

Evaluation of Cytotoxic Activity Alkaloid Fractions of *Zanthoxylum acanthopodium* DC. Fruits

Dina Maya Syari¹, Rosidah Rosidah^{1*}, Poppy Anjelisa Zaitun Hasibuan¹, Ginda Haro², Denny Satria³

¹Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia; ²Department of Biochemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia; ³Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

Abstract

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***Correspondence:** Rosidah Rosidah. Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. E-mail: rosidah@usu.ac.id

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AIM: This study was carried out to investigate cytotoxic activity towards T47D, 4T1, MCF-7, HeLa, and Raji cells of alkaloid fractions of *Zanthoxylum acanthopodium* DC. fruits. *Zanthoxylum acanthopodium* DC.

METHODS: The fruit was extracted by maceration. The ethanol extract was fractionated with liquid-liquid extraction using n-hexane, chloroform at pH 3, 7, and 9 to obtain alkaloid fractions. Cytotoxic activity for fraction chloroform at pH 7 and 9 was determined with MTT assay.

RESULTS: The IC₅₀ of fraction chloroform at pH 7 and 9 was (92.67 ± 1.37; 71.87 ± 1.04; 159.87 ± 0.63; 123.39 ± 0.81; and 103.09 ± 0.58 µg/mL for pH 7) and (451.29 ± 25.48; 247.18 ± 2.82; 318.46 ± 5.40; 303.96 ± 8.75; and 181.45 ± 1.35 µg/mL for pH 9) respectively.

CONCLUSION: The results reveal that alkaloid fractions at pH 7 and 9 of *Zanthoxylum acanthopodium* DC. Fruits have cytotoxic activity. Our further study is to isolate and assess anticancer activity from alkaloid compounds.

Introduction

Alkaloids are compound which contains a nitrogen atom in their heterocyclic ring structure. Grouping of alkaloids based on biosynthetic pathways is widely used to categorise alkaloids. Alkaloids have a broad distribution in the plants and mainly stand in Spermatophyta. Moreover, many alkaloids show prominent pharmacological activities, such as for asthma, analgesic, antibacterial, and cytotoxicity. Alkaloids are either the most important active compounds in natural products, and some of them have successfully improved into anticancer drugs [1], [2], [3], [4], [5].

Zanthoxylum acanthopodium DC. has been used as aromatic substances, tonic, and treat dysentery. Indian people have used *Zanthoxylum acanthopodium* DC. to treat paralysed and skin

diseases such as abscess and leprosy. Andaliman has been used as spices at North Sumatera, especially at North Tapanuli [6], [7], [8]. 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 [9], [10], [11], [12], [13], [14], [15], [16]. Ethylacetate extract of *Zanthoxylum acanthopodium* DC. fruits (EAF) was showed to have cytotoxicity effect against MCF-7 and T47D cell lines. EAF was found to have the synergistic effect when combined with doxorubicin. EAF was showed to have anticancer activity towards mice induced with benzo(a)pyrene, having a cardioprotective effect and active on T47D resistance cells [17], [18], [19]. The purpose of this research was to determine cytotoxicity activity alkaloid fractions of *Zanthoxylum acanthopodium* DC. fruits on cancer cells.

Material and Methods

Materials

Fresh fruits of *Zanthoxylum acanthopodium* DC. were collected from Onan Rungu village, Samosir Regency, Sumatera Utara Province, Indonesia. *Zanthoxylum acanthopodium* DC. was determined in Herbarium Bogoriense, DMSO (Merck), [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma), chloroform (Full Time), n-hexane (Full Time).

Extraction and Fractionation

Zanthoxylum acanthopodium DC. fruits powdered (1.000 g) were extracted with ethanol 96% (3 x 3 d, 7.500 mL). at room temperature with occasional stirring. The filtrate was collected and then evaporated with a rotary evaporator (Stuart, Stone, UK) until obtained viscous extract [19], [20], [21]. The viscous extract was fractionated with n-hexane and continue with chloroform at pH 3, 7, 9 and 11 [22].

Analysis of Alkaloids with Thin Layer Chromatography

The chloroform fractions at pH 7 and 9 were carried out by thin-layer chromatography using silica gel GF₂₅₄ as stationary phase and chloroform: methanol: ammonia (18: 15: 1) as mobile phase. Dragendorff solution was used as a sprayer reagent to identify alkaloid compounds [22], [23].

Cytotoxicity assay

Alkaloid fractions were submitted for cytotoxic examination. Wheresoever (4T1 and MCF-7 cells line) were grown in DMEM medium, (T47D and HeLa) were grown in RPMI medium, Raji was grown in IMDM medium completely. The cytotoxic examination was adopted from Satria, et al., (2017) [21], [23], [24].

Statistical Analysis

The results were interpreted as means \pm SD. The statistical analysis was performed with SPSS edition 21.

Results

Thin Layer Chromatography

The result of analysis of alkaloid compounds with thin layer chromatography using the GF₂₅₄ gel

and the chloroform: Methanol: ammonia in the ratio (18: 15: 1) were shown in Figure 1, and Rf values were shown in Table 1.

Table 1: Rf value of alkaloid spots on TLC analysis and sprayed with Dragendorff

Spot	Rf Value	
	pH 7	pH 9
1	0.06	0.08
2	0.14	0.16
3	0.48	0.44
4	0.61	-
5	0.68	-

The results of the TLC analysis used the stationary phase of silica gel 60 F254 with the appearance of dragendorff spots.

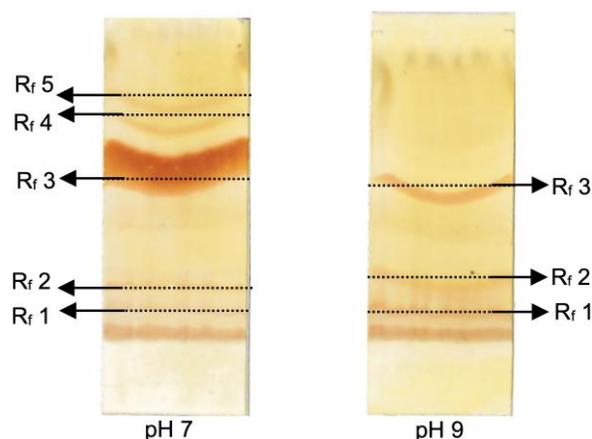


Figure 1: Thin layer chromatography plates after sprayed with Dragendorff

Inhibitory Concentration 50% (IC₅₀)

[3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] assay was used to determine cell viability. In each handling, of fractions were shown to inhibit cells growth. The IC₅₀ value was shown in Table 2.

Table 2: IC₅₀ value of alkaloid fractions at pH 7 and 9 of *Zanthoxylum acanthopodium* DC. fruits towards several cell lines

Cell Line	Chloroform Fraction (μ g/mL)	
	pH 7	pH 9
T47D	92.67 \pm 1.37	451.29 \pm 25.48
4T1	71.87 \pm 1.04	247.18 \pm 2.82
MCF-7	159.87 \pm 0.63	318.46 \pm 5.40
HeLa	123.39 \pm 0.81	303.96 \pm 8.75
Raji	103.09 \pm 0.58	181.45 \pm 1.35

Discussion

The cytotoxic activity from natural materials is correlated with the phytochemical compounds, including in *Zanthoxylum acanthopodium* DC. and alkaloids estimated as active compounds [21], [25]. The majority of studies focus on the induced cytotoxicity of well-known alkaloids such as, taxol,

vincristine and vinflunine that are used clinically in cancer therapy worldwide. Screening for novel agents has led to the discovery of new alkaloid that showed promising anti-neoplastic and apoptotic abilities in several cancer cell lines [1], [4], [5].

Benzophenanthridine and furoquinoline are alkaloids compound from *Zanthoxylum buesgenii*, which has cytotoxic activity in several cell lines [26]. Benzophenanthridine derivates from *Zanthoxylum nitidum* exhibited A549, HeLa, SMMC-7721 and EJ cancer cells with IC₅₀ 27.50; 37.50; 16.95; and 60.42 μM respectively [27]. Acridone alkaloids from *Zanthoxylum leprieurii* Guill. was showed modest cytotoxicity with LD₅₀ 13.1 μg/mL at brine-shrimp (*Artemia salina* Leach) and active towards A549 and DLD-1 cells [28].

In conclusion, the results reveal that *Zanthoxylum acanthopodium* DC. fruits alkaloid fractions provide effective as anticancer towards several cell lines.

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