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# Bioactive Phenolics and Flavonoids, Antioxidants, Anti-Inflammatory, Enzyme-Inhibitory and Cytotoxic Activities of Aerial part of *Trachyspermum roxburghianum* (DC.) Craib.

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**Abstract**

*Trachyspermum roxburghianum* (DC.) Craib is an edible plant found in Northern and Northeastern Thailand. This plant is used in household remedies to relieve carminative and digestive problems, hiccups, vomiting, bladder pain, etc. This study aims to assess the bioactive compounds, consisting of phenolics and flavonoids, and evaluate the biological activities of this plant. The aerial portion was macerated in EtOH1 to obtain EtOH1 crude extracts. Then, the EtOH1 was divided and separated by the solvents' polarity to obtain Petr, EtOAc, and EtOH2 crude extracts. All crude extracts were assessed for their total phenolics and flavonoids and subsequentially evaluated for their antioxidants (using DPPH, ABTS and FRAP), enzymatic inhibitory effects (on tyrosinase and collagenase), anti-inflammatory activity (protein denaturation assay) and cytotoxicity (MTT assay). The results presented that a high amount of phenolics and flavonoids were observed in EtOH2 (56.57 mg GAE/g ext. and 22.75 mg QE/g ext.) followed by EtOAc (57.95 mg GAE/g ext. and 7.41 mg QE/g ext.), EtOH1 (47.14 mg GAE/g ext. and 4.23 mg QE/g ext.), and Petr (37.05 mg GAE/g ext. and 2.08 mg QE/g ext.), respectively. All crude extracts represented antioxidants in all assays; EtOAc presented a high level of antioxidants on ABTS ($IC_{50}$ 54.96 µg/mL) and DPPH ($IC_{50}$ 133.32 µg/mL), while EtOH2 revealed a high ability to reduce the $Fe^{3+}$-TPTZ complex (155.85 $Fe^{2+}$g ext.). Remarkably, for all crude extracts used for anti-collagenase enzyme activity, the percentage of inhibition ranged from 78.35 - 97.96 %. Besides, only Petr and EtOH2 showed the activity of the anti-protein denaturation ($IC_{50}$ 1.926 and 2.578 mg/mL). The cytotoxicity test showed that EtOH1, Petr, and EtOAc exhibited a concentration-dependent manner of inducing cell death in 24 and 48 h, while EtOH2 showed less toxicity to the cell line. This information would be valuable and pave the way for future study and application of this plant to other products.

**Keywords:** *Trachyspermum roxburghianum*, Bioactive content, Flavonoids, Phenolics, Edible plant, ABTS, DPPH, FRAP, Cytotoxicity

## Introduction

*Trachyspermum roxburghianum* (DC.) Craib is an aromatic plant member of the family Apiaceae (Umbelliferae). The common names are Radhudi, Ajmod, Ajamoda, and Kant-Balu, which are extensively cultivated in South Asia and Southeast Asia [1,2]. The seed of this herb was used as a spice and showed its function in treating hiccups, vomiting and bladder pain [1]. Furthermore, it was reputed in the Indian medical system [2]. This plant is well known in Thailand as Hom-Yae or Pak Chi Rai [3]. The seed essential oil was reported to be rich of 2-cyclohexene-1one, 2-methyl-5-(1-methylethenyl) (40.03 %), apiol (18.7 %), limonene (17.1 %), myristicin (12.3 %), dihydrocarvone (7.89 %) and eugenol (1.68 %) [1]. The essential oil extracted from seed cultivated in Bangladesh showed the presence of limonene, 5,7,8-trimethyl-dihydrocoumarin and sabinene as major compounds and exhibited radical scavenger activity on DPPH and cytotoxicity on brine shrimp lethality [4]. In 2018, a novel roxydienone was separated and

reported from the seed extract of this plant, and it also showed cytotoxicity against NCI-H187 and KB cell lines [5].

The aerial portions of *T. roxburghianum*, were extensively used, especially in Northern and Northeastern part of Thailand, as an edible plant and an ingredient in local cuisine. The aroma contained in the plant provided the flavour of unique foods. The essential oil from the aerial part was remarkable and sabinene (28.6 %), a-terpinolene (24.2 %) including 3-n-butylphathalide (23.34 %) were reported as the major components [3]. Additionally, the essential oil presented antioxidant activity on DPPH, ABTS and FRAP assays and also antimicrobial activity on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [3]. However, during the spacious consumption in the northern and northeastern regions of Thailand, not only the crude extract or essential oil from the seed and aerial portion were studied, but the whole aerial part of this plant which commonly used for consumption should also be focused on and examined. Therefore, this study aimed to evaluate the bioactive compounds of the phenolic and flavonoid contents and investigate the biological activities of comprised of antioxidant, enzymatic inhibitor, anti-inflammatory and cytotoxicity effects of crude extracts from the aerial portion of this plant. This information will serve as a guideline for future applications of this plant.

## Materials and methods

### Plant material

The mature aerial portion of *T. roxburghianum* was collected from the local village in Soemngam district, Lampang province, Thailand before it bloomed. The plant identification was recognized by a taxonomist and deposited at CMU Herbarium, Department of Biology, Faculty of Science, Chiang Mai University, Thailand.

### Sample preparation

The aerial portion of *T. roxburghianum* was cleaned and dried at 45 °C. The dried sample was ground and macerated with 95 % ethanol (EtOH1) for 5 days. The extracted sample was evaporated under reduced pressure to obtain crude EtOH1. Then, crude EtOH1 was partially divided for separation by dry column vacuum chromatography technique and eluted by petroleum ether (Petr), ethyl acetate (EtOAc) and ethanol (EtOH2), respectively. The sample solutions were evaporated, and crude Petr, EtOAc and EtOH2 were obtained. All extract samples were kept at 4 °C in a refrigerator for further investigation.

### Determination of phenolic and flavonoid contents

#### *Total phenolic content*

The total phenolic compound of all crude extracts were determined by the Folin-Ciocalteu assay [6] with some modification. The initial sample concentration was prepared at 2.5 mg/mL and dissolved with the appropriate solvent, such as DMSO and ethanol. The Folin-Ciocalteu reagent was diluted with water at a ratio of 1:10 before testing. The sample solution (50 µl) was pipetted and mixed with 100 µl of Folin-Ciocalteau reagent. Then, sodium carbonate 7.5 % w/v (100 µl) was added. Afterwards, the mixture was incubated for 30 min in the dark condition at room temperature. The absorbance was measured at wavelength 750 nm by a Multimode spectrophotometer. Gallic acid was used as a standard. Total phenolic content was calculated and reported in terms of Gallic acid equivalents (mg GAE/g extract).

#### *Total flavonoid content*

The aluminium chloride ($AlCl_3$) colorimetric method [7] was used to determine the total flavonoid content in all crude extracts. The sample solution was identically prepared as the assay above. One hundred microliters of the sample were pipetted and transferred into a microplate with 96-well. Then, 2 % aluminium chloride (100 mL) was added. After that, it was incubated for 10 min in the dark condition at room temperature. The absorbance at wavelength 415 nm was used to detect the presence of flavonoid. Quercetin was used as a comparative standard and total flavonoid content was calculated and expressed as mg QE/g extract.

### Antioxidant activity

#### *DPPH assay*

The capability of the free radical scavenger was assessed using the DPPH (2,2-Diphenyl-l-picrylhydrazyl) assay [8] to assess crude extracts. The DPPH radical solution was prepared in ethanol, and the absorbance at 520 nm was measured (the range of 0.7 ± 0.2) before testing. The sample was dissolved with the appropriate solvent at an initial concentration of 500 µg/mL. Then, 20 µl of the sample was pipetted

and mixed with 180 µl of DPPH radical solution in a microplate 96-well. The mixture was left in the dark at room temperature for 30 min. After that, the absorbance was measured at wavelength 520 nm by a Multimode spectrophotometer. Trolox standard was used for comparison. The percentage of inhibition was calculated as followed:

$$\% \text{ Inhibition} = [(A0-A1)/A0]\times 100$$

where A0 is the absorbance of DPPH and A1 is the absorbance of sample.

***ABTS assay***

All crude extracts were evaluated for antioxidant activity on ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay [8,9]. The samples were arranged similarly to the assay for DPPH. The ABTS radical solution was prepared in a ratio of 1:1 using 4 mM of ABTS solution mixed with 4.9 mM of potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand in the dark at room temperature for 16 h. Then, ethanol was used to dilute at a ratio of 1:50, followed by measurement of the absorbance at a wavelength of 734 nm (which should be $0.7 \pm 0.2$). Twenty microliters of sample were pipetted and mixed with 180 µl ABTS radical solution in a microplate 96-well, then incubated in the dark at room temperature for 6 min. The absorbance was detected at the same wavelength. The assay used Trolox as a comparative standard. The percentage of inhibition was calculated as the above equation.

***FRAP assay***

FRAP method was used to evaluated the ability of the crude extracts to reduce $Fe^{3+}$-TPTZ to $Fe^{2+}$-TPTZ complexes [10]. The FRAP reagent was freshly prepared using 300 mM acetate buffer pH 3.6 mixed with 10 mM of TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) in hydrochloric acid and 20 mM of ferric chloride hexahydrate in a ratio of 10:1:1. The sample was dissolved with the appropriate solvent at an initial concentration of 500 µg/mL. Then, 50 µl of the sample was mixed with 150 µl of FRAP reagent in a 96-well microplate. The mixture was incubated for 8 min in the dark condition at room temperature, and the absorbance at wavelength 600 nm was measured using a Multimode spectrophotometer. Ferrous sulphate ($Fe_2SO_4$) was used as standard, and the FRAP value was calculated and expressed in terms of mg $Fe^{2+}$/g extract.

**Enzymatic inhibitory activity**

***Anti-tyrosinase***

The assay of tyrosinase inhibitors was used to assess the enzymatic inhibitory effect of the crude extracts. The procedure follows Dej-adisai [11] and Piao[12] with some modifications. L-DOPA (3, 4-Dihydroxy-L-phenylalanine ethyl ester) at the concentration 0.85 mM was used as a substrate for testing. The tyrosinase enzymes from mushrooms (500 U/mL) were freshly prepared in 20 mM phosphate buffer pH 6.8 for use in the assay. Before testing, the sample (10 mg/mL) was dissolved with a small amount of DMSO and diluted with phosphate buffer. Then 20 µl of sample solution and tyrosinase enzyme were mixed in a microplate 96-well, and 140 µl of phosphate buffer were added. The mixture was incubated at 37 °C for 10 min, then 20 µl of the substrate solution was added and followed by repeated incubation at 37 °C for 20 min. The absorbance was measured (at 492 nm), and the percentage of inhibition was calculated as the formula mentioned below. Kojic acid was used as a standard.

$$\% \text{ Inhibition} = 100\times[(Ac-(As-Asb)/Ac]$$

where Ac is the absorbance of the solvent that was used to dissolve the sample or standard, mixed with tyrosinase and substrate. As is the absorbance of the tested sample or standard mixed with tyrosinase and substrate. Abs is the absorbance of a sample mixed with substrate without enzyme.

***Anti-collagenase***

All crude extracts were evaluated for collagenase inhibitory activity by following the procedures of Thring [13] and Chattuwatthana [14] with some modification. FALGPA (N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala) was used as the substrate and prepared at 1 in 50 mM of tricine buffer, pH 7.4. The collagenase stock solution (1 U/mL) was freshly arranged at 2 - 8 °C and dissolved in 18.2 MW ultrapure water. The initial sample concentration at 2.5 mg/mL was dissolved in DMSO and diluted with tricine buffer. Then 20 µl of sample solution was pipetted and mixed with 10 µl of collagenase enzyme in a microplate 96-well, and 30 µl of tricine buffer was added. The mixture was incubated for 20 min at 37 °C. Then 40 µl of substrate solution was added and again incubated at the same temperature for 30 min. After that, the

absorbance was measured at wavelength 340 nm using a Multimode spectrophotometer, and the percentage of inhibition was calculated as in the formula above. Epigallocatechin gallate (EGCG) was used as the comparative standard.

**Anti-inflammatory activity**

The bovine serum albumin assay was used to assess anti-inflammatory ability in all crude extracts with some modification [15,16]. The 0.3 % of bovine serum albumin (BSA) was prepared in 1 M TBS (Tris buffer saline), pH 8.5, and then the mixture was adjusted to pH 6.7 by 1 M of HCl or NaOH. The sample was dissolved in an appropriate solvent such as DMSO or ethanol. About 150 µl sample solution was mixed with 2,850 µl BSA solution (the final concentration range was 40 - 0.31 µg/mL). The mixture was incubated at 37 °C for 20 min, and heated at 75 °C for 20 min, then allowed to cool at room temperature. The turbidity of the mixture was measured at 650 nm, and the percentage of inhibition was calculated using the formula below. The results reported in terms of $IC_{50}$ µg/mL and diclofenac sodium were used as a comparative standard.

% Inhibition = 100×[Ac– (As–Asb)/Ac]

where Ac is the absorbance of the solvent that was used to dissolve the sample or standard mixed with 0.3 % BSA. As is the absorbance of a sample or standard mixed with 0.3 % BSA. Asb is the absorbance of the solvent that is used to dissolve the sample of a standard mixed sample or standard solution.

**Cytotoxicity activity**

***Cell and cell culture conditions***

HeLa cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained using Dulbecco's modified eagle medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10 % fetal bovine serum (FBS) (Gibco), 2 mM L-glutamine (Gibco) and penicillin (100 U/mL)-streptomycin (100 µg/mL) (Gibco). Cells were maintained in a 37 °C humidified incubator containing 5 % $CO_2$. In preparing HeLa cells for the cell viability assay, the cells were seeded at $1.5\times10^3$ cells/well in a 96-well culture plate and incubated in a 37 °C humidified incubator containing 5 % $CO_2$ overnight.

***Cell viability assay***

The MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) colorimetric assay was employed to evaluate the viability and proliferation of the cells. The method of Valiulytė [17] was used for evaluation with some modifications. In brief, test samples were prepared by dissolving the crude extracts in DMSO followed by further dilution with a C-DMEM medium to reach the desired final concentration (10, 5, 2.5 and 1.25 mg/mL). The control DMSO in this assay was used at 10 % to ensure that the maximum concentration of diluent had no cytotoxic effect. Test samples of 100 µl containing the desired concentration of the crude extracts were added to the wells. After 24 and 48 h, cell morphology was observed under light microscopy. Cells were then washed with sterile phosphate buffer saline (PBS), and 100 µl of MTT solution (0.5 mg/mL MTT in PBS) was applied to each well and incubated for 2 h at 37 °C. Afterwards, the solution was removed, and 100 µl DMSO was added to each well to solubilize the purple formazan crystals that formed. Subsequently, absorbance was read at 570 nm, and the percentage of cell viability was calculated.

## Results and discussion

**Total phenolic and flavonoid contents**

Phenolics and flavonoids are among the bioactive chemical groups that can be generated from plants and have played an important role in preventing many diseases. The results (**Table 1**) indicated that all crude extracts contained a number of phenolics and flavonoids. Crude EtOAc (57.95 mg GAE/g ext.) and EtOH2 (56.57 mg GAE/g ext.) showed similar levels of total phenolic compounds higher than crude EtOH1 (47.14 mg GAE/g ext.) and Petr (37.05 mg GAE/g ext.), respectively. For total flavonoid content, which was calculated and expressed in terms of milligrams of quercetin equivalents per gram of dry weight extract (mg QE/g ext.). Crude EtOH2 (22.75 mg QE/g ext.) exhibited a significant number of flavonoids higher than crude EtOAc, EtOH1 and Petr (7.41, 4.23 and 2.08 mg QE/g ext.). The results proved that the solvents used for extraction and separation are responsible for dissolving the endogenous compounds in plants. Although crude EtOAc and EtOH2 showed similarities in the number of phenolic compounds, the difference in flavonoids was revealed. Therefore, the difference in polarity of phenolics and flavonoids in

all extracts responded to the solubility of their compounds in the solvents used for extraction. The phenolic compound structures comprise at least one molecule of phenol (C6H5OH) [18] which is an aromatic ring that carries hydroxy group (one or more) substituents, including other functional derivatives, for example, esters, glycosides, etc. [19]. They have involved the oxidation of an alkaline solution in the Folin-Ciocalteu assay [20]. Crude extracts containing the phenolic substances with phenol molecules will react with the Folin-Ciocalteu reagent (a yellow colour solution comprising phosphomolybdic/phosphotungstic acid) and then change to the blue complex. It can be detected at a wavelength of 750 nm [20]. Thus, the quantity of phenolic content in the crude extracts depends on the solubility and variety of phenolic compounds contained in the extracts. Flavonoids content exhibited a high number in crude EtOH2 (22.75 mg QE/g ext.). This extract can estimate flavonoid substances with more polarity, substituting glycoside structures in the molecule. The soluble property of flavonoid glycosides can be extracted by alcohol or a mixture of alcohol-water [21]. In contrast, less polar flavonoids can be separated by non-polar solvents such as chloroform, dichloromethane, ethyl acetate, etc. [21,22]. Crude EtOH1 obtained by maceration using ethanol represented a small number of flavonoids since this EtOH1 extract was a mixture of polar and non-polar bioactive compounds. When crude EtOH1 was eluted by non-polar to polar solvents. The chemical substances will dissolve as the property "like dissolved like" in the separated solvents. Polar flavonoids can dissolve easily in ethanol; therefore, after crude extract (EtOH1) is eluted by petroleum ether and ethyl acetate, it's presented in a high quantity in crude EtOH2.

**Table 1** Total phenolic and flavonoid contents of crude extracts from *T. roxburghianum*.

| Crude extracts | Total phenolics (mg GAE/g ext.) | Total flavonoids (mg QE/g ext.) |
|---|---|---|
| EtOH1 | $47.14 \pm 1.13^{a}$ | $4.23 \pm 0.53^{a}$ |
| Petr | $37.05 \pm 1.74^{b}$ | $2.08 \pm 0.44^{b}$ |
| EtOAc | $57.95 \pm 0.62^{d}$ | $7.41 \pm 0.76^{c}$ |
| EtOH2 | $56.57 \pm 0.82^{cd}$ | $22.75 \pm 0.49^{d}$ |

Note: Different superscript letters (a, b, c, d, and e) in the same columns represent significant differences ($p < 0.05$).

**Antioxidant activity**

The antioxidant effect is usually used to evaluate remarkable biological activity in plants, especially edible plants. This function is well known to prevent many ailments. Crude extracts from *T. roxburghianum* edible plant evaluated the antioxidant effects such as scavenging the DPPH and ABTS radicals and reducing $Fe^{3+}$-TPTZ to $Fe^{2+}$-TPTZ complexes. The results are presented in **Table 2**. Consideration of the trend of antioxidant activity on ABTS and DPPH methods from the separated crude extracts. Crude EtOAc ($IC_{50}$ 54.96 and 133.32 µg/mL) presented the highest antioxidant effect, followed by EtOH2 ($IC_{50}$ 91.32 and 146.16 µg/mL) and Petr ($IC_{50}$ 246.95 and 227.86 µg/mL), respectively, while crude EtOH1 (non-separated extract) showed a higher effect to scavenge ABTS radicals than DPPH radicals. For the FRAP assay, crude EtOH2 exhibited the highest significance in reducing the $Fe^{3+}$-TPTZ complex equivalent to ferrous sulphate as 155.85 mg $Fe^{2+}$/g ext. followed by crude EtOH1 (111.73 mg $Fe^{2+}$/g ext.), EtOAc (110.74 mg $Fe^{2+}$/g ext.) and Petr (80.10 mg $Fe^{2+}$/g ext.), respectively. All crude extracts proved that the antioxidant substances in these extracts provided proton and electron transfer, radical scavenging and a reducing agent that reacted to the free radicals in the assay of testing. The antioxidant activity may result from bioactive compounds, especially phenolics and flavonoids, as well as other chemical constituents contained in crude extracts. The mechanism of action described good electron donors in the hydroxy-functional groups, which are linked to the carbon atoms of the benzene ring (on both phenolic and flavonoid structures), providing high antioxidant activity [23].

**Table 2** Antioxidant activity of crude extract from *T. roxburghianum*.

| Crude extracts | $IC_{50}$ μg/mL | | FRAP assay (mg $Fe^{2+}$/g ext.) |
|---|---|---|---|
| | ABTS assay | DPPH assay | |
| EtOH1 | 177.84 ± 3.97[a] | 237.10 ± 4.76[a] | 111.73 ± 0.55[a] |
| Petr | 246.95 ± 3.86[b] | 227.86 ± 5.37[ab] | 80.10 ± 2.74[b] |
| EtOAc | 54.96 ± 6.92[c] | 133.32 ± 7.60[cd] | 110.74 ± 0.92[ac] |
| EtOH2 | 91.32 ± 0.35[d] | 146.16 ± 18.91[c] | 155.85 ± 2.17[d] |
| Trolox | 7.24 ± 0.18[e] | 8.47 ± 0.05[e] | - |

Note: Different superscript letters (a, b, c, d, and e) in the same columns represent significant differences ($p < 0.05$).

**Enzymatic inhibitory and anti-inflammatory effects**

All crude extracts of *T. roxburghianum* were assessed for enzymatic tyrosinase and collagenase inhibitors and evaluated for their anti-inflammatory effect using the anti-protein denaturation method. The results are shown in **Table 3** and **Figure 1**.

**Table 3** Enzymatic inhibitory and anti-protein denaturation activities of crude extracts from *T. roxburghianum.*

| Crude extracts | % inhibition | | Anti-protein denaturation ($IC_{50}$ mg/mL) |
|---|---|---|---|
| | Tyrosinase | Collagenase | |
| EtOH1 | 9.38 ± 1.32 | 95.39 ± 0.18 | ND |
| Petr | 22.28 ± 2.38 | 95.91 ± 0.83 | 1.926 ± 29.83 |
| EtOAc | ND | 78.35 ± 2.99 | ND |
| EtOH2 | ND | 97.96 ± 0.94 | 2.578 ± 35.56 |
| Kojic | 61.43 ± 0.85 | - | - |
| EGCG | - | 96.96 ± 1.49 | - |
| Diclofenac | | | 0.070 ± 0.46 |

For enzymatic inhibitory effects, all crude extracts showed mild tyrosinase inhibition. Conversely, all crude extracts presented inhibition of the collagenase enzyme compared to the EGCG standard. Crude EtOH2 showed higher anti-collagenase activity, which was 97.96 %, followed by Petr and EtOH1 demonstrated similar activity to inhibit this enzyme (95.91 and 95.39 %). The collagenase inhibitor effect may result from the flavonoids and phenolics in these extracts. Previous information described that some plants had bioactive compounds of phenolics and flavonoids, that inhibited tyrosinase, collagenase and elastase [24,25]. The function of the collagenase inhibitor may have beneficially maintained skin damage and reduced the skin wrinkle that occurred. For the denatured protein effect, only Petr and EtOH2 showed activity for protein denaturation. The $IC_{50}$ were presented at 1.926 ± 29.83 and 2.578 ± 35.56 mg/mL, respectively. The denaturation mechanism may be related to the alternation of electrostatic, hydrogen, hydrophobic and disulfide bonds [16]. Protein denaturation is one of the main factors causing rheumatoid arthritis [16]. Therefore, this information presumes that both extracts may have an anti-inflammatory and could be applied for rheumatoid arthritis treatment in the future.

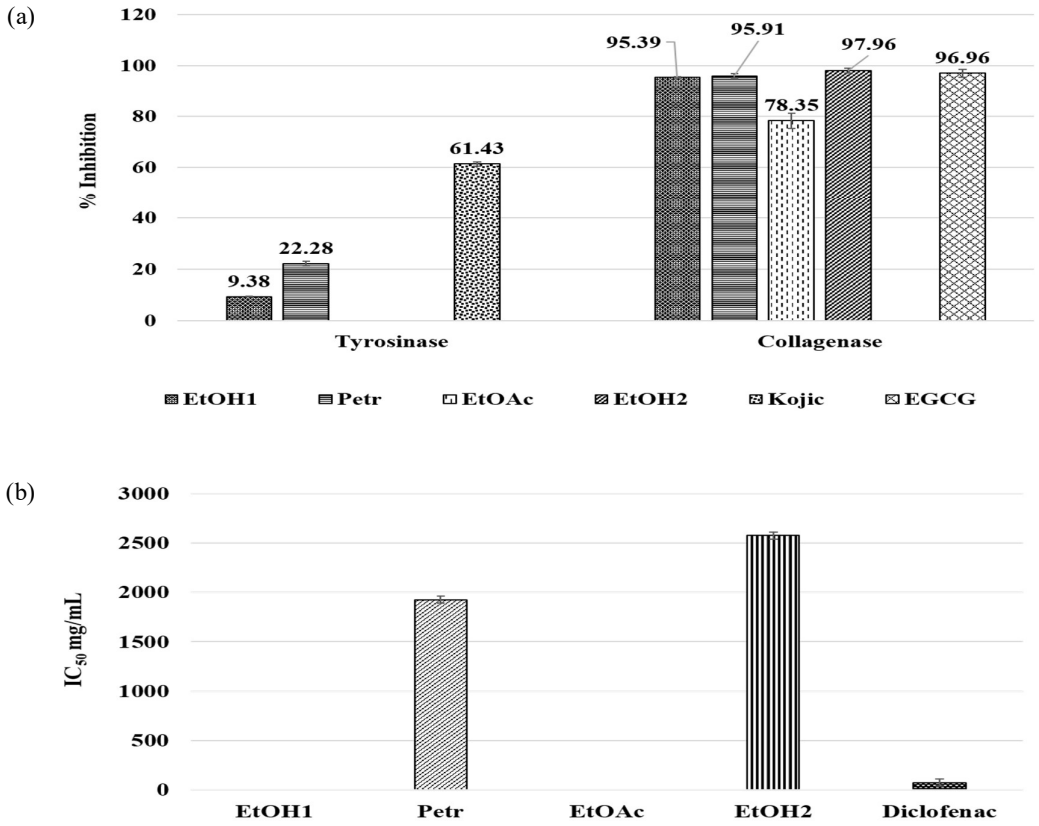


**Figure 1** Enzymatic inhibitory effect (a) and anti-protein denaturation and (b) of crude extracts from *T. roxburghianum*.

**Cytotoxicity activity**

To explore the cytotoxicity effect of *T. roxburghianum* extracts, a human cancer cell line (HeLa cell) was used in this assay. Cell viability associated with crude extracts on the examined cell line is presented in **Figure 2**. The concentration of the crude extracts that inhibit cell viability by 50 % ($IC_{50}$) at 24 h post-treatment was calculated by comparing it with 100 % cell viability provided by the control 10 % DMSO as shown in **Table 4**. The result showed that at 24 h post-treatment, the crude extracts; EtOH1, Petr, and EtOAc induced cell death in a dose-dependent manner. At concentrations greater than or equal to 2.5 mg/mL, cell viability was reduced to the point where more than 50 % of cells were dead. Among the lowest concentrations used in this study, 1.25 mg/mL of these 3 extracts, EtOH1 showed the highest cytotoxic effect on the HeLa cancer cell, followed by EtOAc and Petr, in which percentages of cell viability were 50.57, 61.79 and 67.69 %, respectively. EtOH2, on the other hand, was less toxic to the cell in all concentrations used. DMSO 10 % was used as a diluent control to obtain 100 % cell viability at both 24 and 48 h post-treatment. The $IC_{50}$ of EtOH1, Petr, EtOAc, and EtOH2 extracts was 4.57 ± 0.011, 3.53 ± 0.031, 3.39 ± 0.011 and > 10.00 ± 0.122 mg/mL, respectively. The HeLa cells were further monitored up to 48 h after treatment. The results showed that all of the extracts and all concentrations used caused cell death by more than 50 % (**Figure 2(a) - 2(b)**).

**Table 4** $IC_{50}$ of *T. roxburghianum* extract on HeLa cells at 24 h post-treatment.

| Crude extracts | Cytotoxicity ($IC_{50}$ mg/mL) |
|---|---|
| EtOH1 | 4.57 ± 0.01 |
| Petr | 3.53 ± 0.03 |
| EtOAc | 3.39 ± 0.01 |
| EtOH2 | > 10 ± 0.12 |

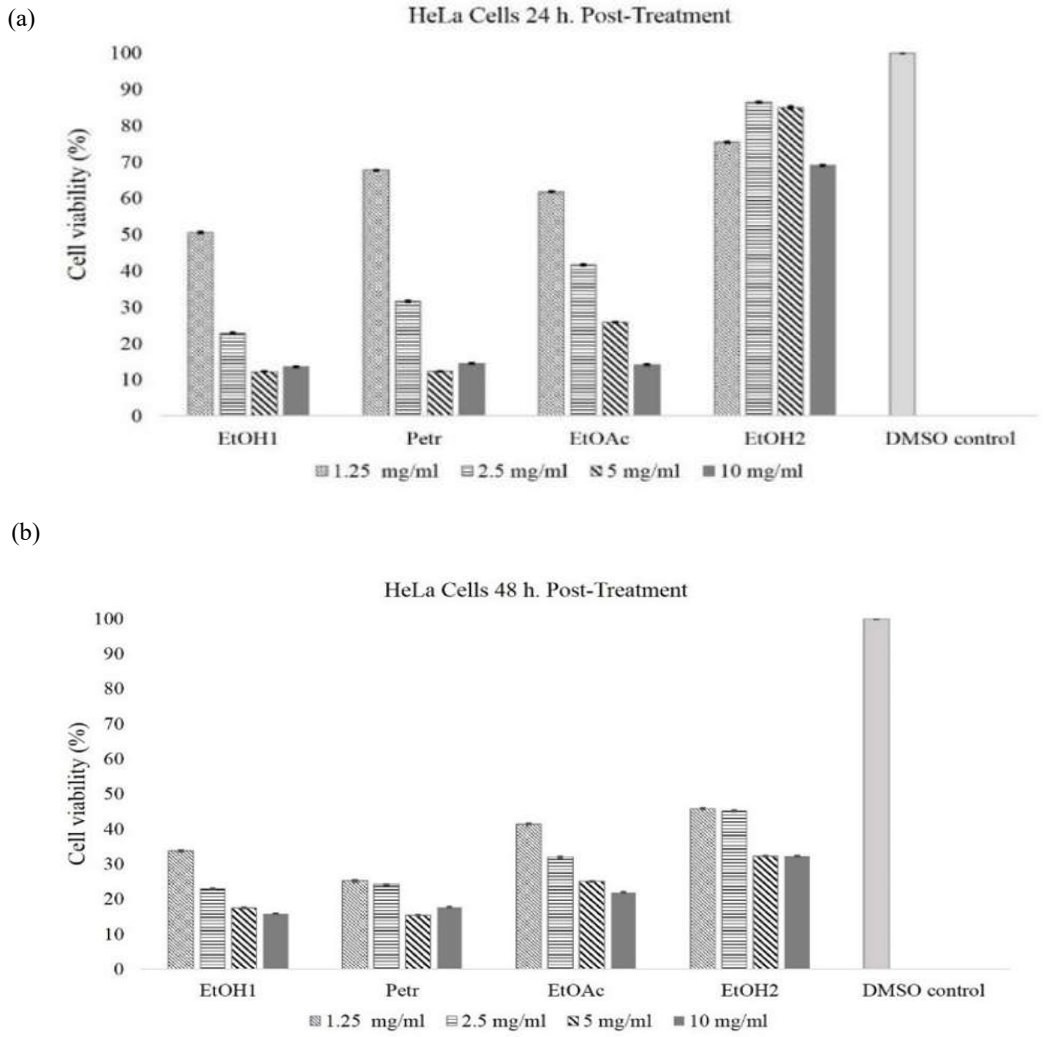


**Figure 2** Cell viability assay (a) and (b) presented the percentage of cell viability at 24 and 48 h post-treatment. Bars represent the mean ± SD.

Cell morphology observed under the light microscope at 24 and 48 h post-treatment is shown in **Figure 3**. Compared to the control 10 % DMSO, the results were correlated to the bar graphs. The crude extracts; EtOH1, Petr, and EtOAc induced cell death in a concentration-dependent manner after 24 h of treatment. Meanwhile, EtOH2 had a less potent cytotoxic effect. However, EtOH2 was the one that had a high level of antioxidant activity. This information implied that EtOH2 contained substance(s) that could reduce cellular stress from free radicals and protect cells from oxidative agents, therefore, retaining cell

viability. In this study, we showed that EtOH2 extract contained phenolics, a member of the non-flavonoid polyphenols and flavonoids higher than others (**Table 1**). This data agreed with several studies on plant polyphenols, including the 2 most common classes, flavonoids and phenolic acids [26], which can be used as antioxidant against a variety of oxidative stress-induced diseases [27-29]. In addition, the natural polyphenols' anticancer efficacy has been attributed to their potent antioxidant and anti-inflammatory and their abilities to modulate targets and signalling pathways. These effects were associated with cell viability, proliferation, differentiation, migration, etc. [30,31]. The advantages of EtOH2, which contains antioxidant effects and anti-collagenase activity, could be beneficial for its application in cosmetic sciences.

The results of the study also indicated that EtOH1, Petr, and EtOAc showed a strong cytotoxicity effect and were able to inhibit HeLa cancer cell growth by more than 50 % in 24 h (**Table 4**). It could be hypothesized that these 3 extracts may have anticancer functions. Therefore, in further study, many different types of cells, both normal and cancer cells, are required to investigate this function.

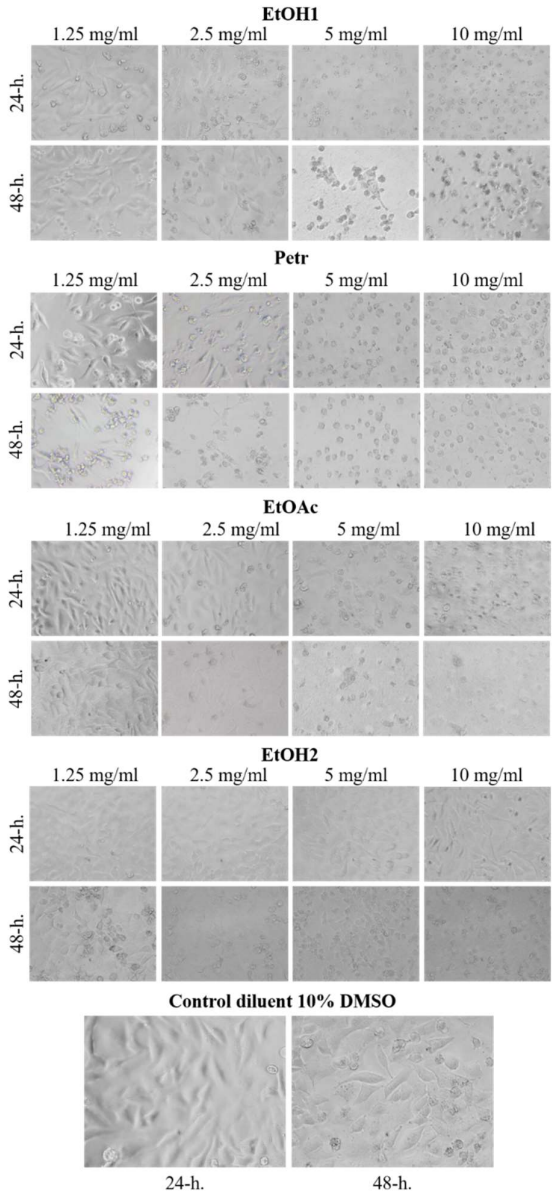


**Figure 3** Light microscopes imaging of HeLa cells treated with different crude extracts and concentrations at 24 and 48 h (200X magnification).

## Conclusions

The aerial portion of *T. roxburghianum* edible plant demonstrated that all crude extracts comprised compounds of phenolics and flavonoids. These contents can be hypothesized to depend on the polarity of the bioactive compounds, which can be dissolved in the extraction solvents. All crude extracts also presented the ability to be a scavenger and reduce free radicals on ABTS, DPPH and FRAP assays and excellent activity inhibited collagenase enzyme. Besides, crude Petr and EtOH2 revealed the activity against protein denaturation, which implies that both crude extracts have anti-inflammatory activity. Furthermore, EtOH1, Petr, and EtOAc showed a strong cytotoxicity effect on HeLa cells, while EtOH2 presented less cytotoxicity but a remarkable in collagenase inhibitory effect. This information would be beneficial for study in depth for the outstanding function of developing this medicinal herb in other healthcare product designs in the future.

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