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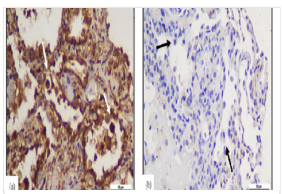
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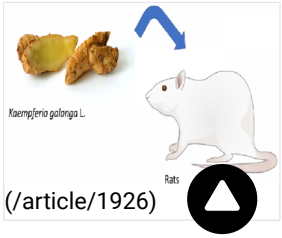

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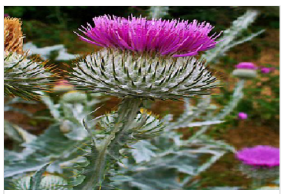

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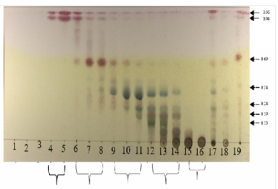

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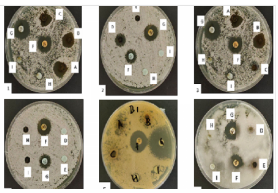

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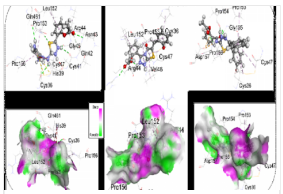

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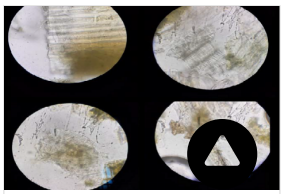

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Research Article

Antioxidant, Antimicrobial, and Antiplasmodial Activities of *Sonchus arvensis* L. Leaf Ethyl Acetate Fractions

Dwi Kusuma Wahyuni[1,2*], Anindya Nariswari[1], Agus Supriyanto[1], Hery Purnobasuki[1], Hunsa Punnapayak[2], Wichanee Bankeeree[2], Sehanat Prasongsuk[1,2,*], Wiwied Ekasari[3,*]

ABSTRACT

Infection is one of the health problems and a disease that mainly causes death. Malaria is a parasitic infection that is transmitted through the *Anopheles* sp. The female then causes infection and besides malaria, other contaminants that caused infection are bacteria such as *Escherichia coli* and *Staphylococcus aureus*. This study aims to determine the antioxidant, antimicrobial, and antiplasmodial activity of *Sonchus arvensis* L. ethyl acetate fractions. *In vitro* antiplasmodial activity was carried out by Rieckman methods against *Plasmodium falciparum* strain 3D7. *In vitro* antioxidant activity was conducted by Prieto method against (2,2-diphenyl-1-picrylhydrazyl (DPPH). Then antimicrobial activity was performed using well diffusion method against *Escherichia coli* and *Staphylococcus aureus*. Maceration of *S. arvensis* L. dried leaves used *n*-hexane and ethyl acetate successively. Then the ethyl acetate extract was fractionated by vacuum column chromatography, using *n*-hexane and ethyl acetate as mobile phases. There are five fraction groups based on thin-layer chromatography (TLC) analysis. The IC_{50} of antioxidant and antiplasmodial activity showed that fraction IV was the lowest value and categorized as active for antioxidant (IC_{50}=22.56 µg/mL), for antiplasmodial (IC_{50}=12.07 µg/mL). Fraction IV also had antimicrobial activity, with diameter of inhibition zone (DIZ) of 19.22 mm against *Escherichia coli* and 17.167 mm against *Staphylococcus aureus*.

Key words: Biological activities, Malaria, *Plasmodium falciparum*, *Sonchus arvensis* L., *Staphylococcus aureus*, *Escherichia coli*.

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INTRODUCTION

Infection is one of the serious global health problems.[1] In Indonesia, as many as 28.1% of the main causes of death in Indonesia are caused by infection.[2] Infections were caused by various microorganisms such as bacteria, viruses, fungi, and protozoa.[3] During global covid pandemic, the number of malaria cases increases by 205 million.[4] Besides malaria, other contaminants that cause infectious diseases are bacteria. *Escherichia coli* and *Staphylococcus aureus* are bacteria that cause diarrheal disease. Malaria and diarrhea are the ten most popular infectious diseases in Indonesia.[2]

Recently, the problem of antibiotic and antimalarial resistance has emerged. The World Health Organization (WHO) has issued a list of priority pathogens to increase research efforts in the search for new antibiotics and antimalarials, to overcome the problem of resistance.[4] One source of searching for new drugs is to use medicinal plants derived from nature. Some studies showed that the active components in medicinal plants have antimicrobial effects that differ in their mechanism of action from antibiotics that have been around so far. This shows that medicinal plants have the potential to overcome the problem of resistance.[5]

One of the medicinal plants that are often used in Indonesia is a plant from the Asteraceae family, a plant from the Asteraceae family which is widely known by the public as a plant that is easy to grow and has been widely used in medicine, *S. arvensis* L.[6,7] Based on the phytochemical screening results from the research of Wahyuni *et al* (2020b) it could be seen that *S. arvensis* L. leaves contain various bioactive components such as flavonoids, terpenoids, and polyphenols that can act as antioxidants, hepatoprotective, diuretic, and have the potential as antimalarial agents.[8,9] *S. arvensis* L. also has antimicrobial activity. This is based on the chemical content of the *S. arvensis* L. flavonoids can affect the polarity of the lipids that make up bacterial DNA. Alkaloids can damage the peptidoglycan constituents of bacterial cells.[10]

S. arvensis L. has potential as an antimalarial agent based on previous research conducted by Wahyuni *et al.* (2020) that the methanol extract from *S. arvensis* L. callus treated with 1-5% sucrose was tested for antimalarial *in vitro* using the Trager and Jensen methods and tested against *Plasmodium falciparum* strain 3D7 has an IC_{50} value of 0.343 g/ml.[8] According to research conducted by Tapan (2016), The (High-Performance Liquid Chromatography (HPLC) on 80% ethanol extract of *S. arvensis* L. leaves produced ascorbic acid, gallic acid, catechin, and kaempferol compounds which are believed to act as antibacterial.[11] Based on research by Xia *et al.* (2011), *S. arvensis* L. methanol extract had a total phenolic value of 417.3 ± 38.3 mg/g GAE dry weight and the antibacterial test had a minimum inhibitory concentration (MIC) value of 9.5-16 mg/ml.[12] Based on research conducted by Kanaani and Sani (2015), *S. arvensis* L. methanol extract has a Minimum Inhibitory Concentration (MIC) value of 50-100 mg/ml which were tested on *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella enterica*, and *Escherichia coli*.[13] Another study also reported that the *n*-hexane extract of *S. arvensis* L. leaves using column chromatography and separated by several solvent fractions was able to inhibit the growth of

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Staphylococcus aureus bacteria by 9 mm, and *Escherichia coli* by 8 mm with a concentration of 1000 ppm.[14]

Although many studies on the bioactivity of *S. arvensis* L have been carried out, there have been no reports on the antiplasmodial activity of the ethyl acetate fraction of *S. arvensis* L. to isolate the target compound. Therefore, this study aims to determine the antioxidant, antimicrobial, and antiplasmodial activities of the ethyl acetate fraction of *S. arvensis* L.. The results of this study are expected to provide basic knowledge as a guideline for isolating compounds that have the potential as antioxidants, antimicrobials, and antiplasmodial from *S. arvensis* L..

MATERIALS AND METHODS

Sample preparation

S. arvensis L. were obtained from the Taman Husada Graha Famili (Medicinal Plant Garden), Surabaya, Indonesia. The plant was identified by the Purwodadi Botanical Gardens Herbarium, East Java, Indonesia. The voucher specimen was collected in Plant Systematic Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga (Voucher No. SA.0010292021). The leaves from vegetative stage were collected, then dried and mashed to form a powder, and then weighed of 1 kg to continue the extraction process.

Extraction

One hundred grams of *S. arvensis* L. leaf powder was extracted by maceration using 1000 mL of *n*-hexane until all the powder was submerged and stirred, then covered and stored for three days. Stirring was done approximately three times a day. Furthermore, filtering was carried out so that only the powder remains. Then the powder was soaked in 1000 mL of ethyl acetate solution for three days. Then the filtrate was concentrated with a vacuum rotary evaporator to obtain a thick extract and then weighed.

Fractionation

Five grams of *S. arvensis* L. ethyl acetate extract was fractionated by vacuum chromatography. The 75 grams of silica gel 60G was added was used as stationary phase. The mobile phase use *n*-hexane:ethyl acetate in 16 combinations (100, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 50:50, 55:45, 40:60, 30:70, 20:80, 10:90, 100). The dried extract was put into a sintered glass and filter paper was placed on top of the extract. The mobile phase was poured into a sintered glass and withdrawn with a suction pump until the liquid did not drip back. Then eluted starting from low polarity then the polarity was increased slowly and the column was sucked dry at each collection of fractions.[15]

Thin Layer Chromatography analysis

GF254 silica plates were used. One hundred milligram fractions were dissolved in 100 µL ethyl acetate. Each fraction was spotted on silica plate at a distance of ± 1 cm from the bottom (± 1.5 cm) with a capillary tube, then dried and eluted with each mobile phase of the compound group. The terpenoid compound group was made into a mobile phase consisting of n-hexane: ethyl acetate (4:1). After the plate was eluted to the mark, taken, and allowed to dry, it was sprayed with sulfuric acid *p*-anisaldehyde reagent, then heated for 5 minutes at 105°C. The presence of terpenoids was indicated by the formation of a blue-violet or red-violet spot.[9]

Antioxidant activity assay

Antioxidant activity was evaluated by a modified method of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay.[16] In brief, 100 µl of methanolic DPPH reagent (0.2 mM) was mixed with 100 µl of each sample in methanol at different concentrations (3.15, 6.25, 10.00, 12.50, 15.00, 25.00, 35.00, 50.00, 75.00, 100.00, 150.00, and 200.00 µg/ml) or methanol as the control. The mixtures were incubated for 30 min in the dark at room temperature and the absorbance was measured at 517 nm. The inhibition of DPPH was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = (A_{control}\text{-}A_{sample})/A_{control} \times 100\% \quad (1)$$

where A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the DPPH reagent at the wavelength of 512 nm. The percentage of inhibition results at different concentrations was then plotted and regressed linearly to obtain the IC_{50} values of DPPH.

In vitro antiplasmodial activity assay

Cultures of *Plasmodium falciparum* strain 3D7 were cultivated using the Trager and Jensen method (1976), as adapted by Ekasari *et al.* (2009),[17] in Roswell Park Memorial Institute 1640 medium (Gibco, Carlsbad, CA, USA) supplemented with human O-type red blood cells, 5% hematocrit, 22.3 mM HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Sigma-Aldrich Corporation, St. Louis, MO, USA), 50 µg/mL hypoxanthine, 2 mg/mL sodium bicarbonate, and 10% human O^+ plasma.[15] The sample was dissolved in 10% dimethyl sulfoxide (DMSO) in various concentrations (0.01, 0.1, 1, 10, and 100 µL). Chloroquine diphosphate was used as a positive control and DMSO as the negative control. An antiplasmodial assay was conducted using a 24-well microplate and incubation at 37°C for 48 h. The incubated materials were then collected, thinly smeared on a glass slide, fixed with methanol, and stained with Giemsa to assess the number of parasites under a microscope as compared with the negative control to determine the IC_{50} value to achieve inhibition of parasitic growth. The data was used to calculate the percent growth and percent inhibition using the following formulas:

$$\% \text{ Growth} = \% \text{ Parasitemia} - D0 \quad (2)$$

$$\text{Percent inhibition} = 100\% - [(Xu/Xk) \times 100\%] \quad (3)$$

Where D0 is the percentage of growth at the 0-hour, whereas Xu and Xk are the percentages of growth in the test solution and negative control, respectively. Based on the percent inhibition data, statistical analysis was carried out using Probit analysis of the SPSS version 20 program to determine the IC_{50} value or the concentration of the test material that inhibits parasitic growth by 50%.

Antimicrobial activity assay

Well diffusion method was used in this study to determine the antimicrobial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The 10 mg of fractions were weighed and dissolved in dimethyl sulfoxide (DMSO). The extract was added with 2 mL of 20% DMSO gradually and 8 mL of sterile distilled water and then homogenized. Chloramphenicol (1000 mg/L) was used as positive control and DMSO (20%) as negative control. Next, mix 1 mL of pure culture into a test tube containing 9 mL of nutrient broth media (peptone 10 mg, yeast extract 2 g, beef extract 1 g, sodium chloride 5 g, pH 6.8 ± 0.2 at 25°C. The mixture was homogenized by vortex and the turbidity was standardized to 0.5 nm Mc Farland using a spectrophotometer with a wavelength of 625 nm. Bacteria that have been standardized were taken as much as 1 mL using a micropipette and put into a petri dish. The warm nutrient agar media (± 60°C, 25 mL) was poured into a plate containing 1 mL of bacteria. Make a well in the agar using a tool that has been sterilized with 70% alcohol and heated on fire. Putting the extract into the wells with a concentration of 25% and 50%, respectively, with positive control of chloramphenicol and negative control of DMSO. Pour plate cultures were incubated at 37°C for 24 hours. Inhibition was revealed based on the diameter of the clear zone formed around the well (diameter of inhibition zone/DIZ). The measurement used a calliper using quantitative observations and expressed in millimetres.[18]

The value of DIZ was used to calculate the percentage of inhibition with the formula:

Percentage of inhibition (%) = (DIZ sample/DIZ control) x 100% (4).

RESULT AND DISCUSSION

The yield of *S. arvensis* L. ethyl acetate fractions

The yield of *S. arvensis* L. *n*-hexane fraction groups were 11.14%, 12.88 %, 20.18%, 24.28%, and 16.44% for fractions no I, II, III, IV, and V respectively. Yield is the ratio of the dry weight of the extract to the amount of raw material. The yield value is related to the amount of bioactive content contained. The higher yield means the higher content of substances that are attracted to raw material. The yield of extract depended on the solvent and the methods of extraction.[19-21] In this study, group fractions IV were the highest yield. They were isolated by the *n*-hexane: ethyl acetate (40:60, 30:70, 20:80). Some studies showed that the more polar solvent resulted in higher yield of extract.[19,20]

Chromatogram profile of *S. arvensis* L. ethyl acetate fractions by Thin Layer Chromatography (TLC)

The chromatogram profile of *S. arvensis* L. ethyl acetate fractions by thin layer chromatography (TLC) showed various spots. There were bioactive compounds that can be seen from the appearance of anisaldehyde stains. The sulfuric acid-anisaldehyde solution was used to detect the presence of terpenoids, steroids, and essential oils. After spraying on the TLC plate, it was heated in an oven at 100°C for 5 -10 minutes. Fraction I (combined extract numbers 4 and 5) showed red color. Fraction II (combined extract numbers 6, 7, and 8) showed purple color. Fraction III (combined extract numbers 9, 10, and 11) showed red, purple, blue, and green colors. Fraction IV (combined extract numbers 12, 13, and 14) shows blue and green colors. And fraction V (combined extract numbers 15 and 16) showed a brown color ring. The significant spots were calculated as the retention factor (Rf). Fraction I had 2 spots (Rf. 1.00 and 0.94), fraction II had one spot (Rf. 0.69), fraction III had one spot (0.34), fraction IV had 4 spots (Rf. 0.34, 0.24, 1.90, and 0.13), and fraction V had no spot (Figure 1). TLC was used to separate plant extracts' secondary metabolites.[22] The Rf value indicated a significant diversity of terpenoid compounds separated from various extracts.[23]

Antioxidant activity

Antioxidant activity of *S. arvensis* L. ethyl acetate fractions showed active to moderate activities. Fraction I-II had moderate activity with the IC_{50} value were 145.07 µg/L and 110.52 µg/L respectively. Fraction III-V had active activity with the IC_{50} value were 46.62 µg/L, 22.56 µg/L, and 22.82 µg/L respectively (Table 2).

Prieto, 2012[24] categorized the antioxidant activity based on the IC_{50} value. The extracts exhibited potent antioxidant activities (IC_{50} < 50 µg/L) and moderate antioxidant activities (101 µg/L > IC_{50} < 250 µg/L). The IC_{50} value (the antioxidant activity) of fraction IV was the lowest. Moreover, compared to the other studies, the ethyl acetate fraction III-V extract from *S. arvensis* L was lower than plants and callus *Trifolium pratense* L.,[25] *Callisia fragrance* leave juice,[26] and *Centella asiatica* L. leave that have been previously reported as high antioxidant compound. The potent antioxidant activity of the *S. arvensis* L. fraction was probably due to the presence of active ingredients with antioxidant activities, such as polyphenols and flavonoids.[26,27]

Antimicrobial activity

The antibacterial activity test used the well diffusion method by calculating the diameter of the inhibition zone (DIZ) against *Escherichia coli* and *Staphylococcus aureus* in the media. Based on the previous report[28], a compound acts as a very potent antimicrobial if the diameter of the inhibition zone value was > 8 mm, potent if the inhibition zone value was 5 mm>DIZ< 8 mm, and not potent if the inhibition zone value was < 5 mm. This study showed that fractions III-V acted as very potent antimicrobials, with DIZ of fraction III, 10.33±2.17 mm (25%) and 12.10±2.33 mm (50%); fraction IV, 16.78±0.70 mm (25%) and 19.22±0.07 (50%); fraction V, 9.53±5.24 mm (25%) and 13.01±5.22 mm (50%) against *E. coli*; fraction IV, 10.37±0.55 mm (25%) and 15.17±1.04 (50%); and fraction V, 0.08±3.02 mm (50%) against *S. aureus* bacteria because they had DIZ value of > 8 mm. The fraction that acted as a potent antimicrobial was fraction III, 5.09±5.31 mm (50%) against *S. aureus* because it had DIZ value of 5 mm >DIZ< 8 mm. Fractions with no antimicrobial activity were fractions I and II. because they had an inhibition zone value of < 5 mm (Table 3).

The percentage of inhibition of the ethyl acetate fractions group IV was the highest, 102.05±15.34 % (25%), 117.38±23.11% (50%) against *E. coli* and 49.35±13.30% (25%), 83.77±17.32 % (50%) against *S. aeureus*. Compared to other studies, the diameter of inhibition zone value was higher than ethanol (15.9 + 0.3 mm) and water (12.2 + 0.7 mm) extract of thyme, water extract of roselle, clove, and rosemary against *E. coli*. The diameter of inhibition zone was also higher than the water extract of thyme, rosemary, and clove against *S. aereus*.[29] They were also higher than ethanolic extract of *Justicia flava*, *Myrianthus arboreus*, and *Momordica charantia*, however lower than aqueous extract, both of *Alchornea cordifolia* and *Psidium guajava* extract against *E. coli*.[30] Some compounds have been industrially applied for antimicrobial activity in doses below 1000 mg.[31]

Antiplasmodial activity

Antiplasmodial activity of *S. arvensis* L. ethyl acetate fractions showed various activity with different IC_{50} against *Plasmodium falciparum* strain 3D7. The IC_{50} value of antiplasmodial activity fraction I-V were 494.95 µg/mL, 51.32 µg/mL, 153.66 µg/mL, 12.07 µg/mL, and 13.34 µg/mL, respectively (Table 3-4). Some studies categorized the antiplasmodial activity based on the IC_{50} value, an extract with an IC_{50} value < 10 µg/mL was classified as very active, an extract's antimalarial activity with a value of 10 µg/mL < IC_{50} < 50 µg/ml was classified as active, and the antimalarial activity of an extract with an IC_{50} value > 50 µg /mL was classified as inactive.[32,33] Based on the criteria, fractions I-III had inactive activity and fractions IV-V had active activity. This is because the content of bioactive compounds in each fraction is different. The content of compounds such as terpenoids, flavonoids, polyphenols, and anthraquinones has potential as antimalarials.[34] The compounds in *S. arvensis* L. that have been analyzed include the flavonoid and sesquiterpene compounds from the terpenoid group.[7] Due to the *in vitro* antiplasmodial activity of leaf extracts from *Vernonia fimbrillifera* Less. (Asteraceae), a bioactivity-guided fractionation was carried out. Three sesquiterpene lactones were isolated, namely 8-(4'-hydroxymethacrylate)-dehydromelitensin, onopordopicrin, and 8α-[4'-hydroxymethacryloyloxy]-4-epi-sonchucarpolide. Their structures were elucidated by spectroscopic methods (1D and 2D NMR and MS analyses) and by comparison with published data. The isolated compounds exhibited antiplasmodial activity with IC_{50} values ≤ 5 µg/mL.[35]

CONCLUSION

This study highlighted the antioxidant, antimicrobial, and antiplasmodial activity of *S. arvensis* L. ethyl acetate fractions. Fraction IV of *S. arvensis* L. ethyl acetate extract had the lowest IC_{50} of antioxidant activity against DPPH and antiplasmodial activity against *Plasmodium falciparum* 3D7. Fraction IV also possessed the highest diameter zone inhibition value zone (DIZ) against *Escherichia coli* and *Staphylococcus aureus*. New findings about the activities of these plant extracts could

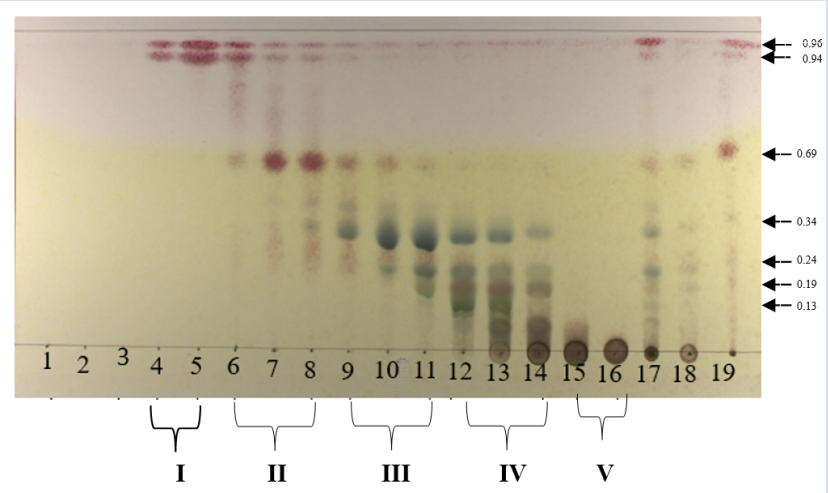

Figure 1: Chromatogram profile of *S. arvensis* L. ethyl acetate fractions by thin layer chromatography (TLC). Mobile phase *n*-hexane:ethyl acetate (4:1). Spot staining: *p*-anisaldehyde sulfuric acid. 1-16: fractions number, I-V: fraction groups number. 17. ethanol extract, 18. ethyl acetate extract, 19. *n*-hexane extract.

Table 1: The yield of S. *arvensis* L. ethyl acetate fractions.

Fractions	Weight (g)	Group Fractions	Final weight (g)	Yield (%)
1	0.08			
2	0.19	-	0.53	10.66%
3	0.26			
4	0.27	I	0.56	11.14%
5	0.29			
6	0.25			
7	0.38	II	0.64	12.88 %
8	0.14			
9	0.36			
10	0.29	III	1.01	20.18%
11	0.36			
12	0.39			
13	0.46	IV	1.21	24.28%
14	0.37			
15	0.33	V	0.82	16.44%
16	0.50			

Note: Five gram of ethyl acetate extract was fractionated to be 16 fractions (Latin number) and then grouped into five fractions (Rome number).

Table 2: The IC_{50} of antioxidant activities of S. *arvensis* L. ethyl acetate fractions.

Sample	IC_{50} (µg/mL)	Activity
I	145.07	moderate
II	110.52	moderate
III	46.62	active
IV	22.56	active
V	22.82	active

Table 3: Diameter of imbibition zone of S. *arvensis* L. ethyl acetate fractions against *Escherichia coli* and *Staphylococcus aureus* (mm).

Fractions	*Escherichia coli* (mm)				*Staphylococcus aureus* (mm)			
	Diameter of inhibition zone (mm)		Percentage of inhibition (%)		Diameter of inhibition zone (mm)		Percentage of inhibition (%)	
	25%	50%	25%	50%	25%	50%	25%	50%
I	0	0	0	0	0	0	0	0
II	3.17±3.01	4.50±4.50	29.15±11.88	41.56±19.71	0	0	0	0
III	10.33±2.17	12.10±2.33	61.81±22.61	72.19±25.11	3.28±3.40	6.00±1.00	22.16±16.07	29.86±16.50
IV	16.78±0.70	19.22±0.07	102.05±15.34	117.38±23.11	10.37±0.55	15.17±1.04	49.35±13.30	83.77±17.32
V	9.53±5.24	13.01±5.22	45.95±24.17	63.17±23.16	5.09±5.31	10.08±3.02	31.87±30.56	60.93±19.25
Chloramphenicol (1000 mg/L)	20.43±1.11				20.67±0.31			

Table 4: Growth and inhibition percentage of *S. arvensis* L. ethyl acetate fractions against *Plasmodium falciparum* strain 3D7.

Concentration (µg/mL)	Fractions	% Parasitemia		Growth percentage	Inhibition percentage
		0h	48h		
control (-)	I	1.02	6.37	5.35	-
	II	1.02	6.48	5.46	-
	III	1.02		5.68	-
	IV	1.02		5.58	-
	V	0.62		3.10	-
100	I	1.02	5.01	3.99	25.42
	II	1.02	3.66	2.64	51.65
	III	1.02		3.06	46.13
	IV	1.02		0.62	88.89
	V	0.62		0.01	99.68
10	I	1.02	5.85	4.83	09.72
	II	1.02	4.29	3.27	40.11
	III	1.02		5.07	10.74
	IV	1.02		3.64	34.77
	V	0.62		1.99	35.81
1	I	1.02	6.59	5.57	00,00
	II	1.02	5.96	4.49	09.52
	III	1.02		5.21	08.27
	IV	1.02		4.54	18.64
	V	0.62		3.15	00.00
0.1	I	1.02	6.82	5.80	00.00
	II	1.02	6.41	5.41	00.92
	III	1.02		5.43	04.40
	IV	1.02		5.95	00.00
	V	0.62		3.22	00.00
0.01	I	1.02	8.12	6.10	00.00
	II	1.02	8.08	6.00	00.00
	III	1.02		6.72	00.00
	IV	1.02		7.16	00.00
	V	0.62		3.52	00.00

Table 5: The IC_{50} value of antiplasmodial activities of *S. arvensis* L. ethyl acetate fractions.

Fractions	IC_{50} (µg/mL)	Activities
I	494.947	Not Active
II	51.323	Not Active
III	153.664	Not Active
IV	12.068	Active
V	13.343	Active

lead to the isolation and identification of active compounds for further pharmaceutical applications.

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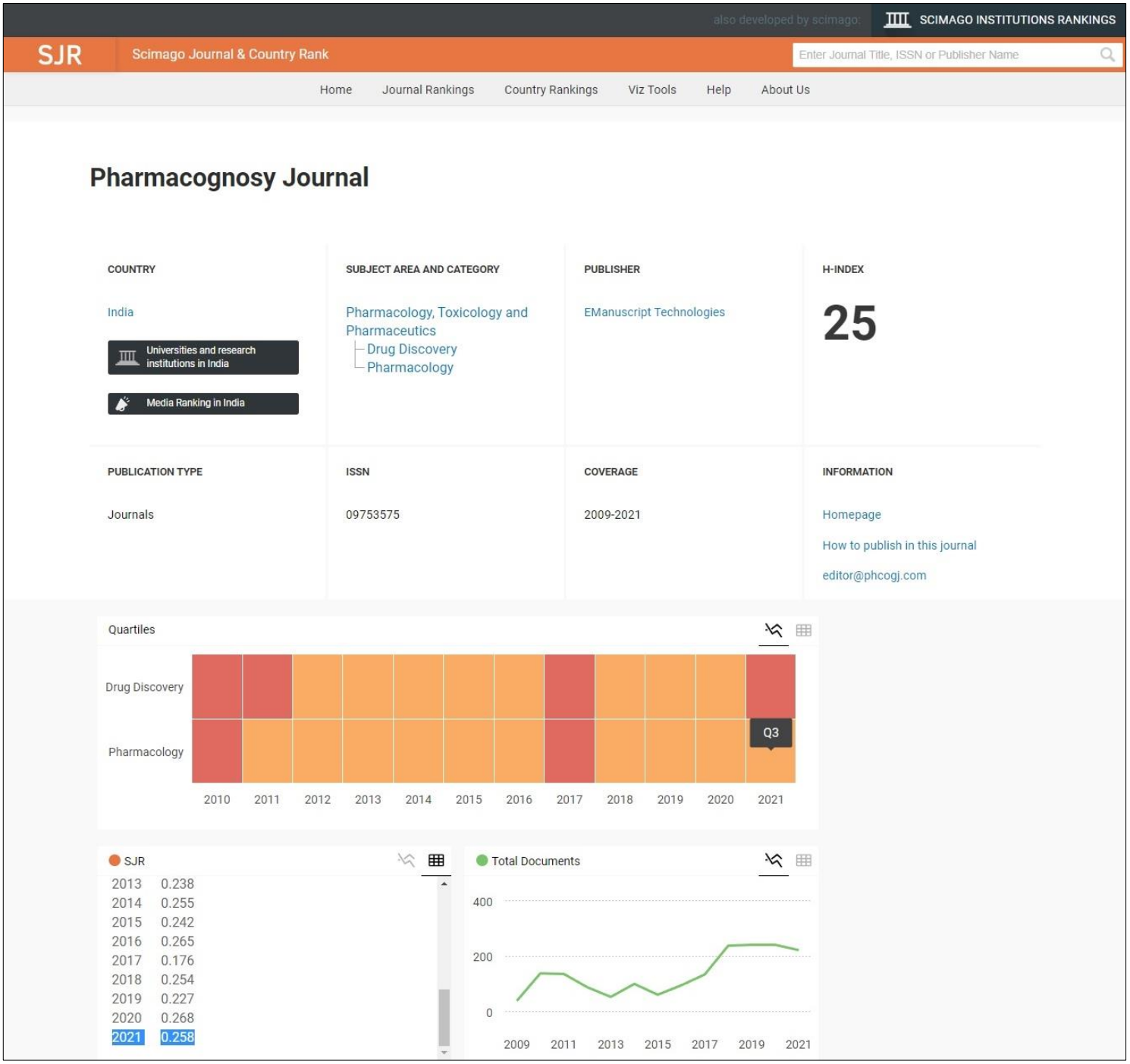

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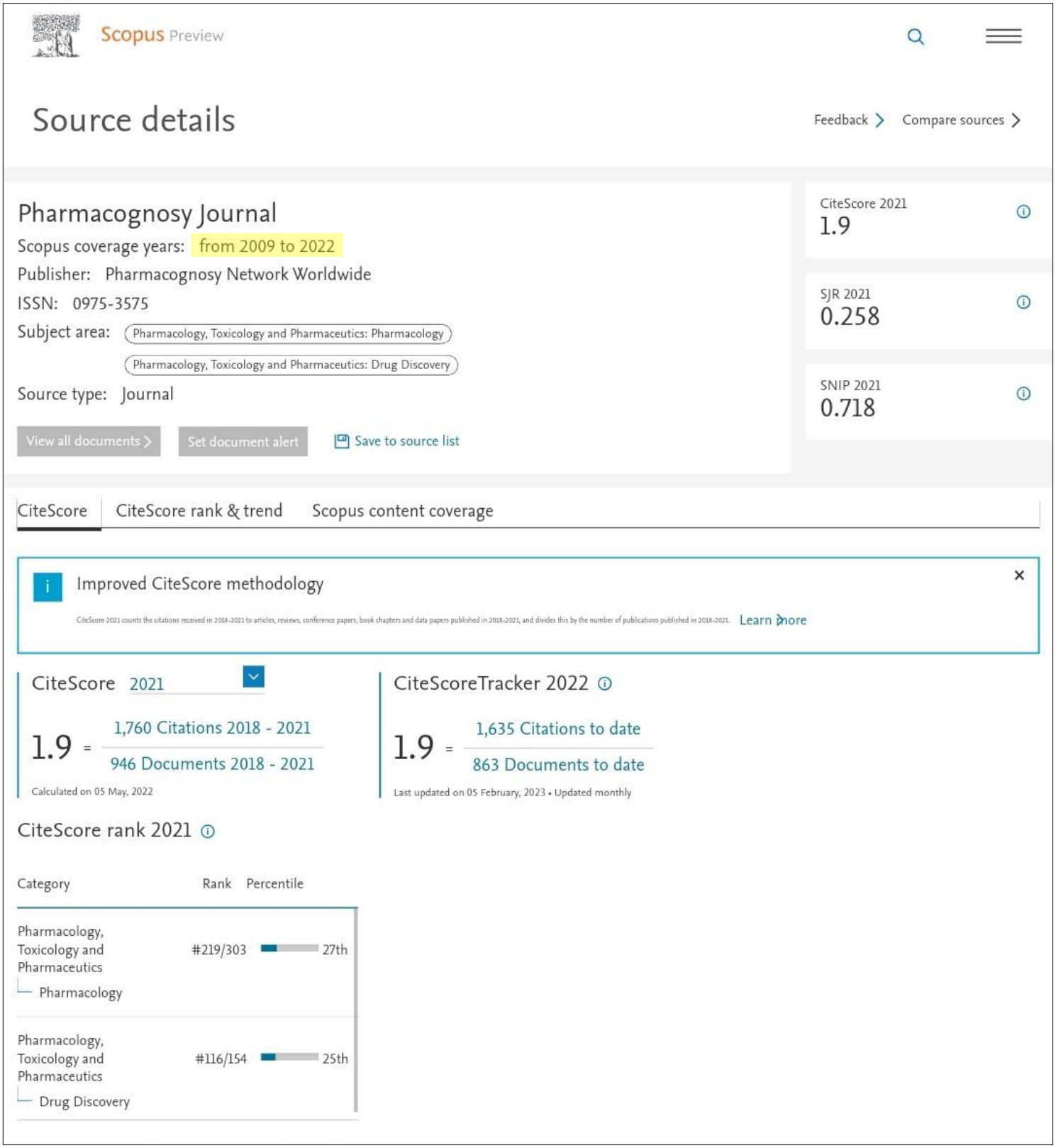

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