatory and Antioxidant Properties of the Extract, Tiliroside, and Patuletin 3-O- *β* -D-Glucopyranoside from *Pfaffia townsendii* (Amaranthaceae). *Evidence-Based Complementary and Alternative Medicine*, *2018*, 1–9. https://doi.org/10.1155/2018/6057579
- Csupor, D., Widowitz, U., Blazsó, G., Laczkó‐ Zöld, E., Tatsimo, J. S. N., Balogh, Á., Boros, K., Dankó, B., Bauer, R., & Hohmann, J. (2013). Anti‐inflammatory Activities of Eleven *Centaurea* Species Occurring in the Carpathian Basin. *Phytotherapy Research*, *27*(4), 540–544. https://doi.org/10.1002/ptr.4754
- Daroui-Mokaddem, H., Kabouche, A., Boutaghane, N., Calliste, C.-A., Duroux, J.- L., & Kabouche, Z. (2017). Antioxidant Flavonoids from *Asteriscus Maritimus*. *Natural Product Communications*, *12*(3), 1934578X1701200. https://doi.org/10.1177/1934578X1701 200319
- Elfahmi, Woerdenbag, H. J., & Kayser, O. (2014). Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. *Journal of Herbal Medicine*, *4*(2), 51–73. https://doi.org/10.1016/j.hermed.2014. 01.002
- Ezzat, S. M., & Salama, M. M. (2014). A new α-glucosidase inhibitor from *Achillea fragrantissima* (Forssk.) Sch. Bip. Growing in Egypt. *Natural Product Research*, *28*(11), 812–818. https://doi.org/10.1080/14786419.2014 .891203
- Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K., & Uma, C. (2015). GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. *Journal of Food Science and Technology*, *52*(2), 1212. [https://doi.org/10.1007/s13197-](https://doi.org/10.1007/s13197-013-1105-9) [013-1105-9](https://doi.org/10.1007/s13197-013-1105-9)
- Guerrero, M. F., Puebla, P., Carrón, R., Martin, M. L., & Román, L. S. (2002). Quercetin 3, 7-dimethyl ether: A vasorelaxant flavonoid isolated from Croton schiedeanus Schlecht. *Journal of Pharmacy and Pharmacology*, *54*(10), 1373–1378.
- Gupta, S. K., Banerjee, A. B., & Achari, B. (1976). Isolation of Ethyl p-methoxycinnamate, the major antifungal principle of Curcumba zedoaria. *Lloydia*, *39*(4), 218–222.
- Ha, S. K., Moon, E., Lee, P., Ryu, J. H., Oh, M. S., & Kim, S. Y. (2012). Acacetin Attenuates Neuroinflammation via Regulation the Response to LPS Stimuli In Vitro and In Vivo. *Neurochemical Research*, *37*(7), 1560–1567. https://doi.org/10.1007/s11064-012- 0751-z
- Han, L., Sumiyoshi, M., Zheng, Y., Okuda, H., & Kimura, Y. (2003). Anti‐obesity action of *Salix matsudana* leaves (Part 2). Isolation of anti‐obesity effectors from polyphenol fractions of *Salix matsudana*. *Phytotherapy Research*, *17*(10), 1195– 1198. https://doi.org/10.1002/ptr.1405
- Hasegawa, T., Hashimoto, M., Fujihara, T., & Yamada, H. (2016). Aroma profile of galangal composed of cinnamic acid derivatives and their structure-odor relationships. *Natural Product Communications*, *11*(10), 1934578X1601101012.
- He, Z.-H., Yue, G. G.-L., Lau, C. B.-S., Ge, W., & But, P. P.-H. (2012). Antiangiogenic effects and mechanisms of transethyl p-methoxycinnamate from Kaempferia galanga L. *Journal of Agricultural and Food Chemistry*, *60*(45), 11309–11317.
- Hsu, J.-Y., Rao Sathyan, A., Hsu, K.-C., Chen, L.-C., Yen, C.-C., Tseng, H.-J., Wu, K.-C., Liu, H.-K., & Huang, W.-J. (2021). Synthesis of Yakuchinone B-inspired inhibitors against islet amyloid polypeptide aggregation. *Journal of Natural Products*, *84*(4), 1096–1103.
- Huang, L., Yagura, T., & Chen, S. (2008). Sedative activity of hexane extract of Keampferia galanga L. and its active compounds. *Journal of Ethnopharmacology*, *120*(1), 123–125.
- Jabeen, A., Mesaik, M. A., Simjee, S. U., Bano, S., & Faizi, S. (2016). Anti-TNF-α and anti-arthritic effect of patuletin: A rare flavonoid from Tagetes patula. *International Immunopharmacology*, *36*, 232–240.
- Jagadish, P. C., Latha, K. P., Mudgal, J., & Nampurath, G. K. (2016). Extraction, characterization and evaluation of Kaempferia galanga L.(Zingiberaceae) rhizome extracts against acute and chronic inflammation in rats. *Journal of Ethnopharmacology*, *194*, 434–439.
- Jang, H.-Y., Ahn, K.-S., Park, M.-J., Kwon, O.-K., Lee, H.-K., & Oh, S.-R. (2012). Skullcapflavone II inhibits ovalbumin-induced airway inflammation in a mouse model of asthma. *International Immunopharmacology*, *12*(4), 666–674.
- Jilan Maulida, F. (n.d.). *Keberadaan Bakteri Escherichia Coli Pada Jamu Gendong Di Jalan Sumatera Kecamatan Sumbersari Kabupaten Jember (The Existence Of Bacteria Escherichia Coli In Jamu Gendong on The Streets of Sumatera, Sumbersari, Jember)*.
- Kim, J. H., Cho, Y. H., Park, S. M., Lee, K. E., Lee, J. J., Lee, B. C., Pyo, H. B., Song, K. S., Park, H. D., & Yun, Y. P. (2004). Antioxidants and inhibitor of matrix metalloproteinase-1 expression from leaves ofzostera marina L. *Archives of Pharmacal Research*, *27*(2), 177–183. https://doi.org/10.1007/BF02980103
- Ko, H.-J., Kim, H. J., Kim, S. Y., Yun, H.-Y., Baek, K. J., Kwon, N. S., Whang, W. K., Choi, H.-R., Park, K.-C., & Kim, D.-S. (2014). Hypopigmentary Effects of Ethyl P‐Methoxycinnamate Isolated from Kaempferia galanga. *Phytotherapy Research*, *28*(2), 274–279.
- Ko, W.-C., Kuo, S.-W., Sheu, J.-R., Lin, C.-H., Tzeng, S.-H., & Chen, C.-M. (1999). Relaxant Effects of Quercetin Methyl Ether Derivatives in Isolated Guinea Pig Trachea and their Structure-Activity Relationships. *Planta Medica*, *65*(03), 273– 275. https://doi.org/10.1055/s-2006- 960776
- Kumar, A. (2020). Phytochemistry, pharmacological activities and uses of traditional medicinal plant Kaempferia galanga L.–An overview. *Journal of Ethnopharmacology*, *253*, 112667.
- Lakshmanan, D., Werngren, J., Jose, L., Suja, K. P., Nair, M. S., Varma, R. L., Mundayoor, S., Hoffner, S., & Kumar, R. A. (2011). Ethyl p-methoxycinnamate isolated from a traditional anti-tuberculosis medicinal herb inhibits drug resistant strains of Mycobacterium tuberculosis in vitro. *Fitoterapia*, *82*(5), 757– 761.
- Lee, H., Lee, D. H., Oh, J.-H., & Chung, J. H. (2021). Skullcapflavone ii Suppresses tnf-α/ifn-γ-induced Tarc, mdc, and Ctss Production in Hacat Cells. *International Journal of Molecular Sciences*, *22*(12), 6428.
- Lee, J., Son, H. S., Lee, H. I., Lee, G.-R., Jo, Y.-J., Hong, S.-E., Kim, N., Kwon, M., Kim, N. Y., Kim, H. J., Lee, Y. J., Seo, E. K., & Jeong, W. (2019). Skullcapflavone II inhibits osteoclastogenesis by regulating reactive oxygen species and attenuates the survival and resorption function of osteoclasts by modulating integrin signaling. *The FASEB Journal*, *33*(2), 2026–2036. https://doi.org/10.1096/fj.201800866R R
- Limboonreung, T., Tuchinda, P., & Chongthammakun, S. (2020). Chrysoeriol mediates mitochondrial protection via PI3K/Akt pathway in MPP+ treated SH-SY5Y cells. *Neuroscience Letters*, *714*, 134545.
- Liou, C.-J., Wu, S.-J., Chen, L.-C., Yeh, K.- W., Chen, C.-Y., & Huang, W.-C. (2017). Acacetin from traditionally used Saussurea involucrata Kar. Et Kir. Suppressed adipogenesis in 3T3-L1 adipocytes and attenuated lipid accumulation in obese mice. *Frontiers in Pharmacology*, *8*, 589.
- Lucini, L., Pellizzoni, M., Pellegrino, R., Molinari, G. P., & Colla, G. (2015). Phytochemical constituents and in vitro radical scavenging activity of different Aloe species. *Food Chemistry*, *170*, 501– 507.
- Muhamad, P., Panrit, L., Chaijaroenkul, W., & Na-Bangchang, K. (2020). Cytotoxicity, cell cycle arrest, and apoptosis induction activity of ethyl-p-methoxycinnamate in cholangiocarcinoma cell. *Asian Pacific Journal of Cancer Prevention: APJCP*, *21*(4), 927.
- Nag, S., & Mandal, S. (2015). Importance of ekangi (Kaempferia galanga l.) As medicinal plants-a review. *Int J Innov Res Rev*, *3*, 99–106.
- Nguyen, T. Y., To, D. C., Tran, M. H., Lee, J. S., Lee, J. H., Kim, J. A., Woo, M. H., & Min, B. S. (2015). Anti-inflammatory Flavonoids Isolated from *Passiflora foetida*. *Natural Product Communications*, *10*(6), 1934578X1501000. https://doi.org/10.1177/1934578X1501 000634
- Nishidono, Y., Fujita, T., Kawanami, A., Nishizawa, M., & Tanaka, K. (2017). Identification of PGC-1α activating constituents in Zingiberaceous crude drugs. *Fitoterapia*, *122*, 40–44.
- Ohishi, K., Aiyama, R., Hatano, H., Yoshida, Y., Wada, Y., Yokoi, W., Sawada, H., Watanabe, T., & Yokokura, T. (2001). Structure-activity relationships of N-(3, 5-dimethoxy-4-n-octyloxycinnamoyl)- N'-(3, 4-dimethylphenyl) piperazine and analogues as inhibitors of acyl-CoA: cholesterol O-acyltransferase. *Chemical and Pharmaceutical Bulletin*, *49*(7), 830–839.
- Parsafar, S., Nayeri, Z., Aliakbari, F., Shahi, F., Mohammadi, M., & Morshedi, D. (2020). Multiple neuroprotective features of Scutellaria pinnatifida–derived small molecule. *Heliyon*, *6*(8).
- Pawłowska, K., Czerwińska, M. E., Wilczek, M., Strawa, J., Tomczyk, M., & Granica, S. (2018). Anti-inflammatory Potential of Flavonoids from the Aerial Parts of *Corispermum marschallii*. *Journal of Natural Products*, *81*(8), 1760–1768. https://doi.org/10.1021/acs.jnatprod.8b 00152
- Pitt, J. J. (2009). Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry. *The Clinical Biochemist Reviews*, *30*(1), 19.
- Punia, R., Raina, K., Agarwal, R., & Singh, R. P. (2017). Acacetin enhances the therapeutic efficacy of doxorubicin in nonsmall-cell lung carcinoma cells. *PLoS One*, *12*(8), e0182870.
- Rauter, A. P., Martins, A., Borges, C., Mota‐ Filipe, H., Pinto, R., Sepodes, B., & Justino, J. (2010). Antihyperglycaemic and protective effects of flavonoids on strep-

tozotocin–induced diabetic rats. *Phytotherapy Research*, *24*(S2). https://doi.org/10.1002/ptr.3017

- Rauwald, H. W., Maucher, R., Dannhardt, G., & Kuchta, K. (2021). Dihydroisocoumarins, naphthalenes, and further polyketides from Aloe vera and A. plicatilis: Isolation, identification and their 5- LOX/COX-1 inhibiting potency. *Molecules*, *26*(14), 4223.
- Rijal, S., Changdar, N., Kinra, M., Kumar, A., Nampoothiri, M., Arora, D., Shenoy, R. R., Ranganath Pai, K. S., Joseph, A., & Mudgal, J. (2019). Neuromodulatory potential of phenylpropanoids; paramethoxycinnamic acid and ethyl-pmethoxycinnamate on aluminum-induced memory deficit in rats. *Toxicology Mechanisms and Methods*, *29*(5), 334–343.
- Roy, M., Chakraborty, S., Siddiqi, M., & Bhattacharya, R. K. (2002). Induction of apoptosis in tumor cells by natural phenolic compounds. *Asian Pac J Cancer Prev*, *3*(1), 61–67.
- Shirota, S., Miyazaki, K., Aiyama, R., Ichioka, M., & Yokokura, T. (1994). Tyrosinase inhibitors from crude drugs. *Biological and Pharmaceutical Bulletin*, *17*(2), 266–269.
- Solnier, J., Martin, L., Bhakta, S., & Bucar, F. (2020). Flavonoids as novel efflux pump inhibitors and antimicrobials against both environmental and pathogenic intracellular mycobacterial species. *Molecules*, *25*(3), 734.
- Song, L., Wu, X., Xie, J., Zhang, H., Yang, H., Zeng, Q., Yang, X., & Xie, W. (2021). Kaempferia galanga Linn. Extract–A potential antibacterial agent for preservation of poultry products. *LWT*, *147*, 111553.
- Srivastava, N., Mishra, S., Iqbal, H., Chanda, D., & Shanker, K. (2021). Standardization of Kaempferia galanga L. rhizome and vasorelaxation effect of its key metabolite ethyl p-methoxycinnamate. *Journal of Ethnopharmacology*, *271*, 113911.
- Surh, Y.-J. (1999). Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic sub-

stances. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *428*(1–2), 305–327.

- Swain, S. S., Hussain, T., & Pati, S. (2021). Drug-lead anti-tuberculosis phytochemicals: A systematic review. *Current Topics in Medicinal Chemistry*, *21*(20), 1832–1868.
- Tanagornmeatar, K., Chaotham, C., Sritularak, B., Likhitwitayawuid, K., & Chanvorachote, P. (2014). Cytotoxic and anti-metastatic activities of phenolic compounds from Dendrobium ellipsophyllum. *Anticancer Research*, *34*(11), 6573–6579.
- Thermo Scientific. (2024). *Liquid Chromatography Mass Spectrometry (LC-MS) Information—ID*. https://www.thermofisher.com/id/en/home/industrial/mass-spectrometry/mass-spectrometry-learning-center/liquid-chromatography-mass-spectrometry-lc-ms-information.html
- Tofighi, Z., Alipour, F., Hadavinia, H., Abdollahi, M., Hadjiakhoondi, A., & Yassa, N. (2014). Effective antidiabetic and antioxidant fractions of *Otostegia persica* extract and their constituents. *Pharmaceutical Biology*, *52*(8), 961–966. https://doi.org/10.3109/13880209.2013 .874463
- Tritripmongkol, P., Plengsuriyakarn, T., Tarasuk, M., & Na-Bangchang, K. (2020). In vitro cytotoxic and toxicological activities of ethanolic extract of Kaempferia galanga Linn. And its active component, ethyl-p-methoxycinnamate, against cholangiocarcinoma. *Journal of Integrative Medicine*, *18*(4), 326–333.
- Ullah, M. A., Johora, F. T., Sarkar, B., Araf, Y., & Rahman, M. H. (2020). Curcumin analogs as the inhibitors of TLR4 pathway in inflammation and their drug like potentialities: A computer-based study. *Journal of Receptors and Signal Transduction*, *40*(4), 324–338.
- Umar, M. I., Asmawi, M. Z., Sadikun, A., Atangwho, I. J., Yam, M. F., Altaf, R., & Ahmed, A. (2012). Bioactivity-guided isolation of ethyl-p-methoxycinnamate, an anti-inflammatory constituent, from Kaempferia galanga L. extracts. *Molecules*, *17*(7), 8720–8734.
- Umar, M. I., Asmawi, M. Z., Sadikun, A., Majid, A. M. S. A., Al-Suede, F. S. R., Hassan, L. E. A., Altaf, R., & Ahamed, M. B. K. (2014). Ethyl-p-methoxycinnamate isolated from Kaempferia galanga inhibits inflammation by suppressing interleukin-1, tumor necrosis factor-α, and angiogenesis by blocking endothelial functions. *Clinics*, *69*, 134– 144.
- Walther, C., Marwa, K. J., Seni, J., Hamis, P., Silago, V., Mshana, S. E., & Jande, M. (2016). Microbial contamination of traditional liquid herbal medicinal products marketed in Mwanza city: Magnitude and risk factors. *Pan African Medical Journal*, *23*(1). https://www.ajol.info/index.php/pamj/article/view/138665
- Wang FangLin, W. F., Luo JianGuang, L. J., Wang XiaoBing, W. X., & Kong LingYi, K. L. (2013). *A pair of sulfonated diarylheptanoid epimers from Kaempferia galanga.* https://www.cabidigitallibrary.org/doi/full/10.5555/2013316291 4
- Wu, H.-J., Wu, W., Sun, H.-Y., Qin, G.-W., Wang, H.-B., Wang, P., Yalamanchili, H. K., Wang, J., Tse, H.-F., & Lau, C.-P. (2011). Acacetin causes a frequencyand use-dependent blockade of hKv1. 5 channels by binding to the S6 domain. *Journal of Molecular and Cellular Cardiology*, *51*(6), 966–973.
- Xue, Y., & Chen, H. (2002). Study on the anticarcinogenic effects of three compounds in Kaempferia galanga L. *Wei Sheng Yan Jiu= Journal of Hygiene Research*, *31*(4), 247–248, 251.
- Yamazaki, R., Hatano, H., Aiyama, R., Matsuzaki, T., Hashimoto, S., & Yokokura, T. (2000). Diarylheptanoids suppress expression of leukocyte adhesion molecules on human vascular endothelial cells. *European Journal of Pharmacology*, *404*(3), 375–385.
- Yang, Y., Zhou, X., Xiao, M., Hong, Z., Gong, Q., Jiang, L., & Zhou, J. (2010). Discovery of chrysoeriol, a PI3K-AKT-mTOR pathway inhibitor with potent antitumor activity against human multiple myeloma cells in vitro. *Journal of Huazhong University of Science and Technology [Medical Sciences]*, *30*(6), 734–740.

https://doi.org/10.1007/s11596-010- 0649-4

- You, K. M., Jong, H.-G., & Kim, H. P. (1999). Inhibition of cyclooxygenase/lipoxygenase from human platelets by polyhydroxylated/methoxylated flavonoids isolated from medicinal plants. *Archives of Pharmacal Research*, *22*(1), 18–24. https://doi.org/10.1007/BF02976430
- Yu, J. G., Yu, D. L., Zhang, S., Luo, X. Z., Sun, L., Zheng, C. C., & Chen, Y. H. (2000).

Studies on the chemical constituents of Kaempferia marginata. *Yao Xue Xue Bao= Acta Pharmaceutica Sinica*, *35*(10), 760–763.

Zhao, N., Dong, Q., Fu, X.-X., Du, L.-L., Cheng, X., Du, Y.-M., & Liao, Y.-H. (2014). Acacetin blocks kv1. 3 channels and inhibits human T cell activation. *Cellular Physiology and Biochemistry*, *34*(4), 1359–1372.