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Bioactive compound profile of sun-dried cubeb (*Piper cubeba*) fruit extract

RENDI FATHONI HADI[1,2], KUSTANTINAH[3], MUHLISIN[3,♥], RONNY MARTIEN[4]

[1]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia
[2]Graduate School of Animal Science, Universitas Gadjah Mada. Jl. Fauna No. 3 Bulaksumur, Sleman 55281, Yogyakarta, Indonesia
[3]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada. Jl. Fauna No. 3 Bulaksumur, Sleman 55281, Yogyakarta, Indonesia. Tel.: +62-274-513363, ♥email: muhlisin.fapet@ugm.ac.id
[4]Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Gadjah Mada. Sekip Utara, Sleman, Yogyakarta, 55281, Indonesia

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Abstract. *Hadi RF, Kustantinah, Muhlisin, Martien R. 2025. Bioactive compound profile of sun-dried cubeb (*Piper cubeba*) fruit extract. Biodiversitas 26: 662-669. Piper cubeba* is a herbal plant that is commonly used to treat digestive and respiratory diseases because it has biological activity related to bioactive compounds. This study aimed to analyze the profile of bioactive compounds in cubeb fruit, focusing on tannins, phenols, and flavonoids. Tannins are a type of polyphenol with antioxidant properties, phenols are organic compounds with various biological activities, and flavonoids are a diverse group of phytonutrients with antioxidant and anti-inflammatory effects. These compounds were extracted using different solvents, and Gas Chromatography-Mass Spectrometry (GC/MS) was employed to profile them. The results indicate that 96% ethanol is more effective at extracting compounds compared to 70% ethanol and distilled water. The percentage yield of the sun-dried cubeb extract varies significantly with different solvents ($P<0.05$). Quantitative analysis of cubeb fruit extracts demonstrated that flavonoid content was highest with 96% ethanol (27.85±0.53 mg QE/g), followed by 70% ethanol (10.84±0.34 mg QE/g) and distilled water (2.39±0.03 mg QE/g). Total phenol content was also greatest with 96% ethanol (45.23±0.23 mg GAE/g), compared to 70% ethanol (36.04±0.33 mg GAE/g), and distilled water (12.60±0.42 mg GAE/g). For tannins, 96% ethanol yielded the highest concentration (27.85±0.25 mg TAE/g), with lower levels in distilled water (7.81±0.32 mg TAE/g) and 70% ethanol (6.56±0.07 mg TAE/g). These significant differences ($P<0.05$) highlight the potential of cubeb fruit as a valuable source of bioactive compounds, suggesting its suitability for functional food applications.

Keywords: Bioactive profiling, cubeb fruit, GC/MS analysis, *Piper cubeba*, solvents

INTRODUCTION

The cubeb plant (*Piper cubeba* L.) in Indonesia, commonly called the *kemukus*, plant grows widely in Java, Kalimantan, Sumatra, and other islands in the Indian and Pacific Oceans. In Indonesia, this plant is traditionally used to treat conditions such as gonorrhea, dysentery, syphilis, stomach aches, diarrhea, colitis, and asthma (Elfahmi et al. 2007; Ahmad et al. 2020; Kumar 2021; Drissi et al. 2022). Because of the antioxidant, antibacterial, anti-inflammatory, and anticancer properties, cubeb plants and isolated compounds have been shown to have diverse applications as herbal and traditional remedies. Essential oils and phytochemicals (phenolic compounds, lignans, and alkaloids) are linked to these biological activities (Hadi et al. 2021b; Drissi et al. 2022). Among the oils found in cubeb, sabinene, and eucalyptol are the most abundant terpenes (Magalhães et al. 2012; Lima et al. 2017). Furthermore, certain extracts from the cubeb plants exhibit insecticidal, antinociceptive, antiasthmatic, anti-diabetic, analgesic, anti-inflammatory, nephroprotective, antidepressant, anticancer, hepatoprotective, antioxidant, analgesic, and antibacterial properties (Ahmad et al. 2020; Drissi et al. 2024)

Bioactive compounds are various chemical compounds naturally produced in plants. These compounds are often used as phytobiotics or herbal medicines that are beneficial for health (Bose et al. 2017; Andriana et al. 2019) but are not considered important. One method to obtain these bioactive components from plant materials is through extraction. As noted by Gaire and Subedi (2014) and Alkaabi et al. (2023), the extraction technique is crucial to determining the acquired bioactive components' quantity and quality. There are numerous extraction methods, either traditional or modern, each with its advantages and disadvantages. According to Mahdi-Pour et al. (2012) and Kaur et al. (2024), the extraction method and solvent impact the extraction outcomes and biological activity of the resultant extract. Various solvents (polar, semi-polar, and non-polar) have been used to extract bioactive compounds from *Phyllanthus emblica* fruit (Albadwawi et al. 2022).

Various studies on plant extracts often use multiple types of polar and non-polar solvents during extraction, focusing on quantitative and qualitative screening of bioactive compounds. Studies on the use of GC/MS of extracts with different solvents have revealed that plant extracts, including those from fruits and leaves, contain many flavonoid compounds, tannins, phenols, alkaloids, essential oils, fatty acids, and various other valuable bioactive compounds (Dahibhate and Kumar 2022). In

addition, the quantity of bioactive compounds can vary depending on the analytical methods and extraction methods employed (Cheong et al. 2018; Dahibhate and Kumar 2022). Therefore, an accurate analytical approach is necessary to determine the quality of effective herbal plant extracts. These may include gas chromatography (GC), capillary electrophoresis (CE), and liquid chromatography (LC) combined with mass spectrometry (MS) and nuclear magnetic resonance (NMR) for monitoring plant-derived bioactive compounds (Wang et al. 2015). Gas chromatography-mass spectrometry (GC/MS) is typically used not only for analyzing volatile compounds but also for non-volatile compounds because of the advancements in derivatization methods. Today, GC/MS is widely applied in metabolomics for the analysis of non-volatile compounds such as sugars, amino acids, and organic acids (Putri et al. 2022).

The cubeb plant has been associated with a pharmacological and biological wide range of activities, including the ability to influence melanogenesis and exhibit antioxidant, anti-inflammatory, anticancer, anti-diabetic, antiparasitic, and antibacterial properties. The plant is primarily cultivated for its berries, which are valued for their high essential oil content (Parreira et al. 2019; Drissi et al. 2022; Maungchanburi et al. 2022). Cubeb plant extract is known to contain several volatile compounds in the form of essential oils, oleoresin, and dichloromethane. The most significant compounds identified in the oleoresin of cubeb include cubebol (26.1%) and beta-cubebene (12.3%). In comparison, essential oil contains various components such as methyl eugenol (41.31%), eugenol (33.95%), beta-cubebene (18.3%), and alpha-cubebene (4.1%) (Zahin et al. 2018). In contrast, dichloromethane is dominated by the propylene glycol compound at 23.82%, a compound not found in the other extracts (Maungchanburi et al. 2022). This research aims to explore the profile of bioactive compounds from cubeb fruit extract, dried using sunlight and extracted with various solvents, to reveal its diverse pharmacological activities.

MATERIALS AND METHODS

Chemicals and materials

The solvents and chemicals used for analysis in this research include 96% ethanol, 70% ethanol, distilled water, Folin-Ciocelteu reagent, 20% Na_2CO_3 (Merck, Germany), aluminum chloride 10%, sodium hydroxide 1 M (Merck, Germany), gallic acid standard, quercetin standard, and tannic acid standard (Sigma Aldrich Company Ltd., England). The equipment used includes Agilent: 8890 GC System, 5977B GC/MSD, 7693A Autosampler (Agilent Technologies, Inc.), rotary evaporator system (BUCHI Laboratory Rotary Evaporator BUCHI R-300), buchner funnel, filter paper (Whatman No. 1), micropipettes, crucibles, and various glassware.

Cubeb fruit extract preparation

Preparations of cubeb fruit extract were collected from cubeb farmers in Kemiriombo Village (7°10'40"S; 110°07'58"E), Gemawang District, Temanggung District, Central Java, Indonesia. The ripe cubeb fruits, identified by the reddish-yellow color, were harvested during the day, cleaned of dirt, and sun-dried at an average daily temperature of 28-30°C. The drying process took 4-5 days until the weight remained constant, as indicated by the cubeb fruit turning black. The dried cubeb fruits were then ground and sieved using a 2 mm screen. The cubeb fruit powder was extracted using three different solvents: 96% ethanol, 70% ethanol, and distilled water, in a ratio of 1:5 (w/v) in a closed container for 3×24 hours. The extraction/ maceration results were first filtered using polyester cloth, then followed by filter paper and Whatman No. 1 paper. Next, the filtrate was concentrated using a rotary evaporator (Winlab, Australia) until a thick extract was obtained. The resulting cubeb plant extract was stored at a minimum temperature of ±4°C until further use (Pan et al. 2017; Ahmad et al. 2019). The following formula was used to calculate the percentage extracted yield.

$$\text{Extract yield percentage (\%)} = \frac{\text{Weight of thick extract}}{\text{Weight of simplicia}} \text{ x } 100\%$$

Qualitative bioactive compound profiling

The qualitative profiling process of cubeb fruit extract was conducted on all macerated extracts using GC-MS analysis. The analysis was performed on an Aligent GC/MS system, which included an Agilent: 8890 GC System, 5977B GC/MSD, 7693A Autosampler (Agilent Technologies, Inc. 5301 Stevens Creek Boulevard Santa Clara, CA 95051). This procedure was based on the method described by Elfahmi et al. (2007), with several modifications.

Determination of total phenols, total flavonoids, and total tannins

Total phenols content

The total phenolic content of cubeb fruit extract was determined using the established Folin-Ciocalteu procedure with slight modifications. A 0.5 mL sample of the extract was mixed with 0.5 mL of Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, Mo., USA) with a series of standard gallic acid solutions (0, 40, 80, 120, 160, and 200 ppm) and 7.5 mL of deionized water was added. The mixture was added with 1.5 mL of 20% (w/v) sodium carbonate and kept for 10 minutes at room temperature. Subsequently, the mixture was heated at 40°C for 20 minutes and then immediately cooled in an ice bath before measuring the absorbance at 760 nm. The results were expressed as milligram equivalents of gallic acid per gram of dry matter (mg GAE/g DM) (Chaovanalikit and Wrolstad 2004).

Total flavonoid content

The technique used to estimate the total flavonoid content of the sun-dried cubeb fruit extracts was slightly modified from that used by (Udayaprakash et al. 2015). In a test tube, 200 µL of the plant extract was added, and the solvent was allowed to evaporate. Next, 5 mL of 0.1 M aluminum chloride was added to the residual and well-shaken. A UV-visible spectrophotometer was used to measure the absorbance value at 415 nm after 40 minutes of room-temperature incubation. The total flavonoid

content of the plant material was calculated using a standard plot of quercetin at different concentrations. The results were represented as milligrams of quercetin equivalent per gram of dry matter (mg QE/g DM).

Total tannins content

Total tannin content was determined by the method (Chanwitheesuk et al. 2005) with slight modifications. A sample of 0.2 mL was mixed with 10 mL of diethyl ether for 20 hours, then filtered. Evaporate the remaining diethyl ether and add distilled water to the sample to a volume of 10 mL. Mix 1 mL of sample solution with 0.1 mL of Folin Ciocalteu reagent and vortex, and wait 5 minutes. Add 2 mL of 20% Sodium Carbonate and vortex, and wait 5 minutes. Add distilled water to a volume of 10 mL and dilute 5 times. Read the absorbance at λ 760 nm after incubation for 30 minutes at room temperature. The results were represented as milligrams of tannic acid equivalents per gram of dry matter (mg TAE/g DM).

Experimental data analysis

The results of maceration and analysis of bioactive compound content (total phenol, total flavonoid, and total tannin) were carried out with five replications and repeated three times. Data were analyzed using one-way analysis of variance (ANOVA) at a significance level of 0.05. Significant differences between treatments were further evaluated using Duncan's Multiple Range Test (DMRT). Data were processed using the R Studio program (R Core Team, Vienna, Austria). In addition, the results of bioactive compound profiling were analyzed descriptively.

RESULTS AND DISCUSSION

The maceration of sun-dried cubeb fruit extract

The extract preparation was performed using the maceration method, with three repetitions for consistency. Maceration is a preferred extraction technique for natural materials as it minimizes damage to chemical components that are sensitive to heat, such as secondary metabolites, where the solvent plays a crucial role in dissolving the desired compounds from the sample. The maceration was conducted using a solvent-to-sample ratio of 1:5 (w/v). The maceration results showed that the sun-dried fruit extract had different colors, viscosity levels, and physical forms (Figure 1). These results indicate that the maceration results using distilled water had a dark brown color, and no oil clumps were visible. Different results were shown from the maceration results using 70% ethanol and 96% ethanol, where the physical form was seen to be yellowish-brown, and there were oil clumps in the maceration results. This is in accordance with the opinion of (Hayati et al. 2022), who used 96% ethanol solvent in extracting *Centella asiatica* leaves and obtained thick and oily maceration results.

Ethanol and distilled water were chosen as solvents because they are polar, universal solvents that are readily available. Polar compounds are those that dissolve in water, making polar solvents ideal for extracting these substances. Since the secondary metabolites from cubeb fruit are polar, polar solvents are used in the extraction process. Ethanol was added to the distilled water to increase the solvent's overall polarity and enhance the extraction efficiency. The secondary metabolite compounds extracted from cubed fruits are polar, necessitating polar solvents for their extraction. Ethanol was subsequently added as a solvent to enhance the polarity of the distilled water. Ethanol is often used to extract secondary metabolite compounds because of its low toxicity (Albadwawi et al. 2022; Nazaruddin et al. 2024). The greater the amount of solvent added, the greater the pressure applied, so the maceration process is greater, and more extract is produced.

For each parameter, the amounts of bioactive compounds, including flavonoids, phenols, and tannins, were measured, and the data were compiled in Table 2. The optimum maceration time, which produced the highest yield of fruit extract, was determined to be 3×24 hours. According to Agu and Agulanna (2020), extending the extraction time increases the likelihood of contact between the material and solvent, thereby enhancing the concentration of bioactive components in the solution until saturation is reached. However, excessively prolonged extraction can negatively impact the extracted materials due to increased oxygen exposure, which raises the risk of oxidation of the secondary metabolite compounds. Table 1 presents the percentage yield (%) of extracts obtained from various solvents.

Table 1. Results of cubeb fruit extraction with different solvents (n=5)

Types of solvent	Percentage yield (%)	Quality standard (%)
Distilled water	23.87[a]	>8.30
70% ethanol	14.13[b]	
96% ethanol	15.92[b]	

Note: [a, b] Different superscripts within the same column indicate significant differences ($p<0.05$)

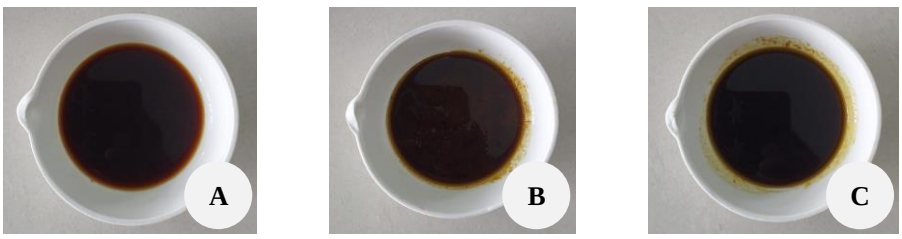

Figure 1. The sun-dried cubeb extract A. distilled water; B. 70% ethanol; C. 96% ethanol

The total yield results show that the results of maceration with different polar solvents will produce different yield amounts. The highest yield percentage was obtained from distilled water solvent of 23.87% compared to the yield results from extraction using 70% ethanol of 14.13% and 96% ethanol of 15.92% (P<0.05). Previously, the results of the Indonesian Herbal Pharmacopoeia Edition II in 2017 showed that the yield percentage for thick kemukus extract (*P. cubeba*) must be at least >8.30% (Ministry of Health of the Republic of Indonesia 2017). Furthermore, the maceration results of kemukus fruit from the Magelang area of Central Java using 70% ethanol with a ratio of 1:10 (w/v) and a maceration time of 1×24 hours produced lower results, namely 9.32%. The yield number describes the ease with which components can be extracted from the sample, with higher yields indicating more efficient extraction (Setyani et al. 2021). The maceration duration can influence the yield percentage; longer maceration allows for greater extraction of compounds due to prolonged contact between the solvent and the sample (Hayati et al. 2022).

Quantitative bioactive compounds of sun-dried cubeb fruit extract

The results of quantitative research showed the presence of phenolic, flavonoid, and tannin content in all sun-dried cubeb fruit extracts with different polarity solvents. Bioactive compounds, including flavonoids, phenols, and tannins, are highly valued for their benefits to humans and animals. Analysis of cubeb fruits revealed the presence of flavonoids, phenols, and tannins. However, there are notable differences in the bioactive content of cubeb extracts depending on the solvent used (distilled water, 70% ethanol, and 96% ethanol), as shown in Table 2.

These results, which show that the value obtained is lower than the reference standard, suggest potential implications for the preservation of bioactive compounds in cubeb fruit. The reduction in the quantity of these compounds due to drying in direct sunlight is a significant finding that could lead to further research. Most of these compounds, being volatile, tend to evaporate when exposed to various treatments, especially heat. The active substances found in cubeb fruit, such as alkaloids, phenolics, and flavonoids, are known for their strong antioxidant properties (Drissi et al. 2022; Qiang et al. 2022). The most prevalent phenolic chemicals found in plants are called flavonoids, which are polyphenolic compounds. Plants contain large amounts of these phytochemicals, which interact with nutrients and dietary fiber (Dahibhate and Kumar 2022).

The total phenolic content with different solvents obtained the highest value, namely with 96% ethanol solvent of 45.23 mg GAE/g compared to the results of 70% ethanol solvent of 36.04 mg GAE/g and distilled water of 12.60 mg GAE/g (p<0.05). Previously, the total phenolic content of cubeb fruit was determined using the Folin-Ciocalteu method, yielding 123.1 and 185.65 mg GAE/g extract for the ethanol extracts and 1,280 mg GAE/g extract for the methanol extract (Mustafa and Hameed 2017; Alminderej et al. 2021). Moreover, Drissi et al. (2024) reported that cubeb fruit extracted with water yielded a total phenols content of 132.39±9.87 mg GAE/g extract. Phenolic compounds are frequently observed in studies in plants, with quercetin being one of the most significant phenolic compounds identified (Shahidi and Ambigaipalan 2015). Moreover, known for their antioxidant properties, phenolic compounds play a crucial role in neutralizing free radicals and have anti-cholesterol effects (Torres et al. 2019; Widyaswari et al. 2024).

The flavonoid content of the extract was obtained with values ranging from 2.39 mg QE/g to 27.85 mg QE/g (Table 2). The flavonoid results with 96% ethanol solvent were higher compared to the other two solvents. This is because 96% ethanol is a universal solvent and is able to bind various active compounds, both non-polar compounds and polar compounds. In addition, 96% ethanol solvent is easier to penetrate the cell walls of the sample than ethanol solvents with lower concentrations, resulting in higher flavonoid compound values. Another study reported that the flavonoid content in cubeb fruit extracted using 96% ethanol was 3.53±0.09 mg QE/g (Salsabila et al. 2024). Flavonoids, natural compounds commonly found in fruits, seeds, roots, stems, and other parts of plants, are important secondary metabolites that play crucial roles in various biological processes and responses to environmental factors in plants. They are currently regarded as essential components in various nutraceutical, pharmaceutical, medicinal, and cosmetic applications, and our research has added a new dimension to their extraction and application (Panche et al. 2016; Shen et al. 2022).

The tannin content of sun-dried cubeb fruit extract with 96% ethanol, 70% ethanol, and distilled water solvents was 20.63±0.42 mg TAE/g; 7.81±0.32mg TAE/g and 6.56±0.07 mg TAE/g, respectively (p<0.05). Based on these results, it can be seen that 96% ethanol extracts higher tannin compounds compared to 70% ethanol and distilled water solvents. This is because 96% ethanol is a universal solvent that can bind polar and non-polar bioactive compounds. Tannin is a polar compound that has a hydroxyl group, so ethanol is very capable of binding tannin compounds in samples during the maceration process.

Table 2. Quantitative bioactive compounds of sun-dried cubeb fruits with different solvents (DM basis)

Types of solvents	Bioactive compounds of sun-dried cubeb fruit extract		
	Total phenols (mg GEA/g)	Total flavonoids (mg QE/g)	Total tannins (mg TAE/g)
Distilled water	12.60±0.43^{c}	2.39±0.03^{c}	7.81±0.32^{b}
70% ethanol	36.04±0.33^{b}	10.84±0.34^{b}	6.56±0.07^{b}
96% ethanol	45.23±0.68^{a}	27.85±0.53^{a}	20.63±0.42^{a}

Notes: $^{a, b, c}$ Different superscripts within the same column indicate significant differences (P<0.05). QE: Quercetin equivalent; GAE: Gallic acid equivalent; and TAE: Tannic acid equivalent

The 96% ethanol solvent penetrates the cell walls of sample plants more easily compared to ethanol solvents with lower concentrations and distilled water, resulting in extracts with high tannin content. According to (Sundang et al. 2012), the tannin compound content of betel leaf (*P. betle*) ranges from 13.33 to 29.33 mg TAE/g. Tannins are complex compounds derived from phenolic acids, often referred to as tannic acid. They are found in many plants and can be categorized into hydrolyzed and condensed tannins. Tannins are known as anti-nutritional compounds because they reduce the quality of ingredients by forming complexes with proteins (Hadi et al. 2021a; Tong et al. 2022). Moreover, variations in the composition of bioactive compounds can result from factors such as the plant's age, climate differences, and the composition and nutrient content of the soil where the plants are grown (Beulah et al. 2021; Dahibhate and Kumar 2022). These differences may also arise from variations in extraction methods, detection techniques, and geographic distribution, as well as genetic chemotypes (Drissi et al. 2022).

The composition of sun-dried cubeb fruit extract using GC/MS analysis

The results of GC/MS analysis showed that the extract of sun-dried cubeb fruit with 96% ethanol solvent against the content of secondary metabolic compounds are given in Figure 2. and all identified compounds are shown in Table 3. Analysis of dried cubeb fruit extract revealed the presence of various sterols, terpenes, fatty acids, and others. In the extraction process using ethanol at 96%, the most abundant compound was cubebol, reaching 39.55% of the extract, followed by nerolidol at 8.04%, δ-cadinene at 7.54%, and lemnalol at 4.83%. When 70% ethanol was used as the solvent, the highest yield was cis-vaccenic acid at 33.72%, followed by benzoic acid, octadecanoic acid, and pyranone, which accounted for 14.08%, 10.16%, and 7.05%, respectively (Figure 3 and Table 4). Cubeb is characterized by a strong aroma, which originates from the essential oils present in its seeds, including sesquiterpene hydrocarbons, β-caryophyllene, δ-sadinene, α- and β-cubebene, along with small amounts of monoterpenes (Salehi et al. 2019).

The results also showed differences related to component retention time (min), match factor (%), and relative abundance (%) from GC/MS analysis between 96% ethanol and 70% ethanol solvents. The results of the extract with 96% ethanol showed a component retention time of between 6.660 to 57.705 min and a match factor of between 85% to 99%, while 70% ethanol ranged from 3.123 to 41.566 min and a match factor of between 67% to 96%. In addition, the relative abundance value ranged from 0.01% - 39.55% for 96% ethanol solvent, and 70% ethanol showed results between 0.37% - 33.72%.

Furthermore, the results of GC/MS analysis with distilled water solvent showed that no metabolic compound components could be found from the analysis using GC/MS (Figure 4 and Table 5). This could be because the volatile compounds from the distilled water maceration results cannot be evaporated, so GC/MS cannot detect these compounds.

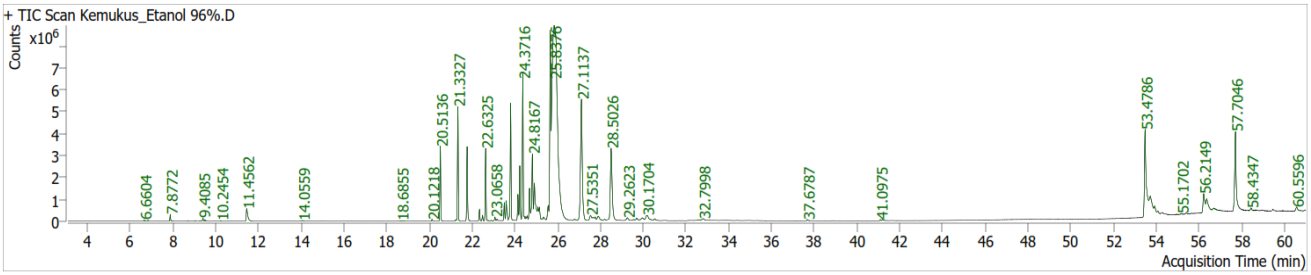

Figure 2. GC/MS profile of sun-dried cubeb fruit extract using 96% ethanol

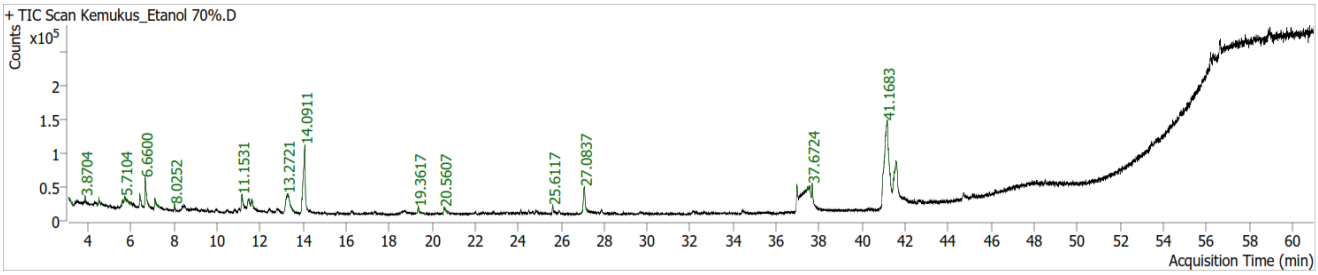

Figure 3. GC-MS profile of sun-dried cubeb fruit extract using 70% ethanol

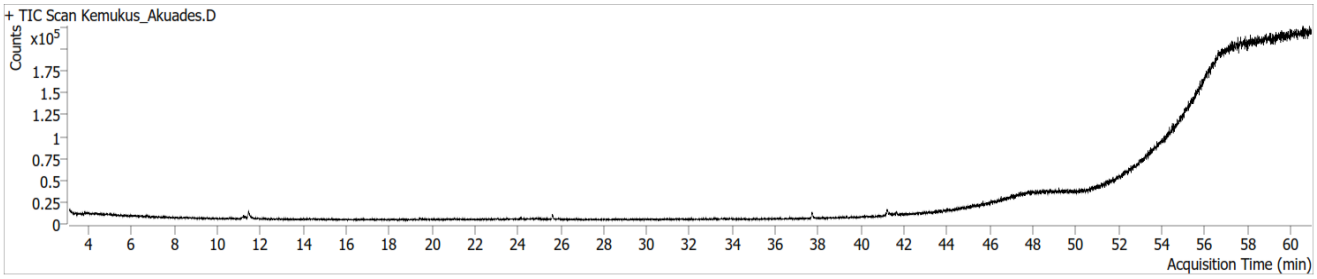

Figure 4. GC-MS profile of sun-dried cubeb fruit extract using distilled water

Table 3. Composition of sub-dried extract from cubeb fruits using 96% ethanol analyzed with GC/MS

Compound name	Component retention time (min)	Match factor (%)	Relative abundance (%)
α-Phellandrene	6.660	88	0.03
Cyclofenchene	6.838	92	0.02
β-Phellandrene	7.877	97	0.15
β-Pinene	9.052	89	0.01
β-Cymene	9.278	90	0.01
ψ-Limonene	9.408	95	0.08
Eucalyptol	9.503	94	0.04
γ-Terpinene	10.245	86	0.01
Linalool	11.456	97	0.71
Benzoic acid	14.056	91	0.04
δ-Elemene	20.122	92	0.06
α-Cubebene	20.514	96	2.28
isoledene	21.238	91	0.05
Copaene	21.333	98	3.56
β-Copaene	21.766	97	2.38
α-Cadinene	22.348	96	0.34
cis-α-Bergamotene	22.484	95	0.16
Caryophyllene	22.633	99	2.20
α-Funebrene	23.066	92	0.10
α-Guaiene	23.161	92	0.04
Cadina-3,5-diene	23.505	94	0.54
α-humulene	23.594	95	0.88
Alloaromadendrene	23.802	98	3.64
δ-Cadinene	24.140	94	0.81
γ-Muurolene	24.229	97	1.79
Lemnalol	24.372	98	4.83
β-Selinene	24.514	87	0.12
β-Guaiene	24.579	89	0.16
epi-Bicyclosesquiphellandrene	24.686	96	1.16
β-Cyclogermacrane	24.817	98	2.67
α-Muurolene	24.912	95	2.33
trimethylcyclododeca-1,5,9-triene	25.262	86	0.55
β-bisabolene	25.143	85	0.56
γ-Cadinene	25.559	85	0.21
δ-Cadinene	25.660	97	7.54
Cubebol	25.838	96	39.55
Nerolidol	27.114	98	8.04
Epicubebol	27.535	91	0.30
Viridiflorol	27.903	89	0.34
Himbaccol	28.503	97	4.88
α-Amorphene	29.262	88	0.16
α-muurolol	29.957	87	0.22
Pogostole	30.170	90	0.54
β-Cubebin	57.705	95	4.68
Total compound (%)			98.76
Unidentified compounds (%)			1.24

Table 4. Composition of sun-dried cubeb fruit extract using 70% ethanol analyzed with GC/MS

Compound name	Component retention time (min)	Match factor (%)	Relative abundance (%)
Alanine	3.123	67	1.35
2(3H)-Furanone, dihydro-4-hydroxy-	3.870	69	0.37
Imidazole	4.511	73	0.78
3-hydroxy cyclohexanone	5.710	69	3.74
Butyrolactone	6.405	85	1.58
1,2-Cyclopentanedione	6.660	91	4.65
2-Pentenoic acid	7.111	69	1.78
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanon	8.025	72	0.59
Methylcatachol	11.153	89	1.93
Linalool	11.456	67	1.60
Hotrienol	11.610	69	1.49
Pyranone	13.272	87	7.05
Benzoic acid	14.091	96	14.08
Varamol	19.362	78	1.04
Dimethoxyphenol	20.561	78	1.93
cis-muurola-3,5-diene	25.612	66	0.72
Isovanillic acid	27.084	91	4.70
Palmitic acid	36.960	73	1.06
Hexadecanoic acid	37.672	75	1.55
cis-Vaccenic acid	41.168	91	33.72
Octadecanoic acid	41.566	80	10.16
Total compound (%)			95.87
Unidentified compounds (%)			4.13

Table 5. Composition of sun-dried cubeb fruit extract using distilled water analyzed with GC/MS

Compound name	Component retention time (min)	Match factor (%)	Relative abundance (%)
Unidentified	-	-	-
Total compound (%)			-
Unidentified compounds (%)			100

GC/MS profiling of the extract revealed the presence of various chemicals, each with distinct biological characteristics, depending on the solvent used. By correlating retention time, match factor, component area (%), and established mass spectrum fragmentation patterns, compounds were identified using the Metabolite library NIST20. L. In the analysis, 44 compounds were identified from the extract using 96% ethanol, 21 compounds were identified with 70% ethanol, and no compounds were detected with distilled water. Metabolic compounds with a similarity index greater than 85% are listed in Table 3. The variation in compound quantities is influenced by the type of polar solvent used (ethanol and distilled water) during the sample evaporation process analyzed with GC/MS. Gas Chromatography-Mass Spectrometry (GC/MS) utilizes both mass spectrometry and gas chromatography to identify compounds based on their retention time and specific fragmentation patterns (mass spectra). This technique is widely utilized for identifying metabolites in diverse biological samples such as plants, fruits, vegetables, blood, urine, milk, and meat (Bose et al. 2017; Sharma and Ramanathan 2021; Putri et al. 2022).

Water extraction found the presence of 43 compounds analyzed using High-Performance Liquid Chromatography (HPLC), including organic acids, phenolic acids, and flavonoids. In contrast, analysis of essential oils (EO) using GC/MS identified 36 volatile compounds, with the main components including Z-isoeugenol, dihydroeugenol, β-pinene, E-caryophyllene, and 1,8-cineole (Drissi et al. 2024). The essential oil also exhibited higher levels of straight-chain hydrocarbons, such as tetracontane and nonacosane, compared to saturated fatty acids like lauric acid, stearic acid, and myristic acid (Casillas-Vargas et al. 2021). Moreover, essential oils are rich in terpenes. Variations observed in this study compared to previous reports (Hadi et al. 2021b; Putri et al. 2022) may be attributed to seasonal variations, differences in sample collection, or variations in experimental methods.

In conclusion, based on the extraction results with various solvents, 96% ethanol is recommended as the optimal solvent for achieving higher levels of polyphenols, flavonoids, and tannins from cubeb fruit. This study underscores the significant potential of cubeb fruit as a rich source of bioactive compounds, which can be utilized in developing new drugs, nutritional supplements, and nutraceuticals.

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