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SECONDARY METABOLITE PROFILE IN STEM AND ROOT OF KEJI PLANT (*Staurogyne elongata* [Blume] Kuntze)

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ABSTRACT

Keji (Staurogyne elongata (Blume) Kuntze) is an herbal medicinal plant that is often found on the Java to Sumatra islands. The leaves and roots of keji (S. elongata) have been used by the community as diuretic drugs, increasing blood pressure and trusted to treat bladder stones, kidney stones, and joint problems. However, research on secondary metabolites in the stems and roots of keji has never been done. The study aimed to identify the secondary metabolite content and marker compounds in the stems and roots of S. elongata. The research stages were drying and sample grinding, extraction, and metabolite compound analysis using Gas Chromatography-Mass Spectrometry (GC-MS). The results showed nine secondary metabolite compounds found in S. elongata stem, such as phenol, terpenoid, and alcohol. Meanwhile, secondary metabolite compounds of S. elongata root were ten compounds from the coumaran, phenols, and ester group. The 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol is proposed as a marker compound in S. elongata stem. Meanwhile, no secondary metabolites can be used as marker compounds in S. elongata roots because octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester is the primary metabolite.

Keywords: *GC-MS, root, secondary metabolites, stem, Staurogyne elongate*

INTRODUCTION

Indonesia is an archipelago state has a high biodiversity of flora, fauna, and microbes. Indonesia is ranked as the country with the second highest diversity, with Brazil as the first. In 2017, there are 31,750 plant species and fungi that have been identified in Indonesia [1]. Only 7,500 species are used as medicinal plants by the society [2]. Based on the diversity of existing plants, society has utilized these plants as herbal medicinal resources. As many as 61% of Indonesians believe by proving that around 117 plant species can be used for treating some disease complaints [3]. Plants can be used as herbal medicines because they contain secondary metabolite compounds, which are natural chemicals that contain bioactive substances that can treat many diseases [4]. Basically, plant extracts are a collection of secondary metabolite compounds that have different types, functions, and levels according to the conditions and needs of each plant. High-level plant extracts, as much as 14-28%, are used as medicines, and 74% of them can be known to have medicinal functions after the process becomes traditional medicine or ethnomedicine [5].

Keji plant (*Staurogyne elongata* (Blume) Kuntze) is one of the plants used by society as a herbal medicine and can be found from Java to Sumatra. The leaves of *S. elongata* are used by society as a vegetable salad, but they can also be trusted to be used as a diuretic drug. People also believe that the leaves and roots of *S. elongata* can be used as blood pressure medicine to increase blood pressure [6]. People also use *S. elongata* leaves to treat kidney stones, bladder stones, and joint problems [7].

Research on *S. elongata* is still quite limited. Additionally, previous research on *S. elongata* mostly only research on the leaves. The metabolite content of *S. elongata* leaves is determined by leaf age [8]. The ethyl acetate extract from leaves of *S. elongata* in a concentration of 580 mg/ml has antibacterial activity against *Escherichia coli* with an inhibition zone of 0.88 cm [9].

The plant organs most widely used as medicine are leaves (49%), rhizomes (24%), fruits (16%), stems (6%), roots (3%), bulbs (2%), and flowers (1%) [10]. The metabolite content of the *S. elongata* stem and root has not been analyzed. It will support the development of medicinal plant products. According to Hakim et al., each plant organ of *Helianthus annuus* L. differs in metabolite content, with four marker compounds in the roots and two marker compounds in the seeds [11]. The study shows that each plant organ has a different compound content. Another study of secondary metabolites of the stem organs, *Punica grantum* shows that pomegranate tree stems contain alkaloids, tannins, phenols, steroids and terpenoid

[11]. In addition, the roots of mangrove plants (*Sonnaratia caseolaris*), and the results showed that mangrove roots contained metabolite compounds of terpenoid and alkaloid groups [12]. The identification of secondary metabolites in stem and root organs showed merung plant (*Coptosapelta tomentosa*), the results showed that the stems and roots contained flavonoid-derived compounds and phenol-derived compounds that have potential as antioxidants [13]. Based on this research, it can be seen that plant organs other than leaves, such as stems and roots, also contain secondary metabolite compounds that have the potential to be used as herbal medicine resources.

The content of metabolite compounds in plants can be determined by the Gas Chromatography-Mass Spectrometry (GC-MS) method. GC-MS is an efficient method with high resolution that can analyze small particles. GC-MS can detect compounds with small concentrations of up to <1 ng/g. In addition, it can identify compounds based on fragmentation reactions so that the class of compounds can be known [14]. The majority of previous research on *S. elongata* only explored the leaf organs. Meanwhile, the research on the stems and roots of *S. elongata* has not been done. The roots of *S. elongata* are known to be useful in herbal medicine, but the secondary metabolite compounds that cause the roots to be used in medicine are not yet known. Therefore, research on the content of secondary metabolites in the stems and roots of *S. elongata* is important to do. The aim of the study is to identify the secondary metabolite content and marker compounds in the stems and roots of *S. elongata*.

METHODS

Sampling. Stems and roots of keji plants (*S. elongata*) were taken from Domiyang Village, Paninggaran District, Pekalongan Regency, Central Java. The simple random sampling technique was used with all *S. elongata* that had the same opportunity to be sampled. The stem and root samples were taken 300-500 g, respectively, and wet sorting was carried out by removing unused plant parts to clean samples from dirt and other foreign materials in plants.

Drying and grinding samples. The sorted stems and roots of *S. elongata* were washed thoroughly with flowing water to remove dirt in the form of soil and other contaminants. Stems and roots that had been cleaned were cut into small pieces and then dried using an oven at 60°C. The dried samples were grindered, and sample powder could be stored in a jar with silica gel.

Extraction. The soxhletation was utilized as an extraction method. Sample powder was taken as 15 g and wrapped in filter paper. Ethanol solvent was poured for two cycles through the top of the extracting column then placed a condenser and adjusted the water rate. Extraction was continued until the solvent became clear, and then the extraction results were concentrated with a rotary evaporator at 40°C with a speed of 90 rpm and then dried with a water bath.

GC-MS analysis. GC-MS analysis was performed with a GC-MS (Thermo Scientific ISQ 7000). The GC was connected to an MS with a TG-5MS semi-polar column of length 30 m, I.D 0.25 mm, and 0.25 µm film. Extracts prepared for GC-MS analysis were prepared with a concentration of 1000 ppm. A total of 1µl of extract was injected at stable machine conditions with an initial temperature of 70°C held for 2 minutes. The column temperature was increased by 5°C/minute until it reached 200°C. The column temperature was increased again to 10°C/minute until it reached a temperature of 250°C and held for 10 minutes. The carrier gas used was helium gas at 1.0 mL/min with splitless injection mode. The chromatogram results and MS data were compared with the NIST Library, and the compound data were manually identified based on the similarity index library, which was above 80%. The principal component analysis (PCA) was performed with the Unscrambler software version 10.4 for score plot and loading correlation analysis [8].

RESULTS AND DISCUSSION

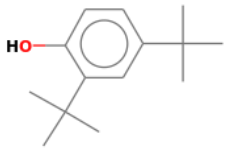
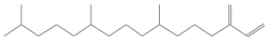
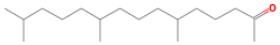
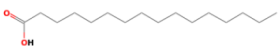
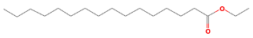
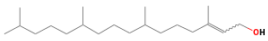
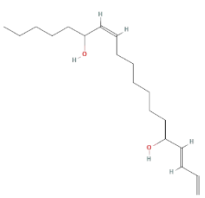
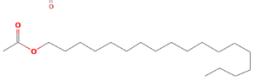
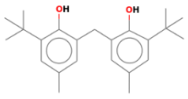
The metabolite compounds in stem and root of keji plant (*Staurogyne elongata* (Blume) Kuntze). Compounds detected during GC-MS running time in stems and roots of *S. elongata* showed different quantities and compound profiles, with stems detecting 155 compounds while the roots detecting 110 compounds. From these compounds, the compound was identified manually based on the similarity index (SI) of the GC-MS library above 80%. The final data compounds in the stem of *S. elongata* were nine secondary metabolite compounds and one primary metabolite compound (hexadecanoic acid). Meanwhile, ten secondary metabolite compounds and two primary metabolite compounds (hexadecanoic acid and octadecanoic acid) were found in the *S. elongata* roots.

The metabolite compounds in the stem of the *S. elongata*. Secondary metabolite compounds in the stem of *S. elongata* consisted of phenol, terpenoid, and alcohol. Compounds classified as phenol compounds were *2,4-di-tert-butylphenol* and *2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol*. Compounds classified as terpenoids are *Neophytadiene; 2-pentadecanone,*

6,10,14-trimethyl-; and 3,7,11,15-tetramethyl-2-hexadecen-1-ol. Alcohol compounds are *E,E,Z-1,3,12-Nonadecatriene-5,14-diol*. Primary metabolite compounds were also detected in the stem of *S. elongata*, which are metabolite compounds from *n-hexadecanoic acid, Acetic acid n-octadecyl ester; n-Propyl 9,12-octadecadienoate;* and *hexadecanoic acid, ethyl ester*. The *n-Hexadecanoic acid* compound in the stem has a relative area value of 3.26%. According to the GC-MS results, the compound with the highest relative area value in *S. elongata* stem is *2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol* with a relative area value of 33.08%. Furthermore, the compound in the stem with the lowest relative area value is *2-Pentadecanone, 6,10,14-trimethyl-* with a relative area of 0.34% which is a terpenoid.

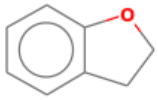
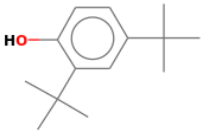
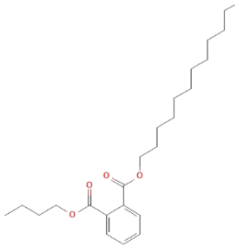
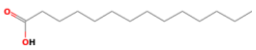
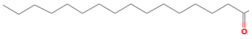
The results of the final GC-MS compound analysis with relative area values of compounds in the stem are shown in Table 1.

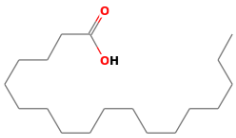
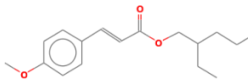
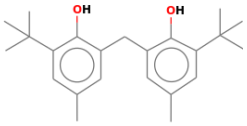
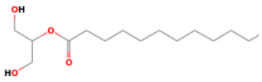
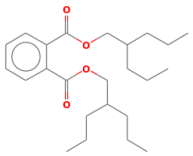
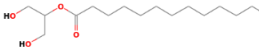
Table 1. Metabolites content of keji (*Staurogyne elongata* (Blume) Kuntze) stem

RT	Name of compound	Compound Structure	Area %	Peak	Biology Activity
18,88	*2,4-Di-tert-butylphenol*		1.47	25	Antibacterial, antiviral, antifungal, anti-inflammatory and antioxidant [15]
26,10	*Neophytadiene*		0.42	47	Antimicrobial, anti-inflammatory, and antifungal [16]
26,21	*2-Pentadecanone, 6,10,14-trimethyl-*		0.34	48	Antibacterial [17]
28,48	*n-Hexadecanoic acid*		3.26	60	Anti-inflammatory, antiandrogenic, antioxidant, hypocholesterol, nematicide, pesticide, and can treat rheumatic symptoms [18]
29,05	*Hexadecanoic acid, ethyl ester*		1.40	64	Antioxidant, hypocholesterolemia, antiandrogenic, nematicide and anticancer [19]
30,78	*3,7,11,15-Tetramethyl-2-hexadecen-1-ol*		0.87	77	Antifungal, antibacterial, anticonvulsant, anticancer, and antiarthritis [20]
31,02	*E,E,Z-1,3,12-Nonadecatriene-5,14-diol*		0.74	78	Antidiabetic and antilipidemic [21]
31,40	*n-Propyl 9,12-octadecadienoate*		1.88	82	Antioxidant [22]
31,94	*Acetic acid n-octadecyl ester*		1.54	86	Antimicrobial [23]
34,32	*2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol*		33.08	111	Antifungal, antibacterial, and germicidal [24]

The metabolite compounds in *S. elongata* roots. The secondary metabolite compounds in *S. elongata* roots were from coumaran, phenol, and ester compounds. The secondary metabolite compounds of coumaran were *2,3-dihydro-Benzofuran,* while phenol group were *2,4-Di-tert-butylphenol; Phthalic acid, butyl tetradecyl ester; 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester; 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol;* and *Phthalic acid, di(2-propylpentyl) ester.* Secondary metabolite compounds of the ester group in *S. elongata* roots were *Dodecyl acrylate.* Besides secondary metabolite compounds in the roots of *S. elongata*, primary metabolite compounds were also detected such as *n-hexadecanoic acid; Octadecanoic acid; Hexadecanoic acid, ethyl ester; Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester;* and *Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl*. The *Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester* from the ester group compound, had the highest relative area value in the roots as 8.13%. The compound with the lowest relative area value in the roots was *Phthalic acid, butyl tetradecyl ester* with a value of 0.44%, which was a compound from the phenol group. The final GC-MS compound analysis on the roots is shown in Table 2.

Table 2. GC-MS analysis compound in *S. elongata* roots

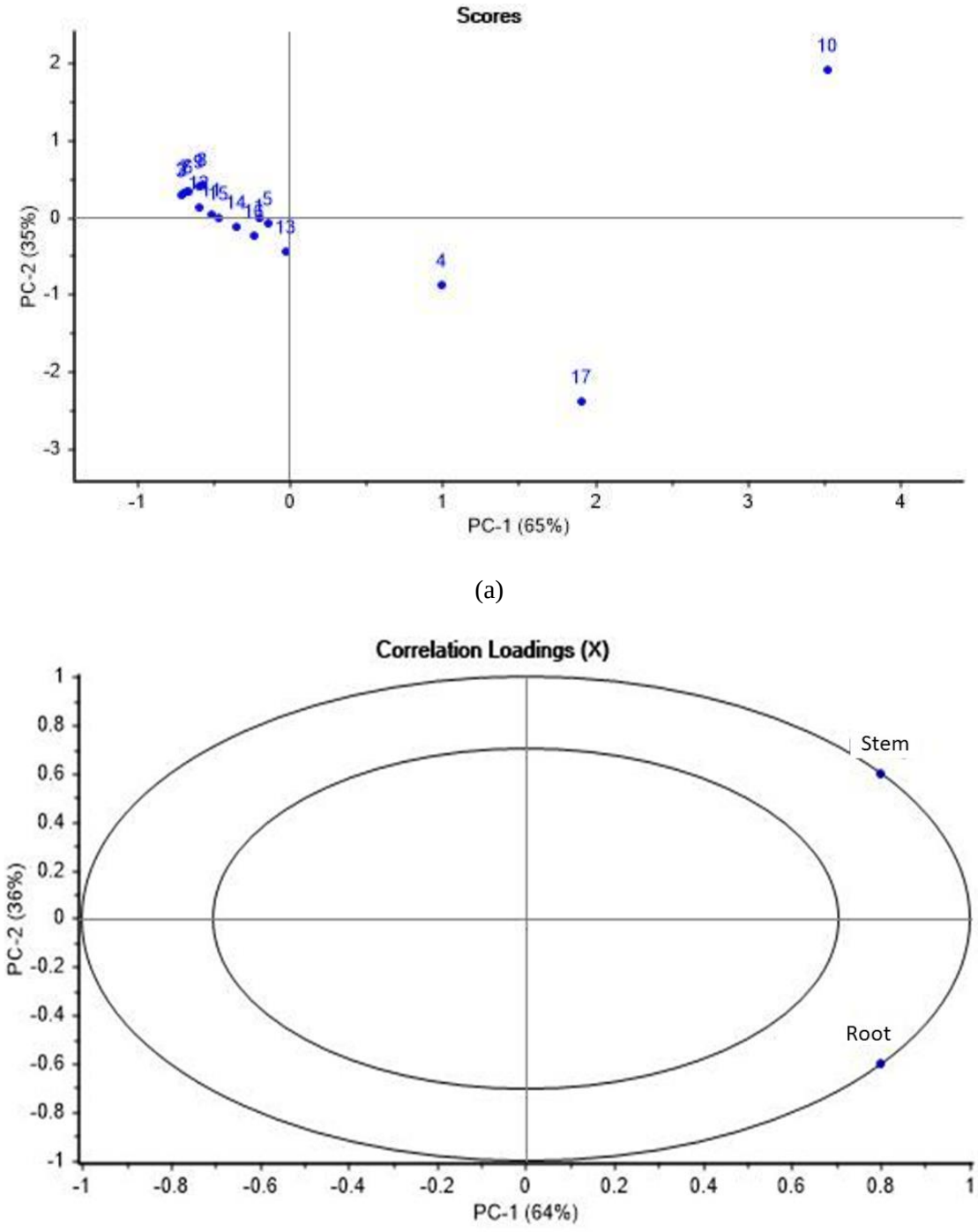
RT	Name of compound	Compound Structure	Area %	Peak	Biology Activity
11,33	*2,3-dihydro- Benzofuran*		0.68	11	Anti-inflammatory, anti-diarrhea and antihelminthic [25]
17,93	*Cyclododecane*		2.84	22	Antiviral [26]
18,88	*2,4-Di-tert-butylphenol*		1.23	25	Antibacterial, antiviral, antifungal, anti-inflammatory and antioxidant [15]
26,71	*Phthalic acid, butyl tetradecyl ester*		0.44	42	Antibacterial [27]
28,47	*n-Hexadecanoic acid*		4.42	46	Anti-inflammatory, antiandrogenic, antioxidant, hypocholesterol, nematicide, pesticide, and can treat rheumatic symptoms [18]
29,04	*Hexadecanoic acid, ethyl ester*		1.44	51	Antioxidant, hypocholesterolemia, antiandrogenic, nematicide and anticancer [19]

RT	Name of compound	Compound Structure	Area %	Peak	Biology Activity
31,36	*Octadecanoic acid*		2.19	63	Antibacterial and antifungal [28]
33,16	*2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester*		1.19	79	Antiprotozoa [29]
34,29	*2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol*		4.01	84	Antifungal, antibacterial, and germicidal [24]
35,34	*Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester*		0.84	89	Antioxidants, pesticides, and hemolytic [8]
35,94	*Phthalic acid, di(2-propylpentyl) ester*		1.55	93	Antioxidant and antimicrobial [30]
38,88	*Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester*		8.13	103	Antimicrobial and anti-inflammatory [31]

Principal component analysis (PCA) of *S. elongata* stem and root. The variables used in the analysis were the final compound data on the stem and roots of *S. elongata*. The analysis carried out was score plot analysis and correlation loading. The score plot and correlation loading analysis results can be seen in Figure 1.

PCA score plot analysis is known to be used for marker compound detection. The marker compounds were grouped into eight, which included (a) therapeutic compounds, (b) bioactive compounds, (c) synergistic compounds, (d) characteristic compounds, (e) major compounds, (f) correlative compounds, (g) toxic compounds, and (h) general compounds [32]. A determination marker compound can also be used in the authentication of the wax apple fruit cultivar [33]. According to the PCA score plot, the compound contained in quadrant I was the major compound in the *S. elongata* stem, which is *2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol* (10). The major compound is a plant compound that is dominant because it has a higher area than other compounds [34]. *2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol* is one of the phenol compounds and has antioxidant activity and can be used as herbal medicines or natural antioxidants for human health [35]. Phenol also contributes to being a plant defensive agent against abiotic environmental stress and inducing plant tolerance [36].

The compounds potentially proposed as markers in *S. elongata* roots were in quadrant IV, which are *n-Hexadecanoic acid* (4) and *Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester* (17). Both compounds had potential as marker compounds in roots because they had a higher relative area than others. Unfortunately, they were fatty acid and fatty acid ethyl ester (FAEE), respectively. It was not specifically included in the major secondary metabolites like phenolic, terpenoid, alkaloid etc. Furthermore, *Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester* (17), was a primary metabolite compound with the highest relative area (8.13%). *Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester* was the major compound in *S. elongata* roots. It is also the main compound in young leaves of *S. elongata* [8]. Meanwhile, *n-hexadecanoic acid* was not classified as the main marker compound because it was not the compound with the highest relative area (4.42%). The *S. elongata* roots can be potentially used as herbal medicine because *Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, is* known to have antioxidant, antimicrobial, and anticancer activities [37]. Antioxidants may be able to inhibit oxidative damage to the kidney tubular cells and have an inhibitory effect on the production of crystals in the urine [38]. Therefore, the stem and root of *S. elongata* can be proposed as herbal medicine.



(a)

(b)

Figure 1. Principal component analysis of keji plant metabolite. Score plot; (b) Correlation Loading Plot

CONCLUSION

Secondary metabolite compounds in *S. elongata* stem were nine compounds from the phenol, terpenoid, and alcohol. Secondary metabolite compounds detected in *S. elongata* roots were ten compounds from the coumaran, phenols, and ester group. The marker compounds in *S. elongata* stem are *2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol*. Meanwhile, no secondary metabolites can be used as marker compounds in *S. elongata* roots because *octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester* is the primary metabolite.

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