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(RESEARCH ARTICLE)

Antimalarial activity test and GC-MS analysis of ethanol and ethyl acetate extract of snake plant (*Sansevieria trifasciata* Prain)

Eva Lestari, Endah Setyaningrum, Sri Wahyuningsih, Emantis Rosa, Nuning Nurcahyani and Mohammad Kanedi *

Department of Biology, Faculty of Mathematics and Sciences, University of Lampung, Bandar Lampung Indonesia.

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Abstract

Snake plant or Mother-in-law's tongue (*Sansevieria trifasciata* Prain) is a plant possessing of many ethnobotanical uses one of which is for medicinal. Previous pharmacological studies showed that this plant has several properties such as healing lesions, anthelmenthic, antimicrobial and cytotoxic. This research was conducted to investigate phytocompounds constituents of ethanol and ethyl acetate of *S. trifasciata* and their antimalarial activity potential. The phytochemical constituents were analyzed using Gas Cromatography and Mass Spectroscopy (GCMS). The antiplasmodial properties of the extract against malaria parasites were determined by calculating the percentage of parasitaemia suppression which was then used as a variable in probit analysis to determine the IC_{50} value of the extract. There were 4 and 24 constituents of compounds detected using GC-MS in the ethanol and ethyl acetate extracts of the plant leaves respectively. Antiplasmodial assay of the extracts showed that ethyl acetate solvent gave stronger suppression against *Plasmodium falciparum* with an IC_{50} value of 21,29 µg/mL compared to ethanol solvent with an IC_{50} value of 21,29 µg/mL In conclusion plant leaf extract of *Sansevieria trifasciata* is potential to be developed as antimalarial ingredient.

Keywords: Snake Plant; Mother-in-Law's Tongue; *Sansevieria trifasciata*; Antimalarial; Antiplasmodial

1. Introduction

Sansevieria trifasciata Prain is a succulent, herbaceous, perennial plant in the Asparagaceae family that is native to Africa with several synonyms namely: *Aletris hyacinthoides* var. zeylanica L., *Sansevieria jacquinii* N.E.Br., *Sansevieria laurentis* De Wild., and *Sansevieria zeylanica* var. laurentii (De Wild.) L.H.Bailey [1]. The plant with the vernacular name snake plant or Mother-in-law's tongue has many ethnobotanical uses, such as for ritualistic purpose, medicinal use, horticultural use, food additives, and materials [2,3]. In addition, this plant is also known to have the potential to be used as raw material for the textile industry because of its high lignocellulosic fiber content [4].

Regarding the medicinal potential of *Sanseviera*, it is known that this plant has several properties such as healing lesions, anthelmenthic, antibacterial and anti-fungal. A study reported by Afrasiabian *et al* (2017) indicated that ointment formulated from this plant extract is efficacious in healing lesions on the skin of the toes [5]. An in-vitro study conducted by Karomo and Rwai (2016) showed that the extract of Mother-in-law's tongue leaves possess ananthelmintic activity against *Fasciola hepatica* [6]. Antimicobial test reported by Febriani et al (2019) revealed that plant extract of *Sansevieria trifasciata* significantly showed inhibition activities against *Escherichia coli* and *Staphylococcus aureus [7].* Another important pharmacological property of this plant is its cytotoxic potential. By using brine shrimp bioassay test of the roots and leaves extracts, Berame at al (2017) suggested that *Sansevieria trifasciata* plant has cytotoxic properties [8].

*Corresponding author: Mohammad Kanedi

Phytochemical screening reported by Kumar et al (2022)indicated that plant extract of snake plant contained alkaloids, carbohydrates and flavonoids [9]. Oomariyah and van Dijk (2022) revealed that plant extract *Sansevieria trifasciata* contained phytochemicals including alkaloids, flavonoids, saponin, steroids, triterpenoids, tannins, and phenolic compounds. By GC-MS analysis, this plant extract showed the present of phytol, stigmasterol, linoleic acid, oleic acid, stearic acid, and palmitic acid [10].

Referring to snake plant extracts which are cytotoxic, anthelmintic, and contain main plant compounds such as alkaloids, flavonoids, tannins, phenols and triperterpenoids, we are compelled to test the antimalarial properties of this plant extract.

2. Material and methods

2.1. Plant samples and extraction

The plant samples of *Sansevieria trifasciata* were collected from suburb of Bandar Lampung, Lampung Province, Indonesia. The leaves were washed and rinsed under running water. Once clean, the samples were cut into small pieces and then air-dried by placing them in the sun covered with a black cloth until completely dry. Furthermore, the dry samples were blended into a fine powder (simplicia). The simplicia was then separated into two groups of 500 g each. The first group was macerated using ethanol solvent and the other one was extracted using ethyl acetate. Maceration was carried out for 3 x 24 hours with a ratio of 1: 10. After filtration the extracts were evaporated in a rotary evaporator until a thick extract was obtained. The thick extracts were then stored at 4˚C as stock extract.

2.2. GC-MS analysis of the extract

For GC-MS analysis each stock extract was redissolved using ethanol and ethyl acetate solvents. The GC-MS analysis was performed on a GC-MS equipment (Shimadzu QP 2010 SE) equipped with Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol) standard capillary column. The identification of components was based on molecular structure, molecular mass, and calculated fragments. Interpretation of data was based on mass spectral matching with standard reference compounds in *Wiley Spectral Library* version 7.

2.3. Assay for antimalarial activity

Both ethanol and ethyl acetate extracts of *Sansevieria trifasciata* were evaluated for their antimalarial activity against *Plasmodium falciparum* strain 3D7 (chloroquine sensitive). The *P. falciparum* isolates were obtained from C-NPMRD (Center-Natural Product Medicine Research and Development), ITD (Institute of Tropical Disease), Universitas Airlangga, Surabaya, Indonesia.

Before being used in testing, plasmodium isolates were subjected to continuous cultivation and maintained using type O-positive human erythrocytes. The antiplasmodial assay was started by diluting 1 mg extract in 100 mL of *dimethyl sulfoxide* (*DMSO)*. Serial concentrations of each seaweed extract were made in 5 levels namely: 100, 10, 1, 0.1 and 0.01µg/ml.

The extract (2 µl) was added to a microplate well which already contained 198 µl of parasite suspension with an initial parasitemia of 1%. The test plates were placed in a chamber containing mix gas (O2 5%, CO2 5%, N2 90%) under controlled condition and incubate at 37°C for 48 hours. After incubation, the cultures were then harvested for preparation of thin blood smears stained using 20% Giemsa dyes on a glass slide.

2.4. Data analyses

2.4.1. Parasitaemia measurement

To determine the number of parasites in the blood smear, the slide was then observed under a microscope. Ten fields on each slide were observed to calculate the percent of parasitaemia using formula below (Equation 1).

$$\text{Parasitaemia (\%)} = \frac{\sum \text{parasitizied RBC}}{\sum \text{ of RBC}} \text{x } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

The data obtained from the Equation 1 was then use to calculate the percentage of parasitemia growth (Equation 2) and parasitaemia suppression (Equation 3) due to the effect of extracts.

$$\text{Growth}(\%) = \%\ \text{parasitaemia} - \text{Do} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where D_0 represents initial 1% parasitaemia prior to incubation.

$$\text{Suppression}(\%) = 100\% - \frac{\text{Treated parasitaemia}}{\text{Control parasitaemia}} \times 100\% \ldots\ldots\ldots\ldots\ldots(3)$$

2.4.2. IC_{50} determination

To determine the seaweeds extract concentration that causes half-maximal inhibition against *Plasmodium falciparum* (IC_{50} values) a probit analysis in Minitab statistical software package *was used.* The IC_{50} values were determined by plotting concentration of extract on X-axis and percentage of suppression on Y-axis with dose-response curves The antiplasmodial activity of the samples extracts categorized into four groups based on the IC_{50} values i.e. very active (IC_{50}< 5 µg/ml); active (5<IC_{50} <50 µg/ ml); weakly active (50<C_{50} <100 µg/ ml); and inactive (IC_{50} >100 µg/ ml) [11].

3. Results and discussion

3.1. GC-MS metabolite profiles

The GC-MS analysis of the ethanol extract of Mother-in-Law's leaves resulted in metabolite profiles as depicted by a chromatogram presented in Figure 1. The metabolite constituents of the ethanol extract based on the GC-MS analysis mentioned above are listed in Table 1.

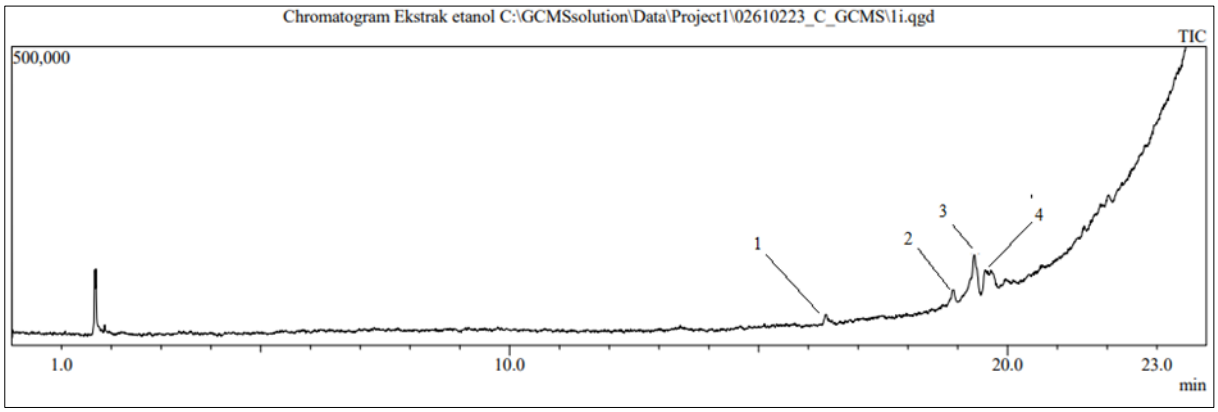

Figure 1 GC-MS chromatogram of ethanol extract of *Sansevieria trifasciata* leaves

Table 1 Constituents of phytocompounds in ethanol extract of *Sansevieria trifasciata* leaves

No	Retention Time(min)	Similarity index (SI)	Compounds	Molecular formula	% of peak area
1	16.351	92	(E) -Phytol	$C_{20}H_{40}O$	7.71
2	18.908	83	N-heterocontanol-1	$C_{41}H_{84}O$	10.45
3	19.339	84	Cyclohexane,1,2,3,5-Tetraisopropyl	$C_{18}H_{36}$	30.82
4	19.555	84	Cyclohexane,1,2,3,5-Tetraisopropyl	$C_{18}h_{36}$	51.03

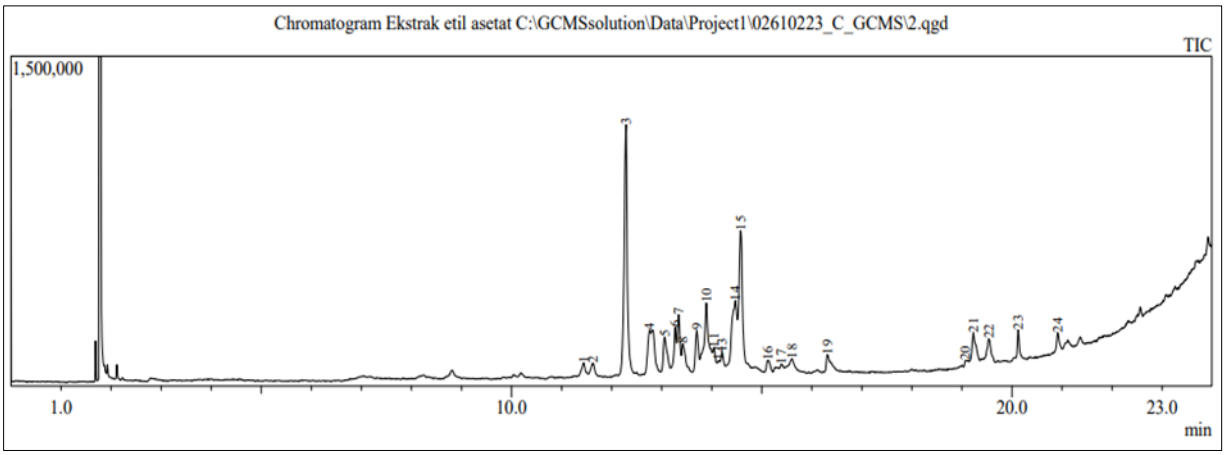

Figure 2 GC-MS chromatogram of ethyl acetate extract of *Sansevieria trifasciata* leaves

Figure 2 is presenting a chromatogram depicting the GC-MS metabolite profile of the ethyl acetate extract of *Sansevieria trifasciata* leaves. The types of constituents of the extract in question based on the GC-MS analysis are presented in Table 2.

Table 2 Constituents of phytocompounds in ethyl acetate extract of *Sansevieria trifasciata* leaves

No	Retention Time(min)	Similarity index (SI)	Compounds	Molecular formula	% of peak area
1	11.441	81	Kuren-19-YL-Acetate	$C_{22}H_{64}O_2$	1.30
2	11.633	82	Kuren-19-YL-Acetate	$C_{22}H_{34}O_2$	1.61
3	12.289	89	A Cymbratrienol	$C_{20}H_{34}O$	22.88
4	12.760	82	3.beta-Bromocholest -5-ene	$C_{27}H_{45}Br$	8.46
5	13.061	83	17-Acetoxy-19-Kaurana	$C_{22}H_{34}O_3$	4.00
6	13.272	86	4,8,13-Duvatriene-1,3-diol	$C_{22}H_{34}O_2$	3.18
7	13.341	82	Dibenzoa[A,H]Cyclotetradecece,2,3,11,12-Tetraethenyl-1,2,3,4,5,6,7,8,9,190,11,12	$C_{30}H_{44}$	4.31
8	13.424	91	2,6,10-Trimehyl,14-Ethylene14-Pentadecn	$C_{20}H_{38}$	2.52
9	13.703	83	9-n- Dodecyltetredecahydrophenantrene	$C_{26}H_{48}$	3.01
10	13.893	87	4,8,13-Duvatriene-1,3-diol	$C_{20}H_{34}O_2$	8.64
11	14.046	87	(11E,13Z)-11813-Labadien-8-1	$C_{20}H_{34}O$	1.58
12	14.133	64	(11E,13Z)-11813-Labadien-8-o1	$C_{20}H_{34}O$	0.46
13	14.209	87	(11E,13Z)-11813-Labadien-8-o1	$C_{20}H_{34}O$	0.90
14	14.474	84	Dibenzoa[A,H] Cyclotetradecece 2,3,11,12-Tetraethenyl-1,2,3,4,5,6,7,8,9,10,11,12,	$C_{30}H_{44}$	9.60
15	14.579	85	(12Z)-Abienol	$C_{20}H_{34}O$	13.52
16	15.122	86	(-)-Sclareol	$C_{20}H_{36}O_2$	1.28
17	15.392	75	Ethyl Iso Allocholate	$C_{26}H_{44}O_5$	0.58
18	15.605	78	endo-isofenchol	$C_{10}H_{18}O$	1.18
19	16.314	94	(E)-Phytol	$C_{20}H_{40}O$	2.00
20	19.067	91	n-Eicosane	$C_{20}H_{42}$	0.70

21	19.232	85	Cyclohexan 1,2,3,5-Tetraisopropyl	$C_{18}H_{36}$	3.33
22	19.540	84	N-Hentetracontanol-1	$C_{41}H_{34}O$	1.99
23	20.127	97	Phthalic acid dioctyl ester	$C_{34}H_{38}O_4$	1.54
24	20.915	95	n-Tetratetracntane	$C_{44}H_9O$	1.43

Based on the data in Figures 1 and 2, as well as Tables 1 and 2, it can be emphasized that ethyl acetate solvent dissolves more phytochemical compounds in *Sansevieria trifasciata* leaves than ethanol. This finding seems confusing considering that ethyl acetate is less polar solvent than ethanol. According to common rules, polar solvents usually dissolve more plant compounds [12].

Even so, there is nothing wrong with the findings of this research because ethyl acetate actually dissolves more components than ethanol for certain compounds in certain plants. Shalmashi and Golmohammad (2010), for example, found that the solubility of caffeine in ethyl acetate was much higher than in ethanol [13].Next, da Nóbrega et al (2022) reported that ethyl acetate solvent produces more betulinic acid from the crude extract of the *Eugenia florida* DC plant leaves [14].Chen et al (2022) also found that the active compound from the *Portulaca oleracea* plant extracted using ethyl acetate showed strong antioxidant activity compared to the ethanol extract [15]. Therefore it is not an exaggeration to say that ethyl acetate is the most preferred solvent for the extraction of active ingredients from certain plant species [16].

3.2. Antimalarial activity

The antiplasmodial properties of the ethanol extract and ethyl acetate extract of *S. trifasciata* against *P. falcifarum* described by percentage of parasitaemia growth (Figure 3) and percentage of suppression (Figure 4). While the IC_{50} values obtained through probit analysis are presented in Table 3.

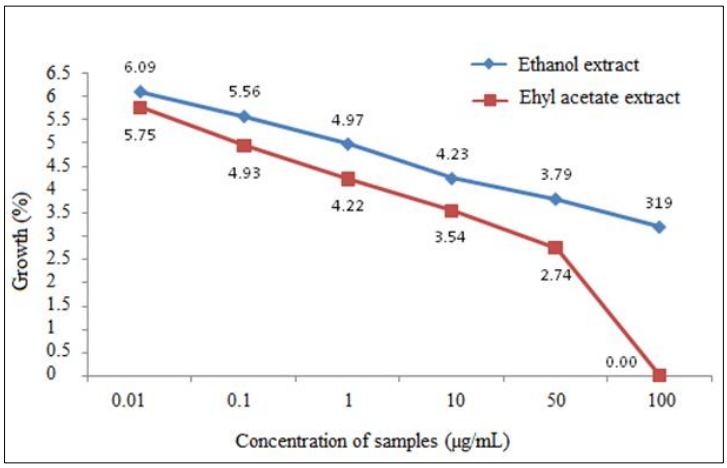

Figure 3 Percent of parasitaemia growth of *P. falciparum* by different concentration of extract

Table 3 IC_{50} of ethanol and ethyl acetate extract of *S. trifasciata* leaves against *P.falciparum* strain 3D7

Sample	**IC_{50}**	**Category**
Ethanol extract	137.73 µg/ml	inactive/not potential
Ethyl acetate extract	21.29 µg/ml	active/potential

Referring to the data in Figures 3 and 4 and Table 3 it can be assumed that the ethyl acetate extract of *S. trifasciata* leaves provides higher inhibition against *P. falciparum* strain 3D7. This finding confirms the results of previous studies suggesting that ethyl acetate is a good solvent for extracting antimalarial phytochemicals. Bagavan et al (2011) found that leaves extract *Phyllanthus emblica* and flower extract *Syzygium aromaticum* showed good antiplasmodial effect against *P. falciparum* [17]. Papaya (*Carica papaya*) leaf extracted using ethyl acetate also revealed to show antimalarial acivity against *P. falciparum* 3D7 and Dd2 [18]. Another study reported by Belay et al (2022) indicates that stem bark

of *Periploca linearifolia* (Asclepiadaceae) extracted using ethyl acetate solvent significantly suppressed parasitemia of *Plasmodium berghei* [19].

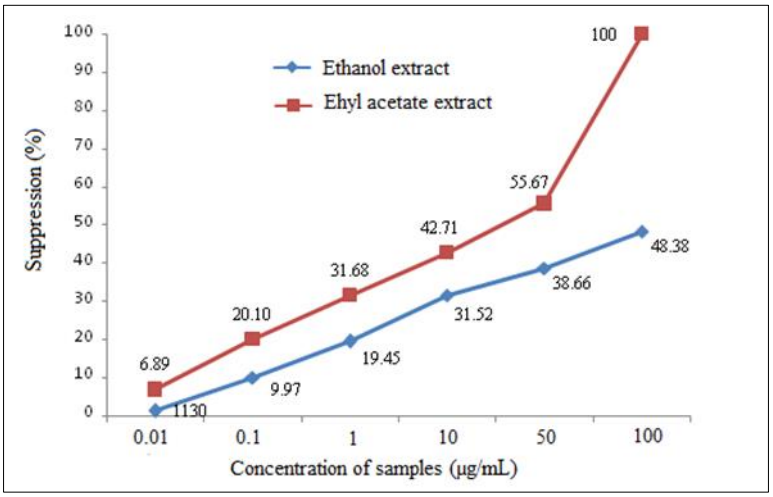

Figure 4 Percent of parasitaemia suppression of *P. falciparum* by different concentration of extract

The results of the GC-MS analysis in this study showed that both the ethanol extract and ethyl acetate extract of snake plant leaves contained phytol ($C_{20}H_{40}O$). As reported by Nwonuma et al (2022) phytol is one of the constituents of the phytocompounds in Cannabis leaf extract which is proven to have antiplasmodial properties on *Plasmodium berghei* NK-65 (a chloroquine-sensitive strain) [20].

4. Conclusion

GC-MS analysis and antiplasmodial assay of ethanol and ethyl acetate extracts of snake plant leaves presented a different result. Ethyl acetate solvent dissolved more constituent of phytocompounds and showed stronger parasitaemia suppression against *Plasmodium falsiparum* than that of ethanol. It suggests therefore that plant leaf extract *Sansevieria trifasciata* is potential to be developed as antimalarial ingredient.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare there is no conflict of interest.

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