

Phytochemical Analysis and Antioxidant Activity of *Zanthoxylum acanthopodium* DC. Essential Oils

Eldiza Puji Rahmi, Feda Anisah Makkiyah¹, Abdi Wira Septama², Aprilia Nur Tasfiyati², Rizna Triana Dewi²

Pharmacy Program, Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jakarta, ¹Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jakarta, Jakarta, ²Research Centre for Pharmaceutical Ingredients and Traditional Medicine, The National Research and Innovation Agency (BRIN), Cibinong, Indonesia

Abstract

Introduction: Andaliman (*Zanthoxylum acanthopodium* DC), which belongs to the *Rutaceae* family, is a plant widely used as a spice in traditional Batak cuisine around the Toba region-Samosir, North Sumatra. The Batak people also use *Z. acanthopodium* to treat digestive problems, reduce pain, relieve diarrhea, as an aromatic ingredient, tonic, and increase appetite. **Methods:** This investigation analyzed the phytochemical content and antioxidant activity of *Z. acanthopodium* fruit essential oil. The phytochemical content was studied using total phenol content (TPC), total flavonoid content (TFC), and gas chromatography mass spectrometry, and the essential oil's antioxidant activities were evaluated *in vitro* against DPPH, FRAP, and ABTS. **Results:** The results showed that the principal constituents of *Z. acanthopodium* fruit essential oil were D-Limonene, Geranyl acetate, -pinene, and α -Ocimene. In addition, the TPC and TFC of the essential oil were relatively low, at 3.81 mg GAE/g sample and 11.42 mg QE/g sample, respectively. According to the DPPH assay results, the essential oil is effective as an antioxidant with an IC₅₀ value of 28.37 μ g/mL. However, the FRAP and ABTS assays did not showed antioxidant activity. The different results from antioxidant assays (DPPH, FRAP, and ABTS) may be due to the various time of conducting the assay. The DPPH assay was done immediately after the essential oil extracting process, while the FRAP and ABTS assays were done 3 months later. **Conclusion:** The results indicated that the essential oil has high antioxidant properties and has the potential to be further studied for its pharmacological properties.

Keywords: Antioxidant, essential oil, *Zanthoxylum acanthopodium* DC

INTRODUCTION

In recent years, it has become increasingly evident that herbal remedies can effectively cure inflammatory and oxidant-related diseases. Oxidative stress contributes significantly to the development of age-related and degenerative diseases, such as cancer, diabetes, cardiovascular disease, atherosclerosis, and neurodegenerative diseases. The body produces enzymatic and nonenzymatic antioxidant defenses, whereas other antioxidants are received through the diet. Together, these defenses transform free radicals into nonreactive forms. Among these, the potential antioxidant properties of plant essential oils have been explored. Plant essential oils contain these capacities, which can be related to the existence of terpenes and phenolic components that contribute to free radical scavenging action.

Terpenes are the principal components of the essential oils derived from medicinal plants, most frequently aromatic

species belonging to the *Rutaceae* family. Terpenes are natural antioxidants with a significant potential for use as dietary supplement additions to avoid oxidative stress that contributes to developing degenerative diseases. There are more than 160 genera containing flowering species within the *Rutaceae* family. *Citrus*, which includes oranges (*Citrus sinensis*), lemons (*Citrus limon*), grapefruit (*Citrus paradisi*), and limes (mostly *Citrus aurantifolia*), is the family's most economically significant genus. *Zanthoxylum*, often known as

Address for correspondence: Dr. Eldiza Puji Rahmi,
Pharmacy Program, Faculty of Medicine, Universitas Pembangunan
Nasional Veteran Jakarta, Jakarta, Indonesia.
E-mail: eldizapr@upnvj.ac.id

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Fagara, and *Agathosma* are a couple of the other economically prominent genera in this family.

When biological resources are employed to resolve health-related issues, it is hoped that a scientific approach will maximize the usage and application of local knowledge for the greater good of the community. Andaliman (*Zanthoxylum acanthopodium* DC), also known as Batak pepper, is a spice utilized extensively by the North Sumatran population. This herb offers traditional health benefits, such as pain alleviation and enhanced stamina.

Z. acanthopodium has various secondary metabolites, including alkaloids, glycosides, carbohydrates, tannin, phenol, flavonoid, steroid, oil, and lipid.^[1] In addition, the saponins, alkaloids, and steroids found in *Z. acanthopodium* leaves are antibacterial against *Staphylococcus aureus*.^[2] Moreover, it is well-documented that *Z. acanthopodium*'s petroleum extract has low antioxidant strength but potent cytotoxic activity.

Exploring and developing the use of *Z. acanthopodium* as a health product is motivated by the fact that *Z. acanthopodium* has long been recognized for its health advantages as a food ingredient (spice). As a nutraceutical for degenerative disorders, *Z. acanthopodium* has not been the focus of substantial research. This research can therefore serve as a foundation for promoting *Z. acanthopodium* as a nutraceutical raw material that can be consumed daily, prevents degenerative diseases, is easily sourced from locals, and enhances local Indonesian potential. *Z. acanthopodium* extract will be examined to assess its chemical composition and antioxidant capacity. Future research will continue to uncover and improve effective neurodegenerative disease nutraceuticals.

LITERATURE REVIEW

Degenerative diseases, such as diabetes mellitus, and neurodegenerative diseases, such as Alzheimer's and Parkinson's, are chronic diseases that have a significant influence on an individual's life quality. As a physical activity, lifestyle, and diet diminish, the prevalence of degenerative diseases increases. Degenerative diseases are associated with a high mortality rate and can significantly affect an individual's quality of life and productivity.^[3]

Antioxidants (endogenous antioxidants) are one of the body's natural defenses against disease, as they can neutralize free radicals that attack body cells and restore injured body cells.^[4] More free radicals are produced as the severity of the disease increases, resulting in a high level of oxidative stress within the body. Consuming antioxidants from sources outside the body, also known as exogenous antioxidants, such as food and medication, is necessary to battle and reduce the effects of oxidative stress.

The terms "nutrition" and "pharmaceutical" are where the phrase "nutraceutical" comes from (Nutraceutical). Products that are considered to be nutraceuticals are chemical substances that offer protection against chronic diseases, delay the aging

process, and enhance life expectancy. Nutraceuticals are currently garnering a tremendous amount of attention as a result of the nutritional potential, safety, and medicinal impacts that they possess.^[5]

Multiple studies have shown that nutraceuticals can prevent and treat numerous degenerative diseases, such as diabetes, atherosclerosis, osteoporosis, cardiovascular disease, cancer, and neurological diseases. Antioxidant characteristics are present in the majority of nutraceuticals. The scientific study has demonstrated that some popular nutraceuticals, including ginseng, Echinacea, green tea, glucosamine, omega-3, lutein, folic acid, and fish oil, have therapeutic properties. When treating degenerative illnesses, the presence of antioxidants in nutraceuticals is vital.^[6]

Indonesia is the second-richest country in the world in terms of biodiversity, after only Brazil. The country is home to around 30,000 plant species, including 940 medicinal herbs. The rise in the consumption of nutraceutical goods in Indonesia can be attributed, in part, to rising health-care expenditures and a heightened public awareness of the significance of maintaining health through healthy consumption patterns and a return to nature.^[7]

Z. acanthopodium DC is one of the natural substances that contain antioxidants. Several studies have been conducted on the potential of *Z. acanthopodium*, including the following: *Z. acanthopodium* contains secondary metabolites in the form of alkaloids, glycosides, carbohydrates, tannins, phenols, flavonoids, steroids, oils, and fats (Panggabean *et al.*, 2020),^[1] while *Z. acanthopodium* leaves contain saponins, alkaloids, and steroids (Batubara *et al.*, 2017).^[2] *Z. acanthopodium*'s possible antioxidant action promotes additional investigation into its ability to prevent and treat degenerative illnesses. It is possible to manufacture nutraceutical preparations with the intention that ordinary people can utilize them to prevent degenerative disorders.

RESEARCH METHOD

Extraction

The plant material was obtained in Indonesia, Toba Samosir-Tapanuli, North Sumatera. In a round flask, 25 mg fresh and washed *Z. acanthopodium* fruits were mixed with 250 mL distilled water and subjected to hydrodistillation using a Clevenger apparatus. The working temperature was 100°C, and the extraction took 3 h. To extract the essential oil, it was separated from the mixture by liquid-liquid partitioning in a separating funnel and then extracted with a micropipette.

Total flavonoid content

The aluminum chloride method was used to figure out the total flavonoid content (TFC), which was then given in terms of quercetin equivalent (mg/g extract) based on the regression equation of the calibration curve. The sample and the quercetin were weighed and dissolved in 4 ml of methanol (1000 g/ml). Standard curve measurement: A series is made of standard solutions of 10 µg/ml, 20 µg/ml, 30µg/

ml, and 50 µg/ml by pipetting the solution quercetin standards (50, 100, 150, and 250 µl) into test tubes. At the same time, sample measurements were carried out by pipetting as much as 250 µL into the tube reaction. Then treated the same as the standard. For the above reaction, 2 ml of distilled water and 150 µl of 5% NaNO₂ were added to each tube. After 5 min, 150 µl of 10% AlCl₃ was added. Six minutes later, 2 ml was added to NaOH 1M. The volume is measured to be 5 ml with the addition of distilled water.

The mixture was homogenized, and its absorbance λ 510 nm was measured with a spectrophotometer ultraviolet-visible. Then, a calibration curve is made by connecting the absorption values as the coordinates (Y) and the concentration of the standard solution as the abscissa (X). The flavonoids (C) levels obtained were equivalent to quercetin (mg/g extract).

Total phenolic content

The total phenol content (TPC) was determined with the Follin Ciocalteu method using gallic acid equivalent (GAE) as standard. Gallic acid standard solutions were prepared in concentration series of 20, 40, 60, 80, 100, and 120 ppm in methanol. Each concentration was taken as much as 0.25 mL, then 2.5 mL of 10% Follin-Ciocalteu reagent was added, mixed using a vortex for about 1 min and left for 5 min, 2.0 mL of 1 M Na₂CO₃ solution was added, and shaken for 15 min until homogeneous. Then left for 30 min at room temperature, measured the absorbance at λ is a maximum of 746 nm.

Gas chromatography mass spectrometry analysis

The chemical composition of the essential oil and significant terpene quantification was analyzed using gas chromatography mass spectrometry (GC-MS) data techniques reported by.^[8] Briefly, 1 µL of the sample was injected into an Agilent Technologies (7890B) GC coupled with a mass detector (Agilent 5977A) and equipped with an Agilent DB-5MS Ultra Inert capillary column (30 m × 250 µm, i. d. 0.25 µm, (5%-phenyl)-methylpolysiloxane). Helium gas was used as a carrier gas with a flow rate of 1 mL/min. A sample of 1 µL was injected into the GC-MS instrument with a splitless injection mode at a temperature of 250°C. The temperature in the GC oven was started at 40°C for one min, then increased by 10°C every min until it reached 300°C, where it remained for 4 min. The transfer line, MS source, and MS quadrupole temperatures were kept at 270, 230, and 150°C, respectively. GC-MS detection utilized an electron ionization system with an energy of 70 eV and a mass range of 30–600 AMU. Comparing the mass spectra of the tested material with a database from the NIST 17 library enabled the identification of the chemical ingredients (National Institute of Standards and Technology, Mass Spectra Libraries).

Antioxidant test

The antioxidant activity of *Z. acanthopodium* fruits essential oil was evaluated by several methods to reach a comprehensive evaluation.

DPPH assay

The antioxidant activity of the extract against DPPH free radicals was measured according to.^[9] The extract solution

in 2 ml of methanol was added to a solution of 0.5 ml DPPH (1 mM in methanol). The mixture was shaken and left at the room temperature for 30 min. The absorption produced was measured at a wavelength of 515 nm. Percent inhibition of the sample is calculated based on a difference in absorption between the blank and the model.

$$\text{Percentage of inhibition} = 1 - \left(\frac{As}{A0} \right) \times 100\%$$

where *As* is the absorbance of the sample; *A0* is the absorbance of the control.

ABTS assay

ABTS radical scavenging activity was measured according to the method of a previous study with some modifications.^[10] The radical of ABTS was produced by reacting 5 mL of 7mM ABTS solution with 88 µL of 140 mM K₂S₂O₈ for 16 h in the dark at room temperature. The stock solution of ABTS radical (1 mL) was mixed with 1 mL of *Z. acanthopodium* fruits essential oil (0.1% (v/v) in methanol). The mixture was incubated for 6 min in the dark. The radical scavenging activity was determined by the absorbance value at 734 nm with the inhibition percentage calculation following Equation 1.

FRAP assay

FRAP test was conducted with some modifications.^[11] FRAP reagent was generated by mixing 300mM acetate buffer (pH 3.6), 20 mM FeCl₃, and 2,4,6-tripyridyl-S-triazine (TPTZ, 10 mM in 40mM HCl) with the ratio of 10:1:1 (v/v/v). This FRAP reagent (1.2 mL) was added to the test tube containing 40 µL of the sample solution (in methanol) and allowed to stand for 30 min at 37°C. Absorbance was measured at 593 nm. FRAP radical scavenging activity was obtained from the calibration curve of Trolox and expressed as µmol of trolox equivalent per gram of extract dry weight.

Data analysis

The data obtained was seen to determine the levels of phytochemical content in the Andaliman fruits. In addition, the data analysis of antioxidant activity was to calculate the IC₅₀ value by comparing it with the control. Statistical tests were carried out using One Way ANOVA followed by the Tuckey *post hoc* test.

FINDINGS AND DISCUSSION

Oxidative stress is the primary factor in cancer, infectious diseases, cardiovascular disorders, and neurological ailments. It occurs from an imbalance between reactive species generation and antioxidant defence. Therapeutically effective pharmacological drugs may exert antioxidant characteristics in target tissues, which may be connected to their mode of action.^[12]

In this study, the hydrodistillation of the fresh fruits of *Z. acanthopodium* gave clear oil with a yield of 8% (v/w). This study showed that the essential oils of *Z. acanthopodium* fruits are composed mainly of hydrocarbon monoterpenes, oxygenated monoterpenes, and hydrocarbon sesquiterpenes [Figure 1]. The

principal constituents of *Z. acanthopodium* fruit essential oil were D-Limonene (47%), Geranyl acetate (24%), α -pinene (8%), and α -Ocimene (3.5%) [Figure 2]. Our results on *Z. acanthopodium* essential oil are consistent with other studies, identifying limonene and geranyl acetate as the most abundant constituents.^[13]

In addition, the TPC and TFC of the essential oil were relatively low, measuring 3.81 mg GAE/g of the sample and 11.42 mg QE/g of the sample, respectively. Therefore, the TPC and TFC of *Z. acanthopodium* fruit essential oil may not considerably contribute to its antioxidant activity. Using three distinct assays, DPPH, FRAP, and ABTS, the antioxidant activity of the fresh fruit essential oil of *Z. acanthopodium* was evaluated. Using the DPPH assay, the essential oils of *Z. acanthopodium*

demonstrated significant antioxidant activity in this study, with an IC_{50} value of 28.37 μ L/mL. While DPPH findings suggest that *Z. acanthopodium* essential oil possesses substantial antioxidant activity, it possesses less radical scavenging activity than the reference chemical, quercetin, with an IC_{50} value of 3.96 μ g/mL. However, neither the FRAP nor the ABTS assays revealed any antioxidant activity. At 100 μ L/mL and 1000 μ L/mL, the essential oil of *Z. acanthopodium* exhibited just 0.83 mg TEAC and 16% inhibition via the FRAP and ABTS assays, respectively. The disparate outcomes of antioxidant assays (DPPH, FRAP, and ABTS) may be attributable to the varying durations of the experiments. The DPPH assay was conducted immediately following the essential oil extraction, whereas the FRAP and ABTS assays were conducted 3 months later.

The antioxidant activity of *Z. acanthopodium* essential oil is likely owing to the high hydrocarbon monoterpenes and oxygenated monoterpene content, under which limonene and geranyl acetate are most prominent. Geranyl acetate is renowned for its powerful antioxidant capabilities due to its ability to decrease the stability of free radicals through electron-or hydrogen-donating processes.^[14] Limonene, a common monoterpene that is a substantial component of the active compound of the genus *Citrus*, has been demonstrated to possess anxiolytic, antinociceptive, antioxidant, and anti-inflammatory properties. Many research demonstrated that D-limonene provides antioxidant capabilities, prevents lipid peroxidation, and fights against free radical-induced cell damage.^[15] *Z. acanthopodium*'s antioxidant activity is enhanced by other terpenes, such as pinene and ocimene.

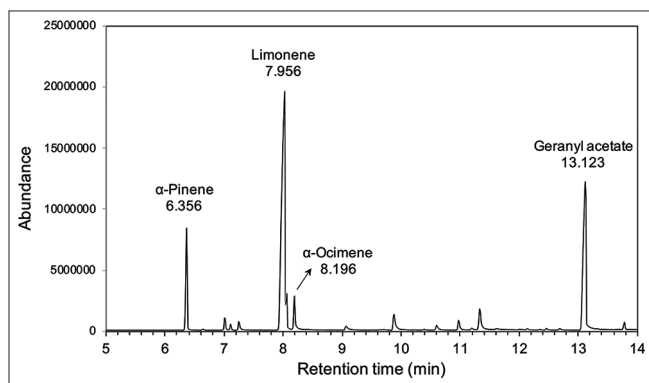


Figure 1: GC-MS chromatogram of *Z. acanthopodium* essential oil. GC-MS: Gas chromatography mass spectrometry, *Z. acanthopodium*: *Zanthoxylum acanthopodium*

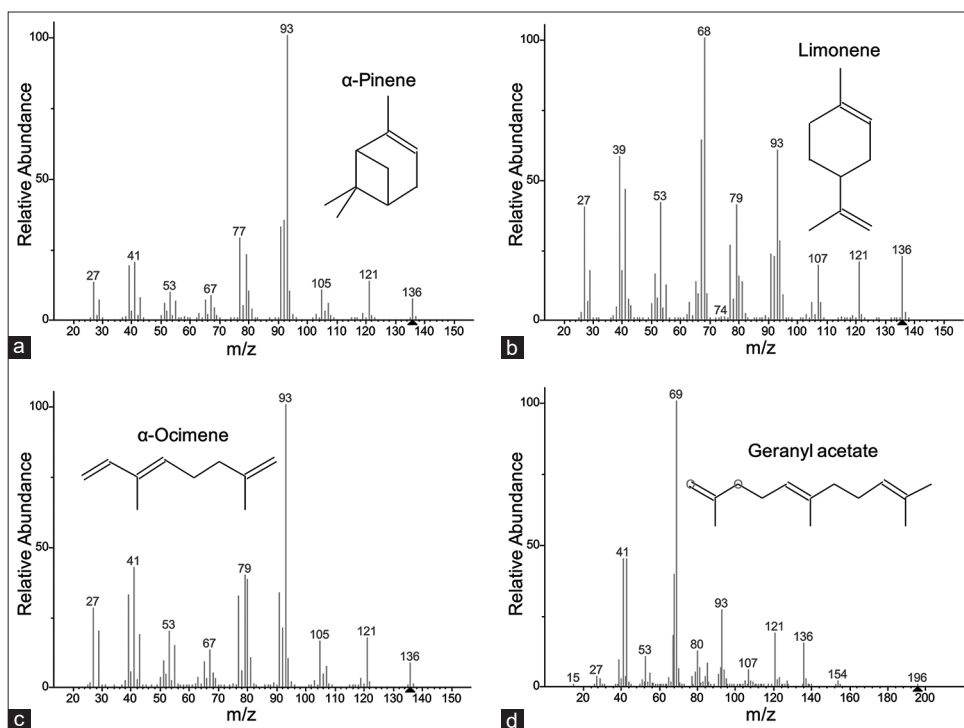


Figure 2: GC-MS fragmentation patterns of the primary compounds in *Z. acanthopodium* essential oil: (a) α -pinene, (b) limonene, (c) α -ocimene, and (d) geranyl acetate. GC-MS: Gas chromatography mass spectrometry, *Z. acanthopodium*: *Zanthoxylum acanthopodium*

CONCLUSION

Based on the results of this study, *Z. acanthopodium* essential oil is an accessible and natural source of terpenes. In addition, due to its antioxidant properties and high limonene content, as well as the presence of geranyl acetate and a series of compounds of terpenic origin, the essential oil of *Z. acanthopodium* fruits has the potential to be utilized in the development of a nutraceutical for neurodegenerative diseases.

Limitations and further research

This study has limitations because DPPH and FRAP/ABTS results did not match. The different results from antioxidant assays (DPPH, FRAP, and ABTS) may be due to the various time of conducting the assay. The DPPH assay was done immediately after the essential oil extracting process, while the FRAP and ABTS assays were done 3 months later. Hence, it is suggested that the FRAP and ABTS assays will be repeated with fresh essential oils. Moreover, we should investigate the phytochemical constituents of old and new essential oils.

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Conflicts of interest

There are no conflicts of interest.

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