ORIGINAL ARTICLE

Cytotoxic activity of *Cyrtostachys renda* extracts and fractions against MCF-7 and HeLa cancer cells through cell cycle arrest and apoptosis induction mechanism

Syamsurizal, Sutrisno[1], Diah Tri Utami

Department of Pharmacy, Faculty of Medicine and Health Sciences, University of Jambi, [1]Department of Chemistry, Faculty of Science and Technology, University of Jambi, Jambi, Indonesia

J. Adv. Pharm. Technol. Res.

ABSTRACT

In traditional medicine, *Cyrtostachys renda* has been used for its many bioactive components that are good for people's health. This research assessed the cytotoxic effects of extracts and fractions against Michigan Cancer Foundation-7 (MCF-7) and Henrietta Lacks (HeLa) cell lines. The extracts and fractions of root and fruit assess cytotoxic activities and inhibitory mechanisms against the MCF-7 and HeLa cancer cell lines, respectively. The fruit and roots of *C. renda* were extracted using the liquid-liquid method. The sample concentrations evaluated included extracts 31.5-1000 μg/mL, fractions 15.625-500 μg/mL, and doxorubicin 2-0.0625 μg/mL. Cytotoxicity was evaluated on MCF-7 and HeLa cells using an MTT assay. Morphological alterations were subsequently discovered utilizing an inverted microscope. Flow cytometry was utilized to find out the cell cycle's distribution and the apoptosis characteristics. The different parts and extracts showed cytotoxic effects on HeLa and MCF-7 cells, with IC_{50} values ranging from 30.69 ± 0.47 to 787.89 ± 1.77 μg/mL. Cell cycle studies showed that fraction A4 inhibited the cell cycle in MCF-7 cells at the G1 phase followed by the G2/M and S phases, while fraction B5 inhibited the cell cycle in HeLa cells at the G2/M phase. Both fractions showed the ability to induce apoptosis against MCF-7 and HeLa cells. The results showed that the fractions A4 and B5 showed cytotoxic activity against MCF-7 and HeLa cells by cell cycle arrest and apoptosis induction.

Key words: Apoptotic, cell cycle arrest, *Cyrtostachys renda,* cytotoxicity, HeLa, MCF-7

INTRODUCTION

The International Agency for Cancer Research reported that among the top 10 deaths globally are non-Hodgkin lymphoma, leukemia, breast, cervical, lung, colon, prostate, ovary, liver, and nasopharynx cancers.[1,2] In Indonesia, breast cancer had the highest number of newly diagnosed cases, totaling 396,914, with 65,858 cases constituting 16.6% of the total. Cervical cancer followed with 36,633 cases, representing 9.2%.[3] Breast and cervical cancer are the most common cancers among women, resulting in a significant number of cancer-related deaths.[4]

Several attempts have been made to improve cancer therapy. However, some adverse effects make treatment less effective.

Address for correspondence:
Dr. Syamsurizal,
Department of Pharmacy, Faculty of Medicine and Health Sciences, University of Jambi, Jl. Letjen Soeprapto No. 33, Telanaipura, Jambi 36122, Indonesia.
E-mail: syamsurizal68@unja.ac.id

Submitted: 03-Jul-2024 **Revised:** 31-Dec-2024
Accepted: 06-Jan-2025 **Published:** 14-Feb-2025

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/JAPTR
	DOI: 10.4103/JAPTR.JAPTR_222_24

How to cite this article: Syamsurizal, Sutrisno, Utami DT. Cytotoxic activity of *Cyrtostachys renda* extracts and fractions against MCF-7 and HeLa cancer cells through cell cycle arrest and apoptosis induction mechanism. J Adv Pharm Technol Res 2025;16:18-23.

Herbal medicine, as a complementary therapy, utilizing natural substances with cytotoxic effects, is one technique to increase therapeutic efficacy while reducing damage induced by chemotherapy medications. *Cyrtostachys renda* is an ornamental and medicinal plant containing several secondary metabolites that can enhance the well-being of humans, one of which is a substance known as bioactive that is toxic to cancer cells. *C. renda* is a plant belonging to a genus that contains arecoline compounds, which inhibit tumor cell growth and proliferation by reducing the activity of the enzyme acetyl-CoA acetyltransferase 1.[5]

In previous research, combining doxorubicin with a fractionated approach induced apoptosis in T47D cells. In contrast, using doxorubicin alone led to necrosis. Therefore, further cytotoxic testing on additional cell types is necessary to assess its effectiveness. This study investigates the cytotoxic activity of extracts and their active fractions on MCF-7 and HeLa cells. Cytotoxic testing of extracts from *C. renda* plant parts has not been previously reported, and no compounds with anti-cancer properties have been isolated.

MATERIALS AND METHODS

Root and fruit specimen

C. renda was harvested in Muaro Bungo, Jambi Province. Samples were collected in May 2023. Drs. Joko Kusmoro, M. P., identified this specimen at the Jatinangor Herbarium, Indonesia, and assigned the herbarium number 33/HB/05/2023.

Extraction

The powder of fruit and root were extracted using the liquid-liquid method with four solvents: Water-methanol (MeOH), water-ethyl acetate (AcOEt) and water-dichloromethane (DCM), and *n*-hexane.

Phytochemical screening

The following methods were employed to screen phytochemicals: Harborne methods, alkaloids by Dragendorff, Mayer, and Wagner, flavonoids by Mg powder + HCl, H_2SO_4, NaOH 10%, saponin by HCl 2N, steroids by Liebermann–Burchard, terpenoids by Salkowski, and phenolic by $FeCl_3$.[6]

Isolation of active fraction using vacuum liquid chromatography

The extracts AcOEt of fruit and DCM extract of root, which exhibited potent cytotoxic activity were subjected to a vacuum liquid chromatography column and eluted with a gradient polarity eluent of DCM-MeOH-H_2O with a ratio of 30:3:1, 15:3:1, 7:3:1, and 3:3:1, respectively, then finally eluted with 100% MeOH, yielding five fractions.

Cytotoxicity test

The cytotoxic effects of extracts and fractions against MCF-7 and HeLa cells were evaluated utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, as referenced by Nordin *et al*.[7] The sample concentrations evaluated included extracts (31.25, 62.5, 125, 250, 500, 1000 μg/mL), fractions (15.625, 31.25, 62.5, 125, 250, 500 μg/mL), and doxorubicin (2, 1, 0.5, 0.25, 0.125, 0.0625 μg/mL).

Cell imaging

Changes in cell morphology after treatment with extracts and active fractions were evaluated using inverted microscope.

Cell cycle arrest and apoptosis induction assay

Cell cycle arrest and apoptosis induction assays of the active fractions used the flow cytometry method, referring to the procedure described by Utami *et al*.[8] The concentration of fraction B5 (145.95 μg/mL) and doxorubicin (1.33 μg/mL) on HeLa cell, and fraction A4 (30.69 μg/mL) and doxorubicin (1.21 μg/mL) on MCF-7 cell.

Statistical analysis

All data values were shown as means with standard deviations (SDs). The student's *t*-test was employed to identify differences of statistical significance, with a limit of $P < 0.05$.

RESULTS

Extraction using liquid-liquid and fractionation using vacuum liquid chromatography

Extraction of the extracts AcOEt of fruit and DCM extract of root resulted in a yield 15.74% and 20.27%, respectively. Meanwhile, the results of the fractionation obtained B5 of 34.01% and A4 yield of 38.64%.

Phytochemical screening of plant material

Alkaloids and flavonoids are present in all extracts. However, not all extracts have saponins, tannins, steroids, and terpenoids [Supplementary Table 1].

The cytotoxicity effect of extracts and fractions

The cytotoxic effect of the extracts and fractions is presented in Supplementary Table 2 and Figure 1.

Cell morphology analysis of extracts and fractions

The results of cell morphology analysis of the extracts, active fraction, and doxorubicin are presented in Figures 2 and 3.

Cell cycle arrest of fractions

The results of the cell cycle modulation of the active fractions are presented in Figure 4.

Apoptosis induction of fractions

The results of the apoptosis induction of the active fractions are presented in Figure 5.

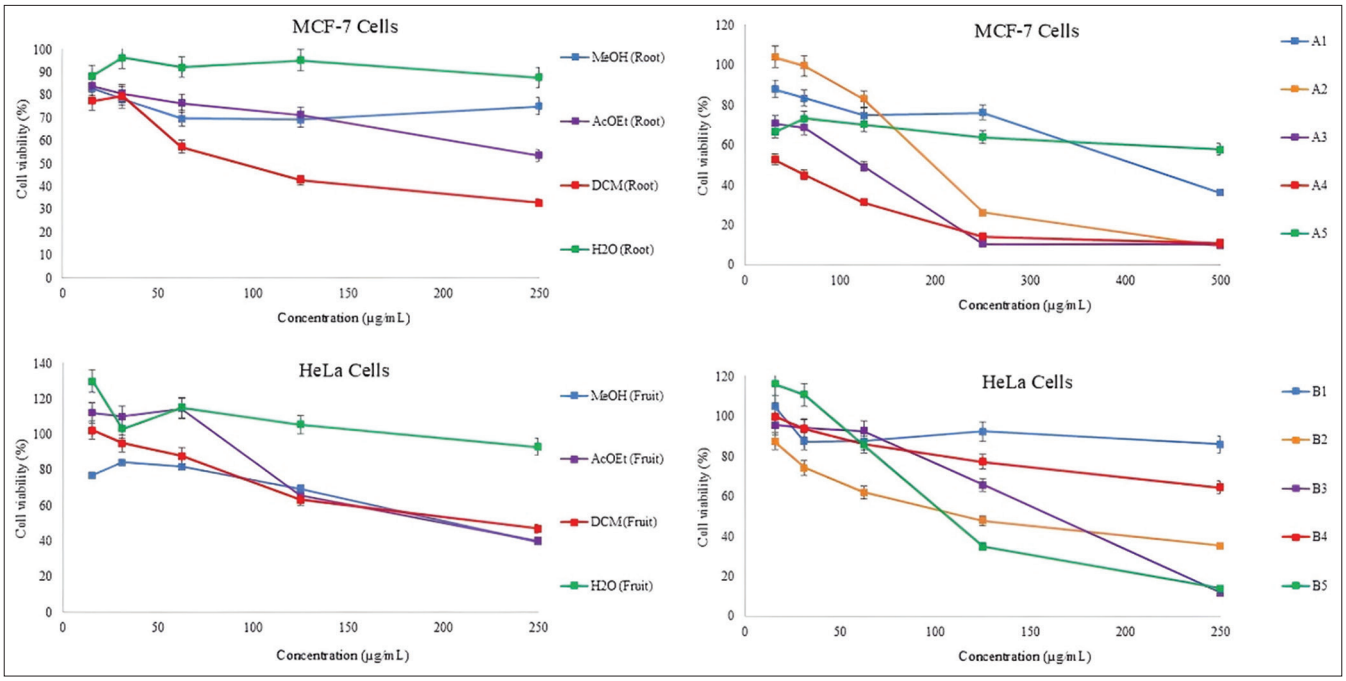

Figure 1: Cytotoxic activity of extracts and fractions *Cyrtostachys renda* against MCF-7 and HeLa cell lines. Percent cytotoxicity was reported as mean values ± standard deviations of three independent assays

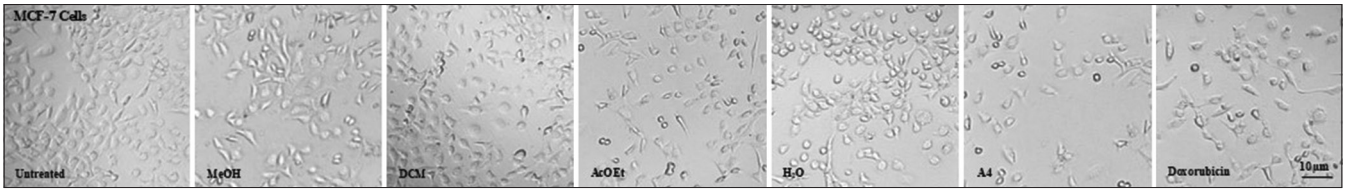

Figure 2: Effect of the doxorubicin, extracts, and fractions *C. renda* against the morphology of MCF-7 cells

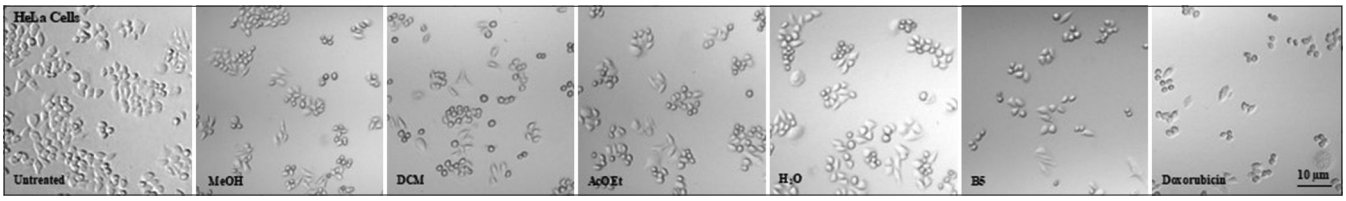

Figure 3: Effect of the doxorubicin, extracts, and fractions *C. renda* against the morphology of HeLa cells

DISCUSSION

Secondary metabolites that appear in the fruit and root include alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids. Previous studies have reported that these metabolites are important in cancer treatment by suppressing the proliferation of several types of cancer cells through various mechanisms, one of which is apoptosis induction.[7] The cytotoxic category was measured in reference to Prayong *et al*.[9] The genus Areca or Cyrtostachys such as *C. renda* contains alkaloid compounds such as guvacine, arecoline, guvacoline, arecaidine, nicotine, and chavibetol.[10,11]

Cytotoxic effect analysis showed that DCM extract from the root showed the strongest activity against MCF-7 compared to AcOEt, MeOH, and H_2O extracts. While, in HeLa cells, AcOEt extract from fruit showed the strongest activity compared to DCM, MeOH, and H_2O extracts. Previous studies have reported that DCM, AcOEt, and MeOH extracts showed cytotoxic activity against T47D cells.[12] Figure 1 and Supplementary Table 2 show that fraction A4 exhibited higher cytotoxic activity against MCF-7 cells with an IC_{50} of 30.69 ± 0.47 µg/mL. According to Figure 1, extracts and fractions inhibit the proliferation of MCF-7 and HeLa cells in a dose-dependent manner. Doxorubicin showed a stronger effect as a positive control, with IC_{50} values of 1.2 ± 0.36 and 1.3 ± 0.74 µg/mL against MCF-7 and HeLa cells, respectively [Supplementary Table 2].

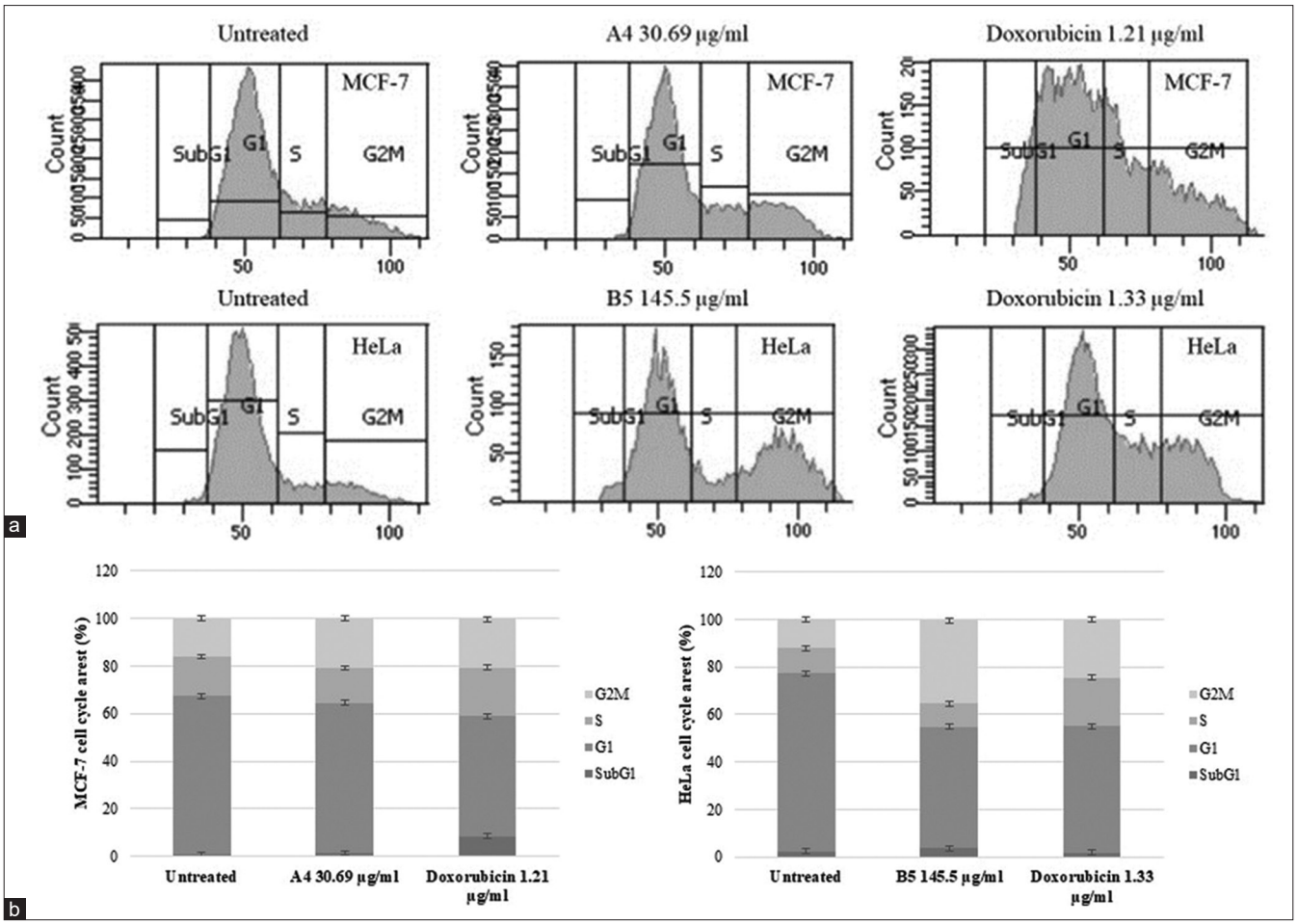

Figure 4: A4 fraction and doxorubicin caused the changing of cell cycle distribution against MCF-7 cell and B5 fraction and doxorubicin caused the changing of cell cycle distribution against HeLa cell. Cells were treated for 24 h with doxorubicin and A4 and B5 fractions and subjected to flow cytometry analysis after stained with propidium iodide/RNase: (a) Flow cytogram of cell cycle profiles, (b) Quantification of cell cycle distribution

Extracts and fractions caused changes in cell morphology, which changed from elongated to round, shrinkage, and irregularity of cell membranes [Figures 2 and 3]. Furthermore, the density of treated cells was lower than that of untreated cells. Living HeLa and MCF-7 cells were characterized by regular and clear morphology, while dead cells were characterized by irregular shape, nontransparent, reduced cell number, separated and small size along with increasing concentration. At low doses, it had no significant effect on cell proliferation, but when the concentration was increased, cell shrinkage, apoptotic bodies, and morphological changes were observed more significantly.[13]

Figure 4 shows that MCF-7 and HeLa cells were arrested in G1 and G2/M phases when exposed to fractions A4 and B5. Treatment of HeLa cells with fraction B5 resulted in a greater accumulation of cells in G2/M phase compared to doxorubicin which resulted in accumulation of cells in G1 phase, followed by accumulation in G2/M and S phases. Whereas treatment of MCF-7 cells with fraction A4 and doxorubicin resulted in accumulation of cells in G1 phase, followed by G2/M and S phases. In contrast, fraction B5 showed substantial capacity to inhibit HeLa cell cycle during G2/M phase, while no significant difference was observed in G1 phase. These findings are consistent with previous studies showing that doxorubicin induces cell cycle arrest at the G2/M and S phases by inhibiting the enzyme topoisomerase II.[8] A substantial decrease in cell viability results from the regulation of either the S or G2/M phases of the cell cycle, as many cells are unable to divide. The average duration for one round of cell division is 24 h, and this experiment was conducted for 24 h.

Figure 5 shows that treatment with fraction B5 can increase HeLa cell death through apoptosis induction, while treatment with doxorubicin will increase HeLa cell death through necrosis induction. Fraction B5 can increase cell apoptosis by 64.6%. The number of cells undergoing apoptosis due to treatment with fraction B5 is higher than treatment with doxorubicin. Figure 5 also shows that treatment with fraction A4 can increase apoptosis in MCF-7 cells by 14.1%, while doxorubicin increases

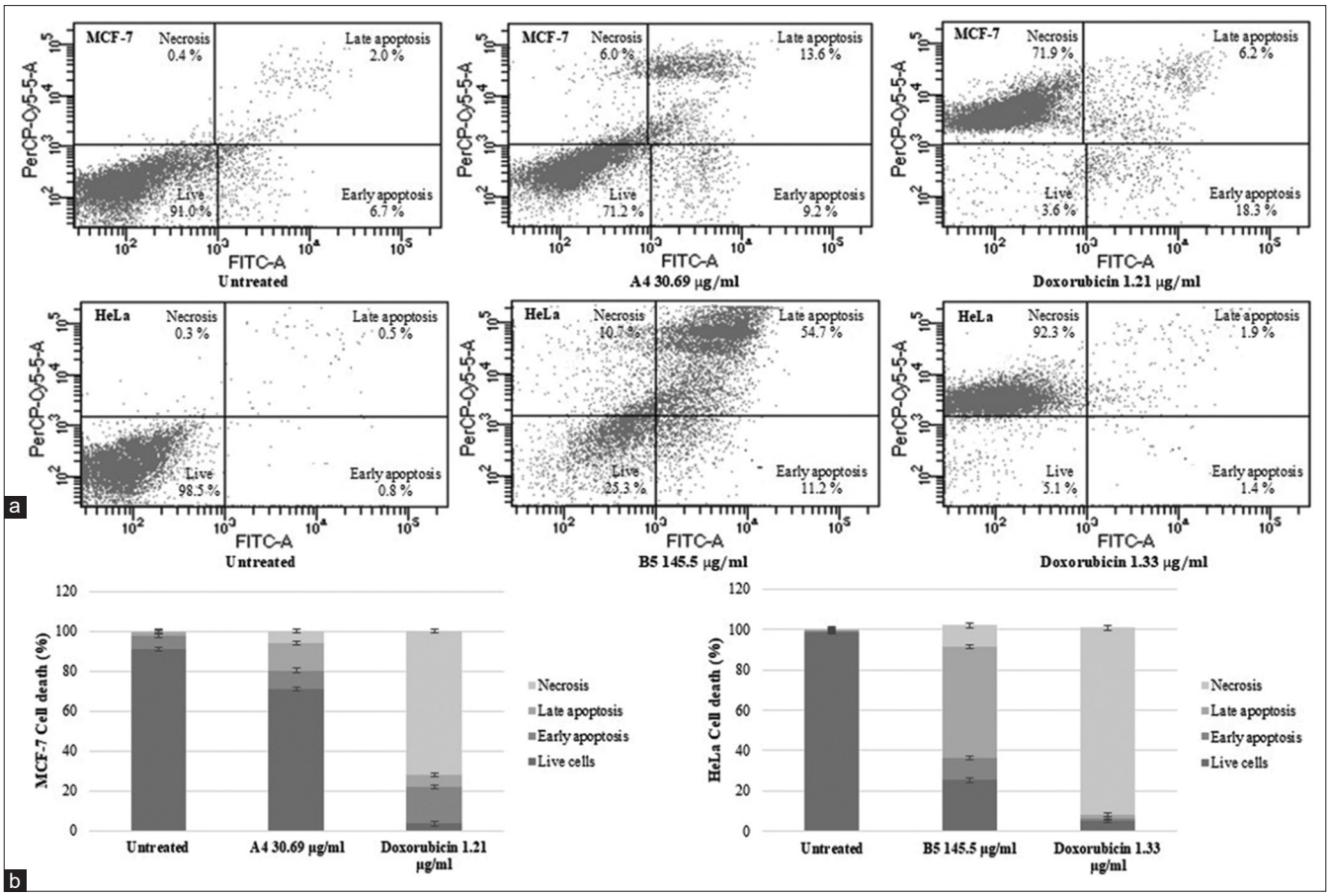

Figure 5: A4 fraction induced apoptosis against MCF-7 cell and B5 fraction induced apoptosis against HeLa cell. Cells were treated for 24 h with doxorubicin and A4 and B5 fractions and at the appropriate concentrations before being stained with FITC/PerCP-Cys5-5-A for cytometry analysis: (a) Flow cytogram profiles of treated cells, (b) Quantification of treated cell death population

necrosis by 71.5%. Fraction A4 and doxorubicin do not differ significantly in inducing apoptosis in MCF-7 cells, but doxorubicin can cause increased cell necrosis. Cell destruction and apoptosis in normal tissues are side effects of doxorubicin toxicity, which affects the heart, kidneys, liver, and brain. Through caspase-3 activation, it triggers cell death.[14,15] In contrast, fraction B5 induced significantly more apoptosis than doxorubicin. Overall, flow cytometry analysis of cell death showed that administration of fractions B5 and A4 induced apoptosis in HeLa and MCF-7 cells.

Previous studies have shown that arecoline is a major compound found in areca nut and other palm species, which can induce cell death in HA22T/VGH cells by activating caspase-3.[16] The mechanism of action of active fractions in inducing apoptosis specifically targets caspase activation. Arecoline induces apoptosis in various cancer cells, such as lung adenocarcinoma A549/8, and various leukemia cells. Through its muscarinic effects, it causes an increase in tumor necrosis factor receptor 2, which causes cancer cell death.[17] Recent studies have shown that arecoline can stop the development of basal cell carcinoma by reducing interleukin-6 levels, increasing p53, and inducing cell cycle arrest and apoptosis.[18] Molecular docking studies have shown that arecoline binds to the muscarinic acetylcholine receptor M3, an essential role in the proliferation, differentiation, and death of leukemia cells.[19] When applied to PC-3 cells, arecoline could also inhibit the expression of several proteins involved in cell cycle regulation, including cyclins D1 and D3 as well as CDK1, CDK2, and CDK4.[20] Cell cycle regulation mainly emphasizes the inhibition of CDK and cyclin protein expression.

The potential of A4 fraction was further studied to isolate and elucidate the bioactive compounds that act as antiproliferatives. Furthermore, more effective cultivation techniques should be applied to enhance the production of bioactive compounds in *C. renda*.

CONCLUSION

The purpose of this study was to isolate the active fractions from the *C. renda* fruit and root extracts and evaluate the cytotoxic effects, and inhibitory mechanisms of active fractions against MCF-7 and HeLa cells. The cytotoxic test against MCF-7 cells using the MTT assay revealed that

fraction A4 had the highest cytotoxic activity, with an IC_{50} value of 30.69 ± 0.47 µg/mL. Furthermore, flow cytometry profiles showed that the A4 fraction's cytotoxic effect was enhanced by modulating the cell cycle at the G2/M phase and inducing apoptosis.

Acknowledgment

The authors thank Ria Novia, Amalia Rani, and Mira Ovita Damayani for their assistance in collecting samples and providing technical support.

Ethical approvals

In this experiment, neither humans nor animals were used.

Financial support and sponsorship

This research was funded by the Institute for Research and Community Service, University of Jambi, Indonesia (award number: 1392/UN21.11/PT.01.05/SPK/2022).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Cao W, Chen HD, Yu YW, Li N, Chen WQ. Changing profiles of cancer burden worldwide and in China: A secondary analysis of the global cancer statistics 2020. Chin Med J (Engl) 2021;134:783-91.
2. Xiang D, Hu S, Mai T, Zhang X, Zhang L, Wang S, *et al.* Worldwide cancer statistics of adults over 75 years old in 2019: A systematic analysis of the global burden of disease study 2019. BMC Public Health 2022;22:1979.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
4. Krishnamoorthy Y, Ganesh K, Sakthivel M. Prevalence and determinants of breast and cervical cancer screening among women aged between 30 and 49 years in India: Secondary data analysis of National Family Health Survey – 4. Indian J Cancer 2022;59:54-64.
5. Goudarzi A. The recent insights into the function of ACAT1: A possible anti-cancer therapeutic target. Life Sci 2019;232:116592.
6. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud 2020;8:603-8. Available from: https://www.chemijournal.com/archives/?year=2020 and vol=8&issue=2&ArticleId=8834&si=false. [Last accessed on 2023 Dec 21].
7. Nordin ML, Abdul Kadir A, Zakaria ZA, Abdullah R, Abdullah MN. *In vitro* investigation of cytotoxic and antioxidative activities of *Ardisia crispa* against breast cancer cell lines, MCF-7 and MDA-MB-231. BMC Complement Altern Med 2018;18:87.
8. Utami DT, Nugraheni N, Jenie RI, Meiyanto E. Co-treatment of Brazilein enhances cytotoxicity of doxorubicin on WiDr colorectal cancer cells through cell cycle arrest. Indones Biomed J 2020;12:376-83. Available from: https://www.inabj.org/index.php/ibj/article/view/1293. [Last accessed on 2023 Oct 18].
9. Prayong P, Barusrux S, Weerapreeyakul N. Cytotoxic activity screening of some indigenous Thai plants. Fitoterapia 2008;79:598-601.
10. Ragab AE, Badawy ET, Aboukhatwa SM, Kabbash A, Abo El-Seoud KA. *In vitro* characterization of inhibitors for lung A549 and leukemia K562 cell lines from fungal transformation of arecoline supported by *in silico* docking to M3-mAChR and ADME prediction. Pharmaceuticals (Basel) 2022;15:1171.
11. Liu YJ, Peng W, Hu MB, Xu M, Wu CJ. The pharmacology, toxicology and potential applications of arecoline: A review. Pharm Biol 2016;54:2753-60.
12. Syamsurizal S, Utami DT. The combination of fraction A3 of *Cyrtostachys renda* Blume with doxorubicin improved cytotoxicity against T47D cell line through cell cycle arrest and apoptotic induction. J Appl Pharm Sci 2024;14:270-8.
13. Tu LY, Bai HH, Cai JY, Deng SP. The mechanism of kaempferol induced apoptosis and inhibited proliferation in human cervical cancer SiHa cell: From macro to nano. Scanning 2016;38:644-53.
14. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: An update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol 2013;65:157-70.
15. Larasati L, Kusharyanti I, Hermawan A, Susidarti RA, Meiyanto E. Naringenin enhances the anti-tumor effect of doxorubicin on HeLa cervical cancer cells through cytotoxic activity and apoptosis induction. Indones J Cancer Chemoprevention 2011;2:325-33. Available from: https://www.ijcc.chemoprev.org/index.php/ijcc/article/view/51. [Last accessed on 2024 Nov 18].
16. Cheng HL, Su SJ, Huang LW, Hsieh BS, Hu YC, Hung TC, *et al.* Arecoline induces HA22T/VGH hepatoma cells to undergo anoikis – Involvement of STAT3 and RhoA activation. Mol Cancer 2010;9:126.
17. Chen YJ, Chang LS. Arecoline-induced death of human leukemia K562 cells is associated with surface up-modulation of TNFR2. J Cell Physiol 2012;227:2240-51.
18. Huang LW, Hsieh BS, Cheng HL, Hu YC, Chang WT, Chang KL. Arecoline decreases interleukin-6 production and induces apoptosis and cell cycle arrest in human basal cell carcinoma cells. Toxicol Appl Pharmacol 2012;258:199-207;258:199-207. Available from: https://www.sciencedirect.com/science/article/pii/S0041008X11004285. [Last accessed on 2023 Oct 18].
19. Siregar P, Audira G, Feng LY, Lee JH, Santoso F, Yu WH, *et al.* Pharmaceutical assessment suggests locomotion hyperactivity in Zebrafish triggered by arecoline might be associated with multiple muscarinic acetylcholine receptors activation. Toxins (Basel) 2021;13:259.
20. Shih YT, Chen PS, Wu CH, Tseng YT, Wu YC, Lo YC. Arecoline, a major alkaloid of the areca nut, causes neurotoxicity through enhancement of oxidative stress and suppression of the antioxidant protective system. Free Radic Biol Med 2010;49:1471-9.

Supplementary Table 1: Secondary metabolites of fruit and root extracts

Secondary metabolites	Fruit extracts				Root extracts			
	DCM	AcOEt	MeOH	H_2O	DCM	AcOEt	MeOH	H_2O
Alkaloids								
Dragendorff	+	+	+	+	+	+	+	+
Mayer	+	+	+	+	+	+	+	+
Wagner	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Saponins	−	−	+	+	−	−	+	+
Tannins	−	+	+	−	−	−	+	+
Steroids	−	−	−	−	−	−	+	−
Terpenoids	−	−	+	−	−	−	+	−

+: Contains secondary metabolites, −: Secondary metabolites are not present. DCM: Dichloromethane, AcOEt: Ethyl acetate, MeOH: Methanol

Supplementary Table 2: The IC_{50} values of doxorubicin, extracts and fractions against MCF-7 and HeLa cells

Sample	IC_{50} (μg/mL)		Cytotoxicity category
	MCF-7	HeLa	
Extracts			
Fruit			
DCM	-	219.86±4.918	Moderate
AcOEt	-	210.63±5.473	Moderate
MeOH	-	209.73±2.102	Moderate
H_2O	-	625.56±5.559	Moderate
Root			
DCM	136.62±1.259	-	Moderate
AcOEt	282.73±2.417	-	Moderate
MeOH	1193.46±2.105	-	Nontoxic
H_2O	2841.57±1.807	-	Nontoxic
Fractionates			
Fruit			
B1	-	265.84±0.254	Moderate
B2	-	152.65±0.893	Moderate
B3	-	156.06±1.487	Moderate
B4	-	336.08±3.406	Moderate
B5	-	145.95±0.282	Moderate
Root			
A1	404.02±2.676	-	Moderate
A2	261.01±1.451	-	Moderate
A3	88.08±1.814	-	Potential
A4	30.69±0.469	-	Potential
A5	787.89±1.773	-	Moderate
Doxorubicin	1.21±0.355	1.33±0.737	Potential

*Cytotoxic category: potential<100 μg/mL, moderate 100–1000 μg/mL, nontoxic >1000 μg/mL). IC_{50} was provided as mean values ± standard deviations from three independent tests. -: Not tested, DCM: Dichloromethane, AcOEt: Ethyl acetate, MeOH: Methanol, MCF-7: Michigan Cancer Foundation-7