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Original Article

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *ZANTHOXYLUM ACANTHOPODIUM* DC. FRUITS USING CUPRAC METHODS

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ABSTRACT

Objective: Free radicals are substances that can be harmful to our bodies if their concentrations are too high. Free radicals are neutralized by antioxidant molecules, which additionally protect cells against damage from oxidation caused by free radicals. This study aims to conduct a phytochemical analysis and quantify the antioxidant capabilities of the methanol extract of *Zanthoxylum acanthopodium* DC. fruits.

Methods: *Zanthoxylum acanthopodium* DC. fruit was extracted by reflux method using methanol. Antioxidant activity for extract was measured with CUPRAC (Cupric Ion Reducing Antioxidant Capacity) method and LC-HRMS (Liquid Chromatography High Resolution Mass Spectrometry) instrument was used for phytochemical analysis of methanol extract.

Results: The IC_{50} of methanol extract was 86.58±1.87 µg/ml. LC-HRMS analysis showed that there were 10 active compounds detected in methanol extract of *Zanthoxylum acanthopodium* DC. The content of secondary metabolite compound was group of flavonoid ("neohesperidin, isorhamnetin, astragalin, quercetin, quercetin-3β-D-glucoside, (2E)-4-Hydroxy-3,7-dimethyl-2,6-octadien-1-yl beta-D-glucopyranoside, and 5,7-Dihydroxy-2-(4-hydroxyphenyl)-6,8-bis[3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl]-4H-chromen-4-one") and phenolic (Chlorogenic acid, quinic acid).

Conclusion: The results show that the methanol extract from the fruit of *Zanthoxylum acanthopodium* DC. has potent antioxidant properties and contains several flavonoid and phenolic components.

Keywords: *Zanthoxylum acanthopodium* DC, Antioxidant, Methanol extract, Phytochemical analysis

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INTRODUCTION

Antioxidant capacity allows to contribute one or more electrons to the cell and be resistant to the harmful effects that free radicals can provide. Free radicals are reactive compounds that have fluctuating quantities of unpaired electrons that strive to liberate or steal from other molecules. These electrons allow them to damage other molecules. Antioxidants are chemicals that have the power to either entirely reverse the oxidation process or to slow its progression toward completion. The majority of chemical plants offer unusually abundant biogenic resources, which can be utilized for the discovery of novel medications hidden among a wide range of secondary metabolites. According to the recent study, more than half of all drugs that are now being tested in clinical trials around the world are natural compounds or their derivatives [1-4].

An ethnic plant from the Rutaceae family and *Zanthoxylum* genus is *Zanthoxylum acanthopodium* DC [5]. In north Sumatera *Z. acanthopodium* DC has been used as a seasoning [6] as a treatment for diarrhea and a tonic. It has also been used as an aromaticum. Indians have been treating skin diseases, including lepsory and abscesses, as well as paralysis. In north Sumatera, specifically in North Tapanuli *Z. acanthopodium* DC has been used as a spice [7-9]. A variety of compounds from *Zanthoxylum*, including volatile oils, flavonoids, steroids or triterpenoids, tannins, alkaloids, amides, lignans, phenol hydroquinones, glycosides, coumarines and terpenes [10-15].

Oxidative stress occurs when there is an imbalance between the presence of free radicals and antioxidants in the body, characterized by an excess of free radicals and a shortage of antioxidants. Oxidative damage may come from cells, tissues, and organs under conditions of oxidative stress. Additionally, oxidative stress causes of accelerated aging [16]. Antioxidants are mostly used as a main therapy or as an adjuvant therapy for diseases, as a prophylaxis against a disease, as a supplement to increase endurance and utilization as a prevention against the aging process. Fruit and vegetables have been the subject of much resarch about their antioxidant content. According to recent resarch, the antioxidant content of fruits, vegetables and seaweed can help either avoid or reduce the severity of disease [17].

The CUPRAC method is employed to quantify the antioxidant capacity against free radicals and assess its efficacy. This involves measuring the absorbance of the sample using a UV-Vis spectrophotometer at a wavelength of 450 nm. The objective of this work is to quantify the antioxidant capacity of the methanol extract obtained from Z. *acanthopodium* DC fruits using the CUPRAC method. Additionally, the phytochemical analysis was conducted using LC-HRMS (Liquid Chromatography High-Resolution Mass Spectrometry) instrument.

MATERIALS AND METHODS

Plant and chemicals material

Fresh fruits of *Z. acanthopodium* DC. were being supllied from Central Parsoburan, Toba Regency, Sumatera Utara Province, Indonesia. Ammonium acetate (Smart lab), distilled water, methanol (Smart lab), neocuproine (Aldrich), $CuCl_2.2H_2O$ (Sigma).

Preparation of methanol extract

For the purpose of extracting Z. *acanthopodium* DC. Powder, the plant material that had been dried in the air was crushed, and then methanol was used to perform a reflux extraction. Following the collection of the filtrate, it was subjected to a rotary evaporator at a lower pressure, which resulted in the production of a crude extract [3].

Free radical scavenging activity test

Preparation of CUPRAC reagent

$CuCl_2.2H_2O$ 0.01 M was made by weighing 0.4262 g, and dissolving in 250 ml of distilled water. Ammonium acetate buffer

(CH_3COONH_4) pH 7.0 was made by dissolving 19.27 g of ammonium acetate (CH_3COONH_4) in 250 ml of distilled water. Neocuproine (Ne) solution at a concentration of 0.0075 M was made by dissolving 0.039 g of neocuproine in 250 ml of methanol [18].

Preparation of sample

A concentration of 500 µg/ml was used to dissolve the *Z. acanthopodium* DC methanol extract. Then, five different of concentration series were made from the main solution.

Absorbance measurement of free radical reduction

1 ml of sample was pipetted, and then 1 ml of each CUPRAC reagent (CuCl2.2H2O 0.01 M, Neocuproin ethanolic 0.0075 M, and Buffer ammonium acetate) and 1 ml of distilled water were added to each volumetric flask. Following an incubation time of thirty minutes, an absorbance measurement was taken with a UV-Vis spectrophotometer at a wavelength of 452 nm [19].

The IC_{50} value of CUPRAC

The value of IC_{50} (Inhibitory Concentration), demonstrated the test compound's ability to capture 50% of free radicals, as determined by the computation employed to assess its free radical scavenging activity [10]. The calculation outcomes are inputted into a regression equation, where the inhibitory values is the ordinate and the sample concentration (µg/ml) is the absorbance. If an IC_{50} value less than 50 µg/ml is called a really strong antioxidant; if it is between 50 and 100 µg/ml is called strong; moderate if it is between 100 and 150 µg/ml and weak if the IC_{50}value is between 151 and 200 µg/ml [20].

$$\text{Percentage of inhibition} = [1-(\frac{A_{control}}{A_{Sample}})]\times 100\ \%$$

Identification of phytochemicals using LC-HRMS

The methanol extract of Z. *acanthopodium* DC was analyzed for the identification of phytochemical components using LC-HRMS with electrospray ionization. The LC-HRMS phytochemical profiling utilized a diode array detector, namely the Agilent 6540 UHD UHPLC system, in conjunction with an ESI-QTOF-MS (electrospray ionization quadrupole time-of-flight mass spectrometer). The column uses an Agilent zorbax SB-C18 (150 × 0.5 mm, 5 µ) for analysis. We make use of the gradients that are listed below: a solution of 0.1% formic acid in methanol (B) and a solution of 0.1% formic acid in water (A). With a flow rate of 0.5 milliliters per minute, the injection volume was 10 microliters. In the process of positive ionization electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS) analysis, the mass range that was utilized was from 100 to 1700 m/z. [21, 22].

RESULTS AND DISCUSSION

Antiradical activity

The reduction of the Cu^{2+} into a Cu^{+} Complex, which is indicated by a blue-to-yellow color change in the spot of compounds that have antioxidant activity, was used to test the antiradical activity of the samples. CUPRAC is a selective reagent with a low value of potential reduction, which is 0.17 V; the color change with the addition of solvents can be visually detected according to the theory that can be detected from the complex color [23].

The CUPRAC reagent is a selective reagent because it has a more stable and accessible potential to reduce antioxidant capacity than the other chromogenic reagents [24]. This method has the advantage of being able to oxidize the antioxidant type of thiols in a relatively short amount of time, which is a significant advantage when compared to other methods for measuring antioxidants. According to the results, table 1 showed the absorbance and percentage of inhibition of methanol extracts of *Zanthoxylum acanthopodium*.

Table 1: Absorbance and % Inhibition of methanol extracts of *Zanthoxylum acanthopodium*

No.	Sample	Concentration (µg/ml)	Absorbance	% Inhibition	Regression equation	IC_{50} (µg/ml)
1.	Methanol extract	400	0.232	83.41	Y= 0.198x+	86.58±1.87
2.		200	0.531	62.04	10.892	
3.		100	0.881	37.02		
4.		50	1.101	21.30		
5.		25	1.341	4.14		

N=3

Based on table 1, it seems that the higher concentration of solution has higher antioxidant activity, and higher concentration of solution has lower absorbance. After obtaining the % inhibition data, the solution concentration (x) and % inhibition (y) are plotted and linear regression equation is obtained. The following is the linear regression equation of *Zanthoxylum acanthopodium* DC.

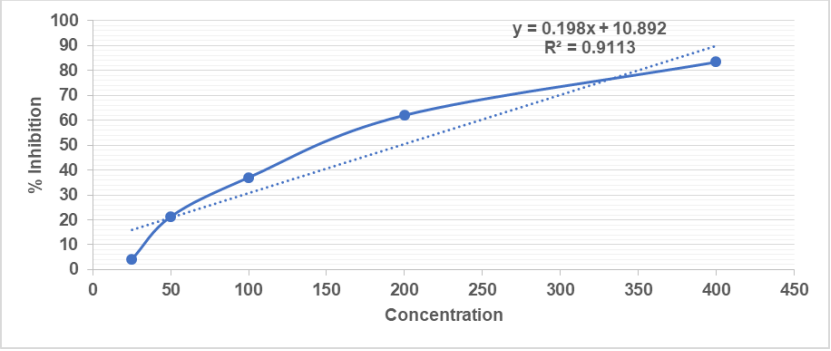


Fig. 1: Linear regression equation of methanol extract of *Zanthoxylum acanthopodium* DC

As seen on table 1, showed that methanol extract of *Zanthoxylum acanthopodium* DC. has an IC_{50}value of 86.58±1.87 µg/ml. The amount of methanol extract of *Z. acanthopodium* DC can 50% block the oxidation process is indicated by the number of IC_{50}. The less IC_{50} value means the higher the antioxidant activity. The extract showed that has a strong (86.58±1.87 µg/ml) antioxidant activity according to the aforementioned categories. According to [17] when the IC_{50} value is less than 50 µg/ml, a subtance is considered

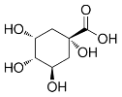
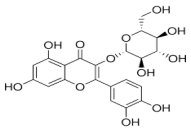
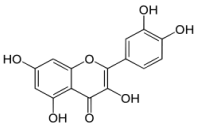
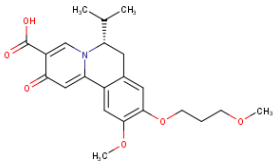
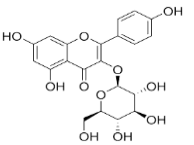
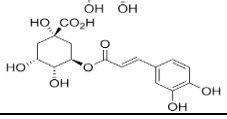
extremely strong when it is between 50 and 100 µg/ml have a strong antioxidant, moderate when the IC_{50} value is between 101 and 150 µg/ml and when the IC_{50} value is >150 µg/ml thats mean has a weak antioxidant.

A few plants have antioxidant activity from multiple processes or mechanisms, for example, preventing of chain initiation, decomposing peroxides, ion catalysts for transition metal binding, preventing hydrogen abstraction and the last one, scavenging radicals [25].

Most of the antioxidant mechanisms is for scavenging free radicals. Reactive chemical compounds called by antioxidants that can protect plant cells, animal, and human from ROS, which can cause oxidative damage. Flavonoids are among the most effective phytochemicals that possess antioxidant properties and can impede disease-causing factors.

The arrangement of the functional groups within the flavan nucleus is responsible for determining the antioxidant activity [26-28].

Table 2: Phytochemicals constituent analysis of methanol extract of *Z. acanthopodium* DC with LC-HRMS

S. No.	Name	Formula	Molecular weight	Retention time (Min)	Structure
1.	"D-(-)-Quinic acid"	$C_7H_{12}O_6$	192.06244	1.104	
2.	"Quercetin-3β-D-glucoside"	$C_{21}H_{20}O_{12}$	464.09514	8.783	
3.	"Quercetin"	$C_{15}H_{10}O_7$	302.04231	11.588	
4.	"(4E)-4-{(2E,4E,6R)-6-[(2R,6S,7R)-1,6-Dimethylspiro[8,9-dioxabicyclo [3.3.1]non-3-ene-2,2'-oxiran]-7-yl]-1-hydroxyl-4-methyl-2,4-heptadien-1-ylidene}-5-hydroxy-2,4-dihydro-3H-pyrrol-3-1"	$C_{22}H_{27}N\,O_6$	401.18336	8.627	
5.	"Astragalin"	$C_{21}H_{20}O_{11}$	448.10011	9.483	
6.	"Chlorogenic acid"	$C_{16}H_{18}O_9$	354.09483	6.182	

According to table 3. The final results of this research showed that methanol extract of *Z. acanthopodium* DC with LC-HRMS analysis revealed presence. The content of secondary metabolite compound was group of flavonoid (neohesperidin, isorhamnetin, astragalin, quercetin, quercetin-3β-D-glucoside) and phenolic (Chlorogenic acid, quinic acid).

Various biochemical and antioxidant activity of flavonoids have been associated with a few diseases such as atherosclerosis, antibacterial, cancer, antimalaria, anxiolytic and the last one, antioxidant [29, 30]. Flavonoids are linked to a wide range of health-promoting effects and essential of many pharmacological, nutraceutical, cosmetic and medical applications [31, 32].

Flavonoids have a wide range of biochemical characteristics, their ability as antioxidants is the one that has been extensively investigated for almost all of them. Flavonoid's antioxidant activity is depend on how their functional groups are arranged around the sructure of nuclear. The substitution, arrangement, and overall quantity of hydroxyl groups significantly impact many mechanisms of antioxidant capacity, including the capacity to scavenge radicals and hydroxide metal ions [30, 31].

CONCLUSION

The final results showed that methanol extract of *Z. acanthopodium* DC. Fruit have strong antioxidant activity and the contain of secondary metabolite of *Zanthoxylum acanthopodium* DC was group of flavonoid and phenolic compounds.

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AUTHORS CONTRIBUTIONS

Assyfa A: Data Curation, Formal Analysis Writing – Original Draft Preparation; Dalimunthe A: Funding Acquisition, Supervision, Validation; Satria D: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, writing – Review and Editing; Yoo C. M: Methodology, Project Administration.

CONFLICT OF INTERESTS

The writers affirm the absence of any conflict of interest among us.

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