Microscopic characteristics, total phenolic content and antioxidant activity of *Zingiber nudicarpum*

Nguyen Dinh Quynh Phu[1*], Doan Quoc Tuan[1], Huynh Van Quynh[1]

(1) Hue University of Medicine and Pharmacy, Hue University

Abstract

Background: *Zingiber* Mill. is one of the most diverse genera in Vietnam with 36 recorded species, many of which have been used as traditional medicine to treat several ailments. Reports of studies on *Z. nudicarpum* D. Fang are relatively scarce. **Objectives:** This study aimed to determine the microscopic characteristics, total phenolic content and antioxidant activity of *Z. nudicarpum*. **Materials and methods**: *Z. nudicarpum* was collected in Phong Dien district, Thua Thien Hue province. Anatomic features and powder characteristics were determined by the microscopic methods. The Folin-Ciocalteau method and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay were used to analysis the total phenolic content (TPC) and antioxidant potential, respectively. **Results:** The microscopic characteristics of the leaves and roots of *Z. nudicarpum* have been reported. The ethanol extract from the aerial part of *Z. nudicarpum* exhibited a notable amount of phenolic content and antioxidant activity than the underground part extract. The total phenolic content in the aerial and underground parts extract were 373.41 ± 1.50 mg GAE/g extract and 61.27 ± 1.65 mg GAE/g extract, respectively. The highest DPPH radical scavenging effect was observed in the aerial part with IC_{50} value of 4.86 ± 0.08 µg/mL, while it was not found in the extract from the underground part (IC_{50} > 500 µg/mL). **Conclusion**: This is the first report on the microscopic features, total phenolic content and antioxidant capacity of *Z. nudicarpum*.

Keywords: *Zingiber nudicarpum, microscopic characteristics, phenolic, DPPH.*

1. BACKGROUND

Zingiber Mill. is the third largest genus of the Zingiberaceae family with over 140 species that are extensively distributed in Asia, South America and Africa. In particular, Southern China and the Indochina peninsula are considered representative biodiversity centers for this genus. Species in the *Zingiber* genus have been used for ethnomedicine, food, and spices in many countries. Recent research has identified a wide variety of chemical components from *Zingiber* plants, including volatile oils, organic acids, flavonoids, terpenoids, etc… Modern pharmacological studies have demonstrated that they possessed enormous pharmacological applications such as antimicrobial, antioxidant, anti-obesity, anti-inflammatory, hypoglycemic, neuroprotective, cardiovascular protective and anti-tumor effects [1].

There are currently at least 36 species of *Zingiber* known to exist in Vietnam. Numerous species in this genus have been widely used as medicinal plants in folk and traditional medicine, as spices, and as a source of raw material for the extraction of essential oil [2]. *Z. nudicarpum* D. Fang has been considered an endemic species in southern China. Recently, this species was discovered in central Vietnam and has been added to Vietnam's flora. *Z. nudicarpum* has been found in Nghe An, Quang Binh, Thua Thien Hue, Quang Nam and Quang Ngai provinces [3]. Literature review showed that studies on this plant mostly focused on the chemical composition of essential oil [4]. In this study, the microscopic characteristics, total phenolic content and DPPH radical scavenging effect *Z. nudicarpum* were investigated.

2. MATERIALS AND METHODS

2.1. Materials

The plant of *Zingiber nudicarpum* D. Fang (Zingiberaceae) (Figure 1) was collected in Phong Dien district, Thua Thien Hue province in July 2023 and identified by Dr. Anh Tuan Le (Mientrung Institute for Scientific Research, Vietnam National Museum of Nature, VAST, Vietnam). Voucher specimen (PD-02) has been deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam.

Corresponding Author: Nguyen Dinh Quynh Phu. Email: ndqphu@huemed-univ.edu.vn
DOI: 10.34071/jmp.2024.6.11

Received: 20/3/2024; Accepted: 10/10/2024; Published: 25/12/2024

Figure 1. Image of *Zingiber nudicarpum*
A: Plant, B: Leaves, C: Inflorescences, D, E: Flower

2.2. Methods

2.2.1. Identification of microscopic characteristics

Anatomical character: Fresh leaves and root were cut into thin slices and soaked in 5% sodium hypochlorite for approximately 30 minutes and washed with water. Sections were submerged in 1% acetic acid about 5 minutes before being washed with water. After that, the pieces were colored with methylene blue and carmine red solution at the appropriate time, and they were repeatedly cleaned with water. The last sections were put on a slide with a few drops of 10% glycerol and observed under the microscope (Eclipse E100, Nikon, Japan) and photographed with an attached camera (Nikon, D5100) [5].

Powder character: The dried aerial and underground parts of the plant were ground into a powder and put through a 0.125-mesh hand sieve. Slides are prepared and observed under the microscope (Eclipse E100, Nikon, Japan) and the images were taken (Nikon, D5100) [5].

2.2.2. Extraction

The aerial and underground parts of *Z. nudicarpum* (10.0 g, each sample) were dried and ground to afford a fine powder. From each sample, the material was macerated with 100 mL ethanol (EtOH) at room temperature for 24 hours and shaken intermittently. The mixture was then filtered through cotton. The process was repeated twice and all filtrates were combined together then evaporated under reduced pressure using a rotary evaporator to obtain the EtOH extracts. These dried extracts were stored at a refrigerator for the following experiments.

2.2.3. Determination of total phenolic content

The total phenolic content (TPC) of EtOH extracts were estimated by the Folin-Ciocalteu method with slight modifications [6]. A 0.2 mL aliquot from each extract was mixed to 0.8 mL distilled water and 1.0 mL of 10% Folin-Ciocalteu reagent and shaken. After 5 minutes, 2.5 mL of 7.5% Na_2CO_3 was added and allowed to react in dark condition at room temperature in 30 minutes. The absorbance of solution was measured at 760 nm by a UV-Vis spectrophotometer. Quantification was done based on a calibration curve of gallic acid. The results was expressed as mg of gallic acid equivalents (mg GAE) per gram of extract using following formula:

$$TPC = \frac{C1 \times V \times k}{m}$$

where TPC = total phenolic content in mg GAE/g extract, C1 = the concentration of gallic acid established from the calibration in mg/mL, V = the

initial volume of the sample test solution in mL, k is the dilution factor and m is the weight of the plant extract in g.

2.2.4. Determination of antioxidant activity

The antioxidant activity was evaluated using the DPPH assay with a few minor modifications [7]. In brief, 2 mL of test sample solution at different concentrations were mixed with 2 mL of 0.135 mM DPPH. The reaction mixture was vortexed thoroughly and then incubated in the dark at room temperature in 30 minutes. Thereafter, the absorbance was read at 517 nm. Quercetin was used as positive control following same procedure as described above. The percentage DPPH radical scavenging ability of extracts were calculated and expressed in IC_{50} value, which is the concentration at which the sample removes 50% of DPPH free radicals:

$$\text{DPPH free radical scavenging activity (\%)} = [(A_c - A_s)/A_c] \times 100$$

where A_c is the absorbance of the control solution containing all reagents except test samples and A_s is the absorbance of the DPPH solution containing plant extract or reference standard.

2.2.5. Statistical analysis

All measurements were performed in triplicates and analyzed by using Microsoft Excel program. Experimental data were expressed as mean *± standard deviation (SD)* of three replicates.

3. RESULTS

3.1. Microscopic characteristics

3.1.1. Anatomy structure

Leaf midrib [Fig. 2A, 2B]: In transverse section, the midrib was concave on adaxial surface and convex on the abaxial surface. The upper and lower epidermis (B1, B6) consisted of a layer of rectangular cells and arranged adjacently with approximately the same size. The parenchyma (B2) comprised of many layers of polygonal cells, which were different sizes and thin-walled. These cells were arranged randomly with many intracellular spaces. There were many large and small vascular bundles. The large bundle (B4) was next to the lower epidermis, while the small bundle (B3) was located in the middle of the midrib. Large bundles were surrounded by sclerenchyma cells (B5).

Leaf blade [Fig. 2C, 2D]: The structure of the upper and lower epidermis (C1, D1 and C4, D5) was comparable to that of the leaf midrib epidermis. The size of the upper epidermis cells was bigger than those of the lower epidermis. The palisade cells (C2, D2) contained an abundance of chloroplasts. Vascular bundles (D3) were surrounded by a parenchymal sheath composed of large cells (C3, D4).

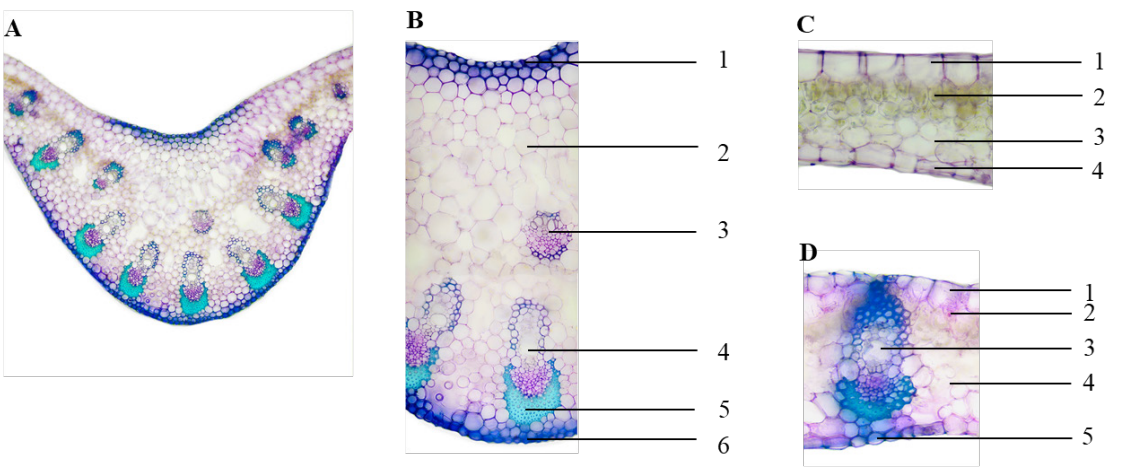

Figure 2. Microscopic characteristics of leaf cross-section of *Z. nudicarpum*
A, B: *Leaf midrib (1. Upper epidermis, 2. Parenchyma, 3. Small bundle of phloem-xylem, 4. Large bundle of phloem-xylem, 5. Sclerenchyma, 6. Lower epidermis);* ***C:*** *Leaf blade (1. Upper epidermis, 2. Palisade parenchyma, 3. Parenchyma, 4. Lower epidermis);* ***D:*** *Leaf blade (1. Upper epidermis, 2. Palisade parenchyma, 3. Bundle of phloem-xylem, 4. Parenchyma, 5. Lower epidermis)*

Root [Fig. 3]: In transverse section of the root of *Z. nudicarpum*, it was oval or broadly elliptical in outline. The cortical area occupied more than half of the microsurgery radius. The layers from outer to inner showed the following features: The piliferous layer (B1) was single layered, with polygonal cells and the presence of unicellular root hairs. The suberoid layer (B2) was composed of 2-3 layers of polygonal, oval or irregularly shaped cells. The cortical parenchyma (B3) comprised several layers

of parenchyma, with polygonal, unequal-sized, and thin-walled cells. The oil cells were subrotund and contained yellow or translucent oil droplets (C1). Some of the cortical cells contained many starch grains (B4, D1). The endodermis (B5) formed a casparian strip with a thick U-shaped layer of cells. The pericycle (B6) was a single layer of thin-walled cells located underneath the endodermis. The vascular tissues consisted of many patches of phloem (B7) and xylem (B8) arranged radially. At the centre of the internal structure was the pith parenchyma (B9) made from thin-walled and polygonal cells.

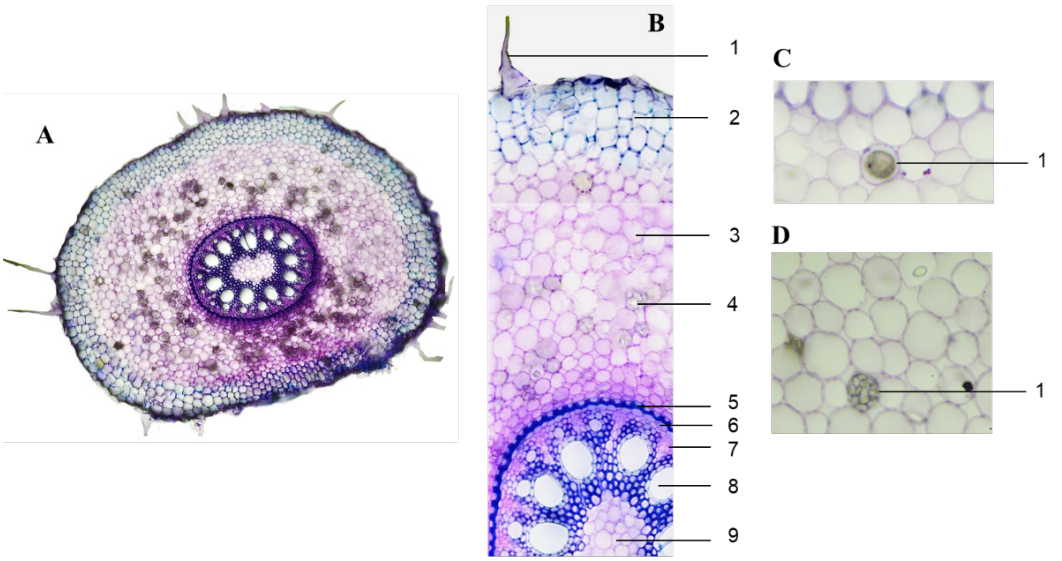

Figure 3. Microscopic characteristics of root cross-section of *Z. nudicarpum*
B1. Piliferous layer, B2. Suberoid, B3. Cortical parenchyma, B4. Starch grain, B5. Endodermis, B6. Pericycle, B7. Phloem, B8. Xylem, B9. Pith parenchyma, C1. Oil cell, D1. Starch grains

3.1.2. Powder character

The aerial part [Fig. 4]: A green powder with a pleasant and aromatic odour was covered in 10% glycerol and observed under a light microscopic at 10X and 40X magnifications. The powder had several microscopic features: fragment of epidermis containing stomata (1), fragment of epidermis (2), fragment of cork (3), bundle of fiber (4), bundle of fiber containing vessel (5) and fragment of vessel (6).

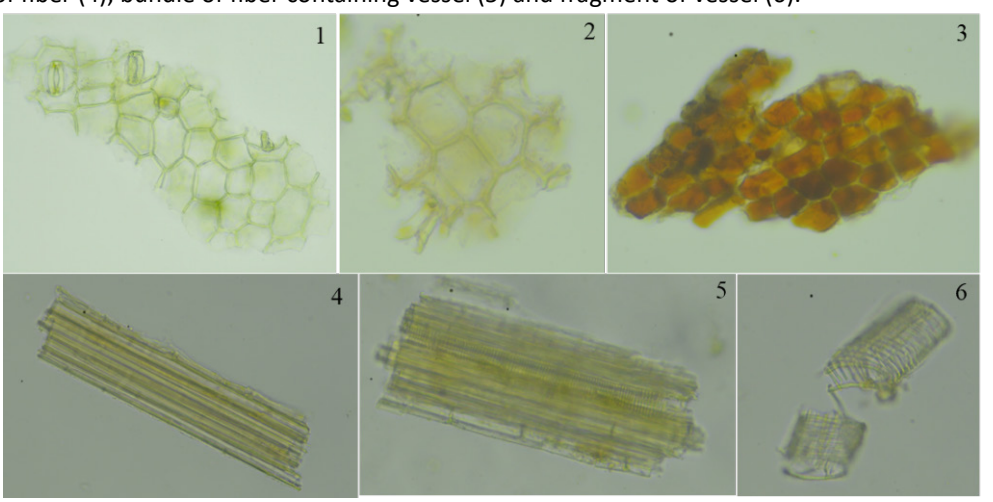

Figure 4. Microscopic features of the aerial part of *Z. nudicarpum*

The underground part [Fig. 5]: A brown-yellow powder had pleasant and aromatic odour. Under the light microscope at 10X and 40X magnifications, some microscopic characteristics were observed: fragment of cork (1), fragment of vessel (2), bundle of fiber (3), fragment of parenchyma containing starch (4), starch grains (5) and fragment of parenchyma (6).

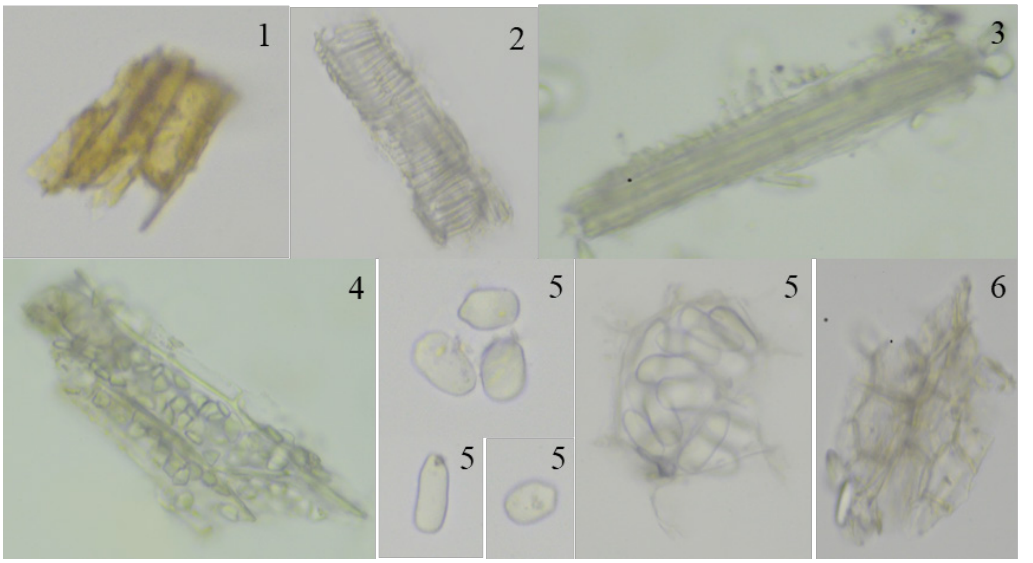

Figure 5. Microscopic features of the underground part of *Z. nudicarpum*

3.2. Determination of total phenolic content

The total phenolic content (TPC) of the samples was calculated from the regression equation of the calibration curve (*y = 3.8958x - 0.0319, R^2 = 0.9898*) and expressed in GAE as miligrams per gram of the extract. The TPC of ethanol extracts from *Z. nudicarpum* was shown in Table 1. The results indicated that the TPC of ethanol extract from the aerial and the underground parts of *Z. nudicarpum* were 373.41 ± 1.50 mg GAE/g extract and 61.27 ± 1.65 mg GAE/g extract, respectively. According to the data, the TPC in the aerial part was found significantly higher as compared to the underground part.

Table 1. Total phenolic content and antioxidant activity of *Z. nudicarpum*

No.	Sample	Total phenolic content (TPC ± SD (mg GAE/g extract))	Antioxidant activity (IC_{50} ± SD (µg/mL))
1	**Aerial part**	373.41 ± 1.50	4.86 ± 0.08
2	**Underground part**	61.27 ± 1.65	> 500
	Quercetin		3.38 ± 0.09

3.3. Evaluation of antioxidant activity

The antioxidant potential of *Z. nudicarpum* on DPPH assay was also summarized in Table 1. The findings revealed that the ethanol extract of the aerial part demonstrated remarkable *in vitro* DPPH radical scavenging activity with IC_{50} value of 4.86 ± 0.08 µg/mL in comparison with quercetin used as positive control (IC_{50} = 3.38 ± 0.09 µg/mL). In contrast, the extract of the underground part was not able to remove 50% of DPPH free radicals at a concentration of 500 µg/mL.

4. DISCUSSION

Studies on the microscopic characteristics of *Zingiber* species are relatively few and focus mainly on morphological descriptions. Microscopic results showed that the anatomy structure of leaf and root of *Z. nudicarpum* have typical characteristics such as many phloem-xylem vasculars in the midrib and the casparian strip, as well as pericycle in the root, besides the usual features such as epidermis, parenchyma, sclerenchyma (in leaf) and piliferous layer, suberoid, parenchyma, starch grains (in root). The anatomical structure of root of *Z. nudicarpum* is found to be quite similar to *Z. officinale*. The powder characters were also comparable, except for the starch grains in the underground part. Starch granules in *Z. nudicarpum* were oblong or rounded, while they were circular or oval in *Z. officinale* [8]. The present study provides a thorough detail of the individual microcharacteristics of *Z. nudicarpum* for the first time. Moreover, it can be used for determine the identification and standardization of medicine and contribute to the efficiency of the microscopic

analysis of *Zingiber* genus.

Based on the results of the conducted research, the total phenolic content of the ethanol extract of the aerial part of *Z. nudicarpum* was noticeably high level with TPC value of 373.41 ± 1.50 mg GAE/g extract. The total phenolic content in *Zingiber* species have been reported in previous studies. The TPC of the water extract of rhizome of *Z. cassumunar* and ethanol extract of rhizome of *Z. officinale* were 42.00 ± 0.45 mg GAE/g extract and 48.56 ± 1.64 mg GAE/g extract, respectively [9, 10]. The total phenolic content ranged from 24.83 ± 0.31 to 71.45 ± 1.45 mg GAE/g extract of hexane, dichloromethane, acetone, ethanol, methanol, 75% ethanol and 50% ethanol extracts of rhizomes of *Z. montanum* [11]. Therefore, it is noticeable that *Z. nudicarpum* has a substantially greater TPC than other *Zingiber* species that have been studied. Phenolic compounds have high pharmaceutical values in preventing and treating for a variety of oxidative stress-related diseases, including cardiovascular, cancer, age-related disorders, diabetes mellitus and neurodegenerative diseases [12]. Thus, *Z. nudicarpum* act as important source of polyphenol with potential therapeutic effects that may be useful for development of nutraceuticals and medicine.

In the DPPH assay, when compared to the underground part, the ethanol extract from the aerial part of *Z. nudicarpum* revealed much stronger activity with IC_{50} value of 4.86 ± 0.08 µg/mL. This might be due to the greater total phenolic content in the extract. Previous studies have already shown that *Zingiber* species have certain antioxidant activity. The methanol extract of rhizome of *Z. zerumbet* was found to possess the antioxidant properties with an IC_{50} value of 305.2 ± 0.243 µg/mL in the DPPH test [13]. The ethanol crude extract and fraction extracts (*n*-hexane, ethyl acetate, *n*-butanol) of the root of *Z. officinale* have DPPH radical scavenging activity with IC_{50} values of 20.18 ± 1.12, 34.91 ± 2.14, 8.89 ± 1.37 and 10.11 ± 1.53 µg/mL, respectively [14]. The effect of the rhizome ethanol extract from 10 *Zingiber* species (*Z. montanum*, *Z. ottensii*, *Z. rubens*, *Z. cornubracteatum*, *Zingiber 'Phlai-chompoo'*, *Z. zerumbet*, *Z. officinale*, *Z. bisectum*, *Z. spectabile*, *Z. barbatum*) on DPPH free radical with IC_{50} values ranged from 4.26 to 60.80 mg/mL, while L-ascorbic acid used as a positive control had an IC_{50} value of 0.024 mg/mL [15]. Hence, it is evident that the aerial part of *Z. nudicarpum* could be potential candidates for natural antioxidants.

5. CONCLUSION

The microscopic features, total phenolic content and antioxidant activity of *Z. nudicarpum* were reported for the first time. According to the study's findings, the aerial part of *Z. nudicarpum* possessed a significant content of phenolic component and antioxidant activity, which could be considered as natural sources for maintaining good health. Further research is required to identify the potential bioactive compounds present in *Z. nudicarpum*.

ACKNOWLEDGMENT

This work was supported by grants from Hue University of Medicine and Pharmacy, Hue University (ID No. 08SV/23).

REFERENCES

1. Miao D, Xuan Y, Shurui R, Zhixing Q, Fenglian L. Plants of the genus Zingiber: A review of their ethnomedicine. Phytochemistry and Pharmacology. Molecules. 2022; 27(2826):1-25.

2. Le TH, Nguyen TC, Trinh TH, Ly NS, Nguyen HH, Isiaka AO et al. Essential oils of Zingiber species from Vietnam: Chemical compositions and biological activities. Plants. 2020; 9(1269):37 pages.

3. Ly NS, Dang VS, Do ĐG, Tran TT, Do NĐ, Nguyen DH. Zingiber nudicarpum D. Fang (Zingiberaceae), a newly recorded species for Vietnam. Bioscience Discovery. 2017; 8(1):01-05.

4. Nguyen DH, Le TH, Ly NS, Tran M, Isiaka AO. Constituents of essential oil of Zingiber nudicarpum from Vietnam. Chemistry of Natural Compounds. 2019; 55(2):361-363.

5. Nguyen VT. Testing medicinal herbs by microscopic method (in Vietnamese). Science and Technology Publishing House. Hanoi. 2003.

6. Milan V, Maitreya B. Qualitative analysis, total phenol content (TPC) and total tannin content (TTC) by using different solvent for flower of Butea monosperma (Lam.) Taub. collected from Saurashtra region. Journal of Pharmacognosy and Phytochemistry. 2019; 8(3):2902-06.

7. Asekun OT, Okoh SO, Familoni OB, Afolayan AJ. Chemical profiles and antioxidant activity of essential oils extracted from the leaf and stem of Parkia biglobosa (Jacq) Benth. Research Journal of Medicinal plant. 2013; 7(2):82-91.

8. Yin YA. Microscopical characters, phytochemical and FTIR studies on rhizome of Zingiber officinale Rosc. (Gyin). University of Mandalay, Research Journal. 2020; 11:10-20.

9. Suriyan S, Khemjira J, Surachai T, Nakuntwalai W, Warachate K. In vitro anticoagulant and antioxidant activities of Prasaplai recipe and Zingiber cassumunar Roxb. extracts. International Journal of Applied Pharmaceutics. 2019; 11(5):26-30.

10. Erdoğan Ü, Sabri E. Phytochemical profile and antioxidant activities of Zingiber officinale (Ginger) and

Curcuma longa L. (Turmeric) rhizomes. Bilge International Journal of Science and Technology Research. 2021; 5(special issue):1-6.

11. Parichat T, Kantika R, Nattharawadi R, Sonchai I, Wankuson C. Effect of extraction solvents on antioxidant and antibacterial activity of Zingiber montanum rhizomes. ASEAN Journal of Scientific and Technological Reports. 2023; 26(3):1-9.

12. Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB. Resources and biological activities of natural polyphenols. Nutrients. 2014; 6:6020-6047.

13. Aita RS, Santosh KR. Phytochemical screening, physico-chemical analysis and antioxidant activity of some ethnomedicinal plants from Sikkim Himalaya. Indian Journal of Natural Products and Resources. 2018; 9(3):235-243.

14. Bui TT, Dang KT, Nguyen TKT, Nguyen TH. Antioxidant and acetylcholinesterase inhibitory activities of gingerroot (Zingiber officinale Roscoe) extract. Journal of Complementary and Integrative Medicine. 2017; 20160116. 7 pages.

15. Vipada K., Yingyong P. Antioxidant activity and selected chemical components of 10 Zingiber spp. in Thailand. Journal of Developments in Sustainable Agriculture. 2012; 7:89-96.