Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society
ANALIS Persatuan Sains Analisis Malaysia

CYTOTOXICITY STUDY, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY AGAINST PLANT PATHOGENIC BACTERIA OF METHANOL EXTRACT FROM *Coscinium fenestratum* (AKAR SEKUNYIT)

(Kajian Ketoksikan Sel, Aktiviti Antioksida dan Sifat Antibakteria Ekstrak Metanol dari *Coscinium Fenestratum* (Akar Sekunyit) Terhadap Bakteria Penyebab Penyakit Pokok)

Aiza Harun[1*], Noorshilawati Abdul Aziz[2], Shaari Daud[1], Khairil Syazwan Salim[1], Norlaili Abu Bakar[3], Siti Zaiton Mat So'ad[4], and Norizan Ahmat[5]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA Pahang, 26400 Bandar Jengka, Pahang, Malaysia*
[2]*Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Pahang, 26400 Bandar Jengka, Pahang, Malaysia*
[3]*Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak, Malaysia*
[4]*Kulliyyah of Pharmacy, International Islamic University Malaysia, 25200 Bandar Indera Mahkota, Kuantan, Pahang, Malaysia*
[5]*Faculty of Applied Sciences, Universiti Teknologi MARA Shah Alam, Selangor, Malaysia*

[*]*Corresponding author: aizaharun@uitm.edu.my*

Received: 4 June 2023; Accepted: 3 October 2023; Published: 29 December 2023

Abstract

Coscinium fenestratum (Gaertn.) Colebr. (Menispermaceae) is a large woody liana found in moist deciduous to the evergreen forest at an altitude of 350–1200m. The stem and root of this species are highly medicinal valuable, and it is believed to be an endangered species in Malaysia. This study was conducted to investigate the reaction of methanol extract from *Coscinium fenestratum* with cancer cell lines, plant pathogenic bacteria, as well as DPPH scavenging and ferric reduction antioxidant power. The antibacterial activity was evaluated from disc diffusion method and microdilution method against soft rot bacteria *Erwinia chrysanthemi* and *Erwinia carotovora*. The cytotoxicity assay was performed using MCF-7 and Caco-2 cancer cell lines. The antioxidant properties of methanol extract were assessed from DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP). The cytotoxicity activity revealed the methanol extract has potential to suppress the Caco-2 cell line with the percentage cell viability at 70% compared to MCF-7 cell line. The antioxidant activity from FRAP assay recorded its value of 47.8080 µg for TE/gDW and 13.8481 µg/mL for the IC_{50} from radical scavenging activity. However, the total flavonoid content (TFC) was not detected in methanol extract of *C. fenestratum*. The antibacterial assay revealed that the extract had moderately inhibited the growth of *E. chrysanthemi* and *E. carotovora* at 400 mg/mL, with inhibition zones of 11.50 mm and 11.83 mm, respectively. Therefore, the methanol extract from *C. fenestratum* has potential to act as antioxidant and anti-soft rot agent but less potential as anti-cancer agent and anti-soft rot agent.

Keywords: antibacterial, antioxidant, cytotoxicity, *Coscinium fenestratum*, plant pathogen

Abstrak

Coscinium fenestratum (Gaertn.) Colebr. (Menispermaceae), pokok liana berkayu besar yang ditemui dalam hutan daun luruh lembap hingga ke hutan malar hijau pada ketinggian 350–1200 m. Batang dan akar spesis ini sangat bernilai dalam bidang perubatan, dan ia dipercayai spesis terancam di Malaysia. Kajian ini bertujuan untuk mengkaji tindakan ekstrak metanol daripada *Coscinium fenestratum* bertindak balas dengan garisan sel kanser, bakteria patogen tumbuhan serta penghapusan DPPH dan kuasa antioksidan penurunan ferik (FRAP). Aktiviti antibakteria dinilai daripada kaedah resapan cakera dan kaedah pencairan mikro terhadap bakteria reput lembut *Erwinia chrysanthemi* dan *Erwinia carotovora*. Ujian ketoksikan sel telah diadakan menggunakan garisan sel kanser MCF-7 dan Caco-2. Sifat antioksidan ekstrak metanol dinilai daripada aktiviti penghapusan radikal DPPH dan kuasa antioksidan penurunan ferik (FRAP)._Aktiviti ketoksikan sel mendedahkan ekstrak metanol berpotensi untuk menindas garisan sel Caco-2 dengan peratusan daya maju sel pada 70% berbanding garisan sel MCF-7. Aktiviti antioksidan daripada ujian kuasa antioksidan penurunan ferik mencatatkan nilainya sebanyak 47.8080 µg TE/gDW manakala 13.8481 µg/mL untuk IC_{50} daripada aktiviti penghapusan radikal._Walau bagaimanapun, jumlah kandungan flavonoid (TFC) tidak dikesan dalam ekstrak metanol *C. fenestratum*. Ujian antibakteria mendedahkan bahawa ekstrak telah menghalang pertumbuhan *E. chrysanthemi* dan *E. carotovora* secara sederhana pada 400 mg/mL dengan zon perencatan masing-masing 11.50 mm dan 11.83 mm. Oleh itu, ekstrak metanol daripada *C. fenestratum* berpotensi bertindak sebagai agen antioksida dan anti reput lembut tetapi kurang berpotensi sebagai agen anti kanser dan anti reput buah.

Kata kunci: antibakteria, antioksidan, ketoksikan sel, *Coscinium fenestratum*, patogen tumbuhan

Introduction

The cytotoxicity evaluation of plants is a vast subject in pharmaceutical studies, especially in cancer research area [1]. Methanol and methanol-water extracts of *C. fenestratum* was reported to exhibit antiproliferative activities in a concentration-dependent manner against human HT-1080 fibrosarcoma cells among the seventy-seven Vietnamese medicinal plants. The induction of apoptosis was the reason of selective activity against lung carcinoma and/or lung metastatic cell lines, A549, LLC and B16-BL6 and characteristic morphological change and DNA fragmentation that indicated the antiproliferative activity [2]. The berberine and its derivatives revealed potent anticancer activity against human fibrosarcoma cells (HT1080) using MTT assay [3]. Thai edible plant *C. fenestratum* which was extracted with 95% ethanol and tested with cytotoxic effects using Hep2 cells showed the remarkable result with high cytotoxic activity against the Hep2 cell lines at a minimum concentration of 0.05% in ethanol extract. In addition, it demonstrated the most potent cytotoxic extract based on its lowest IC50 (5 mg/mL) [4].

Previous study of antioxidant activity indicated that the berberine isolated from stem of *C. fenestratum* has higher IC_{50} value compared to its berberine derivatives contain phenolic groups which have lower IC_{50} values [3]. Recently, it was found that combinations of two or more medicinal plants extracts can effectively increase the anticancer activity of the synergistic effect between the phytochemicals, as they are found in trace amounts, whereas the attainment of a therapeutic effect requires higher doses [5].

The high berberine content in *C. fenestratum* is the main cause of its antimicrobial properties. There is a recent report on the potent antimicrobial activity of *C. fenestratum* extracts against five human pathogenic microbes [6]. Meanwhile, the aqueous extracts of *C. fenestratum* inhibited the in vitro growth of *Clostridium tetani*. Thus, it is consistent with its traditional use as a prophylactic against tetanus. Ethanolic extract of *C. fenestratum* stem has strong inhibitory effects against *Propionibacterium acnes* and *Staphylococcus epidermidis* and this was resulted from the presence of alkaloids berberine, which could be responsible for this activity. Berberine from *C. fenestratum* also showed anti-phytopathogenic fungal activity against various fungi like *Phytophthora parasitica*, *Pythium* spp., *Colletotrichum gloeosporioides, Cercospora* spp., *Fusarium oxysporum,* and *Alternaria porri* [7].

The antibacterial activity of methanol extract of *C. fenestratum* against soft rot bacteria, such as *Erwinia* species and cytotoxicity activity against Caco-2 cell line was not well documented; this is the main reason for conducting this study.

Materials and Methods

Preparation of methanolic extract from *C. fenestratum*

The preparation of methanol extract from *C. fenestratum* (CFM) has already been mentioned in a previous work [6].

Cytotoxicity study: Cancer cell line

The MCF-7 and Caco-2 cells were cultured in complete Dulbecco's Modified Eagle Media (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 atmosphere and sub-cultured every three to four days at approximately 80% to 90% of confluence.

Cell viability assay

The cell viability assay was measured using tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according with some modifications. A total of 1.0–2.0 × 10^5 cells/mL were seeded in a 96-well plate overnight at 37°C with 5% CO_2 atmosphere. Then, the cells were treated with serial dilution concentrations of the extracts (3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) in triplicate for 24 hours at 37 °C with 5% CO_2 atmosphere. Solvent (vehicle) used to dissolve the sample was used at a final concentration of < 0.1%, which does not affect the viability of cells. After 24 hours, all media were discarded and 30 µL of MTT solution (5 mg/mL MTT in PBS) was added into each well. After four hours of incubation with MTT solution, the formazan crystals were dissolved in 100 µL DMSO and left in the dark at room temperature for an additional one hour. The absorbance measured at 570 nm and 630 nm used as reference wavelength was performed using a microplate reader (TECAN infinite M200). The wells with untreated cells were taken as the control and wells with medium with 0.1% (v/v) of DMSO were used as solvent control. Results were expressed as a percentage of the average absorbance of sample treated cells with respect to untreated ones. Percentage of cell viability (%) was calculated using the following equation 1:

$$\text{Cell viability (\%)} = \frac{\text{(Absorbance of treated cell–Absorbance blank)}}{\text{(Absorbance of untreated cell–Absorbance blank)}} \times 100 \quad (1)$$

Statistical analysis

Data were performed in three replicates, where Microsoft Excel was used to perform graphical representation.

Antioxidant activity: Total flavonoid content (TFC)

Total flavonoid content was determined following the method described in literature. Distilled water (100 µL) was added to each of the 96 wells, followed by 10 µL of 50 g/L $NaNO_2$ and 25 µL of sample solution. After five minutes, 15 µL of 100 g/L $AlCl_3$ was added to the mixture. Six minutes later, 50 µL of 1 mol/L NaOH and 50 µL of distilled water were added. The plate was shaken for 30 s in the plate reader prior to absorbance measurement at 510 nm. The sample was measured against a mixture of DMSO instead of the extract as blank, and the absorbance was subtracted from the absorbance of 25 µl of extract and 225 µl of water. Catechin at a final concentration ranging from 2.125–100 µg/mL was used to generate a calibration curve.

DPPH radical scavenging activity

The method of radical scavenging activity assay was adapted from previous report [8]. All the extracts and standard ascorbic acid were two-fold serially diluted from 400 ug/mL to 12.5 ug/mL. The mixture of 1 mL of each extract and 3 mL of 0.004% DPPH solution was prepared and kept in a dark place for 30 min. The absorbance of all samples was measured at 517 nm using a spectrophotometer, and the percentage of scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = A0-A1/A0\times100 \quad (2)$$

where, A_0 represents the DPPH control absorbance, and A1 represents the extracted/standard sample absorbance.

Ferric reducing antioxidant power (FRAP)

An amount of 455.30 mg of anhydrous sodium acetate was dissolved with 50 mL of distilled water. Then, 3.97 mL of glacial acetic acid was added, and finally the

mixture was diluted to 250 mL to prepare acetic acid buffer solution at a concentration of 0.30 mol/L. TPTZ weighing 156.20 mg was taken using a chemical balance, dissolved with distilled water, and 0.17 mL of concentrated hydrochloric acid was added. The solution was diluted to 100 mL to prepare TPTZ solution at a concentration of 10 mmol/L. $FeCl_3·6H_2O$ weighing 270.03 mg was dissolved with H_2O and diluted to 50 mL. The concentration of $FeCl_3$ solution was 20 mmol/L. Acetic acid buffer, TPTZ solution, and $FeCl_3$ solution were mixed according to the ratio (10:1:1). Then, the mixture was incubated at 37°C. The working solution must be used up within 1–2 h. Next, 180.0 µl FRAP working solution was mixed with 5-µl sample in a 96-well plate. The mixture was shaken and incubated at 37°C for 15 min in the dark. The absorbance was measured at a wavelength of 593 nm. Trolox was used as the standard, while distilled water as the blank control. Trolox concentration was selected under the condition of absorbance value ranging from 0.2 to 0.8 to generate a standard curve.

Antibacterial activity against plant pathogen: Disc diffusion method

The standardized cultured bacteria, *E. chrysanthemi* and *E. carotovora*, were prepared at an optical density (OD) of 0.1. An amount of 100 µL of standardized bacteria inoculum were swabbed on the solidified agar surface to initiate bacteria growth on the agar. Then, the sterile impregnated disc of *E. spiralis* leave extracts with concentrations of 400, 200, 100, and 50 mg/mL were aseptically placed onto the inoculated agar surface and incubated at room temperature for 24 hours. The tests were conducted in triplicate for each extract and repeated with standard disc ampicillin. The effectiveness of the extracts to act as antibacterial agent was evaluated based on the diameter of the inhibition zone (mm). The greater the inhibition zone, the higher the effectiveness of the extract towards bacteria.

Microdilution method

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by two-fold serial dilution method as described by Mogana et al. [9]. Standardized bacteria suspension were diluted with the ratio of 1:100 in nutrient broth. An amount of 180 µL of standardized inoculum was inoculated into each well of 96 well plate, followed by 20 µL of diluted extract to give a final volume of 200 µL with final concentration in each well ranging from 50 mg/mL to 0.024 mg/mL. The plates were incubated at room temperature for 24 hours. After incubation at room temperature, 20 µL of 0.5% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was added into each well to indicate bacterial growth. The dark-blue color will indicate microbial growth, whereas yellow color will reveal the inhibition of microbial growth. The concentration at which the yellow color appeared in the well at the first place indicates the MIC value of the sample. The mixture in the MIC well was streaked on fresh agar plate to ensure the disappearance of bacterial growth. The MBC value was determined from the well next to the well of MIC value.

Results and Discussion

***In vitro* cytotoxicity assay**

Cytotoxicity is defined as the toxicity caused due to the action of chemotherapeutic agents (natural or synthetic drug) on living cells. A cytotoxic agent is a substance that kill cells including cancer cell and may stop the cell from dividing and growing abnormal and may shrink the tumor cell. The cell viability assay was performed using MTT on MCF-7 and Caco-2 cells to evaluate the cytotoxic effects of methanol extract of *C. fenestratum* (CFM). The cells were treated with serial dilution concentrations of the extracts at 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL in triplicate for 24 hours. Table 1 shows the cell viability (%) value of the extract for each concentration and standard deviation. Results from Table 1 and Figure 1 indicate that all concentrations treated on MCF-7 cancer cell line was unable to reduce the cell proliferation until at highest concentration 100 ug/mL. However, according to Figure 2, the percentage of cell viability of Caco-2 cell line was slightly reduced to 70%, as the concentration of CFM increased. This salient information reveals CFM as a potent extract to inhibit Caco-2 cancer cell proliferation. The result against Caco-2 cancer cell line might be due to the abundant presence of phytochemical alkaloid in CFM [6], which is also antioxidative. A previous study also found terpenoid, phenolics, and saponin in CFM, where their presence may possibly contribute to the potent

cytotoxicity effect against Caco-2 cell line. Previous investigation by [10] reported the promising cytotoxicity effect of alkaloidal extract from *Peganum harmala* and the findings of the current study agree with their report, as the CFM is also an alkaloidal extract. Unfortunately, the combination of alkaloid, phenolic, terpenoid, and saponin in CFM to provide synergistic effect still cannot inhibit the MCF-7 cell proliferation well enough.

IC_{50} is recognized as the inhibition concentration of substance for 50% of cell growth. According to Table 2, CFM needed high concentration to inhibit 50% of cell growth for both MCF-7 and Caco-2 cell lines at 3381 µg/mL and 176.2 µg/mL, respectively. In comparison between both cell lines, CFM is considered a good inhibitor against the Caco-2 cell line.

Table 1. Cell viability (%) value of CFM extract

Concentration of sample (µg/mL)	**Percentage of Cell Viability for MCF-7 Cell (%) ± SD**	**Percentage of Cell Viability for Caco-2 Cell (%) ± SD**
0 (untreated cells)	100.00 ± 0.287	100.00 ± 0.903
3.125	171.475 ± 1.614	95.709 ± 0.0574
6.25	167.527 ± 7.371	93.830 ± 0.343
12.5	146.327 ± 1.341	90.513 ± 0.308
25	146.148 ± 2.112	87.333 ± 0.298
50	131.089 ± 0.473	85.170 ± 1.426
100	116.583 ± 3.005	70.014 ± 2.572

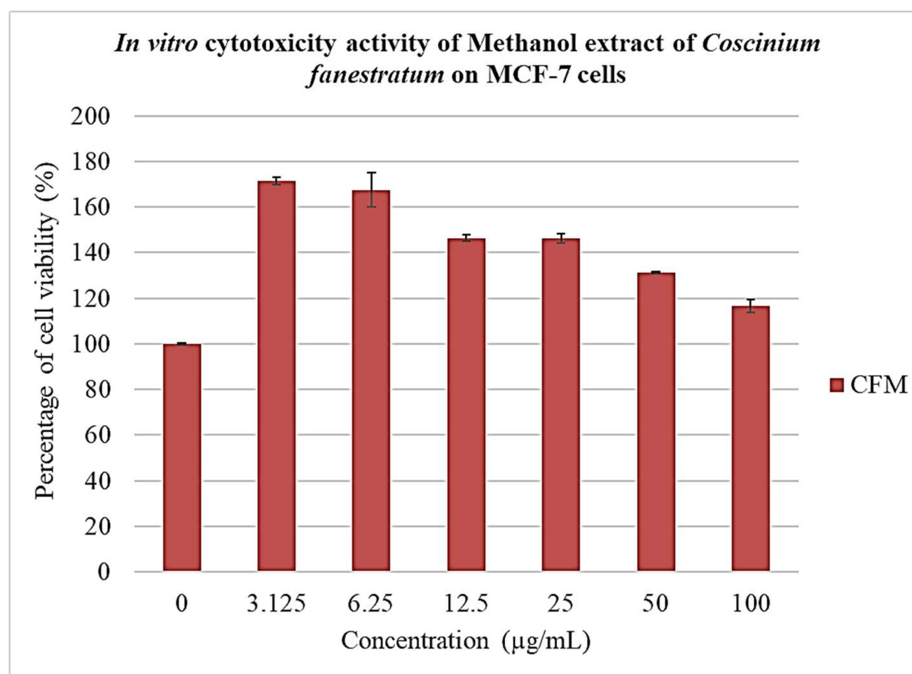

Figure 1. The percentage of cell viability of MCF-7 cells measured by MTT assay treated with CFM extract at the concentration of 3.125 to 100 µg/mL for 24 hours.

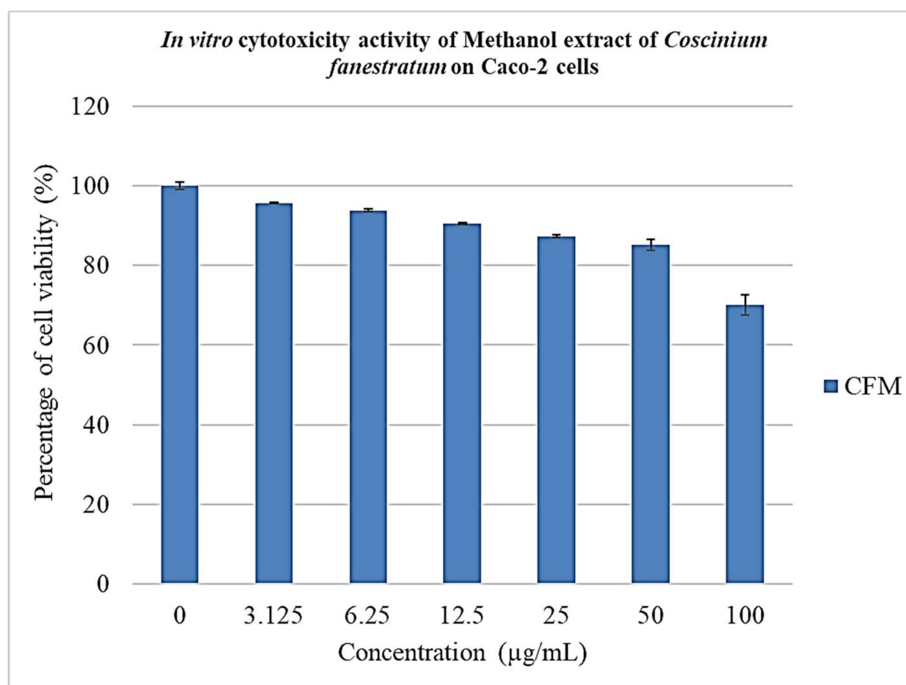

Figure 2. The percentage of cell viability of Caco-2 cell measured by MTT assay treated with CFM extract at the concentration of 3.125 to 100 µg/mL for 24 hours

Table 2. IC_{50} values of methanol crude extract of *C. fenestratum* and standard drug on MCF-7 and Caco-2 cell

Crude	**IC_{50}, ug/mL (MCF-7)**	**IC_{50}, ug/mL (Caco-2)**
CFM	338.1 ± 0	176.2 ± 0
Tamoxifen	0.4 ± 0.3	-

CFM: Methanol extract of *C. fenestratum*

Antioxidant activity

The FRAP assay is used to measure the antioxidant power based on the reduction at low pH of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to an intense blue color ferrous-tripyridyltriazine complex (Fe^{2+}-TPTZ) with an absorption maximum at wavelength 593 nm. Any changes from colorless Fe^{3+} to blue colored Fe^{2+}-tripyridyltriazine solution indicate the action of electron donating antioxidant. The FRAP value of CFM depicted in Table 3 is calculated as 47.8080 ug TE/gDW, and this value demonstrates high antioxidant power of CFM to reduce Fe^{3+} to Fe^{2+} ion. The IC_{50} value indicates the concentration of substance to scavenge 50% DPPH radical. The lower the IC_{50} value, the better the antioxidant activity is. The lower IC_{50} value of CFM at 13.8414 µg/mL revealed the remarkable potential of CFM as antioxidant agent. Moreover, this IC_{50} value is comparable with IC_{50} value of standard ascorbic acid 10.8520 µg/mL. Previous investigation [6] reported the total phenolic content (TPC) of CFM at 12.722 mg GAE/g sample, which could be one of the reasons for antioxidant properties of CFM. However, the presence of major phytochemical alkaloid and other phytochemicals, such as terpenoid and saponin [6], must not be underestimated on their contribution to antioxidant power.

Table 3. Total flavonoid content (TFC), DPPH radical scavenging activity, and ferric reduction antioxidant power (FRAP) of *Coscinium fenestratum* methanol extract

Antioxidant Activity	**Methanol Extract of *C. fenestratum***	**Ascorbic Acid**
Total flavonoid content, (mg CE/g) (TFC)	–1.8595 ± 0.1878 (Not detected)	ND
Ferric reducing antioxidant power (ug TE/gDW)	47.8080 ± 0.4834	ND
DPPH scavenging IC50 (ug/mL)	13.8414 ± 0.1282	10.8520 ± 0.2716

Antibacterial activity

The antibacterial activity of CFM as anti-soft rot agent against two soft rot bacteria *E. chrysanthemi* and *E. carotovora* was illustrated in Table 4 and 5. This antibacterial data is the first time reported using *C. fenestratum* against *E. chrysanthemi* and *E. carotovora*. Generally, CFM extract moderately inhibited the growth of both bacteria in a concentration dependent manner (Table 4). The inhibition zone for both bacteria is not significant as the inhibition zone at 400 mg/mL is recorded as 11.5 mm and 11.83 mm. The presence of phytochemicals terpenoid and saponin [6] are believed to synergistically build the inhibitory effect on both bacteria. According to previous study [11], it was found that the growth of *E. chrysanthemi* was suppressed when using plant species, such as *E. spiralis* with inhibition zone of 16.3 mm, however the effectiveness of this plant towards *E. carotovora* was not reported. According to MIC and MBC value in Table 5, CFM is more effective to inhibit the growth of *E. carotovora* by 50% growth at MIC 15.625 µg/mL compared to *E. chrysanthemi* which is at 125 µg/mL. This means *E. carotovora* was more susceptible towards the CFM compared with *E. chrysanthemi*. The inhibitory effect was attributed with the hydroxyl group from saponin in which this group penetrated and shrunk the cell wall, and finally leading to the death of the cell [12, 13]. Previous report revealed the function of terpenoid as a plant defense to control plants from pathogen and pest and thus, prolonging the plant's lifetime [14,15].

Table 4. Antibacterial bacterial activity of methanol extract of *C. fenestratum* against *E. chrysanthemi* and *E. carotovora* from disc diffusion method

Concentration (mg/mL)	***E. chrysanthemi* inhibition zone ± SD (mm)**	***E. carotovora* inhibition zone ± SD (mm)**
400	11.5 ± 0.5	11.833 ± 0.29
200	6.833 ± 0.29	6.5 ± 0
100	7 ± 0	6. ± 0
50	6 ± 0	6 ± 0

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extract of *C. fenestratum* from microdilution method

Bacteria	**MIC (µg/mL)**	**MBC (µg/mL)**
E. chrysanthemi	62.5	125
E. carotovora	15.625	31.225

Conclusion

The methanol extract from *C. fenestratum* (*akar sekunyit*) is concluded to have good potential as an antioxidant source but less potential as an anti-cancer agent against breast cancer and colon carcinoma cell line, as well as an anti-soft rot agent. Regarding future work, it is recommended to use other types of antioxidant assays, such ORAC, ABTS, and CUPRAC, to establish its antioxidant power. Various anticancer cell lines, as well as plant pathogens, can be applied to evaluate its function in a broad spectrum of bioactivity. Isolation and identification of berberine alkaloid may also be conducted in the future.

Acknowledgment

The authors would like to express their gratitude to the science laboratory staff at Universiti Teknologi MARA (UiTM) Pahang and Assoc. Prof. Dr. Norazian Mohd Hassan from IIUM Kuantan for their assistance throughout this research.

References

1. Cuyacot, A. R., Mahilum, J.J.M. and Madamba, M.R.S.B. (2014). Cytotoxicity potentials of some medicinal plants in Mindanao, Philippines. *Pelagia Research Library*. 4(1): 81-89.
2. Ueda, J., Tezuka,Y., Banskota, A.H., Le Tran, Q., Tran, Q.K., Harimaya, Y., Saiki, I. and Kadota, S. (2002). Antiproliferative activity of Vietnamese medicinal plants. *Biological and Pharmaceutical Bulletin,* 25(6):753-60.
3. Pongkittiphan, V., Chavasiri, W. and Supabhol, R. (2023). Antioxidant effect of berberine and its phenolic derivatives against human fibrosarcoma cells. *Asian Pacific Journal Cancer Preventive*, 16(13): 5371-5376.
4. Kamkaen, N., Jenny, M., Wilkinson J.M., Cavanagh and H.M.A. (2006). Cytotoxic effect of four Thai edible plants on mammalian cell proliferation. *Thai Pharmaceutical Health Science Journal*, 1(3):189-195.
5. Moatasem, M. A., Dhifaf, J. S., Emaduldeen, H. A., Mohammed, M. T. A., Ibrahim, S. A., Dhuha, J. M., and Rana, R. K. The synergistic anticancer effect of some plant extracts in combination against human liver cancer cell line. *Asian Journal of Dairy and Food Research*, 1: 1-6.
6. Harun, A., Abdul Aziz, N. and Daud, S. (2022). The antibacterial, antifungal, antioxidant potential and total phenolic content of Malaysian endangered species *Coscinium fenestratum* from Paya Bungor Pahang. *Malaysian Journal of Analytical Sciences,* 26(4): 734-741.
7. Danapur, V., Haleshi, C. and Sringeswara A. N. (2020). Endangered medicinal plant *Cosciniumfenestratum* (Gaertn.) Colebr. - A review. *Pharmacognosy Journal,* 12(5): 1077-1085.
8. Ilias Fazna, N.F., Harun, A., Daud, S. and Abdul Aziz, N.(2023). Comparative study of antioxidant activity and total phenolic content of stem bark and leaves from *Murraya koenigii* species. *Gading Journal of Science and Technology*, 6(1): 13-24.
9. Mogana, R., Adhikari, A., Tzar, M., Ramliza, R. and Wiart, C. (2020). Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. *BMC complementary medicine and therapies*, 20(1): 1-11.
10. Lamchouri, F., Zemzami, M., Jossang, A., Settaf, A., Israili, Z.H. and Lyoussi, B. (2013). Cytotoxicity of alkaloids isolated from *Peganum harmala* seeds. *Pakistan Journal Pharmaceutical Sciences,* 26(4):699-706.
11. Maha Yudden, N.K., Nordin, N.N., Harun, A., Rosli, N.H., Abdul Aziz, N., Daud, S. (2019). Comparative study of antioxidant activity of stem and leaves of *Entada Spiralis* and their antibacterial properties against *Erwinia Chrysanthemi*. *Science Letters*, 13(2): 37-47.
12. Soetan, K. O. (2008). Pharmacological and other beneficial effects of antinutritional factors in plants-A review, *African journal of Biotechnology*, 7(25): 4713-472.
13. Ezhilan, B. P. and Neelamegam, R. (2012). GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. *Pharmacognosy Research,* 4(1):11-14.
14. Cheng, A., Lou, Y., Mao, Y., Lu, S., Wang, L. and Chen, X. (2007). Plant terpenoids: Biosynthesis and ecological functions. *Journal of Integrative Plant Biology*, 49(2):179-189.
15. Keawpradub, N. and Dej-adisai S. (2005). Antioxidant and cytotoxic activities of Thai medicinal plants named Khaminkhruea: *Arcangelisia flava,Coscinium blumeanum and Fibraurea tinctoria*. *Songklanakarin Journal Science Technology*, 2: 455-467.