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Composition of Metabolic Secondary Compounds in Standardized "Javanese Cardamom" (*Amomum compactum*) Simplicia

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Abstract

Background

Javanese cardamom fruit (*Amomum compactum* Sol. Ex Maton), from the Zingiberaceae family, has long been used as an herbal plant.

Purpose

The aim of the research was to evaluate the standardization of Javanese Cardamom simplicia and the profile of its extract compounds in water, n-hexane and its essential oil.

Methods

Research methods include making simplicia powder, simplicia standardization, phytochemical screening, Total Phenolic Content, Total Flavonoid Content, Total Terpenoid Content, and simplicia extraction in water and n-hexane solvents using maceration-ultrasonic analyzed by LC-MS, and CEO isolation using steam distillation analyzed by GC-MS.

Results

The results of the simplicia standardization research showed Water Content of 7.74 ± 0.11%, Ash Content of 9.69 ± 0.03%, Total Plate Count of $5.4x10^6$cfu/g, Total Fungal Count of $2.3x10^6$cfu/g, Water-Soluble of Extract 1.79±0.11, Ethanol-Soluble Extract of 0.47±0.15, phytochemical screening contained triterpenoids, steroids and alkaloids. Total Phenolic Content of 1.48±0.53 (mgGAE/g), Total Flavonoid Content of 0.53±0.07 (mgQE/g), Total Terpenoid Content 37.12±0.02 (mg/g). The secondary metabolite profile in water includes polyphenols (flavonoids, simple phenols, tannins, oxaspiro-organic), diarylheptanoids and terpenoids . In the n-hexane solvent there are diarylheptanoids, terpenoids (mono-,sesqui-,di-,tri-terpenoids), and polyphenols (flavonoids and simple phenols). CEO contains mono- and sesqui-terpenoid.

Conclusion

The conclusion is that the standardization of simplicia has met FHI standards; Secondary metabolite profile in water solvent extracted polar compounds, i.e. polyphenols group (flavonoids, simple phenols, tannins, oxaspiro-organic) then semi-non-polar, i.e. diarylheptanoids, terpenoids; in n-hexane solvent extracted non-polar compounds, i.e. diarylheptanoids, terpenoids, and CEO only terpenoids.

Keywords: standardization, herbal, secondary metabolic, java cardamom, simplicial

1. Introduction

Cardamom is a member of the Zingiberaceae family and has been used as an herb and spice. The spice is produced from the fruit and can be used whole, or extracted in food (Anwar et al., 2015). The genus Amomum with 108 accepted species is currently distributed in tropical and subtropical Asia (Sabulal & Baby, 2021). In Indonesia, there is Sabrang cardamom (*Elletaria cardamomum* (L.) Maton) and Java cardamom ((*Amomum compactum* Sol. Ex Maton) (Droop et al., 2013). This type of Amomum compactum is a type native to Indonesia which is widely cultivated in Sumatra, Java and the Malay Peninsula (Harist et al., 2024). The type *Elletaria cardamomum* was reportedly imported from India to Indonesia (Ningsih et al., 2023). Several previous studies reported that various parts of cardamom, such as seeds (Arpitha et al., 2019), fruit (seeds and pods) (Abu-Taweel, 2018), rhizomes (Winarsi et al., 2016), and leaves (Winarsi et al., 2014) has been used for the treatment of several diseases.

Cardamom is empirically used to treat asthma, diarrhea, kidney disorders, digestion, gum infections, and nausea (Ashokkumar, Pandian, et al., 2020). Java cardamom extract (*Amomum compactum* Soland. Ex Maton) is active and can be developed as an anti-asthma (J. A. Lee et al., 2010), antioxidant (Amma et al., 2015; Nurcholis et al., 2021; Rini et al., 2022 ; (Juliana et al., 2022), antiatherosclerotic (Winarsi et al., 2016), as an anticancer compound (Ashokkumar, Murugan, et al., 2020); Mahfur et al., 2023; (Arpitha et al., 2019). Various pharmacological properties of cardamom have been reported in various therapies, such as antibacterial (Sukandar et al., 2015) (Rini et al., 2022) (Nofriyaldi et al., 2023), anti-biofilm (Cui et al., 2020), and anti-inflammatory (Rini et al., 2022) ; (Praditha et al., 2020), anti-Covid-19 (Mohammad et al., 2022) and CEO (Cardamom Essential Oils) have potential as nephroprotective agents in aging. doxorubicin induced (Hasanah et al., 2023).

The main components contained in cardamom essential oil (CEO) are α-terpinyl acetate, 1,8-cineole, and linalool (Anwar et al., 2015). GCMS analysis of cardamom essential oil has the constituent components 1,8-Cineole, β-Pinene, α-Fenchone, and alpha-Pinene (Herina & Yulita, 2020). CEOs of *Amomum subulatum, A. tsao-ko, A. kravanh, A. aromaticum, A. compactum, A. korarima and A. verum* are rich in 1,8-cineole. These Amomum species are used as spices and flavors. Bornyl acetate, camphor, methyl chavicol, trans-p-(1-butenyl) anisole, santolina triene, α-pinene and β-pinene are other major constituents in EOs of various Amomum species (Sabulal & Baby, 2021). The phytochemical compounds contained in cardamom fruit (A. compactum) using GC–MS and LC–MS/MS, the content of compounds such as phenolics, flavonoids, terpenoids, alkaloids, polyphenols, and fatty acids (Juliana et al., 2022). GC-MS analysis contained 1,8-cineole (50.82%), ß-pinene (12.43%), α-terpineol (8.50%), fenchone (4.10%), α-pinene (4.00%), sabinene (3.00%), and linalool (1.98%) (Hasanah et al., 2023). Compounds extracted using solvents: ethanol, methanol and water were identified using GC-MS as containing 1,8-cineole, alpha-terpineol and other terpenoid compounds (Tarigan & Saragih, 2023). GC-MS contained alpha-pinene, sabinene, beta pinene, cineol, 3-cyclohexene-1-methanol, 12-chlorobicyclo, 9-octadecenal, 9,12-octadecadienoic acid, methyl ester of ricinoleic acid, 4-cyclopentacycloocten-4-one (Tambunan, 2017). Cardamom has secondary metabolite compounds, such as alkaloids, flavonoids, tannins, polyphenolics and saponins (Ningsih et al., 2023). *Amomum compactum* Sol. Ex Maton extracts originating from different places showed significant differences in total phenolic content and no total flavonoid content was found (Mahfur et al., 2023). Ethanol and ethyl acetate extracts of cardamom have been found to contain flavonoids (Nurcholis et al., 2021)

Several differences in cardamom simplicia varieties, differences in location, differences in extraction, differences in solvents using ethanol and ethyl acetate, essential oils and activities have

also shown differences in content and potential for therapeutic activity. This research will evaluate the standardization of simplification including water content, ash content, Total Plate Count, Total Fungal Count, Water Soluble Extract, Ethanol Soluble Extract. Apart from that, phytochemical screening, Total Phenol Content (TPC), Total Flavonoid Content (TFC), Total Terpenoid Content (TTC) and metabolic secondary profile compounds using LC-MS and GC-MS analysis. The urgency of this research is expected to obtain standardization parameters for Javanese cardamom simplicia and secondary metabolite compound profiles from its extract in water, n-hexane and essential oil which can be used in developing therapies for various diseases through pre-clinical test, i.e. bionformatic, *in-silico, in-vitro and in-vivo* analysis as well as future clinical test.

2. Material and methods

2.1 Preparation of Java Cardamom simplicia

Java Cardamom fruit *(Amomum compactum* Sol. Ex Maton*)* is washed, drained, broken to reduce size, and then dried in an oven at 50°C. Dried *Java Cardamom* fruit is then ground and sieved to form a simplicia powder (Djarot et al., 2023).

2.2 Simplicia standardization

2.2.1 Water content

A 5-gram sample was put into a porcelain cup that had been tared and then dried using an oven at 105°C for 5 hours. The sample was then placed in a desiccator and weighed. Drying and weighing were continued at an interval of 1 hour until the difference between two consecutive weighing's was no more than 0.25%. The water content of simplicia is calculated by the formula:

$$water\ content\ (\%) = \frac{w0-w1}{w0} x100\% \qquad (1)$$

Where w0 is the initial weight of the simplicia and w1 is the final weight of the simplicia (Djarot et al., 2023).

2.2.2 Ash content

A sample of 2 to 3 g is placed in a crucible porcelain that has been incinerated and tared. Next, the sample is incinerated until the charcoal runs out, cooled, and weighed. The ash content of simplicial is calculated by the formula (Djarot et al., 2023):

$$ash\ content\ (\%) = \frac{w1}{w0} x100\% \qquad (2)$$

2.2.3 Total microbial contamination

The number of microbial contaminants was determined by Total Plate Count and Total Fungal Count. Serial dilution of samples was carried out using 0.85% NaCl until a dilution of 10^{-5} was obtained. Each dilution was inoculated on plate count agar (PCA) media for Total Plate Count and potato dextrose agar (PDA) media for Total Fungal Count. Incubation was carried out at 30°C for 24-48 hours. The number of colonies that grew was counted according to standard plate count requirements (Ekawati & Yusmiati, 2018).

2.2.4 Water-soluble extract

The sample was weighed as a total of 5 g, and put into a sealed flask. Then 100 mL of chloroform-saturated water (1mL chloroform:100mL distilled water) was added, shaken several times for the first 6 hours, and left for 18 hours. Then, the mixture was filtered, and 20.0mL of the filtrate was evaporated until dry in a flat-bottomed vaporizer cup heated to 105°C and tared. The remaining was heated at 105°C to a fixed weight. The water-soluble extract is calculated by the formula (Pusmarani et al., 2019):

$$water\ soluble\ extract\ (\%) = \frac{weight\ of\ extract\ (g)}{weight\ of\ sample\ (g)} x \frac{100}{20} x100\% \qquad (3)$$

2.2.5 Ethanol-soluble extract

The sample was weighed, of 5 g, and placed in a sealed flask. Then 100 mL of ethanol was added, shaken several times for the first 6 hours, and then left for 18 hours. To prevent ethanol evaporation, rapid filtration was used. The filtrate (20.0 mL) was evaporated to dryness in a flat-bottomed vaporizer cup heated to 105°C and calibrated. The remainder was heated at 105°C to a constant weight. Ethanol-soluble extract is calculated by the same formula as the calculation of water-soluble extract (Pusmarani et al., 2019).

2.3 Phytochemical screening

Phytochemical screening was conducted on flavonoids, alkaloids, steroids, triterpenoids, saponins, and polyphenols using a standard color reaction test (Wijayanti et al., 2024).

2.4 Sample preparation for quantitative phytochemical analysis

Java Kapulaga fruit powder (2 g) was added to 100 mL of distilled water and sonicated for 30 minutes. The mixture was filtered, and the filtrate was diluted with distilled water to yield a sample with a concentration of 4000 ppm.

2.4.1 Total phenolic content

The Folin-Ciocalteu method was applied to determine the total phenolic content. A 0.5-mL sample was added with 5 mL of 10% Folin-Ciocalteu reagent, followed by 4 mL of 7.5% Na_2CO_3, and left at room temperature for 30 minutes. The absorbance was measured at 758 nm. The calibration curve was based on a standard of gallic acid (20-60 ppm) (Baba & Malik, 2015).

2.4.2 Total flavonoid content

A 1 mL sample was mixed with 3 mL of 96% ethanol, 0.2 mL of aluminum chloride ($AlCl_3$ 10%), 0.2 mL of potassium acetate 1M, and 5.6 mL of distilled water, then shaken and left to stand at room temperature for 30 minutes. The absorbance was measured at 427 nm. The calibration curve used a standard of quercetin (20-100 ppm) (Chandra et al., 2014).

2.4.3 Total terpenoid content

The dried extract was weighed 100mg, added 9 mL of 70% ethanol, then allowed to stand for 24 hours. Extraction was done using a liquid-liquid method using a separatory funnel with 10 mL of petroleum ether. The extract was collected and evaporated using a water bath to dry, then weighed to determine the percentage of total terpenoid content with the following formula (Malik et al., 2017):

$$Total\ terpenoid\ content\ (\%) = \frac{w1}{w0} x100\% \qquad (4)$$

2.5 Extraction and LC-MS analysis

Java Kapulaga fruit (Amomum compactum Sol. Ex Maton*)* weighing 25 grams was placed in a beaker glass, then 250 mL of solvent was added and stirred. The solvents used were water and n-hexane separately. The maceration was carried out for 3 hours at room temperature, then sonicated (200 W, 40 KHz) for 30 minutes, 70 ℃. The extract obtained was filtered and the residue was remacerated with each solvent. The remacerated filtrate was combined with the initial filtrate and then concentrated to a thickness of 50 mL using a rotary evaporator under vacuum at 55℃. The concentrated extract was centrifuged for 10 minutes to remove any solids that may have escaped during filtration. The extract was stored in a freezer until frozen, then lyophilized for 62 hours to obtain dry extract in powder form (Andishmand et al., 2023).

The Shimadzu LCMS 8040 LC/MS was used to conduct high-resolution MS/MS analysis (Teoh et al., 2023). The Shimadzu Shim Pack FC-ODS (2 mm x 150 mm, 3 µm) was used to separate metabolites before analysis. Injection volume: 1 µL (water extract and n-hexane extract), capillary voltage: 3.0 kV, column temperature: 35°C, mobile phase mode: isocratic, flow rate: 0.5 mL/min. Sampling cone voltage is 23.0 V, and the solvent is 90% methanol. MS focused ion mode io type [M]+, collision energy 5.0 V, desolvation gas flow 60 mL/hour, desolvation temperature 350 °C,

fragmentation method: low energy CID, ionization: ESI, scanning 0.6 sec/scan (mz: 10-1000), source temperature 100 °C, and run time 60 minutes.

2.6 Distillation of essential oils and GC-MS analysis

For the extraction of the oil, 100 g of crushed *Java Cardamom fruit (Amomum compactum* Sol. Ex Maton*)* was placed in a distillation flask (1L) then connected to the steam generator with a glass tube and a condenser. The essential oils were volatilized by heating the water at 100°C for 5 to 10 hours. The recovered mixture was left to settle before being extracted for oil. Following the steam distillation process, the product was collected and separated with a separatory funnel. The essential oils settled on the bottom layer of the separatory funnel and were separated several times until no oil remained in the funnel (Wong et al., 2014).

3. Result and Discussion

3.1 Standardizations **of** *Amomum compactum fructus simplicia*

Java Cardamom fruit (*Amomum compactum* Sol. Ex Maton) standardization was performed by analyzing the non-specific and specific parameters, including physical, chemistry, and biological analysis. Non-specific parameters observed include ash content, water content, total plate count, and total fungal count. Specific parameters observed were water-soluble extract, ethanol-soluble extract, and phytochemical analysis. The results are shown in Table 1 and Fig. 1.

Simplicia should have no more than 10% water content, according to the Indonesian Herbal Pharmacopoeia. Kepmenkes RI Number 261/MENKES/SK/IV/2009 states that the extract's ash content should not exceed 10.2%. The BPOM regulation on traditional medicine quality requirements for contamination by microbes should not be exceeding 10^4 cfu/g for total plate count and 10^3 cfu/g for total fungal count (Ngibad et al., 2024). *Amomum compactum fructus* simplicia meets the requirements according to those regulations, where the moisture content obtained is less than 10%, ash content is less than 10.2%, Total Plate Count are less than 10^3 cfu/g, and total fungal contaminants are less than 10^4 cfu/g. These data show that the processing of simplicia was appropriate. Water-soluble extract shows how much polar compound content in simplicial, whereas ethanol-soluble extract shows the number of compounds that are dissolve in ethanol. In terms of quality, measuring the ethanol-soluble extract content less than to determining the water content (Husni et al., 2021). *Amomum compactum fructus* simplicia showed higher water-soluble extract indicating that more content of compounds dissolved in water solvents.

3.2 Composition of Java Cardamom Metabolite Secondary Compounds

Amomum compactum Sol. Ex Maton simplicia was extracted with several solvents to evaluate the profile of active compounds soluble in each solvent. LC-MS identified active compounds on aqueous extracts and n-hexane extracts, while essential oils were analyzed by GC-MS. In Fig. 2 and Fig. 3, and supplementary Table 1, shows the metabolic secondary compound group in Java Cardamom water extract analyzed by LC-MS are **flavonoid glycosides (27%),** which consist quercetin-3-O-rhamnoside 1.84%, kaempferol-3-(6"caffeoylglucoside) 1.79%, kaempferol-3-(5"feruloylapioside) 1.67%, quercetin 4'-methyl ether 3-neohesperidoside 1.65%, kaempferol-3-O-D-glucoside 1.60%, quercetin-3-O-neohesperidoside 1.49%, kaempferol-3-O-rhamnoside 1.47%, isoquercetin 1.47%, kaempferol-3-glucoside-2"rhamnoside-7-rhamnoside 1.27%, kaempferide 3-O-β-D-glucopyranoside 1.27%, kaempferol 7,4'-dimethyl ether-3-neohesperidoside 1.25 %, naringin 1.23%, quercetin 3-glucoside 1.23%, kaempferol-3-sophoroside-7-rhamnoside 1.20%, kaempferol 4'-methyl ether 3-neohesperidoside 1.04%, luteolin-7-glucoside 1.03%, querciturone 1.03%, kaempferol-7-rhamnoside-4'glucoside 0.97%,

rutin 0.87%, kaempferol-4'-rhamnoside 0.71%, hirsutrin 0.61%, and hyperoside 0.58%. **Flavonoid (24%)**, which consist kaempferide 1.52%, quercetin 7,3',4'-trimethyl ether 1.48%, luteolin 1.38%, naringenin 1.26%, quercetin 1.25%, apigenin 1.24%, 3,5,7-trihydroxyflavone 1.10%, chalconaringenin 2'-(6"-pcoumaryl glucoside) 1.09%, 3,7,4'-tri-O-methylkaempferol 1.06%, luteolin 1.01%, 5-hydroxy-2-(3-hydroxy4methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one 0.98%, morin 0.95%, kaempferol 3,7-dimethyl ether 0.88%, 3,7-dihydroxy-5,3',4'trimethoxyflavone 0.81%, 3,5-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-methoxy4H-chromen-4-one 0.77%, catechin 0.76%, alpinetin 0.71%, cardamonin 0.64%, 5-hydroxy-3,7,3',4'-tetramethoxyflavone 0.63%, 3,7-dihydroxy-5,4'dimethoxyflavone 0.63%, luteolin 0.59%, 3,5,7,3',4'-pentamethoxyflavone 0.59%, 3,5,7,4'-tetramethoxyflavone 0.59%, 5-O-methylnaringenin 0.55%, 3,3',5,7-tetrahydroxy-4'-methoxyflavone 0.42%, naringenin 7,4'-dimethyl ether 0.40%, and naringenin 5-methyl ether 0.27%. **Simple phenol (15%)**, which consist ellagic acid 1.85%, ferulic acid 1.45%, 5-O-caffeoylshikimic acid 1.28%, p-coumaric acid 1.27%, vanillic acid 1.20%, salicylic acid 1.20%, gallic acid 1.12%, caffeic acid 0.97%, shikimic acid 0.91%, cinnamic acid 0.79%, syringic acid 0.71%, 3-O-caffeoylquinic acid methyl ester 0.71%, chlorogenic acid 0.63%, and benzoic acid 0.59%. **Diaryheptanoids (14%**), which consist 4-Hydroxycinnamoyl (feruloyl)methane 0.65%, gingerenone-A 0.32%, gingerenone C 0.42%, bis(4-hydroxy cinnamoyl) methane 0.37%, [6]-gingerol 0.94%, [6]-gingerdione 0.9%, [6]-shogaol 0.90%,Galanganol B 0.88%, 1,7-bis(3,4-dihydroxy phenyl) heptan-3-one 0.75%, (R)-1,7-bis(3,4-dihydroxyphenyl)- 5-methoxyheptan-3-one 0.63%, [10]-dehydroshogaol 0.57%,, tsaokoarylone 0.56%, [8]-dehydroshogaol 0.55%, [6]-gingerdiol 0.51%, [10]-gingerol 0.4&%, galanganol A 0.46%, [8]-shogaol 0.40%, [6]-dehydroshogaol 0.37%, [6]-paradol 0.33%, isogingerenone B 0.26%, [8]-gingerol 0.56%, gingerenone B 0.59%, capsaicin 0.53%, gingerenone C 0.42%, and [1,7-bis(4-hydroxy-3-methoxyphenyl)hepten-3-one] 0.22%. **Oxaspirorganic (6%)**, which consist aculeatol-A 1.42%, aculeatol-C 1.30%, aculeatin-D 1.26%, aculeatol-B 1.03%, aculeatol-C 0.65, **Tannin (6%),** which consist prodelphinidin-C2 2.31%, prodelphinidin B1 1.99%, procyanidin 1.58%. And **terpenoids (4%),** which consist zambesiacolactone-A 1.13%, zambesiacolactone-B 0.88%, galanolactone 0.23% and other terpenoid.

The results of LC-MS analysis show the metabolic secondary compounds of Java Cardamom extract, that the compounds extracted in water solvent using the maceration-sonification method are polar compounds, such as polyphenols including flavonoid glycosides, flavonoids, simple phenols, tannins and oxaspiroorganics, but non-polar compounds. such as diaryheptanoids and terpenoids appear to be extracted in small amounts. Extraction in a water solvent using the maceration-sonification method is expected to obtain relatively large amounts of polar compounds in java cardamom as specific therapeutic targets. In the analysis of a natural product compound as a drug candidate, natural materials using QSAR, one of which is determined according to Lipinski's Rules, Supplementary Table 1, also shows that secondary metabolic group compounds from simple phenols, flavonoids, oxaspirorganics, monoterpenoids tend not to violate Lipinski's rules, however flavonoid glycosides and violate Lipinski's rules, because their molecular weight is relatively high (Mr > 500 g/mol) (Lipinski, 2004).

In water java cardamom extract, the main component compounds are prodelphinidin C2 2.31%, prodelphinidin B1 1.99%, procyanidin 1.57%. **Prodelphinidin C2** or Trimer gallocatechin-(4α-8)-gallocatechin-(4α-8)-catechin is a proanthocyanidin consisting of (+)-gallocatechin and (+)-catechin units connected by (4alpha->8)-linkage. This compound was also identified in Eucalyptus ovata. Total synthesis of prodelphinidin C2 has been accomplished. The antitumor effects of synthetic prodelphidin C2 against PC-3 prostate cancer cell lines have been

investigated (S. C. Santos & Waterman, 2001). **Prodelphinidin B1** is a member of the ellagitannins, a class of natural products that are found in plants. It is an antitumor agent that displays antioxidant and anti-inflammatory effects. Prodelphinidin B1 has been shown to inhibit the growth of cancer cells by inducing apoptotic cell death and inhibiting autophagy. This compound also inhibits tumor angiogenesis, which is the formation of new blood vessels in the tumor tissue. Prodelphinidin B1 has been shown to be effective in protecting against cardiovascular diseases such as atherosclerosis and myocardial infarction (Fujii et al., 2013; Makabe, 2013). **Procyanidins**, as a kind of dietary flavonoid, have excellent pharmacological properties, such as antioxidant, antibacterial, anti-inflammatory and anti-tumor properties, and so they can be used to treat various diseases, including Alzheimer's disease, diabetes, rheumatoid arthritis, tumors, and obesity. Given the low bioavailability of procyanidins, great efforts have been made in drug delivery systems to address their limited use. Nowadays, the heavy burden of oral diseases such as dental caries, periodontitis, endodontic infections, etc., and their consequences on the patients' quality of life indicate a strong need for developing effective therapies. Procyanidin is a polyphenolic polymer found in abundance in grapes, cranberries, apples, tea, cocoa, and pine bark. Procyanidin possessed antioxidant, anticancer, antitumor, anti-inflammatory, immunosuppressive, and antiallergy properties and protected against chronic diseases and metabolic disorders, such as cardiovascular disease, cancer, and immune-related diseases. Procyanidin acts as an anti-inflammatory, inhibits adipogenesis, melanogenesis, oxidative stress, and enhances lipid metabolism and macrophage activity (H. Chen et al., 2022). Procyanidin is an abundant polyphenol found in nature. It is composed of flavan-3-ol units, including catechin and epicatechin (Dasiman et al., 2022). **Ellagic acid** (1.85%) is a minor polyphenol, mainly occurs as a constituent of ellagitannins, and is a dimeric derivative of gallic acid present in the group of phenolic compounds. It presents antioxidant, antihepatotoxic, antisteatosic, anticholestatic, antifibrogenic, antihepatocarcinogenic, antiviral antiadipogenic activity, and high potential cancer prevention/treatment (García-Niño & Zazueta, 2015). **Quercetin-3-O-rhamnoside** (quercitrin) is a flavonoid glycoside compound that has the ability to be evaluated for their wound healing activity, including evaluation of wound closure, revascularization, wound re-epithelialization, fibroblast proliferation, and collagen deposition on rat skin samples (Mohammad et al., 2022) and cytotoxic activity and inhibited cell migration against the HeLa cells line, which suggest the potential for therapeutic application in cancer treatment (Herni et al., 2021). Other major components including flavonoid glycosides are kaempferol-3-(6"caffeoylglucoside), kaempferol-3-(5"feruloylapioside), quercetin 4'-methyl ether 3-neohesperidoside, and kaempferol-3-O-D-glucoside. Studies on the activity of **kaempferol-3-(6''caffeoylglucoside), kaempferol-3-(5''feruloylapioside), quercetin 4'-methyl ether 3-neohesperidoside** are still not widely discussed, but studies on kaempferol-3-O-D-glucoside (astragalin) as being able to anti-inflammatory (inhibits pro-inflammatory mediators like IL-1β, NO, PGE2, LTB4 and anti-inflammatory cytokine IL-10), antioxidant, anti-allergic, Inhibits prostaglandin and angiotensin converting enzyme activity, increases estrogen and progesterone, and inhibits CYP1B1. **Kaempferol-3-O-β-d-glucoside** inhibits the activity of cytochrome P450 (CYP) 1B1, which catalyzes estradiol to form 4-hydroxy-estradiol (4-OH-E2) (Meng et al., 2019). In flavonoids group include kaempferide, quercetin 7,3',4'-trimethyl ether, luteolin, naringenin, quercetin, etc. **Kaempferide** is a flavonoid with many biological activities, including anti-inflammatory, antioxidant, anticancer, antidiabetic, antiobesity, antihypertensive, neuroprotective, immunomodulatory, osteogenesis, antioxidant, anticancer, antidiabetic, antiobesity, antihypertensive, neuroprotective, immunomodulatory, and osteogenesis (Song et al., 2024).

Quercetin 7,3',4'-trimethyl ether, also known as 3',4',7-trimethoxyquercetin, has a number of biological activities, including antioxidant, inhibits DNA gyrase and topoisomerase IV, and topoisomerase IV in bacteria (Song et al., 2024). Other secondary metabolic components are minor components including organic oxaspiro such as aculeatol A, aculeatol C, aculeatin D, aculeatol B, aculeatol C. Diarylheptaonoid, i.e 4-hydroxycinnamoyl (feruloyl) methane, gingerenone A, gingerenone C, bis(4-hydroxy cinnamoyl) methane, [6]-gingerol, etc, and terpenoids meliputi zambesiacolactone A, zambesiacolactone B, galanolactone, aframodial, etc. Aculatetol A and B and their derivatives have anti-cancer potential (Chin et al., 2008). Aculeatol C as a fungicide (Chin et al., 2008). **Zambesiacolactone A and zambesiacolactone B** are labdane diterpenoid compounds that have potential as antimalarials. (Kenmogne et al., 2005).

Fig. 4 Fig.-5, and supplementary Table 2, shows the metabolic secondary compound group in n-hexane Java Cardamom extract analyzed by LC-MS are **terpenoids,** i.e. **monoterpenoids:** geranyl acetate 1.40%, neryl acetate 1.20%, bornyl acetate 1.08%, β-pinene 1.06%, camphene 1.06%, etc. **Sesquiterpenoids:** α-zingiberene 3.71%, (3aS,7S,7aR)-7-hydroxy-2,3,3a,6,7,7a-hexahydro-1Hindene-4-carbaldehyde 1.06%, β-caryophyllene 1.05%, δ selinene 1.05%, bisacumol 0.87%. **Diterpenoids:** zambesiacolactone A 2.99%, zambesiacolactone B 2.34%, 16-hydroxylabda-8- (17),11,13-trien-15,16-olide 1.15%, galanal A 0.88%. **Triterpenoids:** β-amyrin 1.12%,β-sitosterol 1.04%, campesterol 0.90%, ergosterol peroxide 0.59%. **Diarylheptanoids:** 1,5-bis(4-hydroxy-3-me-thoxyphenyl)-penta(1E,4E)-1,4-dien-3-one 2.65%, [6]-gingerol 2.50%, [6]-gingerdione 2.38%,[6]-shogaol 2.37%, galanganol B 2.34%, galangin 1.70%, cardamonin 1.70%, gingerenone B 1.58%,[10]-dehydroshogaol 1.51%, 3,5-diacetoxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7(4hydroxy-3-methoxyphenyl)heptane 1.50%, [8]-gingerol 1.48%, [8]-dehydroshogaol 1.466%, capsaicin 1.40%, 3,5-diacetoxy-7-(3,4-dihydroxyphenyl)-1-(4-hydroxy3-methoxyphenyl)heptane 1.345%, [6]-gingerdiol 1.34%, [10]-gingerol 1.23%, (3R,5S)-1,7-bis(4-hydroxy-3-methoxyphenyl) heptane-3,5-diol 1.23%, galanganol A 1.23%, [8]-shogaol 1.06%, 1-dehydrogingerdione 1.14%, gingerenone C 1.12%, meso-3,5-diacetoxy-1,7-bis-(4-hydroxy-3-methoxyphenyl)-heptane 1.08%, bis-(4-hydroxy cinnamoyl) methane 0.99%, 5-hydroxy-7-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-3heptanone 0.98%, 1,7-bis (4-hydroxyphenyl)-1,4,6-heptatrien-3-one 0.98%, [6]-dehydroshogaol 0.97%, galanganal 0.97%, [6]-paradol 0.88%, gingerenone A 0.84% (3S,5S)-3,5-diacetoxy-1,7bis(3,4-dihydroxyphenyl)-heptane 0.83%, 1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxy phenyl)-1,6heptadiene-3,5-dione 0.82%, 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone 0.79%, isogingerenone B 0.69%, 1,7-diphenyl-3,5-heptanedione 0.65%, 1-(4-hydroxy-3-methoxyphenyl)-5-(-4-hydroxylphenyl)-penta-(1E,4E)1,4-dien-3-one 0.58%,[1,7-bis(4-hydroxy-3-methoxyphenyl)hepten-3-one] 0.58%, 5-hydroxy-7-(4-hydroxyphenyl)-1-(4-hydroxy3-methoxyphenyl)-3heptanone 0.53%, 6-dehydrogingerdione 0.46%. Secondary metabolic polar compounds in **n-hexane extract, include: simple phenols,** i.e. syringaldehyde 1.14% and 1-hydroxychavicol acetate 1.13%. **And Flavonoids:** aromadendrin 7,4'-dimethyl ether 1.42%, aromadendrin 3-acetate 1.35%, (2R,3R)-aromadendrin-7-methyl ether 3-acetate 0.98%.

In n-hexane java cardamom extract, the main component compounds are α-zingiberene (3.71%), zambesiacolactone A 2,99%, 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta(1E,4E)-1,4-dien-3-one 2,65%, [6]-gingerol 2,50%, [6]-gingerdione 2,38%, [6]-shogaol 2,37%, galanganol B 2,34%, zambesiacolactone B 2,34%, galangin 1,70%, cardamonin 1,71% gingerenone B 1,58%, [10]-dehydroshogaol 1,51%, 3,5-diacetoxy-1-(4-hydroxy3,5-dimethoxyphenyl)-7-(4hydroxy-3-methoxyphenyl)heptane 1,50%, 1,1-dodecanediol, diacetate 1,49%, [8]-gingerol 1,48%, [8]-dehydroshogaol 1,46%, aromadendrin 7,4'-dimethylether 1,42%, geranyl acetate 1,40%, capsaicin

1,4%, aromadendrin 3-acetate 1,34%, 3,5-diacetoxy-7-(3,4-dihydroxyphenyl)-1-(4hydroxy-3methoxyphenyl)heptane 1,35%, [6]-gingerdiol 1,34%, [10]-gingerol 1,24%, (3R,5S)-1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-diol 1,24%, galanganol A 1,23%, etc. Dalam analisis suatu senyawa bahan alam sebagai kadidat obat, menurut aturan Lipinski, Supplementary Table 2 also shows that secondary metabolic group compounds from diarylheptanoids, flavonoids, monoterpenoids, diterpenoids do not violate Lipinski's rules, while those that violate the rules are several sequiteprneoids and triterpenoids (Lipinski, 2004).

α-zingiberene, a sesquiterpene found in ginger and other plants, has a number of activities, including: anti-inflammatory, anticancer (Seshadri et al., 2022), anti-rhinoviral, neuroprotective, collagen deposition (Raina et al., 2024), and cytotoxic has moderate cytotoxic activity against HeLa, U-87, Siha, and HL60 cell lines (Ferreira et al., 2022)(Mierza et al., 2023). **Zambesiacolactone A** and five other labdane are diterpenoids compounds that can be extracted in n-hexane or water solvents, but are more likely to be extracted in water solvents. This compound has potential as an anti-malarial with an IC_{50} value of 4.97 microM (Kenmogne et al., 2006). **1,5-bis(4-hydroxy-3-methoxyphenyl)-penta(1E,4E)-1,4-dien-3-one** potential as an antioxidant (Mousa, 2012). A series of novel analogues of 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1E,4E)-1,4-dien-3-one were synthesized and evaluated for their cytotoxicities against human colon cancer cell line HCT-116 (Yamakoshi et al., 2010). **[6]-gingerol**, a bioactive compound in ginger families, have many biological activities, including anticancer: pancreatic, gastric, colorectal, renal, and breast cancers (Wang et al., 2014), improves cardiovascular metabolism (Zhang et al., 2023),anti-inflammatory, anti-oxidative stress, antiviral, antiemetic, improves gastrointestinal motility (Promdam & Panichayupakaranant, 2022). **[6]-gingerdione** has biological activities, including cell-free antioxidant (Manjunathan et al., 2021) and decreases ROS and cell death (Li et al., 2012). **[6]-shogaol** is a compound found in ginger that has a variety of biological activities, including anti-inflammatory (Ling et al., 2010), anticancer, antioxidant (Bischoff-Kont et al., 2022), and inhibits enzymes (Jacob, 2016). **Galanganol B** and its derivatives as anti-inflammatory (Aryasa & Sugianta, 2023). Galangin has potential advantages in the treatment of neurodegenerative diseases and cardiovascular and cerebrovascular diseases, which are common in the elderly. In addition, it also showed that galangin had prospective activities in the treatment of tumors, diabetes, liver injury, asthma and arthritis (F. Zhang et al., 2023). **Cardamonin** is a naturally occurring flavonoid (chalcone) abundantly present in plants (Badroon et al., 2020). Cardamonin, a natural chalcone found in spices like cardamom, has many pharmacological activities, including anti-inflammatory, anti-oxidant, anti-microbial (Mehmood et al., 2023), anti-emetic, anti-hypoglycemic, vasorelaxant, anti-melanogenesis, anti-infectious and anti-cancer (Badroon et al., 2020). Cardamonin can inhibit cancer cell growth and induce apoptosis, or programmed cell death, in malignant cells. It can also inhibit cell proliferation and decrease the phosphorylation of mTOR and S6K1 (Niu et al., 2020). Gingerenone B and its derivative showed the highest binding affinities for CpsA, CpsB and CpsD. These findings suggest that gingerenone A, B and C are potential inhibitors of S. pneumoniae-conserved capsule-synthesizing proteins (Azmi et al., 2024). [10]-dehydroshogaol (DHSG) is a minor component of ginger rhizomes and its derivate that has several activities (Imm et al., 2010), including anti-inflammatory, antioxidant (Li et al., 2022), and inhibitor of nitric oxide synthesis (Rahmani et al., 2014). 1,1-dodecanediol, diacetate suggested antidiabet suggest the antidiabetic and cytotoxic effects of C. volubile leaves (Erukainure et al., 2018). [8]-gingerol, [10]-gingerol and its derivate can raised levels of death receptor (DR) 5 in a p53-dependent manner. Besides, it lowered the abundance of anti-apoptotic proteins (survivin, c-FLIP, Bcl-2, and XIAP) and increased pro-

apoptotic proteins, Bax and truncate Bid, by developing ROS. We also found that gingerol's sensitizing effects were blocked in TRAIL-induced cell death by scavenging ROS or overexpressing anti-apoptotic protein (Bcl-2). Henceforth it has also been shown to play a role as a sensitizing agent in inducing TRAIL-resistant glioblastoma cell death (Tagde et al., 2021). 8-gingerol also reduced the effective dosage of 5-fluorouracil and, thereby, the toxicity of drug combination therapy. These data suggest that 8-gingerol may be a promising candidate for the development of novel anticancer agents against CRC. **Aromadendrin 7,4'-dimethylether, aromadendrin 3-acetate, and** its derivative has potential as an immunosuppressive antiproliferative, anti-inflammatory, gastroprotective, antibacterial, trypanocidal, anticandidal, antioxidant and cytotoxic in medicine (Patel & Patel, 2024).**Geranyl acetate** is an epoxide derivative and its hydroperoxide has antifungal action, especially Microsporum gypsum, Trichophyton vercossum and Candida tropicalis. Geranyl acetate shows significant anticancer activity against the colo-205 cancer cell line with an IC50 value of 30 microM. In cell research, activity also showed antiproliferative properties determined by the MTT test. Apoptosis was assessed by DAPI staining and DNA damage was examined by comet assay. Cell cycle analysis was performed by flow cytometry and protein expression was examined by western blotting (Remonatto et al., 2022). **Capsaicin** has the ability to act as a local analgesic, also showing antioxidant, anticancer, antiobesity and gastroprotective activities. The longer half-life of capsaicin in the lungs and skin suggests that it may have a stronger effect on these tissues. **[6]-gingerdiol** and its derivatives have ability antibiofilm, antivirulence (J. H. Lee et al., 2018), anti-inflammatory (Alharbi et al., 2022), antioxidant, anti-tumor, anti-nausea, antiemetic (Promdam & Panichayupakaranant,2022).**3,5-diacetoxy-7-(3,4-dihydroxyphenyl)-1-(4hydroxy-3-methoxy-phenyl)-heptane** is a compound that has been successfully synthesized in total, the first example of the natural product diarylheptanoid (5S)-5-acetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-3-heptanone and (3S,5S)-3,5-diacetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-heptane 2 isolated from Zingiber officinale rhizomes was carried out using Sharpless epoxidation and cross-metathesis reactions as the key steps (Sabitha et al., 2011).

Fig. 4, Fig. 5, and supplementary Table 3, as shown Java Cardamom Essential Oil compounds analyzed by GC-MS including monoterpenoids, i.e.: Eucalyptol/ 1,8-cineol 63.21%, beta.-Pinene 10.49%, o-Cymene 2.75%, (+)-Sabinene 2.37%, pseudolimonene 1.27%, beta-Myrcene 1.11%, terpinen-4-ol 0.89%, D-Fenchone 0.70%, gamma.-Terpinene 0.52%, camphene 0.19%, beta-Ocimene 0.17%, D-Camphor 0.14%, alpha.-Phellandrene 0.13%, alpha-thujene 0.11%, 3-Carene 0.10%, exo-2-Hydroxycineole 0.07%, carvacrol 0.07%, alpha-Terpinene 0.07%, and isoascaridol 0.05%; monoterpenoid alcohol, i.e.: alpha.-terpineol9.07%, linalool 1.69%, 4-Thujanol 1.34%, delta-terpineol 1.22%, (-)-cis-Sabinol 0.10%, (-)-Myrtenol 0.08%, pinocarveol 0.06%, p-Cymen-8-ol 0.05%, isopinocarveol 0.05%, and p-Menth-2-en-1-ol 0.04%; sesquitepenoids, i.e.: (+)-gamma-cadinene 0.06%, beta.-Bisabolene 0.32%, (-)-alpha.-panasinsen 0.17%, bicyclo[5.3.0]-decane-2-methylene-5-(1-methylvinyl)-8-methyl 0.29%, germacrene D 0.14%, beta-Selinene 0.13%, gamma-Selinene 0.08%, gamma-Selinene 0.05%, sesquithujene 0.04%, and 7-epi-cis-sesquisabinene hydrate 0.06%; and simple phenolic, i.e: Cinnamaldehyde, (E) 0.37%.

Java cardamom Essential Oil, the main component compounds including eucalyptol/ 1,8-cineol, beta-pinene, alpha.-terpineol, o-Cymene, (+)-sabinene, linalool, 4-Thujanol, pseudolimonene, delta-Terpineol, beta-Myrcene, and terpinen-4-ol, etc. Eucalyptol, also known as 1,8-cineole, that has many biological activities antimicrobial, bronchodilatory, analgesic, insect repellent (Hoch et al., 2023), antioxidant, and anti-inflammatory. It can also inhibit the production of proinflammatory cytokines like TNF-α, IL-1β, IL-4, and IL-5 (Seol & Kim, 2016). Eucalyptol is

used in many mouthwashes and cough suppressants. It can also be used to treat conditions like respiratory disease, pancreatitis, colon damage, and cardiovascular and neurodegenerative diseases (Seol et al., 2016). **alpha.-Terpineol** and its derivative are found in many plant essential oils and natural monoterpene alcohol with a lilac-like scent that has many biological activities, including anti-inflammatory, antioxidant, anticancer (Khaleel et al., 2018), analgesic, gastroprotective: cardioprotective: neuroprotective, antidiarrheal, insecticidal, skin penetration enhancing, and antibacterial (Yang et al., 2023)(Y. Chen et al., 2023). **Linalool** has many activities, including anti-inflammatory, anticancer, anti-hyperlipidemic, antimicrobial, antinoceptive, analgesic, anxiolytic, antidepressive and neuroprotective (Pereira et al., 2018). Linalool can enhance the cell wall permeability of drugs through mucus membranes and skin. This can make it useful as an absorption promoter in topical formulations. (É. R. Q. dos Santos et al., 2021). Linalool is the main constituent of some essential oils from aromatic plants and has evidence of activity on the central nervous system, mainly acting as an antidepressant agent. Linalool is an effective antibacterial compound that can also inhibit biofilm formation. For example, linalool can inhibit biofilm formation in Shigella flexneri at a concentration of 3% (v/v) and in Listeria monocytogenes at a concentration of 0.5% (v/v) (Gao et al., 2019). **4-Thujanol** has a number of activities, including chemopreventive effects, cancer therapy, flavoring agent, anti-attractant, and bio-active fibrous assemblies. 4-Thujanol may have chemopreventive effects against genetic damage caused by anticancer drugs like cyclophosphamide (CP) and mitomycin C (MMC). It may also reduce the harmful side effects of CP without reducing its antiproliferative activities (Kocaman et al., 2013). Cis-4-thujanol may be effective as a cancer therapy because it inhibits cyclin-dependent kinases (CDKs), which regulate the cell cycle and promote cell division (Kocaman et al., 2013). 4-Thujanol is an aromatic ingredient used in food as a flavoring substance with a taste similar to fresh minted thyme. It is found in small percentages in essential oils like marjoram and thyme. (E)-(R)-4-thujanol can be produced in kilogram-scale crystals and may have applications as bio-active fibrous assemblies (Morvan et al., 2022). **Terpinen-4-ol** is a monoterpene found in many plants and is a key ingredient in tea tree oil. It has many activities, including anti-inflammatory, anti-cancer in leukemia and non-small cell lung cancer (NSCLC) cells, antioxidant, anti-arthritic, anti-tumorous, antidiabetic (Deen et al., 2023), anticonvulsant, cardiovascular (Deen et al., 2023), and antimicrobial. Terpinen-4-ol has antibacterial and antifungal properties, and can inhibit the biofilm formation of bacteria. It's effective against gram-positive and gram-negative bacteria, as well as fungi like Aspergillus and Fusarium (Kamiya et al., 2024).

Standardization of Jaya Cardamom simplicia, such as determining the parameters of ash content, water content, water soluble essence content, total polyphenols content, total flavonoid content will influence the quality of the herbal simplicia produced, in accordance with the standards of the Indonesian Herbal Pharmacopoeia. The quality of the simplicia will determine the guarantee of the product when used in herbal medicine, herbal or phytopharmaceutical preparations. This simplest standardization will also affect the content of secondary metabolite compounds extracted in water solvents, n-hexane solvents or volatile oils produced from steam distillation. The application of simplicia standardization and extraction standardization is also adjusted to the target compounds that will be developed and adapted to the target mechanism of disease therapy in pre-clinical and clinical research in the development of medicinal compounds.

Conclusion

Jaya Cardamom simplicia standardization, such as determining the parameters of ash content, water content, water soluble essence content, total polyphenols content, total flavonoid content in accordance with the standards of the Indonesian Herbal Pharmacopoeia. Secondary metabolite

profile in water solvent extracted polar compounds, i.e. polyphenols group (flavonoids, simple phenols, tannins, oxaspiro-organic) then semi-non-polar, i.e. diarylheptanoids, terpenoids; in n-hexane solvent extracted non-polar compounds, i.e. diarylheptanoids, terpenoids, and CEO only terpenoids. The application of simplicia standardization and extraction standardization is also adjusted to the target compounds that will be developed and adapted to the target mechanism of disease therapy.

Declaration of competing interest

All the authors have no conflict of interest to declare.

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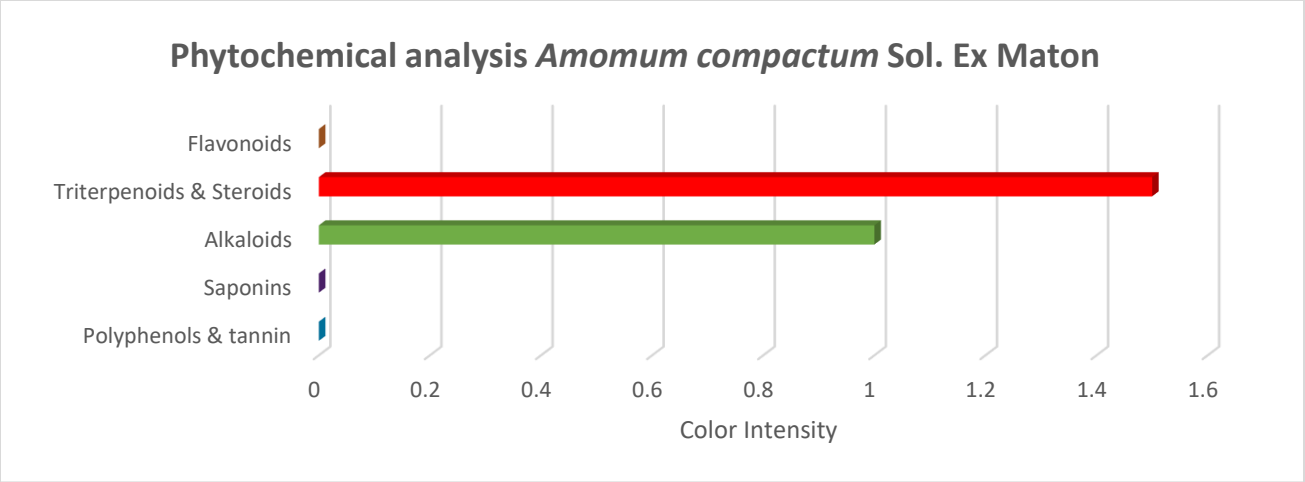
Table 1. Analysis of specific and non-specific parameters of simplicia

Standardization parameter		Results
Non-specific	Water Content	7.74±0.11%
	Ash Content	9.69±0.03%
	Total Plate Count	$5,4x10^2$ cfu/g
	Total Fungal Count	$2,3x10^3$cfu/g
Specific	Water-soluble Extract	1.79±0.11
	Ethanol-soluble Extract	0.47±0.15



(a)

(b)

Parameters	Results
Total Phenol Content	1.48±0.53 (mg GAE/g),
Total Flavonoid Content	0.53±0.07 (mgQE/g)
Total Terpenoid Content	37.12±0.02 (mg/g)

Fig. 1. Qualitative (a) and Quantitative (b) Phytochemical Analysis of *Amomum compactum* Sol. Ex Maton simplicia

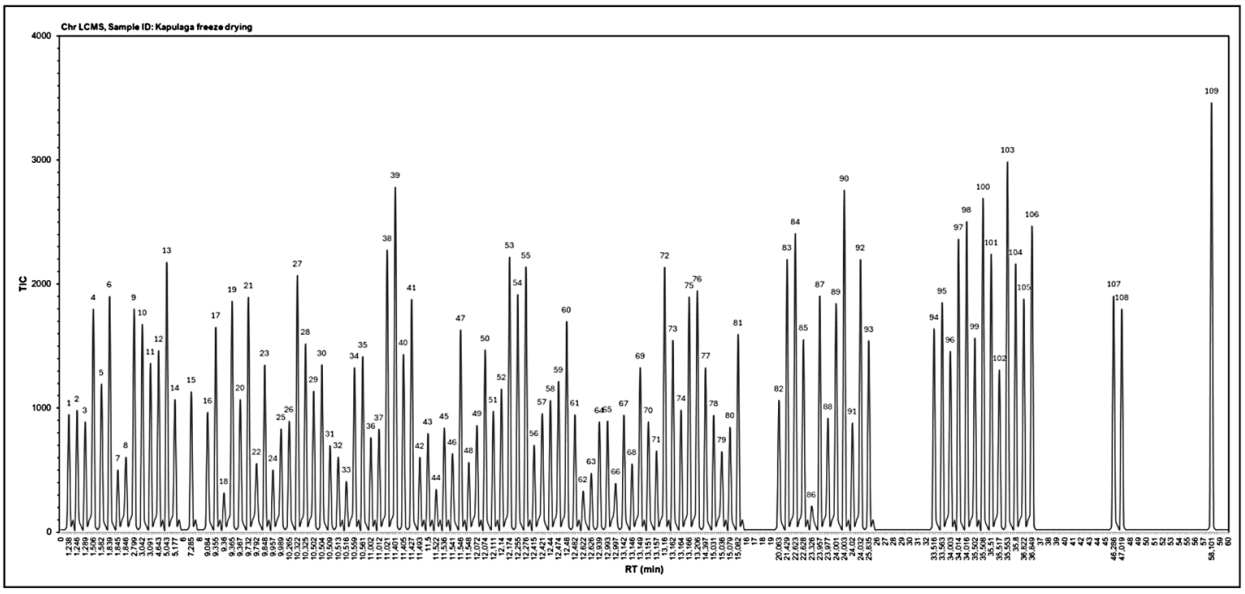


Fig. 2. TIC Water Java Cardamom Extract analyzed by LC-MS

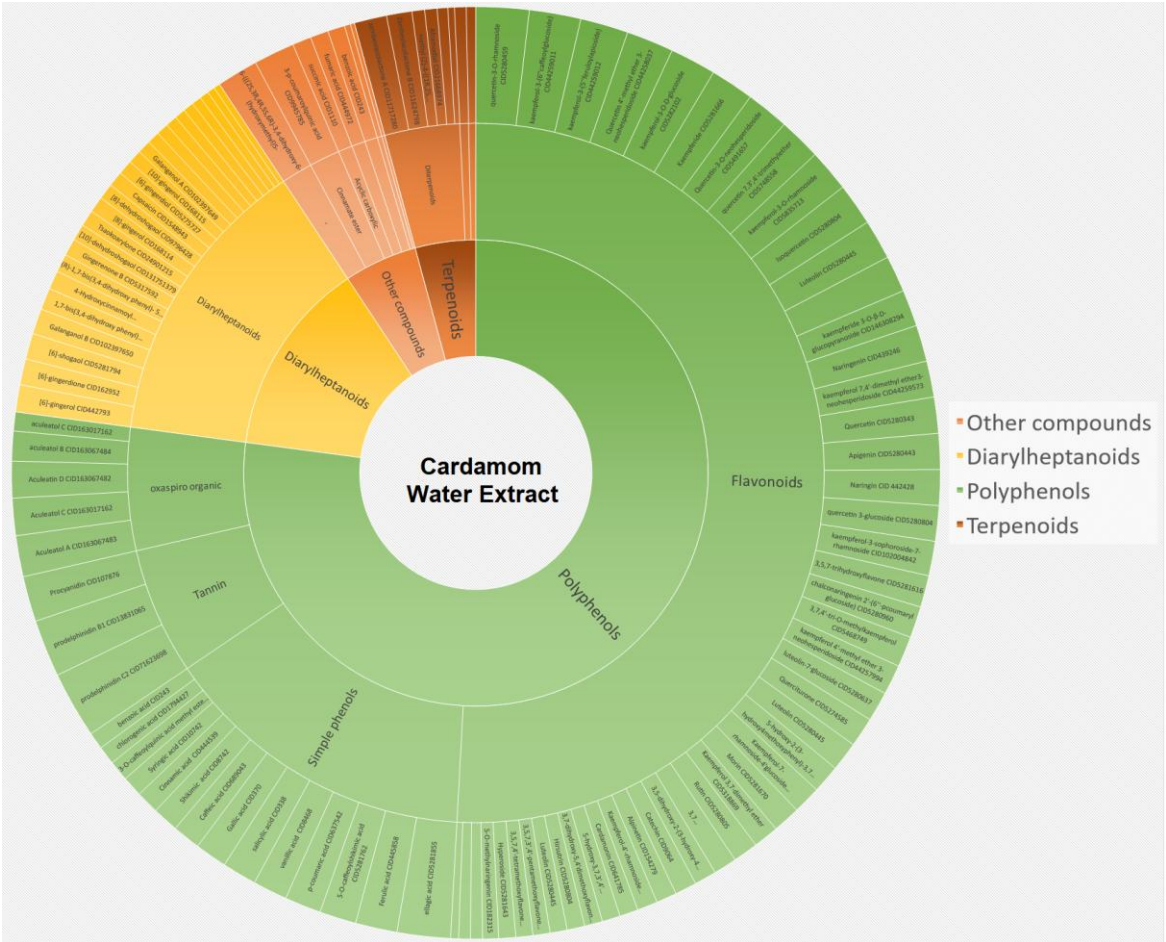


Fig. 3. Composition metabolic secondary Water Java Cardamom Extract analyzed by LC-MS

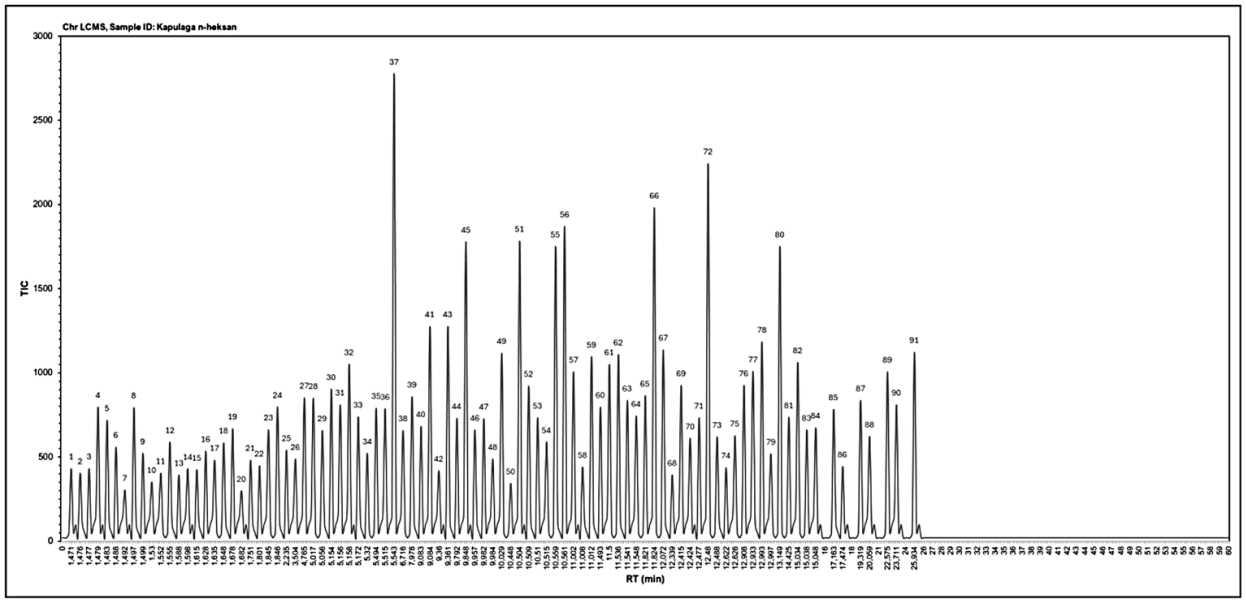


Fig. 4. TIC n-hexane Java Cardamom Extract analyzed by LC-MS

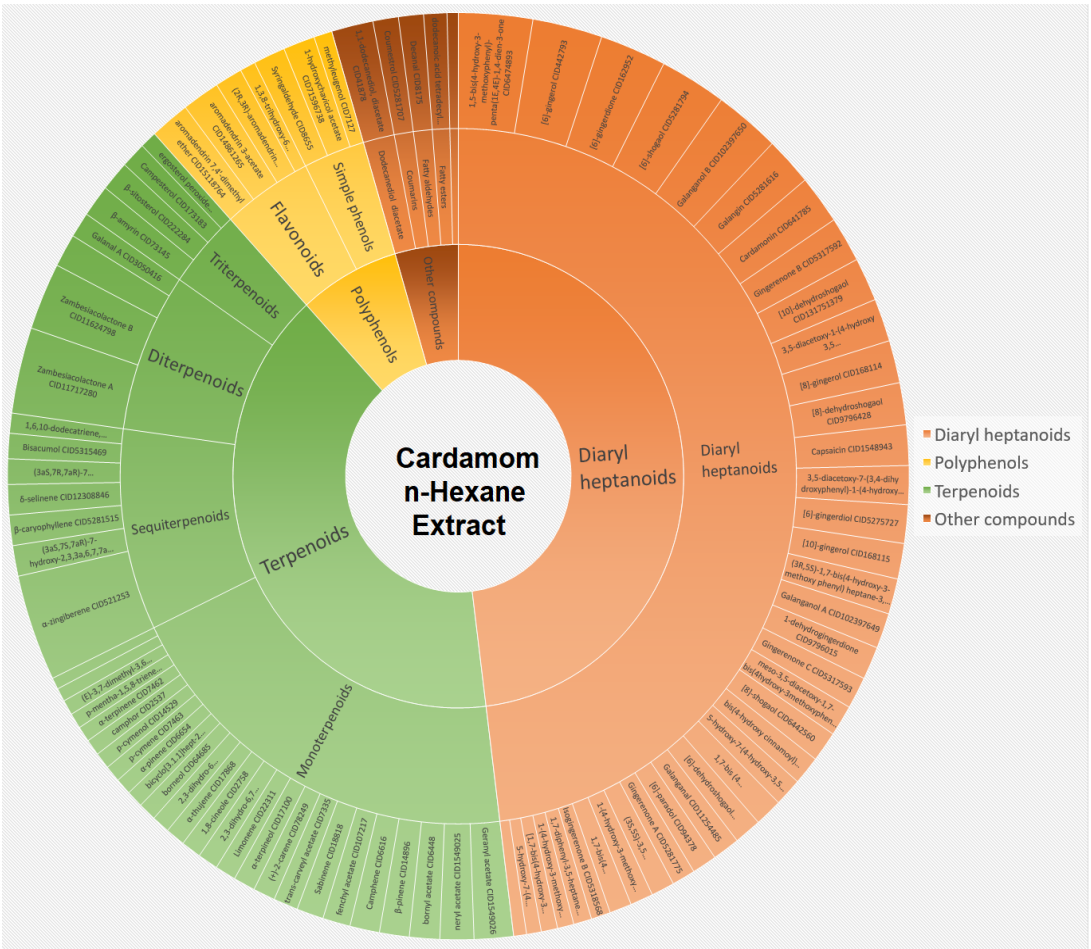


Fig. 5. Composition metabolic secondary n-hexane Java Cardamom Extract analyzed by LC-MS

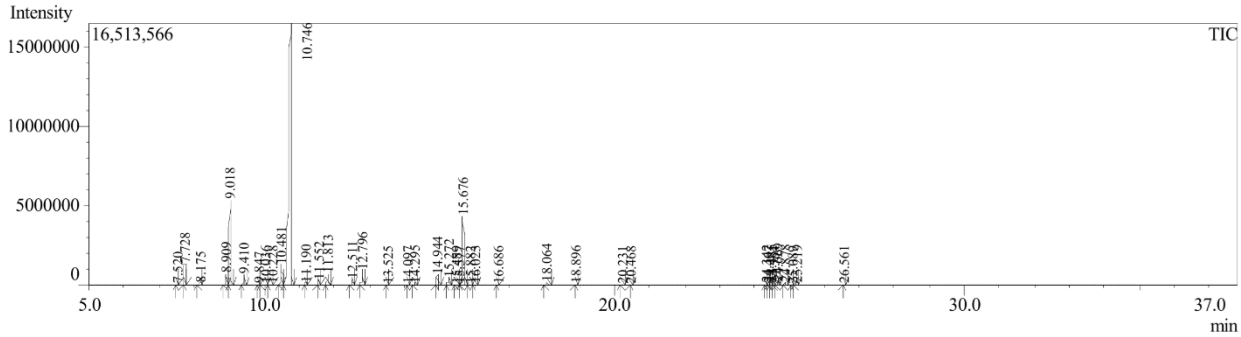


Fig. 6. TIC of Java Cardamom Essential Oil analyzed by GC-MS

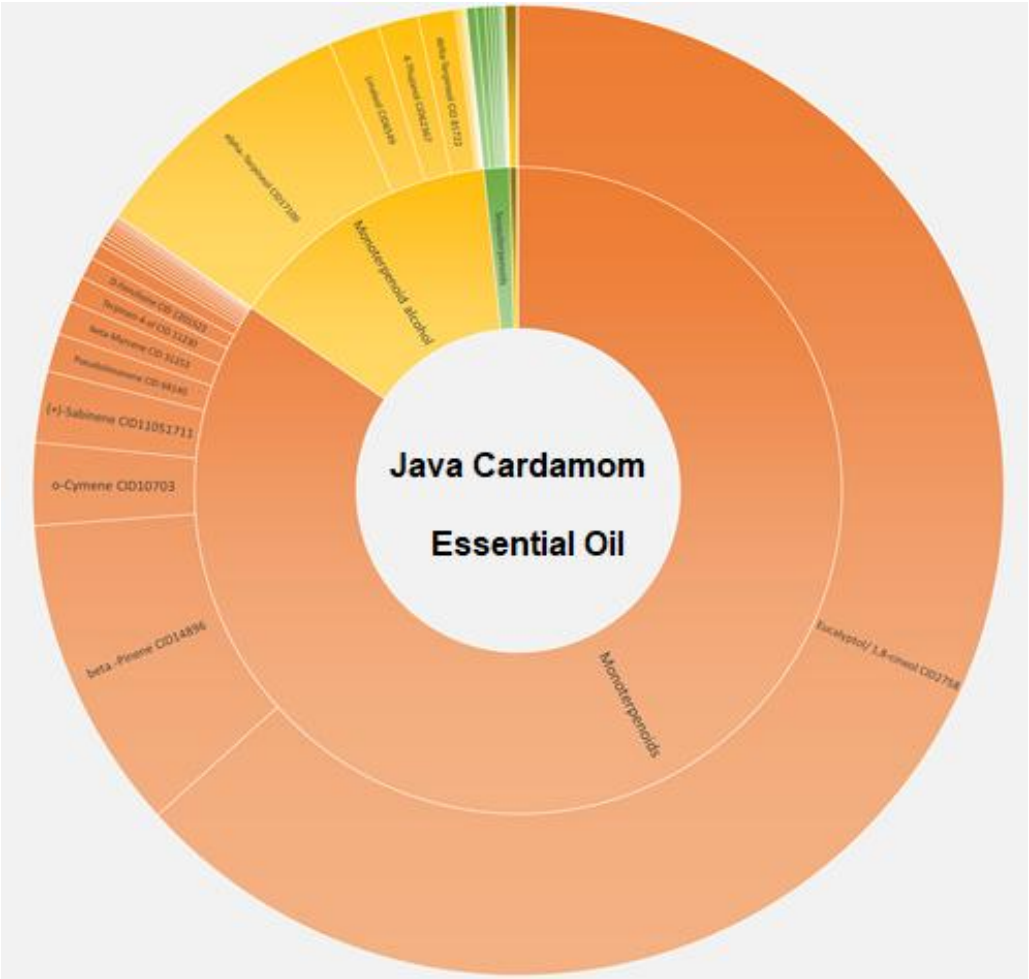


Fig. 7. Composition metabolic secondary n-hexane Java Cardamom Essential Oil analyzed by GC-MS