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RESEARCH ARTICLE

Activity of Antimalarial Compounds from Ethyl Acetate Fraction of Sunflower Leaves (*Helianthus annuus* L.) against *Plasmodium falciparum Parasites* 3D7 Strain

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ABSTRACT:
Antimalarial study using methanol extract and ethyl acetate fraction of sunflower leaves had been done. The study was conducted to know antimalarial activity between methanol extract and ethyl acetate fraction at *Plasmodium falciparum* parasites 3D7 strain. Separation of compounds was done by maceration using methanol and partition using ethyl acetate. Identification of ethyl acetate fraction was done by using UPLC-MS instrument. The results shown ethyl acetate fraction obtained the highest peak based on database were artemisinin, heliangolide, linolenic acid, linoleic acid eupalinolide C. The results of antimalarial study shown that methanol extract had IC_{50} 22.18 mg / mL, and ethyl acetate fraction had IC_{50} 16.68 mg / mL. Antimalarial activity between methanol extract and ethyl acetate fraction showed no significant difference.

KEY WORDS: Sunflower leaves, Antimalarial, *Plasmodium falciparum* Strain 3D7, UPLC-MS

1. INTRODUCTION:

Malaria is an infectious disease that remains a serious and complex health problem complex until recently. The disease is caused by four species of protozoan parasites from Plasmodium type that is transmitted to humans through mosquito bites (Guerra et al. 2006). *Plasmodium falciparum* (*P. falciparum*) parasites is a type of the most lethal malaria parasites (WHO, 2015).

One of the malaria's increasing factors is the resistance of the malaria parasite *P. falciparum* particularly against synthetic drugs such as chloroquine and amodiaquin. Alternative usage can be used to overcome drug resistance by using natural materials in the form of herbs and phytopharmaca. The study of antimalarial drugs from natural materials have been widely applied. Alkaloids, terpenoids, coumarins, lignans, anthranoid, khalkon, flavonoid compounds that can be isolated from several plants, is known to have activity as an antimalarial against *P. falciparum* in vitro (Saxena et al., 2003). Sunflowers plant (*Helianthus annuus* L.) is a plant that cultivated by the people as an ornamental has a high aesthetic value. These plants belong to a family with Artemisia annua is Asteraceae. Besides as an ornamental plant, the use of sunflower on the flowers and seeds are a rich source of linoleic acid oils, while the

leaves have not been utilized optimally. Its existence is abundant, easily obtained and cultivated into an opportunity to increase the point value as a source of antimalarial drugs (Nengatik, 2011). The results of research conducted by Haniah, et al., (2015) showed that the identification of the methanol extract of sunflower leaves using thin layer chromatography (TLC), UV-Vis spectrophotometer instrument and FTIR derived alkaloid and triterpenoid compounds.

This is reinforced by research Triastutik (2013) which shows the results of the methanol extract of sunflower leaves positive terpenoid, sesquiterpene, triterpene and steroid compounds, while the ethyl acetate extract fraction positive terpenoid, sesquiterpene and triterpene compounds. Based on previous research, the notion of active compounds in sunflower leaves potential as an antimalarial is sesquiterpene. This is supported by research paitan plants are same family with Helianthus annuus L. plants are Compositae, it has been reported that the compound of interest methanol fraction paitan give positive test is sesquiterpene lactone group. This sesquiterpene lactones have an important role in killing the malaria parasites (Sari, Hafid, and Widyawaruyanti, 2015).

Other studies that have been done related to the plants that are one family (Asteraceae) is a compound artemisinin is a sesquiterpene lactone that can be used as an antimalarial, where hemozoin malaria can be inhibited by artemisinin. In addition, artemisinin derivative artesunate sesquiterpene lactones of plants Arthemisia annua L can be used as an antimalarial with a low concentration of 10 μm (Simamora and Fitri, 2007). Plants that are in the same family (Asteraceae) have active sesquiterpene lactone compounds, as well as on a sunflower leaf that has sesquiterpene lactone. Potential sesquiterpene lactones, used a compound artemisinin and its derivatives are used as an antimalarial artesunate until now (Macias, 1998).

The research antimalarial in vitro against *P. falciparum* from other plants that have been made at sernai leaves. This plant comes from the Asteraceae family is still a family with a sunflower leaves. The content of secondary metabolites contained in sernai leaves are compound terpenoids. The methanol extract of the leaves as antiplasmodium sernai showed activity in cultured 32 hours with a value Inhibition Concentration (IC_{50}) of 5.253 ug/ml (Veterinaria, Isa, and Aceh, 2013). Based on these studies, it can be concluded that the antimalarial activity of sunflower leves against test animals infected *P. berghei* or in vivo has been done and proven to have a high percent inhibition as antimalarial, so this study was conducted to determine the antimalarial activity of methanol extract and fractions ethyl acetate sunflower leaves in vitro using parasite *P. falciparum* 3D7 strain. The content of active compound contained in the sample determined by testing using UPLC-MS instrument.

2. MATERIALS AND METHODS:

2.1. MATERIALS:

The materials used in this study is powder sunflower leaves dried, *P. falciparum* 3D7 strain parasites, methanol pa (Merck), ethyl acetate pa (Merck), H_2SO_4 pa (Merck), medium RPMI 1640, Serum human blood cells type O, solution giemasa dye.

2.2. Sample Preparation:

Sunflower leaves samples taken from the area Temas, Batu much as 1 kg washed with water and then cut into small pieces. This piece is dried by aerated and then crushed using a blender and sieved using a 60 mesh sieve.

2.3.Sample Extraction:

Sunflower leaves powder weighed 200 g and was put in a 2 erlenmeyer. The results of weighing extracted by soaking using a 300 mL methanol p.a every erlenmeyers until 24 hours and shaker for 3 hours at room temperature with a speed of 120 rpm. Then filtered with a Buchner funnel and pulp macerated again with the same solvent until the filtrate translucent color. The filtrate was combined and concentrated by rotary evaporator vacuum to obtain a concentrated methanol extract. Methanol extract taken 10 g of concentrated and liquid-liquid extraction with 50mL ethyl acetate until clear. Ethyl acetate fractions are combined and concentrated by rotary evaporator vacuum to obtain the ethyl acetate fraction. Methanol used in the process as much as 1800mL maceration with the acquisition of 16.78 g extract weight gain. Liquid-liquid extraction process or partition using the necessary 250mL ethyl acetate extract weighing 3.53 g.

2.4 Phytochemical Test:

Method of phytochemical test is performed to know the content of secondary metabolites in extracts of methanol and ethyl acetate fraction. Each sample is inserted in a 50mL beaker glass. Samples were taken a little later added 2mL of chloroform. Added 3mL of sulfuric acid. Terpenoids positive if it shows a brownish red color, to test seskuiterpen taken a bit of each sample was then dissolved in petroleum ether. Evaporated to dryness and vanillin reagent is added 10% sulfuric acid. Seskuiterpen positive if it shows turquoise color. Taken 5 mg for each sample in a steroid test is then dissolved in 0.5mL of chloroform was then added 0.5mL of acetic acid anhydride and 0.5-1mL of concentrated sulfuric acid. Positive steroid represented by the color blue to green. The identification results phytochemical content in the

methanol extract and ethyl acetate fraction showed positive terpenoid, sesquiterpene and steroid compounds.

2.5. Antimalarial test in vitro:
Test activities are conducted with several stages are thawing parasites, culture monitoring, synchronization parasites, parasite suspension preparation, preparation of test materials, test antimlaria activity and probit analysis of results with SPSS.

2.6. Compounds identification using UPLC-MS:
Ethyl acetate fraction samples taken 5 mL and injected directly into the eluent flow under pressure towards the column. The column used was Acquity C18 with a size of 1.7 µm, 2.1x50 nm. Eluent used is water + acetonitrile + formic acid and formic acid, with 30% water, 0.1% formic acid, and 70% acetonitrile.

3. RESULTS AND DISCUSSION:

3.1 Antimalarial test in vitro:
Antimalarial test do to know activities of ethyl acetate fraction and crude methanol extract in inhibiting the growth of *P. falciparum* parasites using varying concentrations of 100, 10, 1, 0.1, and 0:01 µg / mL. Antimalarial activity test performed in vitro against of *P. falcirum* parasites 3D7 strain. The test results of *P. falciparum* parasites is available from the calculation of the number parasites that grow in wells for ± 48 hours. After that, made thin blood smear slide with colored Giemsa. Giemsa coloring is intended to give color to the parasites, so it will be easier in the observations. After that observed using a microscope with a magnification of 1000x. Number of infected erythrocytes are calculated based on the number of infected erythrocytes were selected from some of the visual field swabs based on the number of infected erythrocytes were selected from some of the visual field swabs monolayer. Antimalarial activity can be determined by calculating the percent parasitaemia has been obtained on the sample testing, resulting in a percent growth, percent inhibition, and the percent inhibition of the average summarized in Table 1. Acquisition of data from Table 1 can be made a graph showing the value of concentration and percent barrier shown in Figure 1.

IC_{50} (Inhibitory Concentration) was determined based on the curve of the relationship between the value of the log probit concentration. Based on Figure 1, the graph shows the relationship between concentration and% barrier proportional. The higher the concentration, so the value% barrier also higher. How to know value of 50% inhibition of the parasite is definitely probit analysis is carried out using SPSS 16, so obtain IC_{50} values. IC_{50} value of the methanol extract and ethyl acetate fraction, respectively for 22.18 ug/mL and 16.68 mg /mL. IC_{50} value according to the category of Gessler (1994), said that the test substance antiplasmodium activity in vitro is divided into three are the test substance with the best activity when $IC_{50} \leq 10$ mg /mL, a good activity when IC_{50} values between 10-50 mg /mL, and less good activity when $IC_{50} \geq 50$ mg /mL. This proves that the sample fraction of ethyl acetate had better IC_{50} than the methanol extract samples.

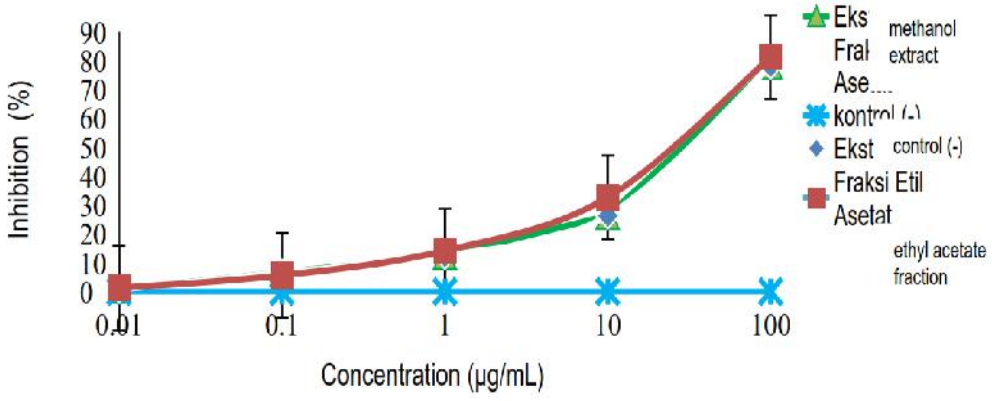

Figure 1 . Graph of the relationship between the percent inhibition and concentration

Table 1 Percentage barriers due to *Plasmodium falciparum* treatment of methanol extract and ethyl acetate fraction sunflower leaves (*Helianthus annus* L.)

Sample name	Concentration (µg/mL)	R	Parasitemia %		Growth %	Inhibition %	mean inhibition %
			0 jam	48 jam			
Methanol extract	**Control (-)**	1	1.28	6.14	4.86	-	-
		2	1.28	6.17	4.89	-	
	100	1	1.28	2.34	1.06	78.19	78.15
		2	1.28	2.35	1.07	78.12	
	10	1	1.28	4.86	3.58	26.34	26.26
		2	1.28	4.89	3.61	26.18	
	1	1	1.28	5.53	4.25	12.55	12.41
		2	1.28	5.57	4.29	12.27	
	0.1	1	1.28	5.81	4.53	6.79	6.67
		2	1.28	5.85	4.57	6,54	
	0.01	1	1.28	6.11	4.83	0.62	0.72
		2	1.28	6.13	4.85	0.82	
Etyl acetate fraction	Control (-)	1	1.28	6.14	4.86	-	-
		2	1.28	6.17	4.89	-	
	100	1	1.28	2.18	0.90	81.48	81.23
		2	1.28	2.21	0.93	80.98	
	10	1	1.28	4.56	3.28	32.51	32.62
		2	1.28	4.57	3.29	32.72	
	1	1	1.28	5.45	4.17	14.20	
		2	1.28	5.48	4,20	14.11	
	0.1	1	1.28	5.87	4.59	5.56	5.64
		2	1.28	5.89	4.61	5.73	
	0.01	1	1.28	6.09	4.81	1.03	1.23
		2	1.28	6.1	4.82	1.43	

3.2. Compounds identification using UPLC-MS:

UPLC-MS chromatogram of ethyl acetate fraction samples sunflower leaves is shown in Figure 2. The results of using UPLC-MS analysis in Figure 2 shows there are 16 peaks. This shows that the ethyl acetate fraction may contain 16 compounds. Five of these were thought to be the compound contained in the leaves of sunflower, which are summarized in Table 2. Besides the five compounds identified in Table 2, there are other compounds that appear in the data chromatogram in Figure 2. These compounds are in the range of time retention of 4.94 to 8.49 minutes.

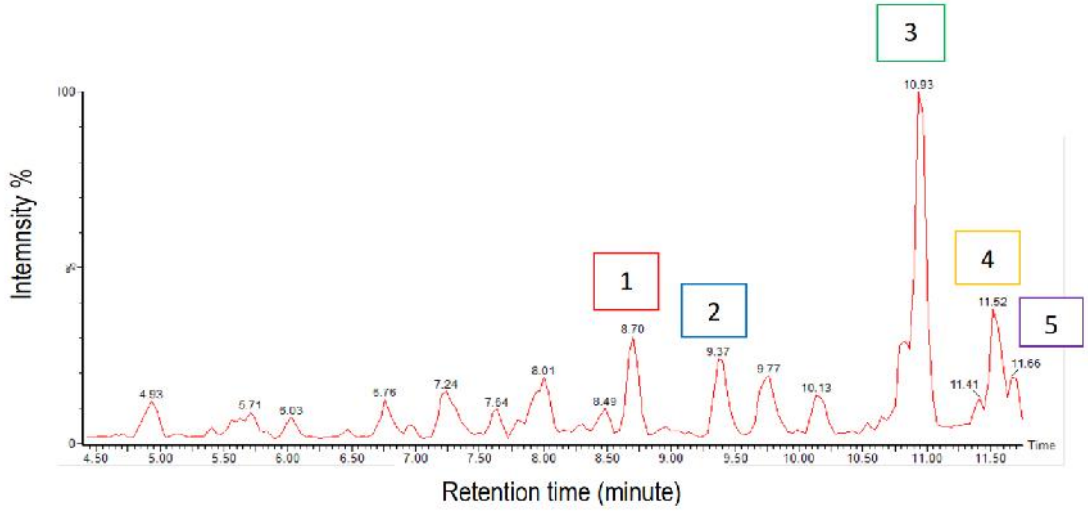

Figure 2. UPLC-MS chromatogram compounds separation results of ethyl acetate fraction sample of sunflower leaves

The peak number 1 is Artemisinin compound has a m/z 283. The molecular formula is $C_{15}H_{23}O_5$. The peak number 1 is Artemisinin compound has a m/z 283. The molecular formula is $C_{15}H_{23}O_5$ fragmented into m/z 265 by releasing the M-18. The difference between the results of the spectra with m/z assumed to release water molecules (H_2O), then fragmented into m/z 247 by releasing the hydroxyl group (OH) and the release of water molecules into m/z 229. (Nieuwerburgh, et al., 2006).

The peak number 2 is Heliangolide compound has a molecular formula C20H22O6 with m/z 358. The fragmentation results showed m/z 276 with the release of molecules C_2H_5-CCH_3CO (M-82), then fragmented into m/z 258 (Spring, et al., 1982). Heliangolide compound is compound sesquiterpene lactone group that has the molecular formula $C_{20}H_{22}O_6$ with m/z 358 is fragmented into m/z 276 with the release of molecules C_2H_5-CCH_3CO (M-82), then fragmented into m/ z258 (Spring, 1982).

The peak number 3 is linolenic acid compound has m/z 278 with molekul$C_{18}H_{30}O_2$ formula. Fragmentation early (M-1) release of the hydrogen atom attached to the carboxylic group into m/z 277 with the intensity of the highest peak, then become m/z 233 with the release of the carboxylic group (M-45). Further releases 5 CH_2 molecules (M-70) to m/z 163 and fragmented again to 139 with the loss of a molecule $(CH_2)_2$ (M-24) Dewi, 2014).

The peak number 4 is Eupalinolide C compound has m/z 443, which is fragmented into m/z 383 with the release of methyl acetate (CH_3COOH, M-60), then fragmented into molecules simpler by letting go (M-116, $HOCH_2$-CH and C (CH_3) COOH into m/z 267 and m/z 237 by releasing (M-30, HCHO) (Yang, Duan, Shang, and Tian, 2010).

The peak number 5 is linoleic acid compound has m/z 280, which fragmented into m/z 235 by releasing the carboxylic group (COOH, M-45). Intensity owned peak m/z 235 is the highest peak representing fragments of linolenic acid, then fragmented back by releasing the five molecules CH_2 (M-70) to m/z 165 and release the $(CH_2)_2$ CH (M-41) to m/z 124 (Dewi, 2014).

Table 2 Compounds that allegedly contained in the leaves of sunflower

Peak number	Retention time (minute)	Area wide	Area %	Compound names
1	8.70	54834	9.80 %	*Artemisinin*
2	9.37	34341	6.14 %	*Heliangolide*
3	1.93	179175	32.02 %	*Asam Linolenat*
4	11.52	42714	7.63 %	*Eupalinolide C*
5	11.66	37507	4.31 %	*Asam Linoleat*

4. CONCLUSIONS:

Methanol extract of sunflower leaves inhibit the growth of Plasmodium falciparum 3D7 strain with IC_{50} value 22.18 mg/mL, while the ethyl acetate fraction has IC_{50} value 16.68 µg/mL. The identification of ethyl acetate fraction using UPLC-MS acquired the five highest peaks of compounds such as artemisinin, heliangolide, linolenic acid, linoleic acid eupalinolide C, and from MS spectra shown m/z at 282, 358, 278, 443, and 280.

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