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Inventory, Morphological and Antioxidant Profile of the Sumatera Sidaguri (*Sida* Spp.) Plants

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ABSTRACT

Sidaguri plant is a wild and potential plant that is promoted as a good challenging medicinal plant in the future. However, information related to the diversity of these plants in Indonesia has not been widely exposed, and there are several species of this sidaguri found in Indonesia that are still unknown to the public. In this paper, five species of sidaguri which are quite widely grown and used for various traditional medicines in Indonesia, especially in West Sumatra and South Sumatra, will be explored. They were : *Sida acuta, Sida rhombifolia, Sida cordifolia*, *Sida retusa*, and *Sida scabrida*. This study has successfully indicated each species' different morphology and demonstrated good antioxidant activity against free radicals. The antioxidant activity test results using the TLC Bioautography method show six active spots from each organ. The sample that produces yellowish spots on a purple background on the silica gel plate is an antioxidant compound that is visualized after being sprayed with a DPPH reagent. The root organ showed the most intense antioxidant active spot, which was thought to offer the best activity. The results showed that the ethanolic extract of root organ sidaguri possessed the highest antioxidant activity among the selected organ sidaguri species.

Keywords: *Sida* spp, *inventory, morphological, antioxidant, TLC Bioautography*

1. INTRODUCTION

The genus Sida L. is one of the most diverse plants in the Malvaceae family. About 200 species of this herbaceous plant are distributed worldwide, 189 of which are found in the Americas and 112 in Brazil [1]. Recently, Yoshikawa et al. (2019) found a new species from Brazil, *S. unaristata* Gonçalez & Yoshikawa, increasing the number to 113 species [2]. In Indonesia, especially on the island of Sumatra, several species of the genus Sida are found, such as *S. acuta, S. rhombifolia, S. retusa, S. subcordata, S. scabrida,* and *S. cordifolia*. Traditionally, local people use various organs of this plant such as gout, rheumatism, anti-inflammatory, swelling, ulcers, scabies, eczema, ringworm, itching, diarrhea, dysentery, jaundice, toothache, and facilitate childbirth [3,4 ,5]. other than that, this sidaguri plant is also used to treat influenza, fever, diphtheria, malaria, urinary stones, stomach pain, bleeding hemorrhoids, vomiting blood, intestinal worms, asthma, pain relievers, urinary laxative, menstrual laxative, skin softener, and abortivum [6,7 ,8].

The chemical content of several sidaguri species has been widely studied, including phenolic compounds, flavonoids, alkaloids, ecdysteroids, terpenoids, tocopherols, lignans, coumarins, steroids, aliphatic and amino acids [9]. These compounds are found in leaves, stems, fruits, flowers, and roots.

From previous research, sidaguri is also a potential source of natural antioxidants. Two phenolic compounds, flavonoids glutinoside and chrysin isolated from *S. glutinosa* showed significant antioxidant activity by DPPH assay [10]. The methanol extract of the roots of 8 sidaguri plants in India (*S. acuta, S. cordata, S. cordifolia, S. indica, S. mysorensis, S. retusa, S. rhombifolia, S. spinosa*), was tested for antioxidant activity in vitro with DPPH, FRAP and ABTS methods. As a result, the roots of *S. cordifolia* had the highest antioxidant activity [11].

Determination of the antioxidant activity of *S. rhombifolia* leaf extract in Brazil, using the DPPH & ABTS method, has been shown to have potential as an antioxidant [12]. From this study, no one has compared the antioxidant activity of each organ of various species, including leaf, stem, and root organs.

Given the many species and potential of sidaguri plants in Indonesia, this study will discuss the morphological characteristics of five sidaguri species that were successfully explored and inventoried in West Sumatra and South Sumatra, *S. acuta, S. rhombifolia, S. cordifolia, S. retusa.* and *S. scabrida.* To obtain new sources of antioxidants and information about the potential antioxidant activity of each organ of this plant, this study also compared the antioxidant activity of each organ of the leaves, stems, and roots of this sidaguri plant.

2. METHODOLOGY

2.1. Inventory, Morphological and Characteristics of Sidaguri Plants in West Sumatra and South Sumatra.

The research method used is exploration, sampling by Purposive Sampling. Morphological observations included plant shape and height characteristics, stem, leave, flower, and fruit morphology organs.

2.2. Material Plants, Chemical and Reagent

The five sidaguri species studied included: four West Sumatran Sidaguri plant species (*S. acuta, S. rhombifolia, S. cordifolia,* and *S. retusa*); and four species of South Sumatran Sidaguri plants (*S. acuta, S. rhombifolia, S. cordifolia,* and *S. scabrida*). The eight Sida species originating from 2 different places were identified at the Andalas University Herbarium (ANDA) Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University, Limau Manis, Padang, Indonesia. Methanol, ethanol, ethyl acetate, chloroform (Merck®), aquabidest (Ikapharmindo), DPPH (2,2-diphenyl-1-picrylhydrazil) (Sigma-Aldrich®), Silica gel 60 (Merck®), Layer Chromatography plate Thin (TLC) precoated silica gel GF254 (Merck®).

2.3. Sample Preparation

Each sample of 8 sidaguri plants was sorted, cleaned, and separated between leaves, stems, and roots. It was then air-dried for two weeks. Then each sample (24 samples) that had been dried was sorted and then mashed using a grinder.

2.4. Extraction of Sidaguri Plant Organs

Extraction was carried out on 24 samples of sidaguri plant organs from West Sumatra and South Sumatra using the maceration method according to the Indonesian Herbal Pharmacopoeia (2017) [13]. Fifty grams of each sample has macerated with 70% methanol as much as 50 ml. Soak for the first 6 hours, stirring occasionally, then let stand for 18 hours. First, separate the macerate by filtering, then the pulp is macerated again with 25 ml of 70% methanol. The obtained macerate was combined and then concentrated with a rotary evaporator until a thick extract was formed. Then tested the antioxidant activity of each sample extract using the TLC-Bioautography method.

2.5. Separation of Compounds by Thin Layer Chromatography (TLC)

Separation of the compounds of each extract by TLC method. The mobile phase used was chloroform-ethyl acetate-methanol in a ratio (65:20:15). The test extract solution (10 mg/ml) was added as much as 5µL on the plate using a capillary tube.

2.6. Antioxidant Activity Test with TLC-Bioautography

The antioxidant activity test for this screening used the TLC-Bioautography method with DPPH reagent based on Wang et al., 2012. First, each extract was separated by TLC as in method 2.4. Then the plate was sprayed with 0.05% (w/v) DPPH reagent. The spot color changes were observed for about 10 minutes. A positive result of DPPH radical capture by antioxidant compounds was indicated by a change in the spot area from purple (after spraying with DPPH) to yellow. Spots that change color are recorded for their Rf values[14].

3. RESULTS AND DISCUSSION

Researchers managed to collect and inventory eight species of sidaguri plants from West Sumatra and South Sumatra. The eight species of Sida, originating from two different places, were identified at the Andalas University Herbarium (ANDA) in the Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University, Limau Manis, Padang. According to the sampling location and habitat, this sidaguri plant was verified to be five different species. Four West Sumatran Sidaguri species were verified as *S. acuta* Burm. f; *S. rhombifolia*. L; *S. cordifolia.* L and *S. retusa.* L. Four verified South Sumatran Sidaguri species as *S. acuta* Burm. f; *S. rhombifolia*. L; *S. cordifolia.* L and *S. scabrida* Wight & Arn. This collection adds data on several sidaguri plant species found in West Sumatra and South Sumatra (Table 1).

3.1 Morphology

The morphological characteristics obtained during the study showed that the genus *Sida* spp. as a whole generally grows wild on roadsides, lawns, forests, fields and places with bright sun or little shade. This plant is spread in the tropics from the lowlands to 1,500 m below sea level. Perennial herbs or subshrubs, erect or prostrate, glabrous or pubescent, sometimes viscid. Leaves simple, rarely divided, subsessile to petiolate, blade ovate, elliptic, rhomboid or linear, usually serrate or dentate, without abaxial nectarines. Flowers usually small, axillary, solitary or clustered or in dense or open terminal racemes or panicles. Pedicels slender, articulated. Involucral absent. Calyx 5-lobed, widely campanulate, often 10-ribbed at the base and plicate in bud. Corolla orange-yellow or white often with a dark center. Staminal column included, antheriferous at apex. Styles 5–12; stigmas capitate. Fruits schizocarpic, glabrous or pubescent; Pepperrps differentiated in to a lower, one seeded, indehiscent cell ant an upper empty, dehiscent portion that is often ornamented with a pair of spines. Seeds solitary, glabrous or pubescent [15,16].

3.2 Extract

According to the Indonesian Herbal Pharmacopoeia (2017), the extraction process using the maceration method obtained the yield of each plant organ, namely the leaves, stems, and roots of each species. From the percent yield data obtained, there is a percent yield that is not too different in the same species, but there is also a difference in the same species, which is 1 to 3 percent. This shows that the greater the yield produced, the more efficient the treatment applied without overriding other properties. Based on the yield results, it can be assumed that the bioactive components contained in the species with high yield percentages are higher than other species. Therefore, the high yield percentage value indicates the large number of bioactive components contained in it.

Table 1. Verification of Sidaguri Plants in West Sumatra and South Sumatra

NO	Species	Sample Code
1	*Sida acuta* Burm.f West Sumatra (Sa.WS)	ANDA 00038430
2	*Sida acuta* Burm.f South Sumatra (Sa. SS)	ANDA 00038438
3	*Sida rhombifolia* L. West Sumatra (S.Rh. WS)	ANDA 00038436
4	*Sida rhombifolia* L. South Sumatra (S.Rh. SS)	ANDA 00038440
5	*Sida cordifolia* L. West Sumatra (Sc. WS)	ANDA 00038432
6	*Sida cordifolia* L. South Sumatra (Sc. SS)	ANDA 00038439
7	*Sida retusa* L. West Sumatra (S.Re. WS)	ANDA 00038434
8	*Sida scabrida* Wight & Arn South Sumatra (Ss. SS)	ANDA 00038441

Table 2. Percentage of Sidaguri Plant Organ Yield in West Sumatra and South Sumatra.

No	Sidaguri Species	% Yield of Plant Parts (% w/w)		
		Leaves	Stems	Roots
1	*Sida acuta* West Sumatra	13, 41	6, 36	1, 55
2	*Sida acuta* South Sumatra	11, 64	4, 87	2, 71
3	*Sida rhombifolia* West Sumatra	8, 26	3, 95	1, 75
4	*Sida rhombifolia* South Sumatra	11, 93	3, 15	1, 73
5	*Sida cordifolia* West Sumatra	8, 97	1, 86	1, 69
6	*Sida cordifolia* South Sumatra	5, 34	4, 17	1, 84
7	*Sida retusa* West Sumatra	7, 41	3, 93	1, 13
8	*Sida scabrida* South Sumatra	7, 94	8, 97	0.36

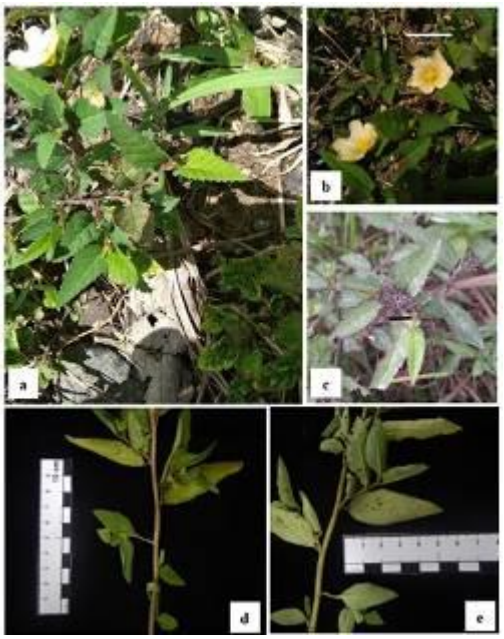

Figure 1. a. Plant habitat; b. Flower; c. Fruit; d. Stem; e. Leave of *Sida acuta* in West Sumatra Plant.

Figure 2. a. Plant habitat; b. Stem; c. Fruit; d. Leave; e. Flower of *Sida acuta* in South Sumatra Plant.

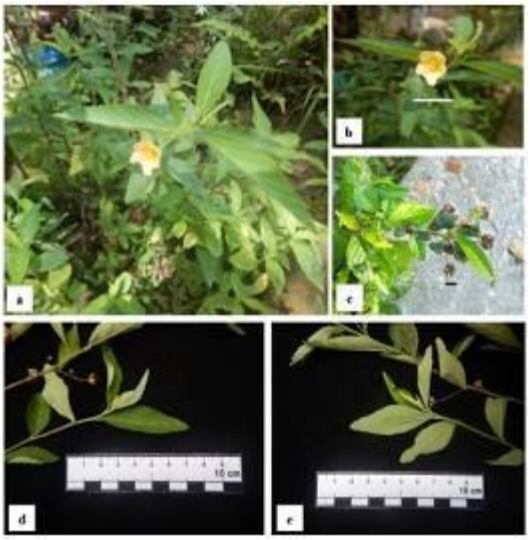

Figure 3. a. Plant habitat; b. Flower; c. Fruit; d. Leave; e. Stem of *Sida rhombifolia* in West Sumatra Plant.

Figure 4. a. Plant habitat; b. Leave c. Flower; d. Fruit of *Sida rhombifolia* in South Sumatera Plant.

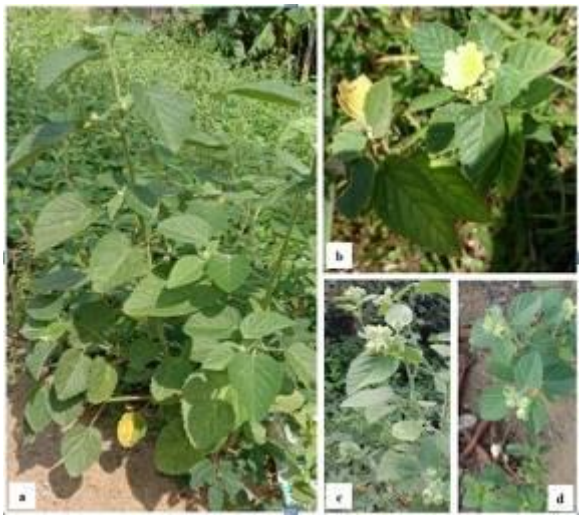

Figure 5. a. Plant habitat; b. Flowers c. Fruit; d. Stem e. Leaves of *Sida cordifolia* in West Sumatra Plant.

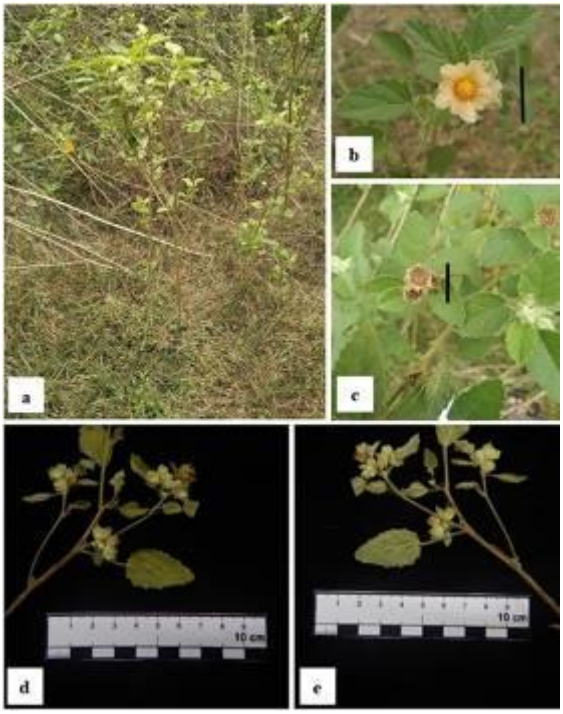

Figure 6. a. Plant habitat; b. Flowers and Leaves c. Stem; d. Fruit of *Sida cordifolia* in South Sumatra Plant.

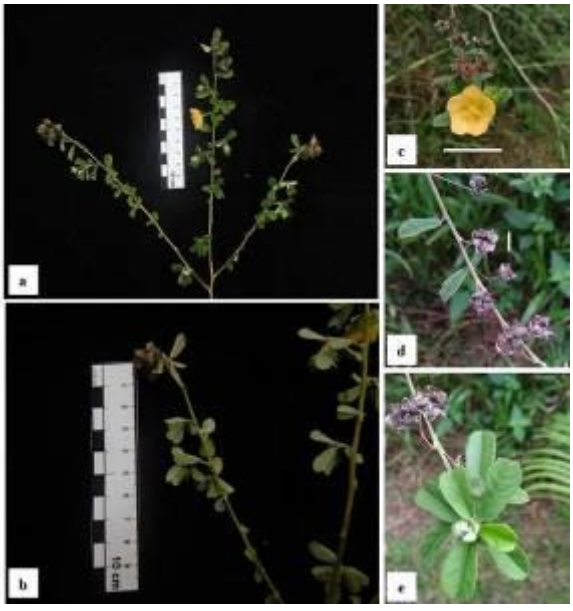

Figure 7. a. Plant habitat; b. Stem c. Flower; d. Fruit e. Leave of *Sida retusa* in West Sumatra Plant.

Figure 8. a. Plant habitat; b. Flower and leave; c. Fruit of *Sida scabrida* in South Sumatra Plant.

Table 3. Morphological Characteristics of Different Parts of *Sida* spp. West Sumatra & South Sumatra Plants

No	Sida Species	Morphological characteristics				
		Plant Shape & Height	**Stems**	**Leaves**	**Flowers**	**Fruits**
1	*Sida acuta* Burm. f West Sumatra	Erect herbs or undersrubs, up to 20 cm high	Stems pubescent with simple and minute stellate hairs	alternatus leaves, Leaves ca 6 × 2.5 cm, lanceolate to linear, elliptic-lanceolate, serrate	Short flower stalks, diameter of open flowers 12.0-15.0, petals yellow	trigonous, Glabrous, short seed stalk, fruit diameter 4.0-5.7 mm
2	*Sida acuta* Burm. f South Sumatra	Erect herbs or undersrubs, up to 80 cm high	Stems pubescent with simple and minute stellate hairs	Alternatus leaves, oval leave shape, uneven leave edge, pointed leave tip (acutus), leave length 5-7 cm, leave width 4 cm	Short flower stalks, diameter of open flowers 12.0-15.0, petals yellow	trigonous, Glabrous, short seed stalk, fruit diameter 4.0-5.7 mm
3	*Sida rhombifolia.* L West Sumatra	Erect branched herbs or undershrubs up to 60 cm high	Stem cinereous with stellate hairs	Leaves blades ca 6 × 3 cm, elliptic to rhomboid, serrate-crenate	Long flower stalk, diamater of open flower 16.0-22.0 mm, petals pale yellow	Long fruit stalk, reniform, blackish, fruit diameter 5.2-6.2 mm
4	*Sida rhombifolia.* L South Sumatra	Erect branched herbs or undershrubs up to 70 cm high	Stem cinereous with stellate hairs	Alternatus leaves, oval leave shape, uneven leave edge, pointed leave tip, leave length 2-4 cm, leave width 2 cm	Long flower stalk, diamater of open flower 16.0-22.0 mm, petals pale yellow	Long fruit stalk, reniform, blackish, fruit diameter 5.2-6.2 mm
5	*Sida cordifolia.* L West Sumatra	Erect, shrubs or subshrubs up to 1 m high	Stem branched with stellate and simple hairs	oval-ellipticus leaves, leaves ca 6 × 5 cm, ovate to suborbicular, upper and lower leave surfaces hairy	Diameter of open flower 15.0-24.0 mm, petals white to purplish-yellow	Long ovoid, apex hairy, fruit diameter 5.8-6.9
6	*Sida cordifolia.* L South Sumatra	Erect, shrubs or subshrubs up to 60 cm high	Stem branched with stellate and simple hairs	oval-ellipticus leave	Diameter of open flower 15.0-24.0 mm, petals white to purplish-yellow	Long ovoid, apex hairy, fruit diameter 5.8-6.9
7	*Sida retusa.* L West Sumatra	Erect subshrubs up to 80 cm high	Stem branched, purplish, stellate hairy	Obovate leaves, apex retusus, leaves ca 4 × 6 cm, rhomboid to lanceolate, obovate or suborbicular, stellate-tomentose beneath	Diameter of open flowers 12.0-14.0 mm, petals deep yellow	Fruit diameter 4.2-5.0 mm
8	*Sida scabrida* Wight & Arn South Sumatra	Erect subshrubs up to 15 cm high	Stem branched, pubescent with stellate and simple hairy	Ovate ellipticus leaves, crenate to serrate, acute	Diameter of open flowers 22-25 mm, petals white-yellow, anthera yellow	Fruit diameter 4.8-5.7 mm

3.3. Antioxidant Activity with TLC-Bioautography Method

A large number of TLC techniques have been developed and successfully applied for qualitative and quantitative analysis of antioxidant activity [17,18], and the free radical stabilizer 2,2-diphenyl-1-picrylhydrazyl (DPPH) is often used as a derivatization reagent for this purpose [19]. The bioautographic TLC test is the method of choice in the antioxidant activity screening method because of several advantages, such as flexibility, simplicity, and good separation results. [20,21,22]. To screen for the antioxidant activity of each organ of this sidaguri plant, it was tested using the bioautography TLC method. After the compounds were separated on the TLC plate, the compounds having free radical scavenging activity were determined with DPPH reagent, then the plates were observed. The following is a TLC profile of the leaves, stems, and roots of several species of sidaguri plants, which were tested for antioxidant activity using DPPH reagent (Figure 9).

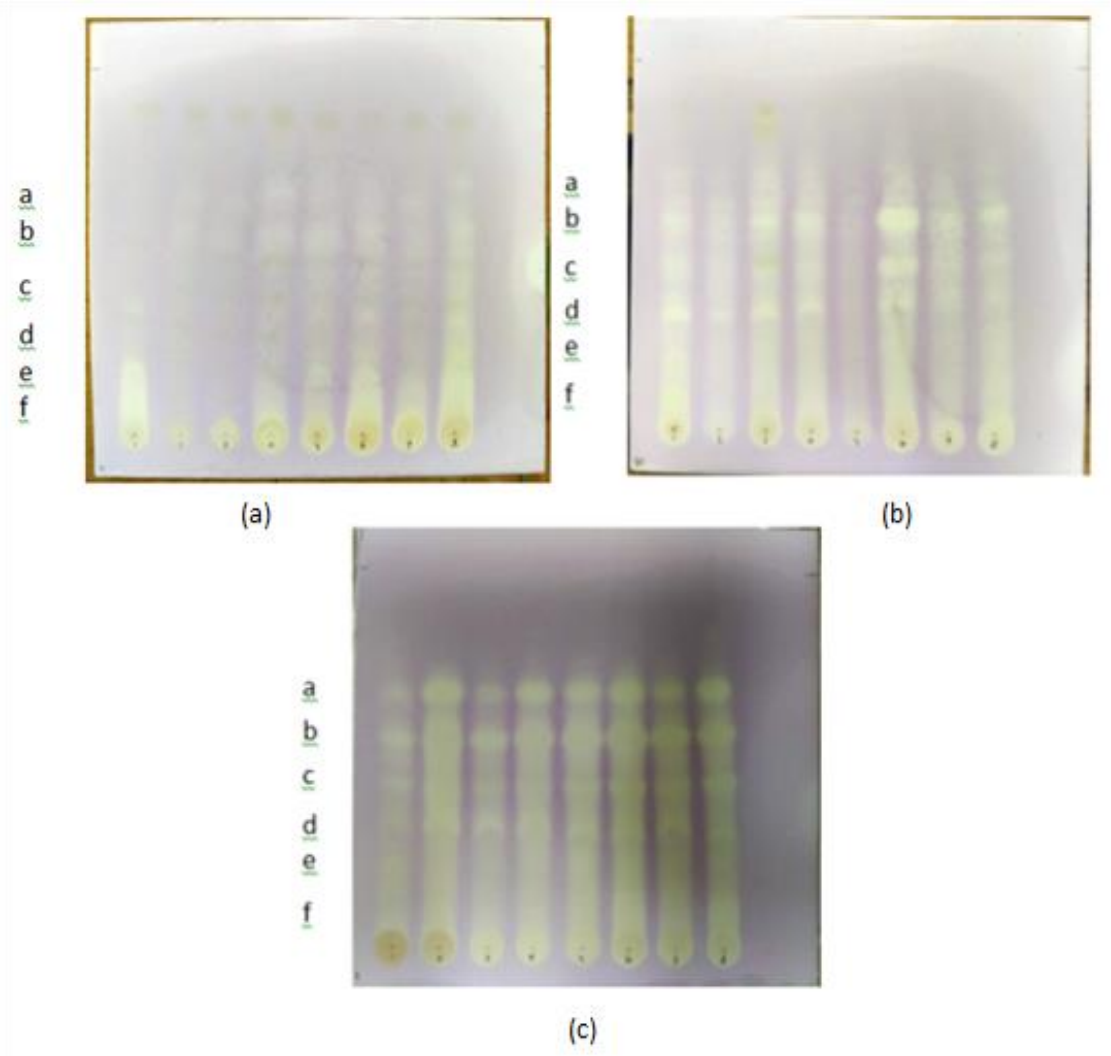

Figure 9. TLC Chromatogram-Bioautography Antioxidant Activity of Leave (a), Stem (b) and Root (c) Organs of *S. scabrida* SS (1), *S. cordifolia* WS (2), *S. acuta* WS (3), *S. acuta* SS (4), *S. rhombifolia* SS (5), *S. rhombifolia* WS (6), *S. cordifolia* SS (7) and *S. retusa* WS (8) with DPPH Reagent.

Table 4. Rf Value of Spots with Antioxidant Activity of Leave, Stem and Root Organs of Various Species of Sidaguri Plants (Sida spp) Using DPPH Reagent.

Sidaguri species	Code	Rf value of spots on leaf organs (a)						Rf value of spots on stem organs (b)						Rf value of spot on root organ (c)					
		a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
Ss. SS	1	-	-	-	-	0.17	0.08	0.68	0.56	0.44	0.44	0.23	0.10	0.66	0.55	0.45	0.32	0.23	0.09
Sc. WS	2	0.80	0.67	-	-	-	-	-	0.56	0.44	0.44	-	-	0.66	0.55	0.45	0.32	0.23	0.09
Sa. WS	3	-	-	-	-	-	-	0.68	0.56	0.44	0.44	0.23	0.10	0.66	0.55	0.45	0.32	0.23	0.09
Sa. SS	4	0.80	0.67	-	-	-	0.08	0.68	0.56	0.44	0.44	0.23	0.10	0.66	0.55	0.45	0.32	0.23	0.09
S.Rh. SS	5	-	0.67	0.42	-	-	0.08	0.68	0.56	-	-	-	-	0.66	0.55	0.45	0.32	0.23	0.09
S.Rh. WS	6	-	0.67	0.42	-	0.17	0.08	0.68	0.56	0.44	0.44	0.23	0.10	0.66	0.55	0.45	0.32	0.23	0.09
Sc. SS	7	0.80	0.67	-	-	0.17	0.08	-	0.56	0.44	0.44	-	-	0.66	0.55	0.45	0.32	0.23	0.09
S.Re. WS	8	-	0.67	0.42	0.29	0.17	0.08	-	0.56	0.44	0.44	0.23	0.10	0.66	0.55	0.45	0.32	0.23	0.09

Figure 9. and Table 4. show the profile and Rf of compounds that have antioxidant activity, samples that produce yellowish spots on a purple background of silica gel plates show antioxidant compounds which are visualized after being sprayed with DPPH reagent [14]. From the data obtained, each organ of each species has antioxidant activity, with the formation of 6 active spots from each organ. Root organs show the most intense spotting, characterized by more pronounced yellowish patches. It is suspected that the root organ of the sidaguri plant has more significant antioxidant activity than other organs. Antioxidant screening by bioautography method is a rapid method on various plant extracts. DPPH in methanol solvent will produce a purple color and is reduced to yellow diphenylpicryl hydrazine. Antioxidant compounds will react with DPPH, a stable nitrogen-centered free radical, and convert it into .α-diphenyl-β-picryl hydrazine. The intensity of the color change indicates the potential for scavenging free radicals by antioxidant extracts [23].

4. CONCLUSION

Sidaguri plant has great potential against various diseases, such as gout, rheumatism and inflammation, diarrhea and dysentery, nasal congestion and bronchial asthma, childbirth and miscarriage problems, weight loss, etc. From the inventory and research that has been carried out, these five species of sidaguri have different morphological characteristics, which allows different chemical content and pharmacological effects. All five species were verified as *S. acuta* Burm. f; *S. rhombifolia*. L; *S. cordifolia.* L, *S. retusa.* L. and *S. scabrida* Wight & Arn. The potential as an antioxidant of this plant is a very interesting thing to study. The root organs of this plant are thought to have the best antioxidant activity compared to the leaf and stem organs of various species.

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