International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Special Issue 4, 2024

Original Article

TOTAL PHENOLIC, FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF STANDARDIZED EXTRACT OF *GAGATAN HARIMAU* LEAVES (*VITIS GRACILIS* BL)

CHEMAYANTI SURBAKTI[1*], LISDA RIMAYANI NASUTION[2], SINGGAR NI RUDANG[3], HENNI CINTYA[4], VANY INDARTI[5], P. A. T. AGNES[6], S. E. S. ELSA[7]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Medan, Universitas Sumatera Utara, Indonesia. [2,4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medan, Universitas Sumatera Utara, Indonesia. [3]Department of Pharmacology, Faculty of Pharmacy, Medan, Universitas Sumatera Utara, Indonesia. [5,6,7]Medan, Universitas Sumatera Utara, Indonesia
[*]Corresponding author: Chemayanti Surbakti; [*]Email: chemayantisurbakti@usu.ac.id

Received: 27 Apr 2024, Revised and Accepted: 12 Jul 2024

ABSTRACT

Objective: The purpose of this research was to standardize the specific and nonspecific simplicia of *Gagatan Harimau* leaf, determine the total phenolic and total flavonoid contents of *Gagatan Harimau* leaf ethanol extract, and evaluate the antioxidant activity of *Gagatan Harimau* leaf ethanol extract.

Methods: Total phenolic content was determined using the Folin-Ciocalteu method and gallic acid as standard comparison. Total flavonoid content using the aluminum chloride method with standard quercetin as a comparison. Antioxidant activity was analyzed using the DPPH method by measuring synthetic radical scavengers in polar solvents.

Results: Standardization of specific and nonspecific simplicia results were pointed leaf tips and bases, pinnate bone placement, jagged and rough leaf margins, smooth, hairy undersides, 14-24 cm long, width 6-11 cm, color green, characteristic odor and sour taste, Cu content<150 mg/l, bacterial contamination ≤ 10.000 colonies/g. The total phenolic and flavonoid content of the ethanol extract of *Gagatan Harimau* showed 207.6695±0.2056 mg GAE/g and 23.2883± 0.0556 mg QE/g. Antioxidant activity with a concentration of 10; 20; 30; 40; 50 µg/ml gives an IC50 value of 34.79 µg/ml.

Conclusion: *Gagatan Harimau* leaves have good antioxidant activity, so they can be used as a source of natural antioxidants.

Keywords: Vitis gracilis BL, Standardization, Antioxidant, Phenol, Flavonoid

DOI: https://dx.doi.org/10.22159/ijap.2024.v16s4.07 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

As plants that produce many of antioxidants to control oxidative stress caused by sunlight and oxygen, which can be a source of new compounds with antioxidant activity. Some of the non-nutritive antioxidants of plants are phenolic compounds, flavonoids, coumarins, benzyl isothiocyanate, etc [1]. Antioxidants play a better role in counteracting free radicals than artificial antioxidants. Antioxidants play an important role in protecting cell membranes from free radical damage [2]. Free radicals are chemical species that can exist independently and have one or more unpaired electrons that give them enormous reactivity. This reactivity is inversely proportional to their stability [3]. Antioxidants can fight the harmful effects of free radicals, which are formed as results of oxidative metabolism [4]. Antioxidants are molecules that slow or prevent oxidation (other chemicals). Known as reducing agents, antioxidants influence cell differentiation and proliferation, block nitrosamine production, stimulate the immune system, maintain cell membrane and matrix integrity, and maintain normal DNA repair [5]. In recent years, research into antioxidants has continued to grow. Various studies have found a link between increased oxidative cell damage due to an imbalance between free radicals and natural antioxidants in the body. It is therefore an important factor in several diseases, such as cardiovascular disease, cancer and ageing [6]. Antioxidants act as a major defence against radical-mediated toxicity by protecting the damages caused by free radicals [7]. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical and is widely used to test the entrapment activity of an antioxidant [8].

Gagatan Harimau leaves (*Vitis gracilis* BL) is a climbing shrub, up to 10m long, with round leaves with serrated edges and sharp tips. The flowers are arranged in panicles. The fruit is round or slightly oval, ±2 cm, smooth skin, various colours, sweet-sour flesh containing 2-4 seeds. In the culture of the Karo people, who live in the area around Mount Sibuatan, it is usually used to treat various types of illness [9]. Traditionally *Gagatan Harimau* leaves (Vitis gracilis BL) is a traditional medicinal plant of the Karo people, which is efficacious as a medicine for diarrhea, strong medicine, treating wounds, infectious diseases, diabetes. *Gagatan Harimau* leaves (Vitis gracilis BL) have been shown to have antibacterial activity on a range of test bacteria and are a source of flavonoids [10].

Primary metabolites are essential necessary for plant growth and development. The most important secondary metabolites are saponins, alkaloids, tannins, flavonoids and cardiac glycosides. Qualitative phytochemical screens help us understand the different compounds produced by plants, and chemical quantification of these metabolite aids in the extraction, purification, and identification of bioactive compounds [11]. The ethanolic extract of *Gagatan Harimau* leaves contains several secondary metabolites, namely alkaloids, glycosides, tannins, saponins and flavonoids. The benefits of flavonoids as antioxidants are not in doubt. These compounds are thought to act as antioxidants because they can rapidly donate hydrogen atoms to radical compounds or convert them to a more stable form [12].

Plants that have the potential to become natural medicinal ingredients need to be standardized to ensure pharmaceutical quality. The subject of standardization of herbal medicines is very broad and profound. There are so many contradictory theories about herbal medicines and their function on human physiology and mental function. Indonesia needs to explore medically important plants. This can be achieved only if herbal products are evaluated and analyzed using standardized techniques [13]. Standardization is carried out with two parameters, namely specific and non-specific parameters. Determination of specific parameters, namely organoleptic (shape, odor taste and color), water-soluble extract, ethanol soluble extract. Determination of non-specific parameters, namely drying shrinkage, specific gravity, residual solvent, microbial contamination, ash content, acid insoluble ash content, water

content and contamination of heavy metals Pb, Cd, and Cu. It is hoped that the standardization parameter determination can be used as a reference for extract quality standard parameters in supporting health because it has not been registered in the Indonesian Materia Drugs book and Monographs of Medicinal Plant Extracts [14, 15].

Based on this, it is necessary to conduct research on *Vitis gracilis*, so that researchers carry out standardize specific and non-specific simplicia, determine the total phenolic and flavonoid content and determine the antioxidant activity of the ethanol extract of *Gagatan Harimau* leaves, to determine their bioactivity so that they can be used as a source of medicine.

MATERIALS AND METHODS

The material used for this research is *Gagatan Harimau* leaves extract (*Vitis gracilis* BL).

Chemical and reagents

A solvent used were distilled water, 96% ethanol, DPPH, amyl alcohol, acetic anhydrous acid, aluminium chloride, concentrated hydrochloric acid, concentrated nitric acid, concentrated sulfuric acid, trichloro acetaldehyde hydrate, chloroform, ethyl acetate, folin-ciocalteu, gallic acid, magnesium powder, isopropanol, methanol, sodium hydroxide, sodium chloride, sodium carbonate, sodium acetate, toluene and quercetin.

Simplicia of the harimau gagatan leaves

Sample was taken from Sukaribu, Telagah Village, Sie Bingei District, Langkat Regency, North Sumatra. The sample used in this study was young and fresh leaves of *Gagatan Harimau*. The leaves are washed thoroughly to remove soil and amounts of impurities, then drained and weighed. Wet weight is obtained. Furthermore, the leaves is reduced the humidity in the drying cabinet using a heat rating of 400–500 °C until the leaves are dry (indicated when crushed brittle). The dried simplicia was weighed then blended into powder.

Formation of the ethanol extract of *Gagatan Harimau* leaves

500 g of *Gagatan Harimau* leaf simplicia powder were weighed, added to a glass container with 75 parts (96%) ethanol, covered, and allowed to step for five days while being kept out of light. The remaining material was then squeezed out. Dregs washed with solvent as much as 25 parts to obtain 100 parts. Then macerate is accommodated in a vessel that is shut, left in a cool and protected 2 d of darkness followed by filtering. A maserate is separated from the solvent using a rotary evaporator with a temperature of ±50 °C until the solvent gradually evaporates. Then the concentration activity was carried out use a stove at 50 °C heat until a concentrated extract is obtained.

Standardization of simplicia

Standardization was carried out with two parameters, namely specific parameters (identity, organoleptic, extracts that can be mixed with distilled water, and extracts which can be incorporated in ethanol and non-specific parameters (drying shrinkage, specific gravity, water percentage, ash percentage, residual solvent, heavy metal contamination, microbial contamination) [16].

Phenolic and flavonoid contents analysis

Value the overall phenolic content, 10 mg of the extract sample was weighed and dissolved in 10 ml of methanol (1000 µg/ml). An aliquot of 0.1 ml of extract solution was mixed with 7.9 ml of water and 0.5 ml of Folin-Ciocalteu, vortexed for ±1 min and added sodium carbonate (20%) up to 10 ml. Incubated for 60 min and absorbance was measured using UV-Vis spectrophotometer at 769 nm. As for total flavonoid content, 10 mg of the extract sample was weighed and dissolved in 10 ml methanol (1000 µg/ml). An aliquot of 0.5 ml of the extract solution was added with 1.5 ml of methanol, 0,1 ml $AlCl_3$ (10%), 0.1 ml of sodium acetate 1 M, and water up to 5 ml. Leave it for about 30 min and absorption can be known using UV-Vis spectrophotometry at 437 nm17,18.

Antioxidant test

Antioxidant test was carried out using the DPPH method. The ethanol extract of *Gagatan Harimau* leaves was added to a 200 µg/ml DPPH solution in ethanol. The control used was 40 µg/ml DPPH solution. The mixture of extract and DPPH solution was vortexed for 1 min and incubated at room temperature (25 °C) for 30 min. Absorbance was measured at a wavelength of 516 nm. Antioxidant activity test was carried out with concentrations of 10, 20, 30, 40 and 50 µg/ml at the maximum wavelength [28].

RESULTS AND DISCUSSION

Organoleptic

Fresh macroscopic examination *Gagatan Harimau* leaf consists of size, hue and flavor. The results of macroscopic analysis of new *Gagatan Harimau* leaves were single leaves having an oval-elliptical size, tapered top and bottom, pinnate bone arrangement, serrated and rough leaf edges, smooth-haired underside of leaves, 14-24 cm long, 6-11 cm wide, green in color, has a distinctive smell and a tart flavor.

Microscopy

Microscopy results of *Gagatan Harimau* leaves simplicia showed the presence of stomata, cuticle, parenchyma, epidermis, and glandular hairs.

Table 1: The result of characterization simplicia *Gagatan Harimau* leaves

No.	Parameters	Results
1.	Water percentage	7.93%
2.	Percentage dissolved in water	16.93%
3.	Percentage dissolved in ethanol	15.37%
4.	Percentage of total ash	2.37%
5.	Acid insoluble ash content	0.43%

Water content

The results of the simplicia characterization of *Gagatan Harimau* leaves showed that the results for determining the water content were obtained at 7.93%. The water content requirement for plant simplicia is less than 10% so that the water content in *Gagatan Harimau* leaves meets the requirements. The percentage of water that exceeds 10% is likely to be a good place for microbial growth, the presence of fungi and insects, and destruction due become hydrolysis process.

Content at water-soluble extract and ethanol

Two solvents, specifically water and ethanol, were used to determine the extract content. The concentrations of polar chemical compounds found in the simplicia were measured in the water-soluble essence content, whilst the concentrations of the ethanol-soluble extract were used to determine the amounts of polar chemical compounds. Both polar and non-plar molecules that are soluble in ethanol were measured for their concentrations. The outcomes of the simplicia characterization of *Gagatan Harimau* leaves showed a water-soluble extract content of 16.93%, while the ethanol-soluble extract content was 15.37%.

The content of the water-soluble essence is greater due to the fact that polar molecules are more soluble in water than ethanol and compounds that are insoluble in water will dissolve in ethanol. Water can dissolve other substances that are not needed such as gum, starch, protein, fat, mucus and others. This causes high levels of water-soluble extracts from dissolved plant [19].

Ash content and acid insoluble content

Determination of the ash content of the simplicia of *Gagatan Harimau* leaves showed percentage of total ash content of 2.37% and the percentage that does not combine in the acid 0.43%. In general, the total ash content for each simplicia is not the same. Generally, the ash content insoluble in acid is<1%, and meets the requirements. The ash content was measured in order to identify the sample's exterior (non-physiological ash), such as sand and dirt, and internal mineral content (physiological ash), which comes from the plant tissue itself. The acid-insoluble ash content shows the amounts of silicates, especially the sand present in the simplicia by dissolving the total ash in hydrochloric acid.

Drying shrinkage test

Drying shrinkage is one of the non-specific parameters that aims to provide a maximum limit (range). In determining the drying shrinkage parameter of *Gagatan Harimau* leaf simplicia, the drying shrinkage value is 7.47%. Based on research by Sutomo *et al.* (2019), in special cases (if the material does not contain essential oils and the remaining organic solvents evaporate), the drying shrinkage rate is identical to the water amount. The value of the water amount is related to the purity and contaminants in the simplicial [20].

Specific gravity test

The purpose of determining specific gravity is to provide a limit on the amount of mass per unit volume which is a special parameter for liquid extracts to concentrated (thick) extracts that can still be poured. Measurement of the specific gravity of the ethanol extract of *Gagatan Harimau* leaves was determined using a pycnometer. Based on the measurements, the specific gravity of the extract was 0.995675.

Solvent residual test

This parameter aims to provide assurance that during the process it does not leave crumb solvent, which should not be present. Based on the research, it was found that the residual solvent value was<1%, which fulfilled the requirements the crumbs solvent extract test with ethanol solvent, which was below 1%.

Metal contamination test

The meaning of this parameter is in order to guarantee that the extract doesn't include specific heavy metals such as Pb, Cd, Cu, etc. Which exceeds the set value because it will cause toxic effects that are harmful to health [21].

Table 2: The result of metal contamination test from Simplicia *Gagatan Harimau* leaves

No.	Parameters	Concentration (mg/l)
1.	Pb	0.204
2.	Cd	0.033
3.	Cu	0.089

Output of metal contamination the test gives result Pb (lead) metal content in the samples showed a value of 0.204 mg/l, which still met the requirements stated regarding Safety and Quality Requirements for Traditional Foods, BPOM Regulation No. 32 of 2019 concerning Medicines, namely <10 mg/l. The value of Cd (Cadmium) metal contamination in the samples detected was 0.033 mg/l where this value still meets the requirements listed regarding Safety and Quality Requirements for Traditional Medicines, BPOM Regulation NO.32 of 2019 states. which is less than 0.3 mg/l. The value of Cu (Cupri) metal contamination in the samples detected was 0.089 mg/l where this value is still in accordance with the WHO range of 150 µg/ml [22].

Microbial contamination test

One of the tests for extract purity is the bacterial contamination test. The purpose is to guarantee that the extract doesn't have microbial contamination that exceeds the set limits.

Table 2: The result of metal contamination test from simplicia *Gagatan Harimau* leaves

Dilution	Repetition			Average
	I	II	III	(colonies/g)
10^{-1}	38	28	52	39.33
10^{-2}	19	25	20	21.33
10^{-3}	17	24	15	18.66
10^{-4}	17	8	24	16.33
10^{-5}	8	12	9	9.66
10^{-6}	10	4	7	7

The results showed that bacterial contamination in the ethanol extract of *gagatan harimau* leaves was ≤ 10.000 colonies/g. These results are in accordance with the regulations of the Head of the Drug and Food Control Agency of the Republic of Indonesia regarding the requirements for Quality of Traditional Medicines that the maximum limit for bacterial contamination is ≤ 10.000 colonies/g.

Total phenolic content

Determination of total phenolic content was used according to the principle of the folin-ciocalteu method and gallic acid was used as the standard solution. The principle of the folin-ciocalteau method is a colorimetric oxidation and reduction reaction to measure all phenolic compounds in the sample. The folin-ciocalteu reagent is a solution of a polymeric ion complex formed from phosphomolybdic acid and heteropolyphosphotungstic acid. This reagent oxidizes the phenolic hydroxyl groups (alkaline salts), reducing heteropolyacids to a molybdenum-tungsten complex. Phenolic compounds only react with the folin-ciocalteau reagent in an alkaline state so that proton dissociation occurs in phenolic compounds into phenolic ions, where this alkaline state is achieved by adding sodium carbonate, which will give blue solution 23. In determining the total content of phenolic compounds, the variable concentrations of the standard solutions used were 200, 250, 300, 350 and 400 µg/ml. Measurements were made at a wavelength of 769 nm and at 60 min. The gallic acid standard curve obtained for measuring the total phenol content is y = 0.0018878x+0.0137 with R2 = 0.9971. The results can be seen in fig. 1.

The total phenolic content of the ethanol extract of *gagatan harimau* leaves is expressed as gallic acid equivalent (GAE) as the number of milligrams of gallic acid equivalent per g of sample. The results of the determination of the total phenol content in the ethanol extract of *gagatan harimau* leaves were 207.6695±0.2056 mg GAE/g.

Total flavonoid content

Determination of the total flavonoid content is used on the principle that AlCl3 will form a complex because it has a keto group C-4 then

with a neighboring C-3 or C-5 hydroxyl group leading to a wavelength shift to the visible direction as seen from the yellow color in the solution [24]. In determining the total flavonoid content, the concentration variation of the standard solutions used was 40, 50, 60, 70 and 80 µg/ml. Measurements were made at a wavelength of 437 nm and at 30 min. The quercetin standard curve obtained for measuring the total flavonoid content is y = 0.010457x – 0.014333 with R2 = 0.9973. The results can be seen in fig. 2.

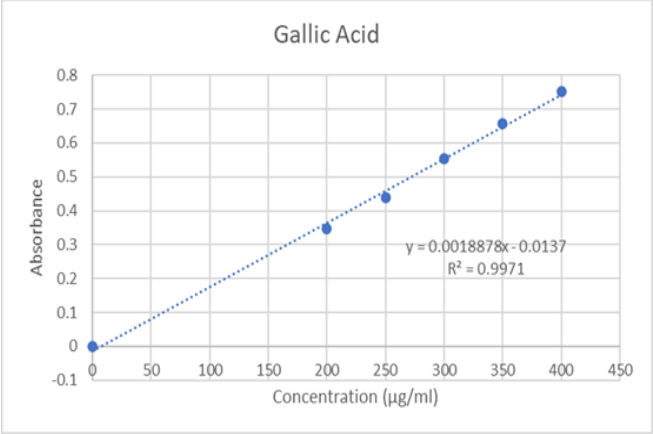

Fig. 1: The relationship of concentration to the absorbance of gallic acid

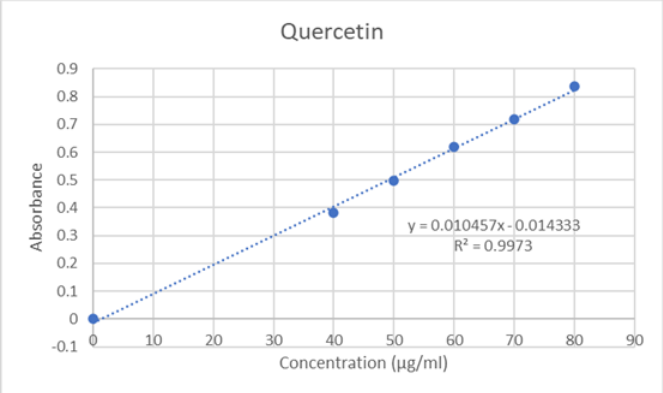

Fig. 2: The relationship of concentration to the absorbance of quercetin

The total flavonoid content in the ethanol extract of *gagatan harimau* leaves is expressed as quercetin equivalent (QE), which is equivalent to the number of milligrams of quercetin per g of sample. The results of determination of total flavonoid content of ethanol extract of *gagatan harimau* leaves were 23.2883±0.0556 mg QE/g. Phenolic compounds have also been reported to play an important role in scavenging free radicals. The correlation between antioxidant activity and total flavonoid is still under discussion, a good linear relationship has been observed in several published works [25].

Antioxidant test

The antioxidant activity measurement of the samples was carried out at 516 nm which is the maximum wavelength of DPPH. The presence of antioxidant activity in the sample causes the color of the original methanol-DPPH solution to change from dark purple to pale yellow 26. Each concentration is obtained later measured on UV-Vis spectrophotometer with quercetin as a comparison (control positive). Antioxidant activity was expressed as IC50 value (50% inhibitory concentration). IC50 value is the value of antioxidant concentration for reduce 50% of free radical activity.

Antioxidant or inhibitory activity assay on free radicals using the DPPH method showed that the ethanol extract of *Gagatan Harimau* (Vitis gracilis BL) leaves has antioxidant activity. The results of testing the antioxidant activity can be seen in fig. 3 and fig. 4.

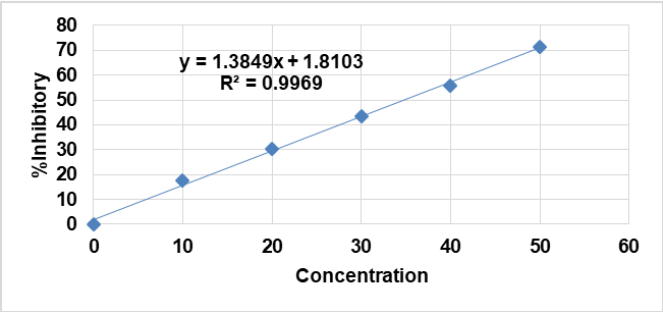

Fig. 3: The relationship between the concentration of ethanol extract of *gagatan harimau* leaves and the percentage of attenuation

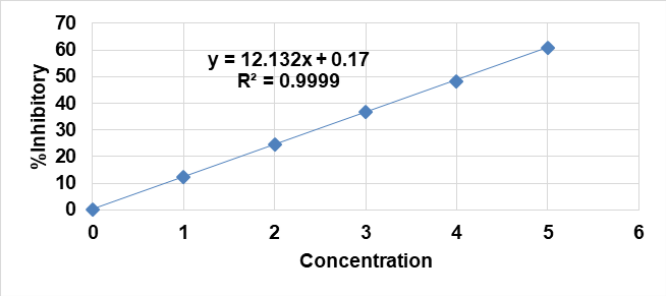

Fig. 4: The relationship between the concentration of quercetin and the percentage of attenuation

Based on data analysis using probit analysis, the IC50 value of the ethanol extract of *Gagatan Harimau* leaves was 34.79 µg/ml and quercetin as a comparison had an IC50 of 4.10 µg/ml. Antioxidant activity of the ethanol extract of *gagatan harimau* leaves was analyzed by DPPH method with UV-Vis spectrophotometer at 516 nm. The amount of antioxidant activity is expressed as the IC50 value, which is the concentration of the sample solution required to inhibit 50% of DPPH free radicals. The smaller the IC50 value, the greater the antioxidant activity of the tested material26. The IC50 value of the ethanol extract of *gagatan harimau* leaves is weaker than the pure antioxidant quercetin. This could be because quercetin is a very pure compound, while compounds suspected of having antioxidant activity in the ethanol extract of *Gagatan harimau* leaves are flavonoids and phenolic compounds. The proton radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. This test determines the scavenging of DPPH-stabilizing free radicals by antioxidant compounds present in the extract. The rates of DPPH scavenging activity of extracts are probably due to the presence phenolic compounds27. According to Kahkonen (1999), phenolic compounds have antioxidant activity due to their reducing properties. Flavonoids can act as antioxidants by scavenging free radicals by donating hydrogen atoms to these radicals28. In general, the ability of flavonoids to scavenge radicals depends on the substitution of hydroxy groups and the stabilization ability of phenolic radicals through hydrogen bonding or through electron delocalization. In addition, flavonoid phenoxy radicals are stabilized by delocalizing unpaired electrons around the aromatic ring. Stabilization of flavonoid phenoxy radicals (reactive oxygen) will reduce the propagation (propagation) rate of the auto-oxidation chain [29].

CONCLUSION

Based on the results of research conducted, it can be concluded that the specific and non-specific standardization of simplicia are oval-elliptical shape, tapered tip and base of leaves, pinnate bone arrangement, serrated and rough leaf edges, smooth haired the underside of leaves, 14-24 cm long, 6-11 cm wide, green in color, characteristic smell, and sour taste, water content <10%, water soluble essence content 16.93%, ethanol soluble essence content 15.37%, total ash content 2.37%, acid insoluble ash content 0.43%, shrinkage content drying 7.47%, specific gravity is 0.99, residual solvent content<1%, Pb content<10 mg/l, Cd content<0.3 mg/l, Cu content<150 mg/l, bacterial contamination ≤ 10,000 colonies/g. Based on the total phenolic content of the ethanol extract of *gagatan harimau* leaves, the total phenolic was 207.6695±0.2056 mg GAE/g, while the total flavonoid obtained was 23.2883±0.0556 mg QE/g. Based on the antioxidant test, it was concluded that ethanol extract of *gagatan harimau* leaves has a very strong antioxidant activity with an IC50 value of 34.79 µg/ml.

FUNDING

The authors are grateful to the talenta program of the Institute of Community Service/Services to the Universitas Sumatera Utara for providing research grants for lecturers of basic research programs so that the authors can carry out research well.

AUTHORS CONTRIBUTIONS

Conceptualization: CS, LRN; table Work: P. A. T; Supervision: CS, SNR, HC; Revisions: CS, LRN; Writing and Editing: CS, VI, SES, PAT; Proofreading: CS, SNR, HC.

CONFLICT OF INTERESTS

There is no conflict of interest in writing this article.

REFERENCES

1. Tiwari P. Phenolics and flavonoids and antioxidant potential of Balarishta prepared by traditional and modern methods. Asian J Pharm Ana. 2014;4(1):2231.
2. Ashfaq MH, Siddique A, Shahid S. Antioxidant activity of cinnamon zeylanicum: (A Review). Asian J Pharm Res. 2021;11(2):106-16. doi: 10.52711/2231-5691.2021.00021.
2. Samal PK. Antioxidant activity of strobilanthes asperrimus in albino rats. Asian J Pharm. 2013;3(2):2231.
3. Yun ZF, Yang S, Wu. Free radicals, antioxidants and nutrition. Nutrition. 2022;18(10):872-9.
4. Saha D, Tamrakar A. Xenobiotics, oxidative stress, free radicals vs. antioxidants: dance of death to heaven's life. Asian J Res Pharm Sci. 2011;1(2):36.
5. Thakur R. Study of antioxidant, antibacterial and anti-inflammatory activity of Cinnamon (Cinamomum Tamala), ginger (Zingiber officinale) and turmeric (curcuma longa). Am J Life Sci. 2013;1(6):273. doi: 10.11648/j.ajls.20130106.16.
6. Muthukumaran P, Salomi S, Umamaheshwari R. *In vitro* antioxidant activity of Premna serratifolia Linn. Asian J Res Pharm Sci. 2013;3(1):15.
7. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. Afr J Microbiol Res. 2009;3(13):981.
8. Silaban EE, Yunus A, Ridwanti B. Tingkat kekuatan antioksidan dan kesukaan masyarakat terhadap the daun gaharu (Aquilaria malaccensis Lamk) berdasarkan pohon induksi dan non-induksi. Sci J. 2015;4(2)1-11.
9. Tita J, Jahro IS, Sari SA, Rukayadi Y. Phytochemical, antibacterial, antioxidant and anticancer activity study of m. candidum leaf acetone extract. J Chem Environ. 2020;24(1):2278.
10. Rangasamy P, Hansiya VS, Maheswari PU, Suman T, Geetha N. Phytochemical analysis and evaluation of *in vitro* antioxidant and anti-urolithiatic potential of various fractions of Clitoria ternatea L. blue flowered leaves. Asian Jour Pharmac Anal. 2019;9(2):67-76. doi: 10.5958/2231-5675.2019.00014.0.
11. Wasnis NZ, Ilyas S, Hutahaean S, Silaban R, Situmorang PC. Effect of vitis gracilis Wall (*gagatan harimau*) in the recovery of gastrocnemius muscle cells and cytochrome c expression of Mus musculus. J Pharm Pharmacogn Res. 2022;10(2):303-9. doi: 10.56499/jppres21.1208_10.2.303.
12. Vakhariya RR, Talokar S, Dhole AR, Mohite SK, Magdum CS. Comparative standardization study of two marketed shatavari churna formulation. Asian Jour Pharmac Anal. 2016;6(1);1-6. doi: 10.5958/2231-5675.2016.00001.6.
13. Department of Health Republic Indonesia. General Standard parameters of medicinal plant extracts. Jakarta; 2000. p. 9-11.
14. Department of Health Republic Indonesia. Indonesian medical materials. Jakarta; 1995. p. 299-304, 321-5, 333-5.
15. Department of Health Republic Indonesia. Indonesian herbal pharmacopoeia. 2nd ed. Jakarta; 2017. p. 311.
16. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants. 2019;8(4):96. doi: 10.3390/plants8040096.

17. Stanojevic L, Stankovic M, Nikolic V, Nikolic L, Ristic D, Canadanovic Brunet JC. Antioxidant activity and total phenolic and flavonoid contents of Hieracium pilosella L. extracts. Sensors (Basel). 2009;9(7):5702-14. doi: 10.3390/s90705702, PMID 22346723.
18. Rasyid R, Oktavia Y, Ismet F, Rivai H. Characterization of simplicia and ethanol extracts of Bark of Asam Kandis (Garcinia cowa Roxb). J Pharm Sci Med. 2018;3(2):1-9.
19. Sutomo S, Lestari HD, Arnida A, Sriyono A. Simplicia and extracts standardization from jualing leaves (Micromelum minutum Wight & Arn.) from South Kalimantan. Borneo J Pharm. 2019;2(2):55-62. doi: 10.33084/bjop.v2i2.898.
20. Adie GU, Adekunle A. Evaluation of potentially toxic metal contamination of local medicinal plants and extracts sold in Ibadan, Nigeria. J Health Pollut. 2017;7(14):23-9. doi: 10.5696/2156-9614-7.14.23, PMID 30524819.
21. Jaiswal S, Chavhan SA, Shinde SA, Wawge NK. New tools for herbal drug standardization. Asian J Res Pharm Sci. 2018;8(3):161-9. doi: 10.5958/2231-5659.2018.00029.2.
22. Matic P, Sabljic M, Jakobek L. Evaluation of the antifungal activity of propolis extracts from stingless bees on phytopathogenic fungi. J AOAC Int. 2017;100:6.
23. Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: a critical evaluation. LWT. 2021;150:111932. doi: 10.1016/j.lwt.2021.111932.
24. Vijayabhaskar K, Venkateshwarlu G, Bhaskar J, Srisailam K, Swapna M. Antioxidant and hepatoprotective effects of the methanol extract of the flowers of Tamarindus indica. Asian J Pharm Technol. 2011;1:3.
25. Molyneux PJ. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Sci Technol. 2004;26(2):211-9.
26. Roy A, Bhoumik D, Sahu RK, Dwivedi J. Phytochemical screening and antioxidant activity of sesbania grandiflora leaves extracts. Asian J Res Pharm Sci. 2014;4(1):16-21.
27. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem. 1999;47(10):3954-62. doi: 10.1021/jf990146l, PMID 10552749.
28. Marios C, Christodoulou CJ, Golnaz H. Spectrophotometric methods for measurement of antioxidant activity in food and pharmaceuticals. J Food Sci Technol. 2022;11:2213.