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

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Abstract

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

| Spot | pH 7 | pH 9 |
|------|------|------|
| 1    | 0.06 | 0.05 |
| 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.

| Cell Line | Chloroform Fraction (µg/mL) | pH 7 | pH 9  |
|-----------|-----------------------------|------|-------|
| T47D      | 92.97 ± 1.37                | 451.29 ± 25.48 |
| 4T1       | 71.87 ± 1.04                | 247.18 ± 2.82 |
| MCF-7     | 159.87 ± 0.63               | 318.46 ± 5.40 |
| HeLa      | 123.39 ± 0.81               | 303.96 ± 8.75 |
| Raji      | 103.09 ± 0.58               | 181.45 ± 1.35 |

Statistical Analysis

The results were interpreted as means ± 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.

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].

Benzophenanthidine and furoquinoline are alkaloids compound from Zanthoxylum buesgenii, which has cytotoxic activity in several cell lines [26]. Benzophenanthidine derivatives 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 LD5013.1 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 alkaloids fractions provide effective as anticancer towards several cell lines.

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