

ANTIOXIDANT ACTIVITY OF ALKALOID FRACTIONS OF *ZANTHOXYLUM ACANTHOPODIUM* DC. FRUITS WITH 1,1-DIPHENYL-2-PICRYLHYDRAZYL ASSAY

ROSIDAH¹, POPPY ANJELISA ZAITUN HASIBUAN^{1*}, GINDA HARO², PUTERI MASRI³, DENNY SATRIA³

¹Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. ²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. ³Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. Email: poppyanjelisa@usu.ac.id

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ABSTRACT

Objective: Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. The purpose of this study is to determine antioxidant activities of alkaloid fractions of *Zanthoxylum acanthopodium* DC. fruits.

Methods: *Z. acanthopodium* DC. fruit was extracted by maceration. Ethanol extract was fractionated with liquid-liquid extraction using n-hexane and chloroform at pH 3, 7, 9, and 11 to obtain alkaloid fractions. Antioxidant activity for extract and fractions was determined with 1,1-diphenyl-2-picrylhydrazyl.

Results: The IC₅₀ of extract and fractions was 101.11±0.11, 273.24±0.12, 116.62±0.04, 61.12±0.01, 23.15±0.01, and 112.39±0.04 µg/mL, respectively.

Conclusion: The results reveal that alkaloid fractions of *Z. acanthopodium* DC. fruit have very strong antioxidant potential. Our further study is to isolate the alkaloid compounds.

Keywords: Antioxidant, *Zanthoxylum acanthopodium* DC., Fruits, Alkaloid, Fractions.

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INTRODUCTION

The information about antioxidant properties is needed to evaluate the potential activity of the drug candidate as anticancer. The antioxidant possesses the ability to protect the cell from the damage caused by free radical because it donates one or more electrons to the free radical. Free radicals are the reactive species that have one or more unpaired electron and attempt to get an electron from another molecule or it can release the unpaired electron [1]. In general, the antioxidants are compounds that could inhibit and delay the oxidation process. Most of the plants' compounds that have secondary metabolites including alkaloids, phenolics, and terpenoids represent extremely rich biogenic resources for the novel drug discovery. The recent study found that natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world [2].

Zanthoxylum acanthopodium DC. has been used as aromatic substances, tonic, and treat dysentery. Indian people have used andaliman to treat paralyzed and skin disease such as abscess and leprosy. Andaliman has been used as spices at North Sumatera, especially at North Tapanuli [3-5]. The plants from *Zanthoxylum* genus contain many compounds such as phenol hydroquinones, flavonoids, steroids/triterpenoids, tannins, glycosides, volatile oils, alkaloids, coumarins, lignans, amides, and terpenes. Ethyl acetate extract of andaliman fruits (EAF) was shown to have cytotoxicity effect against MCF-7 and T47D cell lines. EAF was found to have a synergistic effect when combined with doxorubicin. EAF was shown to have anticancer activity toward mice induced with benzo(a)pyrene, having cardioprotective effect and active on T47D resistance cells [6-14]. The aim of this study was to determine antioxidant activity alkaloid fractions of *Z. acanthopodium* DC. fruits using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.

METHODS

Plant and chemicals material

Fresh fruits of *Z. acanthopodium* DC. were collected from Onan Rungu village, Samosir Regency, Sumatera Utara Province, Indonesia. Chemicals used were distilled water and DPPH (Sigma).

Preparation of extract and fractionation

The air-dried and powdered fruits of *Z. acanthopodium* DC. (1 kg) were repeatedly extracted by cold maceration with ethanol 96% (3 × 3 days, 7.5 L) at room temperature with occasional stirring. The filtrate was collected and then evaporated with rotary evaporator (Stuart, Stone, UK) under reduced pressure to give a viscous extract and then freeze-dried to dry [15]. Viscous extract was fractionated with n-hexane and continued with chloroform at pH 3, 7, 9, and 11.

Free radical scavenging activity test

The DPPH assay was carried out according to the previous study with some modifications [16]. 0.2 mM solution of DPPH• in methanol was prepared, and 100 µL of this solution was added to various concentrations. After 60 min, absorbance was measured at 516 nm. Percentage of inhibition was calculated by comparing the absorbance values of the control and test samples [17,18].

$$\text{Percentage of inhibition} = \frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}} \times 100\%$$

Statistical analysis

Data were expressed as mean ± SD which were analyzed using the SPSS 22 software.

RESULTS AND DISCUSSION

Antiradical activity

Antiradical power of the plant samples was measured in terms of hydrogen-donating ability using DPPH which is a stable, nitrogen-centered free radical and produces deep purple color in methanol solution [19]. DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action [20] and has been largely used as a quick, reliable, and reproducible *in vitro* antioxidant activity assay [21]. The reducing capacity of compounds could serve as an indicator of potential antioxidant property [17,18,22,23]. IC₅₀ for extract and fractions in DPPH assay is shown in Table 1.

Table 1: The linear regression and IC₅₀ analysis of crude extract and fraction of *Z. acanthopodium* DC

Sample solution	Regression equation	IC ₅₀ (ppm)
<i>n</i> -hexane crude extract	Y=0.2134x+1.998	273.20
Chloroform fraction pH 3	Y=1.2756x+6.1406	116.6215
Chloroform fraction pH 7	Y=0.7646x+20.914	61.10
Chloroform fraction pH 9	Y=2.133x+3.487	23.15
Chloroform fraction pH 11	Y=0.4489x+1.5756	112.40
Water fraction	Y=0.3184x+2.8334	172.45

As seen on Table 1, seems that alkaloid compounds containing in the fractions are capable of scavenging the free radical DPPH. The pH 7 chloroform fraction showed strong antioxidant and the pH 9 showed the strongest antioxidant. A compound has antioxidant properties if the IC₅₀ value is <200 ppm [24]. The percentage inhibition of free radical DPPH by *Z. acanthopodium* DC. fruit extract and fractions may be due to the structure and high percentage of alkaloids containing in this fruits. It probably that the alkaloids of *Z. acanthopodium* DC. fruits act as lipid-soluble antioxidant which protects the fatty membrane cellular of the cell from free radicals striking [25]. Lipid-soluble antioxidant such as vitamin E acts as neutralizing antioxidant that plays the role in inhibiting lipid peroxidase (RO₂*) [26]. The antioxidant activity of some plants has some mechanisms such as (1) prevention of chain initiation, (2) binding of transition metal ion catalyst, (3) decomposition of peroxides, (4) prevention of hydrogen abstraction, and (5) radical scavenging [1]. According to the results obtained, the chloroform fraction of pH 7 and 9 is potential as antioxidant.

CONCLUSION

The results reveal that *Z. acanthopodium* DC. fruits alkaloid fractions provide effective as an antioxidant.

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