

COMPARATIVE STUDY OF *Sida rhombifolia* FROM TWO DIFFERENT LOCATIONS

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ABSTRACT

Sida rhombifolia or sidaguri, was taken from two regions in East Java Province, Indonesia, namely Batu (SB) and Ngawi (SN), was investigated in this study. The two samples were determined the content of secondary metabolites by using LC-MS/MS. They were also evaluated for their phytochemical content, total phenolic content (TPC), and antioxidants activity, using the DPPH and ABTS methods. The highest TPC (126.93 ± 0.63 mg GAE / g) was observed in the ethyl acetate fraction of SN. The antioxidant activity against DPPH and ABTS from crude extracts and fractions showed that SB was more potent than SN, although the values were both less active. A total of 54 secondary metabolites were identified by the LC-MS-MS method from both species, with SB samples producing more secondary metabolites (48 compounds) than SN (37 compounds). These compounds are included in lipids, alkaloids, ester lactones, benzoic acid derivatives, steroids, carboxylic acid derivatives, flavonoids, and phenyl propanoic.

Keywords: *Sida rhombifolia*, Profiling, LC-MS/MS, Antioxidant, Comparative Study, medicinal plant

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INTRODUCTION

Quality consistency, safety, and efficacy of herbal medicines can be achieved if the raw materials and production processes are standardized. Standardization is essential because medicinal plants have a high diversity of metabolites. The composition and concentration of bioactive metabolites of medicinal plants will be influenced by several factors such as the environment in which they are grown, planting and harvesting times, post-harvest processes, varieties, etc.^{1,2}

The genus *Sida* has about 200 species scattered throughout world's tropics and subtropics regions.³ The original *Sida* genus comes from Brazil, and in Brazil this genus has an extensive distribution in the Northeast and South and a lesser extent in the North, Midwest and Southeast.^{4,5} Secondary metabolite compounds that have been isolated from the genus *Sida* show antimicrobial, anti-inflammatory, analgesic, hepatoprotective, antiulcer, cytotoxic, cardioprotective, neuroprotective, antitubercular, antioxidant, nephroprotective, antidiabetic and antiobesity activity, abortifacient, and antipyretics.^{6,7} Some of the other pharmacological activities are antibacterial and wound healing, and anti-arthritis.^{8,9} The chemical constituents of *Sida rhombifolia* L (Sidaguri) include alkaloid, phenolic, coumarin, tannin, saponin,

anthraquinone, terpenoid, cardiac glycoside, steroid, calcium oxalate, phytosteroid, saponin, essential oil, and amino acids.¹⁰⁻¹³ *Sida rhombifolia* has been used to treat stings and bites of scorpions, snakes and wasps (flowers), skin diseases and ulcers (stems), to treat stomach disorders, stomach aches, digestive problems (root), malaria, flatulence, diarrhea (root decoction), dysentery (root), irritable bowel syndrome, gastritis, enteritis, hemorrhoids (roots and leaves), diabetes (leaf), chickenpox, blood cleansing and fatigue, migraine headaches, and headaches (fruits), eye problems, conjunctivitis, toothaches (roots), fever, gum infections, swelling, tonics, wounds (roots and leaves), ophthalmia and swelling (leaves), cuts and wounds (leaves).^{14,15} Sidaguri is also known to inhibit the xanthine oxidase activity, so it is used to reduce uric acid levels.¹⁶ This species also belongs to one of the Indonesian herbal medicine scientific programs from the Indonesian Ministry of Health.

Phytochemical screening of *S. rhombifolia* revealed the presence of saponins, tannins, amino acids, fatty acids, sterol compounds, alkaloids, terpenoids, carbohydrates, lignans, glycosides, phenolics, steroids, and flavonoids.¹⁷⁻²⁰

This study results can later be applied in standardizing sidaguri through its metabolite profile and biological activity based on the origin of the growing location, and the extracting solvent.

EXPERIMENTAL

Sample Collection

Sidaguri samples were collected from two areas on the island of Java, namely Kebun Materia Medika, Batu District, East Java Province (SB), and Umbulrejo village, Jogorogo Sub-District, Ngawi District, East Java Province, Indonesia (SN).

Extraction

Ten grams of samples were extracted three times with 100 mL of methanol. The precipitate and the filtrate are filtered and then concentrated with a rotary vacuum evaporator to obtain methanol extract. The extract was partitioned with n-hexane three times. The two fractions are separated and concentrated. Then, the methanol fraction was partitioned with ethyl acetate with the addition of 10% water. It generated two fractions, namely ethyl acetate fraction and methanol residue. Each fraction was evaporated using a rotary vacuum evaporator.

Sidaguri Chemical Component Profiling by LC-MS / MS

The chemical composition analysis of sidaguri analyzed using LC-MS / MS. Metabolite data evaluation was carried out by processing the initial data using MZmine to identify chemical components.

Determining the Total Phenol Content Test

The total phenolic content of crude extract and fractions was determined by using Folin-Ciocalteu reagent.²¹ Briefly, 25 μ L of standard and samples were made up to each well. Then, 75 μ L of distilled water and 25 μ L of Folin-Ciocalteu were added. The plate was incubated for 6 minutes and 100 μ L of Na_2CO_3 solution was added. The mixture was incubated for 90 minutes. The absorbance of the solution was measured at 765 nm. Gallic acid is used as a standard solution with various concentrations. TPC is expressed in mg / g equivalent to GAE (gallic acid equivalent).^{22,23}

In-vitro Test of Antioxidant Activity

Antioxidant Activity Test using the DPPH Method

DPPH radical scavenging activity was carried out by the procedure performed by Salazar et al., 2011.²⁴ Briefly, 40 μ L of the extracts (50, 25, 12.5, 6.25, 3.125, and 1.56 ppm) were mixed with 125 μ mol / L DPPH solution into the 96 well microplate. The solution was incubated for 30 minutes at room temperature. The absorbance of the solution was measured at a wavelength of 515 nm. Vitamin C, vitamin E, BHT, and gallic acid were used as positive controls.

Antioxidant Activity Test using the ABTS Method

ABTS method was carried out according to the previous method.²⁵ Briefly, 5 mL of ABTS solution (7 mM) were mixed with 88 mL of potassium persulfate (140 mM) and stand for 16 hours at darkroom temperature.

The absorbance value of the working solution was modified between 0.7-0.8 at 734 nm. 100 μ L of sample solutions (1000, 500, 250, 125, and 62.5 ppm) were mixed with 100 μ L of ABTS working solution and the absorbance was measured at 734 nm for 6 minutes. Ascorbic acid, vitamin E, gallic acid (25, 12.5, 6.25, 3.125, 1.562 ppm) were used as a positive control. The measurement was taken in triplicate.

RESULTS AND DISCUSSION

Percentage of Extract and Fraction Yield

The extract yield of SB and SN are shown in Table-1. This table showed that the crude extract, n-hexane, and ethyl acetate fraction of SN has a higher percentage than the SB. But the methanol residue fraction of SN has a lower percentage than SB.

Table-1: Percentage of Crude Extract and a Solvent Fraction of SB and SN

Sample	Crude Extract (MeOH)	n-Hexane Fraction	Ethyl acetate Fraction	Residue methanol Fraction
SB	12.988%	1.688%	2.258%	4.393%
SN	18.063%	3.189%	2.290%	1.967%

Phytochemical Content based on LC-MS

Phytochemical screening results of crude extracts and three fractions of SB and SN are presented in Table 2. The presence of alkaloids, benzoic acid derivatives, and phenyl propanoid was detected in both crude extracts and all fractions. In addition, lipids and ester lactones were detected in both crude extracts, hexane and ethyl acetate fractions, while MeOH residue fractions were not detected. Triterpenes showed positive results only in ethyl acetate fraction from both samples, whereas the other fractions were negative. Steroids showed positive results in all fractions of SB, but SN showed negative results except for ethyl acetate extract. Flavonoids showed positive results for all fractions of SB, but for SN, only positive results for crude extract and ethyl acetate fraction, negative results for hexane extract and MeOH residue. Secondary metabolite contents of SB and SN were analyzed and profiled by using LC-MS / MS. Metabolite data evaluation was carried out by processing the initial data by MZmine. The analysis results showed that SB contained 48 compounds and SN contained 37 compounds. These compounds are lipids, alkaloids, ester lactones, benzoic acid derivatives, steroids, carboxylic acid derivatives, flavonoids, and phenyl propanoid.

Table-2: Phytochemical Analysis of Crude Extracts and Three Fractions from Two Different Regions

Test	Sample sidaguri Batu (SB)				Sample sidaguri Ngawi (SN)			
	Crude extract	n-hexane fraction	Ethyl acetate fraction	Residue methanol fraction	Crude extract	n-hexane fraction	Ethyl acetate fraction	Residue methanol fraction
Lipid	+	+	+	-	+	+	+	-
Alkaloid	+	+	+	+	+	+	+	+
Benzoic acid derivative	+	+	+	+	+	+	+	+
Phenyl propanoid	+	+	+	+	+	+	+	+
Triterpenoids	-	-	+	-	-	-	+	-
Steroid	+	+	+	+	-	+	-	-
Flavonoid	+	+	+	+	+	-	+	-
Ester lactone	+	+	+	-	+	+	+	-

Lipids, alkaloids, benzoic acid derivatives, and steroids are found in n-hexane extract; benzoic acid derivatives and lactone esters are found in n-hexane and ethyl acetate extracts; whereas flavonoids and phenyl propanoid are mostly found in ethyl acetate extract. Secondary metabolite compounds from SB and SN are shown in Fig.-1 to 7. Phytochemical profiling data of SB and SN based on LC-MS is shown in Table-3.

Table-3: Phytochemical Profiling Data of Sidaguri Extracts and Fractions from Two Different Sites based on LC-MS/MS

No	Compound	Group	Molecular Formula	MS and MS/MS	Rt	SB	SN
1	Vomifoliol	Lipid	C ₁₃ H ₂₀ O ₃	225, 207, 189	5.91	√	√
2	Vasicinol	Alkaloid	C ₁₁ H ₁₂ N ₂ O ₂	205, 188, 169	3.84	√	√
3	Vanillic acid	Derivative acid benzoate	C ₈ H ₈ O ₄	167, 152, 123, 108, 96, 83, 81, 63	5.48	√	-
4	Umbelliferone	Phenyl propanoid	C ₉ H ₆ O ₃	163, 135, 107, 79	5.47	-	√
5	Taraxasterone	Triterpenoid	C ₃₀ H ₄₈ O	425, 407, 325, 271, 257, 217, 215, 187, 147, 119, 67	32.51	√	√
6	Syringic acid	Derivative acid benzoate	C ₉ H ₁₀ O ₅	197, 153, 151, 139, 123	5.63	√	-
7	Syringaldehyde	Derivative acid benzoate	C ₉ H ₁₀ O ₄	183, 155, 140, 123, 95	6.34	√	√
8	Stigmasterol	Steroid	C ₂₉ H ₄₈ O	412, 397, 217, 177, 163, 99, 97, 95	30.67	√	√
9	Sterculic acid	Lipid	C ₁₉ H ₃₄ O ₂	295	31.25	√	√
10	Sinapic acid	Phenyl propanoid	C ₁₁ H ₁₂ O ₅	225, 209, 207, 179	4.53	-	√
11	Sinapaldehyde	Phenyl propanoid	C ₁₁ H ₁₂ O ₄	209, 191, 177, 149, 121, 107	6.78	√	√
12	Scopoletin	Phenyl propanoid	C ₁₀ H ₈ O ₄	191, 161, 147	7.23	√	-
13	Salicylic acid	Derivative acid benzoate	C ₇ H ₆ O ₃	139, 121, 113, 111, 109	4.21	√	-
14	Quercetin	Flavonoid	C ₁₅ H ₁₀ O ₇	303, 285, 257, 229, 201, 165, 153, 137, 121	6.38	√	-
15	Polypodine B	Steroid	C ₂₇ H ₄₄ O ₈	497, 443, 425, 99, 81	5.57	√	-
16	<i>p</i> -Coumaric acid	Phenyl propanoid	C ₉ H ₈ O ₃	165, 147, 123, 103, 91	5.67	√	-
17	Palmitoleic acid	Lipid	C ₁₆ H ₃₀ O ₂	255, 109, 81, 69	27.07	√	√
18	Palmitic acid	Lipid	C ₁₆ H ₃₂ O ₂	255	32.14	√	-
19	Oleic acid	Lipid	C ₁₈ H ₃₄ O ₂	283	32.28	√	√
20	N-trans-feruloyltyramine	Alkaloid	C ₁₈ H ₁₉ NO ₄	314, 177	10.28	√	-
21	N-Methyl ephedrine	Alkaloid	C ₁₁ H ₁₇ NO	180, 165, 135, 107	20.41	√	√
22	Naringenin	Flavonoid	C ₁₅ H ₁₂ O ₅	273, 153, 147, 123, 119, 91	13.98	√	√
23	Methoxyphenylacetic acid	Derivative acid benzoate	C ₉ H ₁₀ O ₃	165, 147, 121, 119	6.29	√	√
24	Loliolide	Ester lactone	C ₁₁ H ₁₆ O ₃	197, 179	7.23	√	√
25	Linoleic acid	Lipid	C ₁₈ H ₃₂ O ₂	281	28.19	√	-
26	Kaempferol-3-O-D-glucose-6'''-D-rhamnose	Flavonoid	C ₂₇ H ₃₀ O ₁₅	595, 287	7.18	-	√
27	Kaempferol	Flavonoid	C ₁₅ H ₁₀ O ₆	287	11.55	√	√
28	Isoquercetin	Flavonoid	C ₂₁ H ₂₀ O ₁₂	465, 303, 153	6.74	√	√
29	Isoorientin	Flavonoid	C ₂₁ H ₂₀ O ₁₁	449	5.96	-	√
30	Hypaphorine	Alkaloid	C ₁₄ H ₁₈ N ₂ O ₂	247, 188	4.82	-	√
31	Hispidulin	Flavonoid	C ₁₆ H ₁₂ O ₆	301	15.03	√	√
32	Gallic acid	Derivative acid benzoate	C ₇ H ₆ O ₅	169, 125, 107, 97, 79, 69	1.76	√	√
33	Ferulic acid	Phenyl propanoid	C ₁₀ H ₁₀ O ₄	193, 149, 134	8.80	√	√
34	Evofolin-B	Flavonoid	C ₁₇ H ₁₈ O ₆	319	6.80	√	√
35	Evofolin-A	Flavonoid	C ₁₀ H ₁₂ O ₄	197	5.03	√	√
36	Ecdysone	Steroid	C ₂₇ H ₄₄ O ₆	465, 429, 301	8.92	√	-

37	Coniferaldehyde	Phenyl propanoid	C ₁₀ H ₁₀ O ₃	177, 162, 145, 117	11.89	√	√
38	Chlorogenic acid	Phenyl propanoid	C ₁₆ H ₁₈ O ₉	353, 191, 179	-	√	-
39	Caffeic acid	Phenyl propanoid	C ₉ H ₈ O ₄	179, 135	5.06	√	√
40	Betaine	Alkaloid	C ₅ H ₁₁ NO ₂	118	1.07	√	√
41	Acacetin	Flavonoid	C ₁₆ H ₁₂ O ₅	285, 270	8.77		√
42	9-hydroxy-cis-11-octadecenoic acid	Lipid	C ₁₈ H ₃₄ O ₃	297, 279, 183	28.20	√	√
43	4-ketopinoresinol	Phenyl propanoid	C ₂₀ H ₂₀ O ₇	373	5.66	-	√
44	Vanillin	Derivative acid benzoate	C ₈ H ₈ O ₃	153, 125, 93, 65	6.09	√	√
45	4-hydroxy-methylbenzoic acid	Derivative acid benzoate	C ₈ H ₈ O ₃	153	5.62	√	√
46	4-aminobenzoic acid	Derivative acid benzoate	C ₇ H ₇ NO ₂	138, 94	1.05	√	√
47	2-Deoxy-20-hydroxyecdysone-3-O-D-Glucopyranoside	Steroid	C ₃₃ H ₅₄ O ₁₁	627, 447, 429	6.92	√	-
48	25-Acetoxy-20-hydroxyecdysone-3-O-D-glucopyranoside	Steroid	C ₃₅ H ₅₆ O ₁₃	463, 445, 685	8.92	√	-
49	24(28)-Dehydromakisterone A	Steroid	C ₂₈ H ₄₄ O ₇	491	19.22	√	-
50	20-Hydroxyecdysone-3-O-D-Glucopyranoside	Steroid	C ₃₃ H ₅₄ O ₁₂	643, 445	7.47	√	-
51	20-Hydroxyecdysone	Steroid	C ₂₇ H ₄₄ O ₇	481, 445, 427	6.56	√	-
52	2-(3H-Indol-3-yl-methyl)butan-1-ol	Alkaloid	C ₁₃ H ₁₇ NO	204, 189	5.47	√	√
53	α-tocospiro	Lipid	C ₂₉ H ₅₀ O ₄	463	32.55	√	√
54	Phenethylamine	Alkaloid	C ₈ H ₁₁ N	122, 105, 95, 79	3.33	√	√
Total Compound						48	37

Lipids

Eight lipids (1, 9, 17-19, 25, 42 and 53) were identified by LC-MS (Fig.-1)

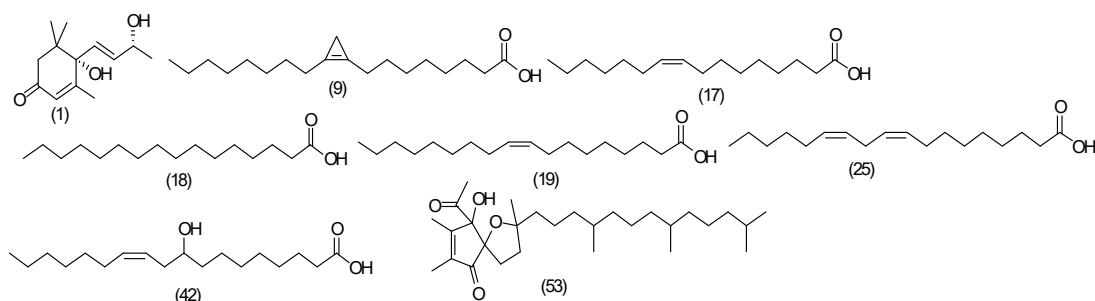


Fig.-1: Chemical Structures of Lipid Compounds

Triterpenes and Steroids

Nine compounds belonging to the triterpenes and steroids groups (5, 8, 15, 36, and 47-51) were identified by LC-MS (Fig.-2).

Alkaloids

Seven alkaloids (2, 20-21, 30, 40, 52, and 54) were identified by LC-MS (Fig.-3).

Carboxylic Acid Derivatives

In the sidaguri sample, 8 carboxylic acid derivatives were identified by LC-MS (Fig.-4).

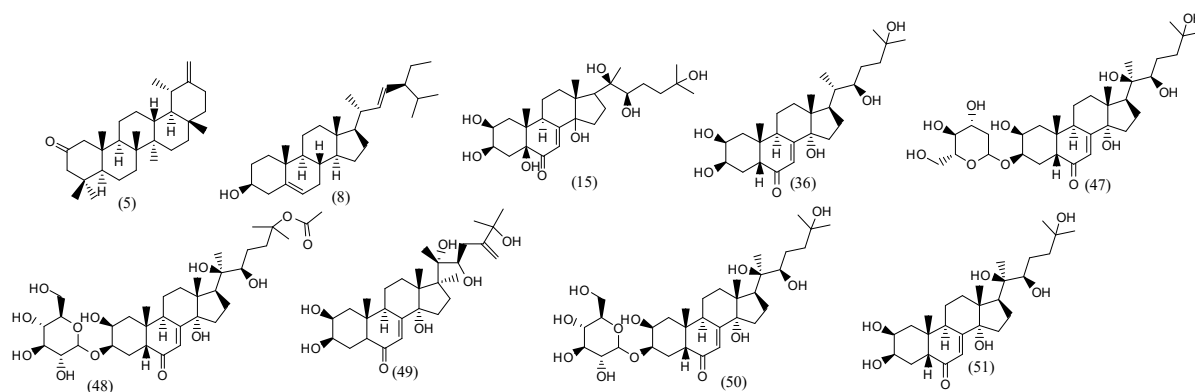


Fig.-2: Chemical Structures of Triterpene and Steroid Compounds

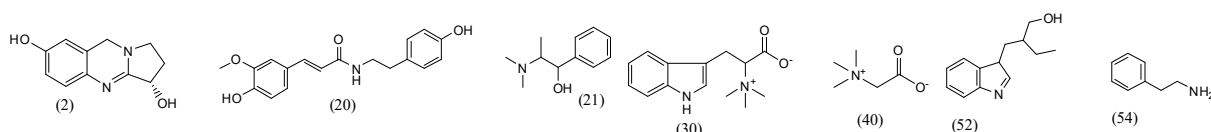


Fig.-3: Chemical Structures of Alkaloid Compounds

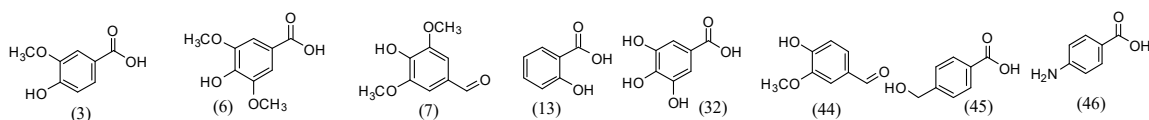


Fig.-4: Chemical Structures of Carboxylic Acid Derivatives

Other Compounds

Two other compounds (23 and 34) were identified by LC-MS (Fig.-5).

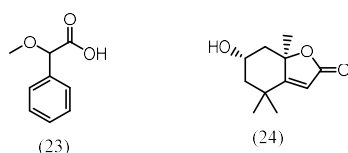


Fig.-5: Chemical Structures of Other Compounds

Phenyl Propanoid

Ten phenyl propanoid compounds (4, 10-12, 16, 33, 37-39, and 43) were identified in the sidaguri sample (Fig.-6).

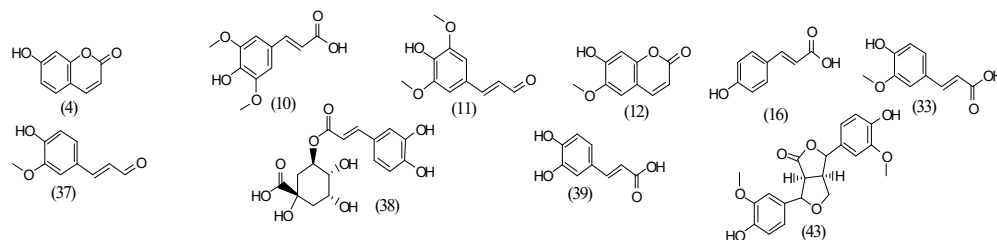


Fig.-6: Chemical Structures of Phenyl propanoid Compounds

Flavonoids

Ten flavonoids compounds (14, 22, 26-29, 31, 34-35, and 41) were obtained in the sidaguri sample (Fig.-7).

Total Phenolic Content (TPC)

The TPC contents of crude extract and three fractions of SB and SN are presented in Table-4. This table showed that EA fraction of SB obtained the highest yield for polyphenol levels (126.93).

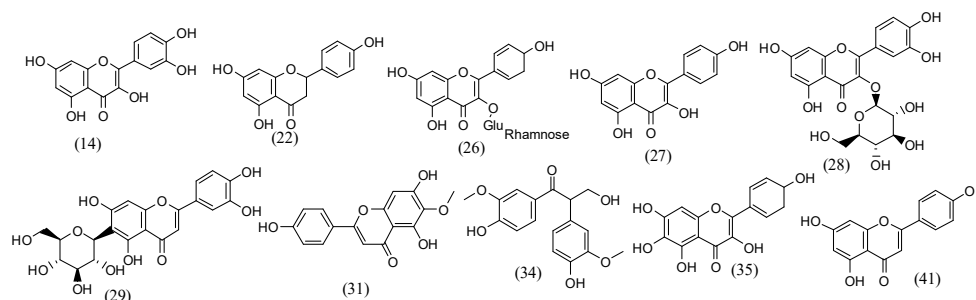


Fig.-7: Chemical Structures of Flavonoid Compound

Table-4: Total Phenolic Data of Sidaguri Extract Samples

No.	Extract Name	Polyphenol Levels	TPC Calculation	mg / g of Sample
1	MeOH Extract of SB	108.2	0.5409	108.19
2	MeOH extract of SN	88.8	0.4442	88.84
3	n-Hexane Extract of SB	38.9	0.1945	38.89
4	n-hexane extract of SN	55.9	0.2799	55.98
5	EA extract of SB	126.9	0.6346	126.93
6	EA extract of SN	108.1	0.5406	108.12

Antioxidant

Test with DPPH Method

DPPH inhibition activities of crude extract and three fractions from both samples are shown in Figure 8. The IC_{50} values of SN crude extract, hexane, ethyl acetate, and the MeOH residue fractions were found 798.22, 2329.33, 1053.47, and 1395.26, respectively. Meanwhile, The IC_{50} values of SB crude extract, hexane, ethyl acetate, and the MeOH residue fractions were found 547.44, 1650.71, 820.33, and 1052.92. The graph for comparison between extracts and fractions is shown in Figure 8. Molyneux (2004) states that an extract has antioxidant properties when the IC_{50} value is less than 200 ppm. If the IC_{50} value obtained is between 200-1000 ppm, then the substance is less active but still has the potential as an antioxidant.²⁶⁻²⁷

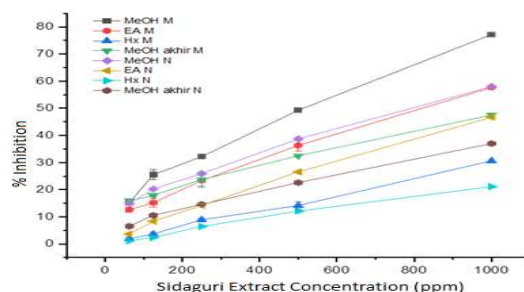


Fig.-8: Graph of Antioxidant Test Results of DPPH Method

Test with ABTS Method

Figure 9 provides the results of ABTS radical scavenging activity of crude extract and three fractions of both samples. Accordingly, this study proves that the ABTS assay IC_{50} values of crude extracts, hexane fractions, ethyl acetate fractions, and MeOH residue fractions of both SB and SN were 12.13, 37.66, 256.35, 193.97, 13.54, 28.49, 28.49, and 43.03, respectively.

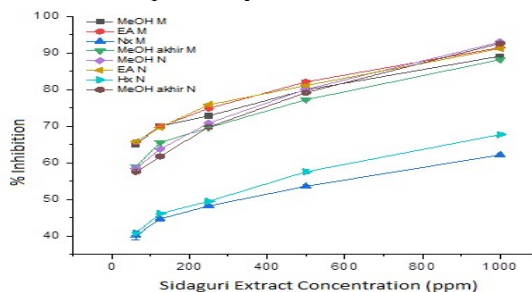


Fig.-9: Graph of the Antioxidant Test Results of the ABTS Method

CONCLUSION

Sida rhombifolia in the LC-MS/MS results obtained identification of 54 compounds, namely sidaguri from Batu (SB) containing 48 compounds and Ngawi (SN) 37 compounds. These compounds are included lipids, alkaloids, lactone esters, benzoic acid derivatives, steroids, carboxylic acid derivatives, lactone esters, flavonoids, and phenyl propanoid. The highest TPC results (126.93 ± 0.63 mg GAE / g) were in the ethyl acetate fraction taken from the Ngawi area. The antioxidant activity results with the DPPH method and ABTS with the highest IC₅₀ results obtained from Batu crude extract, which was 547.44 for DPPH method, and 12.13 for ABTS method.

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