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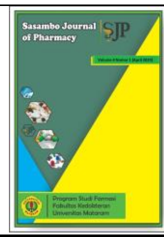

Comparison of the total phenolic and flavonoid contents of *Amomum compactum* Sol. Ex Maton from districts Linggo Asri and Paninggaran, Pekalongan Regency

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Abstract : Cardamom (*Amomum compactum* Sol. Ex Maton) is one of the largest contribution in biopharmaceutical production plants in Indonesia that is equal to 12.22% and yield reaching 105.735 kg/year in Pekalongan Regency. The largest cardamom production in Pekalongan Regency is in 2 districts, namely Linggo Asri and Paninggaran. Cardamom is used in traditional medicines, because it has chemical compounds such as flavonoids and phenolics. The aim of this study was to compare total flavonoids and total phenolics content in *Amomum compactum* Sol. Ex Maton extract originates from Linggo Asri and Paninggaran district, Pekalongan Regency. The samples were extracted by water extraction, and then phytochemical contents were identified with HCl and $FeCl_3$ reagent. The samples were calculated of total flavonoids and total phenolics content by spectrometric using aluminum chloride colorimetric assay and Folin–Ciocalteu method. The result showed a significant difference in the levels of total phenolic content between *Amomum compactum* Sol. Ex Maton extract origin from Linggo Asri district 41.43±0.54 µg/mg and Paninggaran district 120.55±1.89 µg/mg, but it was not found in total flavonoid content where from Linggo Asri district 289.06±4.1 µg/mg and Paninggaran district 303.81±0.5 µg/mg. The conclusion is *Amomum compactum* Sol. Ex Maton extract originates from Linggo Asri and Paninggaran district of Pekalongan city showed a significant difference in the levels of total phenolic content, but it was not found in total flavonoid content.

Keywords: Flavonoid, Phenolic, *Amomum compactum* Sol. Ex Maton, Linggo Asri, Paninggaran

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Introduction

Pekalongan Regency is one of 35 regencies/cities in Central Java Province, which is located in the western part of the Pantura area along the north coast of the Java Sea extending to the south. The geography and topography of Pekalongan Regency make this area have very good potential for development. One of them is the potential of biopharmaceutical and plantation products (Lestari et al., 2019). Cardamom is one of the biopharmaceuticals that has good potential with yields reaching 105,735 kg/year. The largest cardamom production in Pekalongan Regency is in 2 sub-districts, namely Linggo Asri and Paninggaran. Linggo Asri is a sub-districts of Pekalongan regency located at an altitude of 500 meters above sea level while the location

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of Paninggaran sub-district is 850 meters above sea level. Most of them are forests, so a lot of medicinal plants thrive in these places (Rahmawati et al., 2013).

Cardamom (*Amomum compactum Sol*. Ex Maton) belonging to the Zingiberaceae family is a spice plant native to Indonesia that is widely used. Cardamom seeds are part of cardamom which is often used for cooking spices and medicines. Previous research showed that cardamom is one of the commodities with a contribution largest production of the total production of biopharmaceutical plants in Indonesia which is equal to 12.22% (Ashokkumar et al., 2020; Munadi, 2017). Cardamom is 1-2.5 m long, the base of the stem is reddish-green, the leaves are lancet-shaped, the flower stalks are very small as if there were no stalks at all, the fruit is reddish-white, and when dry and pod-shaped or round and slightly flattened, becomes brownish black. The fruit at the bottom falls out of the stem and crawls to the ground. Like a shell, the fruit is grayish-gray and hairy. The diameter of the fruit is ± 10 mm. The seeds are reddish and smell like fragrant camphor (Alkandahri et al., 2021). Cardamom is used as an active compound or ingredient in traditional medicines, including pain relievers, asthma, bad breath, cough and itching in the throat, indigestion, vomiting, antifungal, antibacterial, antioxidant, treat flatulence, anti-inflammatory, immunomodulatory, anticancer, and asthma (Alkandahri et al., 2021; Ashokkumar et al., 2020). The biological activity of cardamom seeds can't be separated from the content of the chemical compounds they have. Among them are flavonoid compounds and phenolic compounds (Kurmukov, 2013).

Flavonoid and phenolic compounds are found in almost all parts of plants including fruit, roots, leaves, and outer bark of stems (Tungmunnithum et al., 2018). Flavonoids and phenolics have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer activities (Sulaiman & Indira, 2012). Numerous epidemiological studies confirm a significant relationship between the high dietary intake of flavonoids and phenolics with the reduction of cardiovascular and carcinogenic risk (Marinova et al., 2005). The concentration of secondary metabolites such as flavonoids and phenolics is influenced by internal factors (genetic, plant health conditions, age) and external factors (environment, care with drugs) (Ali et al., 2018; Mahfur, 2018). The purpose of this study was to determine the comparison of total flavonoid and total phenolic levels in cardamom seeds from Paninggaran and Linggo Asri districts, Pekalongan Regency.

Materials and Methods

Ethanol 70 % (Merck), ethyl acetate (J.T Barker), $FeCl_3$ (*Nitra Kimia*), Gallic acid (*Nitra Kimia*), Folin-Ciocalteau (*Nitra Kimia*), aluminum foil, $NaNO_2$ (Nitra Kimia), $AlCl_3$ (*O*), Dagendrof reagent (Merck), Mayer reagent (Merck), Wagner reagent (Merck), spectrophotometry Visible (Genesys IOS VIS). *Amomum compactum* Sol. Ex Maton species were collected manually from districts Paninggaran and Linggi Asri, Pekalongan, Central Java, Indonesia.

The plant material was identified by Laboratorium Biologi Fakultas MIPA Universitas Ahmad Dahlan. Fruit of *Amomum compactum* Sol. Ex Maton material was dried and extracted using aquadest (H_2O, macerating, and re-macerating over 3 days. After that, the crude extract was concentrated using a rotary evaporator. The plant extract was assessed for the existence of phenols and flavonoids bases by the phytochemical analysis (screening) using typical standard methods (with HCl and $FeCl_3$ reagent) and TLC with silica GF254 as stationary phase and mobile phase for flavonoid is methanol: ethyl acetate (1:9), phenolic BAW (4:1:5).

The total flavonoid compound was measured by the aluminum chloride colorimetric assay based on the work by(Theresa et al., 2015). The total flavonoid compound of the extract was expressed as micrograms of quercetin equivalent/mg extract (µg/mg). The 10 ml of 30% (v/v) ethanol was mixed with 0.7 ml of 5% (w/w) sodium nitrite and a dilute solution of the extract and 0.7 ml of 0% aluminum chloride (w/w) was added, and then 5ml of sodium hydroxide added. The mixture was diluted with 5 ml of 30% (v/v) of ethanol and left standing for 10 mins. This mixture was then placed in a UV spectrophotometer and the absorbance reading was taken at a wavelength of 510 nm. Quercetin was used as the standard and different concentrations of it were prepared and the absorbance readings were obtained at 510 nm. Total flavonoid contents were expressed as quercetin (µg/mg) using the following equation based on the calibration curve: $y = 0.0008x + 0.0294$, where y was the absorbance and x is concentration.

The total phenolic compounds were determined according to the Folin–Ciocalteu method. Results of total phenolic contents were expressed as micrograms of gallic acid equivalents (GAE) per mg extract, Estimation of the total flavonoids in the extracts was carried out using the method of (Theresa et al., 2015). A mixture of 2.5 ml of diluted Folin-Ciocalteau reagent and 2.0 ml of sodium carbonate (7.5%) was added to 1 ml of the diluted extract and incubated for 30 mins at 40^oC. It was then transferred to a spectrophotometer and the absorbance was measured at 760 nm. A calibration curve was constructed, using gallic acid as standard. Total phenolics contents were expressed as gallic acids (μg/mg) using the following equation based on the calibration curve: $y = 0.0171 x + 0.0832$, where y was the absorbance and x is the concentration.

All the methods were carried out in triplicate. The results were expressed as mean ± standard deviation (SD). Experiment data were statistically analyzed using SPSS version 19 and the results were expressed values Statistical significance was established at $p < 0.05$.

Result and Discussion

In this research, the extract of *Amomum compactum* Sol. Ex Maton was prepared with the maceration method. The water was used as the extraction solvent because with water distillation, the extract of *Amomum compactum* Sol. Ex Maton has a good antioxidant comparing ethanol extract (Alkandahri et al., 2021), it was chosen by considering the correlation between antioxidant activity and flavonoids and phenolics content (Nurcholis et al., 2021). The yield of the extract was 15.81%, which is slightly different from the previous study results of 3.08% (Sukandar et al., 2016). The yield of the extract is closely related to the effectiveness of the extraction process which is influenced by the type of solvent used, particle size, extraction method, and length of the extraction process (Salamah *et al.*, 2017). Furthermore, the yield of extraction can be influenced by biological factors such as plant parts, plant species, harvesting time, and location of growth (Mahfur, 2018). The result of phytochemical identification can be seen in **Table 1**.

Table 1. Qualitative Identification of Cardamom Extract

No.	Secondary metabolites	Reagent	Result reaction	Conclusion
1.	Flavonoid content	Wilstater reagent	Yellow	+
2.	Phenolics content	$FeCl_3$ reagent	Black	+

The number of flavonoids in an extract of *Amomum compactum* Sol. Ex Maton was quantified using spectrophotometry, a quantitative technique that relies on the creation of colors. The creation of a complex between $AlCl_3$ and a keto group at C-4 as well as with a hydroxyl group on the C-3 or C-4 atom near flavones and flavanols is the basic idea behind the spectrophotometric method of detecting flavonoid compounds. . Aluminum chloride can also combine with ortho hydroxyl groups on flavonoid compounds' A or B rings to generate stable acid complexes (Nurcholis et al., 2021; Sulaiman & Indira, 2012).

The $AlCl_3$, which can create a complex color when added to the sample solution, causes a shift in wavelength toward the visible (visible), which is shown by the solution's formation of a more yellow color. Potassium acetate is added to keep the wavelength in the visible range. The maximum wavelength obtained in this study is 510 nm, which means the complex absorbance between quercetin and $AlCl_3$ maximum readability at that wavelength. This matter is slightly different from the maximum wavelength study conducted by (Theresa et al., 2015), which obtained a length of the quercetin maximum wavelength at 500 nm.

In the measurement of total flavonoid compounds, a standard curve of quercetin in the range of 280-600 μg/mL with a coefficient determination (r2) value of 0.976 was obtained (**Figure 1**). The total flavonoid content of the sample can see in **Figure 2**. The total flavonoid compound of the *Amomum compactum* Sol. Ex Maton extract origin from Linggo Asri district has 289.06±4.1 μg quercetin equivalent/mg extract, and the sample origin from Paninggaran district has 303.81±0.5 μg quercetin equivalent/mg extract. The sample data analysis results didn't show a significant difference $p > 0.05$.

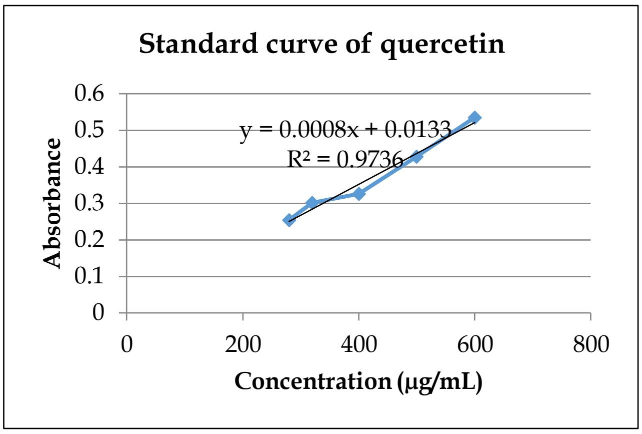

Figure 1. Standard curve of quercetin

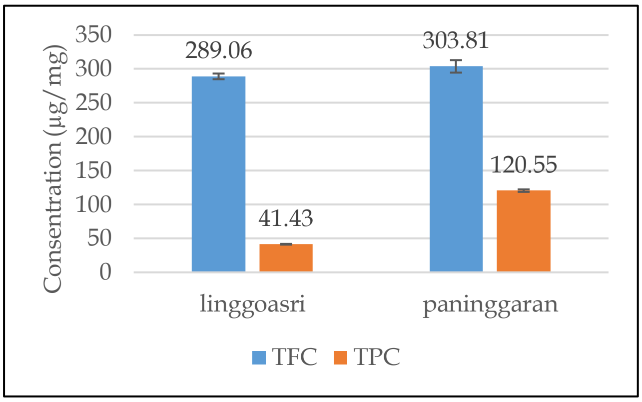

Figure 2. TFC and TPC of the sample

Determination of total phenolic content was carried out using the Folin-Ciocalteu reagent. Folin-Ciocalteu reagent is employed because phenolic chemicals can interact with folic to produce a solution color that can be used to gauge absorbance. The creation of a blue complex chemical, which can be detected at a wavelength of 760 nm, is the basis of the Folin technique ciocalteu. This reagent converts hetero-poly acids (phosphomolybdate-phosphotungstic acid) found in the Folin-Ciocalteu reagent to a molybdenum tungsten complex by oxidizing phenolics (alkali salts) or phenolic-hydroxy groups. Phenolic compounds react with Folin-Ciocalteu. reagent only under alkaline circumstances for proton dissociation to occur in compounds phenolic compounds into phenolic compounds, to produce alkaline conditions used 10% Na_2CO_3 (Yunita et al., 2021).

The hydroxyl group in phenolic compounds reacts with the reagent Folin-Ciocalteu forms a colored molybdenum-tungsten complex which can be detected by a spectrophotometer. The greater of phenolic compounds concentration, the more phenolic ions will reduce hetero-poly acids to molybdenum-tungsten complexes. So that, it results blue color is getting darker (Marjoni et al., 2018). In the measurement of total phenolic compounds, a standard curve of gallic acid in the range of 18-48 µg/mL with a coefficient determination (r2) value of 0.990 was obtained (**Figure 3**). The total phenolics compound of the *Amomum compactum* Sol. Ex Maton extract origin from Linggo Asri district has 41.43±0.54 µg gallic acids equivalent/mg extract, and the sample origin from Paninggaran district has 120.55±1.89 µg gallic acids equivalent/mg extract. The sample data analysis results show a significant difference of $p < 0.05$.

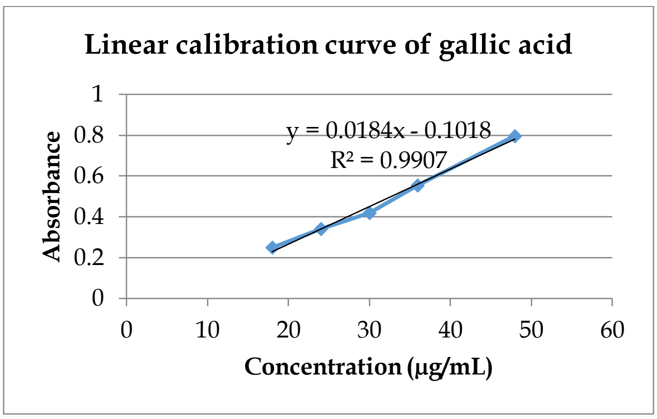

Figure 3. Standard curve of gallic acid

The difference between TFC and TPC in the sample is influenced by several factors, one of which is the place of growth (Mahfur, 2018). The Paninggaran and Linggo Asri districts are included highlands/mountain areas in the Pekalongan Region. Mountains feature more rainfall, lower temperatures, quicker organic matter breakdown, and slower weathering of minerals. On the other hand, organic matter breakdown and mineral weathering happen quickly in the lowlands. When compared to soil in the lowlands, the soil in the mountainous region is substantially more fertile, rich in organic matter, and nutrient-rich. Effect of location's height on the warmth and brightness of the light, the temperature and light intensity will decrease as a location becomes higher. The availability of food is directly affected by light. Chlorophyll is produced as a byproduct of photosynthesis and directly influences the development of every organ or of the entire plant (Alfian & Susanti, 2012). In terms of land, the land in Linggo Asri district is an alluvial soil type, this soil has a texture that tends to be rough with organic compounds and elements lower in nutrients compared to fine-textured soils (Hartiati & Mulyani, 2009). Because of this, the *Amomum compactum* Sol. Ex Maton extract origin from Linggo asri total phenolic content is smaller than samples of Paninggaran district. although in general *Amomum compactum* Sol. Ex Maton can grow on all types of soil as long as the soil is rich in humus, loose, and has good drainage (Dinata et al., 2021).

Conclusion

There was a significant difference in the levels of total phenolic content between Amomum compactum Sol. Ex Maton extract originated from Linggo Asri district and Paninggaran district, Pekalongan Region, but it was not found in total flavonoid content.

Acknowledgments

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References

Alfian, R., & Susanti, H. (2012). Penetapan Kadar Fenolik Total Ekstrak Metanol Kelopak Bunga Rosella Merah (*Hibiscus sabdariffa* Linn) dengan Variasi Tempat Tumbuh Secara Spektrofotometri. *Pharmaciana*, 2(1). https://doi.org/10.12928/pharmaciana.v2i1.655

Ali, A. M. A., El-Nour, M. E. A. M., & Yagi, S. M. (2018). Total phenolic and flavonoid contents and antioxidant activity of ginger (Zingiber officinale Rosc.) rhizome, callus, and callus treated with some elicitors. *Journal of Genetic Engineering and*

Biotechnology, *16*(2), 677–682. https://doi.org/10.1016/j.jgeb.2018.03.003

Alkandahri, M. Y., Shafirany, Z., Rusdin, A., Agustina, S., Pangaribuan, F., Fitrianti, F., Kusumawati, H., Sugiharta, S., Arfania, M., & Mardiana, L. (2021). Mini-review Article Amomum compactum: a Review of Pharmacological Studies. *Journal of Plant Sciences*, *22*(May), 61–69.

Ashokkumar, K., Murugan, M., Dhanya, M. K., & Warkentin, T. D. (2020). Botany, traditional uses, phytochemistry and biological activities of cardamom [Elettaria cardamomum (L.) Maton] – A critical review. *Journal of Ethnopharmacology*, *246*(March 2019), 112244. https://doi.org/10.1016/j.jep.2019.112244

Dinata, D. I., Maharani, R., Muttaqien, F. Z., Supratman, U., Azmi, M. N., & Shiono, Y. (2021). Flavonoids from the Roots of Amomum compactum Soland Ex Maton (Zingiberaceae). *Jurnal Kimia Valensi*, *7*(2), 142–149. https://doi.org/10.15408/jkv.v7i2.21599

Hartiati, A., & Mulyani, S. (2009). Pengaruh Preparasi Bahan Baku Rosella Dan Waktu Pemasakan Terhadap Aktivitas Antioksidan Sirup Bunga Rosella (Hisbiscus sabdariffa L.). *Agrotekno*, *15*(1), 20–24.

Kurmukov, A. G. (2013). Phytochemistry of medicinal plants. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, *1*(6), 13–14. https://doi.org/10.1007/978-1-4614-3912-7_4

Lestari, I., Murningsih, & Utami, S. (2019). Keanekaragaman jenis tumbuhan paku epifit di Hutan Petungkriyono Kabupaten Pekalongan, Jawa Tengah. *Niche Journal of Tropical Biology*, *2*(2), 14–21. https://doi.org/10.14710/niche.2.2.14-21

Mahfur. (2018). Profil Metabolit Sekunder Senyawa Aktif Minyak Atsiri Jinten Hitam (Nigella Sativa L.) dari Habasyah Dan India. *Pharmacy: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, *15*(01), 90–97. http://dx.doi.org/10.30595/pharmacy.v15i1.2274

Marinova, D., Ribarova, F., & Atanassova, M. S. (2005). Total phenolics and flavonoids in Bulgarian fruits and vegetables Total phenolics and flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy*, *40*(03), 255–260.

Marjoni, M. R., Nofita, D., Rahmi, N., Saifullah, & Najla, N. A. (2018). Phenolic compounds, flavonoids, and antioxidant activity methanol extract of arum manis leaves (Mangifera indica L. Var. Arumanis). *International Journal of Green Pharmacy*, *12*(3), S651–S656. https://doi.org/10.22377/ijgp.v12i03.2034

Munadi, E. (2017). *Tanaman obat, sebuah tinjauan singkat Info Komoditi Tanaman Obat*. Badan Pengkajian dan Pengembangan Perdagangan Kementerian Perdagangan Republik Indonesia .

Nurcholis, W., Sya'bani Putri, D. N., Husnawati, H., Aisyah, S. I., & Priosoeryanto, B. P. (2021). Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of Amomum compactum fruits. *Annals of Agricultural Sciences*, *66*(1), 58–62. https://doi.org/10.1016/j.aoas.2021.04.001

Rahmawati, N., Saputra, R., & Sugiharto, A. (2013). Sistem Informasi geografis Pemetaan dan Analisis Lahan Pertanian di Kabupaten Pekalongan. *Journal of Informatics and Technology*, *2*(1), 95–101.

Sukandar, D., Hermanto, S., Amelia, E. R., & Zaenudin, M. (2016). Aktivitas Antibakteri Ekstrak Biji Kapulaga (Amomum compactum Sol. Ex Maton). *Jurnal Kimia Terapan Indonesia*, *17*(2), 119–129. https://doi.org/10.14203/jkti.v17i2.28

Sulaiman, & Indira, B. (2012). Total Phenolics and Total Flavonoids in Selected Indian Medicinal Plants. *Indian Journal of Pharmaceutical Sciences*, *74*(03), 258–260.

Theresa, E., Stephen, O., Oladipupo, S., & Oyelakin, R. A. (2015). Determination of the Total Phenolic, Flavonoid Contents; Antioxidant Activity and GC-MS Study of the leaves of the Medicinal Plant Sarcocephalus latifolius. *Int. J. Pharm. Sci. Rev. Res.*, *34*(12), 82–86.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines*, *5*(3), 93. https://doi.org/10.3390/medicines5030093

Yunita, E., Destasary, E. M., & Wicaksana, F. H. (2021). The Effect Of Different Solvent Extraction On Chemical Content And Quercetin Levels Of Ketapang (Terminalia cattapa L.). *Jurnal Farmasi (Journal of Pharmacy)*, *2*(1), 1–4.