Original Article

Metabolite profiling, total polyphenolic contents and in vitro antioxidant properties of Sidaguri (*Sida retusa* L.)

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ABSTRACT

Sida retusa L. is a plant belonging to the Malvaceae family native to the tropical and subtropical plains grossing abundantly in Indonesia. Traditionally this plant is used for arthritis, asthma, cough, flatulence, colic, burning sensation, hemorrhoids, intermittent fever, and general debility. This study evaluates the chemical content in metabolite profiling, total polyphenolic contents, and in vitro antioxidant activity from *Sida retusa* L. leaves extract from West Sumatra, Indonesia. Metabolite profiling determination of *Sida retusa* L. extract using LC-MS/MS method, total phenolic and flavonoid contents, and antioxidant activity was determined by spectrophotometry. The results of the chromatogram interpretation revealed five main components of *Sida retusa* L. from West Sumatra were epoxypheophorbide A, scortechinone X, stigmastane-3β,5α,6β-triol, shidasterone B, and linolenic acid. Total phenolic and flavonoid contents from *Sida retusa* L. were 31.848 mg GAE/g and 12.242 mg QE/g). *Sida retusa* L. from West Sumatra had an antioxidant activity with IC_{50} value 468,137 ± 13,319 µg/mL. *Sida retusa* L. contains various potential secondary metabolites and sources of polyphenols and antioxidants and is a promising source of Indonesian natural ingredients.

Keywords: *Sida retusa* L, Metabolite profiling, Polyphenolic, Antioxidant

Introduction

Sidaguri plant (Sida spp) is a plant in the Malvaceae family that is often used as traditional medicine against various diseases in various countries [1]. This plant can grow in tropical and subtropical areas consisting of about 200 species spread throughout the world [2]. Several species of Sida, such as *Sida acuta, Sida cordifolia, Sida rhombifolia, Sida spinosa*, and *Sida veronicaefolia* are widely used as traditional medicine in India (including Ayurvedic and Siddha), America, Africa, China, and Indonesia [3]. In Indonesia, especially on the island of Sumatra, several species of the genus Sida have been found, such as *Sida acuta, Sida rhombifolia, Sida subcordata, Sida scabrida, Sida cordifolia*, and *Sida retusa*. *Sida retusa* is one of the Sida species that grow quite a lot in West Sumatra. Traditionally, this herb is often used as a remedy for rheumatism, neurological disorders (epilepsy), diuretics and fever, asthma [4], cough, flatulence, colic, burning sensation, hemorrhoids, and fatigue [5], Tonic, aphrodisiac, diuretic and inflammation [6]. The chemical content of the *Sida retusa* plant includes ecdysteroids, alkaloids, steroids, phenolics, and flavonoids [7].

Several studies have been conducted on this species of *Sida retusa*, among others *Sida rhombifolia* ssp. *retusa* seed extract inhibits DEN-induced murine hepatic preneoplasia and carbon tetrachloride hepatotoxicity was investigated in rats [8]. *S. rhombifolia* subsp. *retusa* exhibits strong cytotoxic, antibacterial, antitubercular, and antimycotic activities [9]. Aqueous extract of *Sida rhombifolia* ssp. *retusa* leaves have hypoglycemic and hypolipidemic effects in diabetic-induced animals [10]. Research on this species of *Sida retusa* has not been done much, so we are interested in conducting metabolite profiling, testing the total phenol and total flavonoid contents, and testing the antioxidant activity of this plant.

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Materials and Methods

Sample and plant identification

Sida retusa L was collected on Perumdos Kampus Universitas Andalas Kel. Limau Manih, Kec. Pauh Padang, West Sumatra, Indonesia **(Figure 1)**. Identification of *Sida retusa* L plants at the Andalas University Herbarium (ANDA) Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University Padang, West Sumatera, Indonesia, with collection number ANDA 00038434.

Figure 1. Sidaguri plant from West Sumatra (*Sida retusa* L.)

Chemicals, reagents, and materials

All the chemicals and reagents used in this study were analytical grade and were purchased from Merck and Sigma-Aldrich. Spectrophotometry microplate reader (BioRad Xmark®), UV-Vis Spectrophotometer (Shimadzu PHarmspec 1700®), LC-MS instrument consisting of LC system (Waters Acquity UPLC I-Class), LC column (ACQUITY UPLC®) BEH C8 1.7 m 2.1 x 100 mm, Mass spectrometer (XEVO G2-XS QTof).

Sample preparation

The leaves of *Sida retusa* L West Sumatra are cleaned and air-dried for 2 weeks. Then it is sorted again and then mashed utilizing a grinder.

Extraction

Extraction was carried out using the maceration method according to the Indonesian Herbal Pharmacopoeia. A total of 50 grams of finely powdered leaves of *Sida retusa* L were macerated with 50 ml of 70% methanol. Soak for the first 6 hours, stirring occasionally, then let stand for 18 hours. Separate the macerate by filtering then the pulp is macerated again with 25 ml of 70% methanol. The obtained maserate was combined and then concentrated with a rotary evaporator until a thick extract was formed [11].

Determination of total phenolic level

Determination of total phenol content by colorimetric method referring to the procedure of Subramanya *et al*. (2015) with some modifications with gallic acid (GAE) as standard. A 50 µL sample (1,000 µg/mL) was pipetted into a 96-well plate. Then 50 µL of aquabidest was added, Folin-Ciocalteu reagent 7.5% 50 µL and 1% NaOH 50 µL, then incubated for 60 minutes. The absorbance was measured using a microplate reader at 730 nm. The test was repeated 3 times. Gallic acid was used in the calibration curve to determine the total phenol content [12]. The total phenol content in the extract was expressed as milligram gallic acid equivalent/gram extract (mgEAG/g) [13].

Determination of total flavonoid level

Determination of total flavonoid content by colorimetric method referring to the procedure of Sato *et al*. (2020) with some modifications with quercetin (QE) as standard. A 50 µL sample (1,000 µg/mL) was pipetted into a 96-well plate. Then $AlCl_3$ 10% (w/v) 30 µL, NaNO3 5% (w/v) 30 µL, aquabidest 50 µL, then incubated for 5 minutes. Then add 40 µL of aquabidest again, and incubate again for 30 minutes. The absorbance was measured using a microplate reader at 430 nm. The test was repeated 3 times. The total flavonoid content in the extract was expressed as the milligram equivalent of quercetin extract/ gram extract (mgQE/g) [14].

Antioxidant activity test

Antioxidant testing using the DPPH Free Radical Attenuation method [15]. One hundred microliters of extract (range concentration 62.5 – 500 µg/mL) was put in a 96-well plate, and then 100 µL of DPPH solution in methanol was added. As a standard, gallic acid was used with the same treatment as the sample [16]. This mixture was stored at room temperature in a dark place for 30 minutes. The absorbance was measured at 517 nm. Each extract test was carried out 3 times. The IC_{50} value is calculated using a linear regression equation [17].

Results and Discussion

Plant inventory of sida retusa L

The main characteristics and geographic origin of this Sidaguri plant (*Sida retusa* L) are shown in **Table 1**. Next for identification, plant samples were prepared under dry herbarium conditions. The determination was made at the Andalas University Herbarium, Padang. The morphology of *Sida retusa* L West Sumatra verified at Andalas University, Padang Herbarium is shown in **Table 2**.

Identify the chemical content profile

To identify the chemical content profile of *Sida retusa* L leaf extract, metabolite profiling was carried out using the LC-

MS/MS method with specifications LC System (ACQUITY UPLC®H-Class System (waters, USA); LC Column (ACQUITY UPLC® HSS C18 (1.8 m 2.1). x100 mm) (waters, USA) and Mass Spectrometer Xevo G2-S QTof (waters, USA) at the Bogor Police Forensic Laboratory. Based on the data from the interpretation of compound content analysis using UPLC-QToF-MS, it can be seen that 5 main compounds from *Sida retusa* L leaf extract as shown in **Table 3**.

From the LC-MS/MS data, the interpretation of the five main chemical constituents of *Sida retusa* L, namely epoxypheophorbide A (24.63 %), scortechinone X (12.07 %), stigmastane-3β,5α,6β-triol (8. 25%), shidasterone B (8.11 %) and linolenic acid (6.99 %) **(Figure 2)**. From the five main chemical constituents of this species, there are several compounds that have been isolated from previous studies. epoxypheophorbide A is a class of phaeophytins compounds isolated from *Sida rhombifolia* species [18]. Shidasterone B is a class of ecdysteroid compounds, which have been isolated from species *Sida cordifolia* and *Sida rhombifolia*. Meanwhile, linolenic acid is a class of aliphatic compounds that have been isolated from species of *Sida acuta* and *Sida rhombifolia* [3].

Table 1. Main characteristics and geographic origin of the plant *Sida retusa*. L

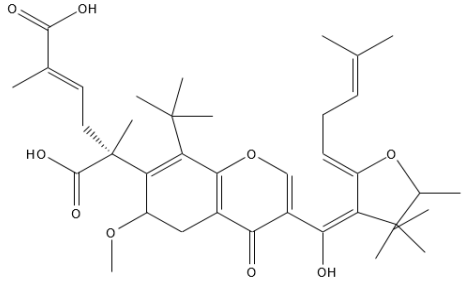
Characteristics	Specification
Locality	Perumdos Kampus Universitas Andalas Kel. Limau Manih, Kec. Pauh Padang
Habitat	Meadow
Altitude	300-500 m asl
Code Specimen	ANDA 00038434
Coordinate	0°55'18.9"S 100°27'24.7"E

Table 2. Morphological characteristics of various organs of the *Sida retusa*. L

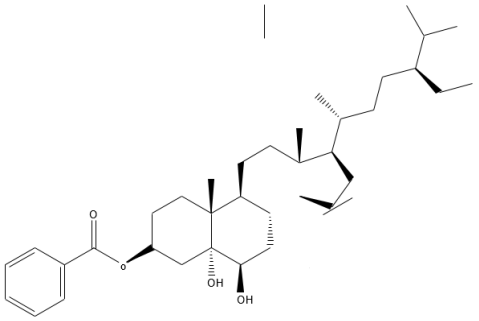
Morphological characteristics	Specification
Plant shape & height	Erect subshrubs up to 80 cm high
Stem	Stem branched, purplish, stellate hairy
Leave	Obovate leaves, apex retusus, leaves *ca* 4 × 6 cm, rhomboid to lanceolate, obovate or suborbicular, stellate-tomentose beneath
Flower	Diameter of open flowers 12.0-14.0 mm, petals deep yellow
Fruit	Fruit diameter 4.2-5.0 mm

Table 3. Interpretation of the five main chemical constituents of Sidaguri leaf extract (*Sida retusa*. L)

Retensi Time (Minute)	Area (%)	Theoretical mass (m/z)	Molecule Formula	MoleculeWeight (g/mol)	Measured mass $[M-H]^+$	Ion Fragment (m/z)	Tentative Identification
15,32	24,63	609.2735	$C_{36}H_{32}N_8O_2$	609.69	608,26	591,2615; 610,2758; 611,2784	Epoxypheophorbide A
15, 03	12,07	609.2670	$C_{34}H_{40}O_{10}$	608,68	608,26	591.2615; 610,2707; 611,2733	Scortechinone X
11,83	8,25	553.4249	$C_{36}H_{56}O_4$	552.827	552.418	507, 2278; 291, 1946; 277, 2165	Stigmastane-3β,5α,6β-triol
5,10	8,11	463.3088	$C_{27}H_{42}O_6$	462,62	462,30	427,2870; 445.2979; 464,3121	Shidasterone B
12,48	6,99	279.2327	$C_{18}H_{30}O_2$	278,43	278,22	275,2010; 280,2361	Linolenic acid



a)



b)

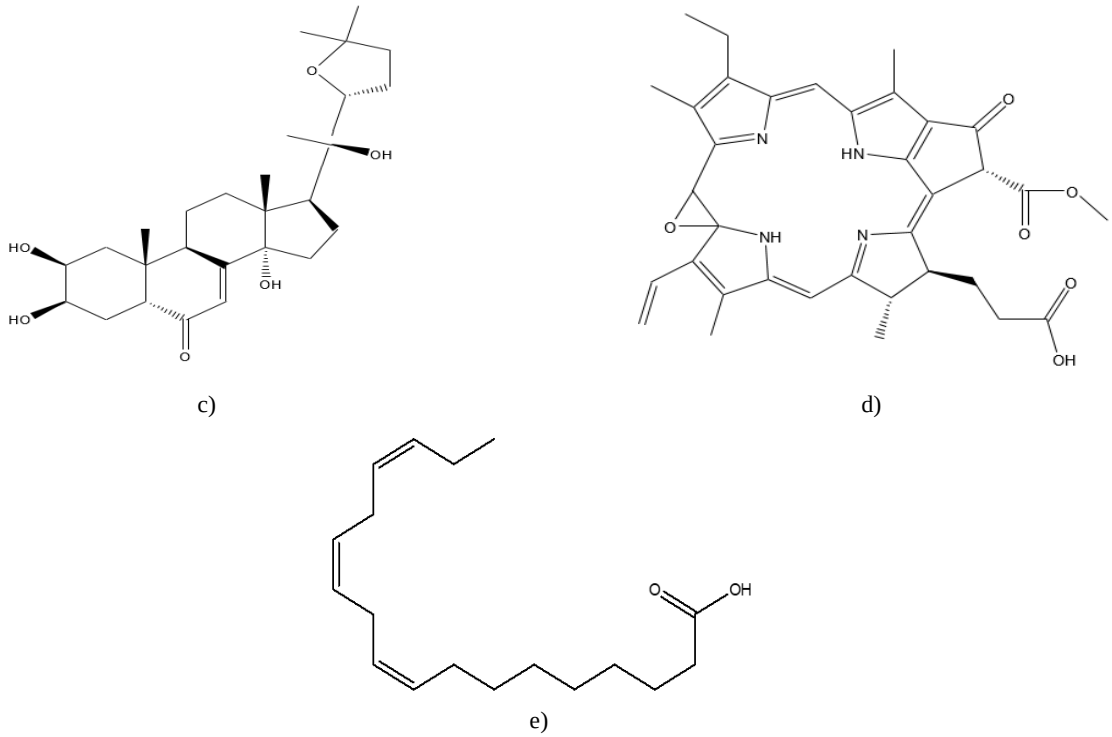


Figure 2. Structure of the five main chemical constituents of the plant extract of *Sida retusa*. L by LC-MS/MS: (a) Epoxypheophorbide A, (b) Scortechinone X, (c) Stigmastane-3β,5α,6β-triol, (d) Shidasterone B, (e) Linolenic acid.

Total phenol and total flavonoid content

Testing the total phenol and flavonoid content of *Sida retusa* L leaf extract by UV Spectrophotometry method using Well 96 Plate and absorbance measured by Microplate Reader. The following is data on the total phenol and flavonoid content of *Sida retusa* L leaf extract using gallic acid and quercetin as standards **(Table 4)**.

Table 4. Total phenol and total flavonoid contents of sidaguri leaf extract (*Sida retusa*. L)

Species	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Sida retusa L	31,848	12,242

QE : Quercetin equivalent.
GAE : Gallic acid equivalent

From the results of testing the total phenol content, *Sida retusa* species has a total phenol content of 31,848 mg GAE/g. Measurement of the total phenol content using the Folin-Ciocalteu method which is based on the reducing power of the phenolic hydroxyl group using gallic acid standard [19]. Gallic acid was chosen because it is a pure and stable substance. All phenolic compounds including simple phenols can react with the Folin-Ciocalteu reagent although they are not effective radical scavengers. The presence of an aromatic nucleus in phenolic compounds can reduce phosphomolybdate phosphotungstate to molybdenum tungsten. Phenolic compounds only react with the Folin-Ciocalteu reagent in an alkaline environment so that proton dissociation occurs in phenolic compounds into phenolic ions [20]. In the measurement of total flavonoids, standard quercetin was made as a comparison. Quercetin was chosen because it is a flavonoid (flavonol) group. The total flavonoid content of *Sida retusa* was 12,242 mg QE/g.

Antioxidant activity

The antioxidant activity of *Sida retusa* leaf extract was tested using UV spectrophotometry and absorbance was measured at a wavelength of 517 nm. The results of the antioxidant analysis can be seen in **Table 5**. Based on **Table 5**. it is known that *Sida retusa* leaf extract has antioxidant activity with an IC_{50} value of 468.137 ± 13.319 µg/mL.

Table 5. Data on IC_{50} antioxidant activity of sidaguri leaf extract (*Sida retusa*. L)

Sample	Range concentration (µg/mL)	IC_{50} (µg/mL)
Sida retusa L	62,5 – 500	468,137 ± 13,319
Gallic acid	0,15625 - 1,25	0,670 ± 0,209

IC_{50}: Inhibitory Concentration Fifty

In a previous study, Dhawal *et al.* (2007), evaluated the antioxidant activity of the extracts of the leaves, stems, roots, and herbs of *Sida retusa* L, which had IC_{50} values of 852.8 ± 15, 1,222.5 ± 11, 46.1 ± 12. and 983.8 ± 16 µg/ml [21]. From this data, the IC_{50} value of *Sida retusa* originating from West Sumatra, has a smaller value indicating greater antioxidant activity than previous studies.

Conclusion

Sida retusa L is one of the Sidaguri plant species that can be a potential source of phenolic and flavonoid compounds with contents of 31,848 mg GAE/g and 12,242 mg QE/g,

respectively. *Sida retusa* is also a source of antioxidant compounds in the future.

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Conflict of interest: None

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Ethics statement: None

References

1. Kitcher C, Mireku-Gyimah NA, Sarkodie JA, Bekoe EO, Asafo-Agyei T, Agyei PA, et al. Pharmacognostic standardization of the leaf and stem bark of millingtonia hortensis linn. (Bignoniaceae). Int J Pharm Res Allied Sci. 2021;10(1):42-9.
2. Brandao JL, Baracho GS, Sales MD, Viegas Filho MP. Synopsis of Sida (Malvaceae, Malvoideae, Malveae) in the state of Pernambuco, Brazil. Phytotaxa. 2017;307(3):205-27.
3. Dinda B, Das N, Dinda S, Dinda M, Silsarma I. The genus Sida L. - A traditional medicine: Its ethnopharmacological, phytochemical, and pharmacological data for commercial exploitation in the herbal drugs industry. J Ethnopharmacol. 2015;176:135-76.
4. Belal SHM, Jaha MJM, Alzahrani MKM, Alyamani AMA, Munshi AHA, Kalantan AAI, et al. Asthma: Overview on diagnostic and management approach in primary health care. Arch Pharm Pract. 2022;13(1):11-4.
5. Madharia P, Jahan A. Ethnomedicinal plants and their conservation in chhattisgarh state: Review and perspectives. IOSR J Environ Sci Toxicol Food Technol. 2015;1(4):46-50.
6. Dhalwal K, Shinde V, Mahadik KR, Kadam SS. Against thioacetamide and allyl alcohol intoxication in rats. Pharmacologyonline. 2006;3:259-66.
7. Nimmy VS, Jayasreee P, Deepa MS. Comparative evaluation of preliminary pharmacognosy and phytochemistry of two source plants of bala (Sida cordifolia Linn. and Sida retusa Linn.). Int J Ayurveda Pharma Res. 2017;5(8):10-5.
8. Poojari R, Gupta S, Maru G, Khade B, Bhagwat S. Sida rhombifolia ssp. Retusa seed extract inhibits DEN-induced murine hepatic preneoplasia and carbon tetrachloride hepatotoxicity. Asian Pacific J Cancer Prev. 2009;10(6):1107-12.
9. Poojari R. Phytochemical fingerprinting, cytotoxic, antimicrobial, antitubercular, antimycotic potentials of Sida rhombifolia subsp. retusa and Embelia tsjeriam-cottam. Asian Pasific J Inf Syst. 2011;4(3):107-34.
10. Dhalwal K, Shinde VM, Singh B, Mahadik KR. Hypoglycemic and hypolipidemic effect of Sida rhombifolia ssp. retusa in diabetic-induced animals. Int J Phytomedicine. 2010;2(2):160-5.
11. Republic of Indonesia Ministry of Health. Indonesian Herbal Pharmacopoeia. II. Jakarta; 2017. 531 p.
12. Sarvananda L, Premarathna AD. Investigation of total phenolic, tannins, flavonoid contents, and antioxidant activity of pisonia alba. Pharmacophore. 2021;12(6):43-9.
13. Subramanya MD, Pai SR, Upadhya V, Ankad GM, Bhagwat SS, Hegde HV. Total polyphenolic contents and in vitro antioxidant properties of eight Sida species from Western Ghats, India. J Ayurveda Integr Med. 2015;6(1):24-8.
14. Sato VH, Chewchinda S, Parichatikanond W, Vongsak B. In vitro and in vivo evidence of hypouricemic and anti-inflammatory activities of Maclura cochinchinensis (Lour.) Corner heartwood extract. J Tradit Complement Med. 2020;10(1):85-94.
15. Benguiar R, Benaraba R, Abdellah F, Hammou ASAIT, Bouriah M, Moulayat KE. Effect of green tea "Camellia sinensis" Extract on antioxidant activity of fresh-cut apple during cold storage. J Biochem Technol. 2021;12(3):91-5.
16. Saravanakumar V, Masi C, Neme I, Arjun K, Dinakarkumar Y. Geographical comparison of phytoconstituents in euphorbia hirta: A pilot study in ethiopia and India. Bull Pioneer Res Med Clin Sci. 2022;1(2):34-41.
17. Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating anti-oxidant activity. Songklanakarin J Sci Technol. 2004;26(2):211-9.
18. Chaves OS, Gomes RA, De Andrade Tomaz AC, Fernandes MG, Das Graças Mendes L, De Fátima Agra M, et al. Secondary metabolites from Sida rhombifolia L. (Malvaceae) and the vasorelaxant activity of cryptolepinone. Molecules. 2013;18(3):2769-77.
19. Nagarajan S. Polyphenolic compounds - A promising leads for antiviral therapy. Pharmacophore. 2022;13(1):36-47.
20. Adjdir S, Benariba N, Adida H, Kamila G, El Haci IA, Terki M, et al. Phenolic compou nds and antimicrobial activity of ziziphus jujuba mill. Fruit from Tlemcen (Algeria). J Biochem Technol. 2021;12(1):40-4.
21. Dhalwal K, Deshpande YS, Purohit AP. Evaluation of in vitro antioxidant activity of Sida rhombifolia (L.) Ssp. retusa (L.). J Med Food. 2007;10(4):683-8.