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CHEMICAL ANALYSIS AND CYTOTOXIC ACTIVITY OF N-HEXANE FRACTION OF *Zanthoxylum acanthopodium* DC. FRUITS

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ABSTRACT

Zanthoxylum acanthopodium DC. the fruit is a potential anticancer agent. This study was evaluated the anticancer activity of n-hexane fraction (nHF) of *Zanthoxylum acanthopodium* DC. fruit towards T47D cell line. nHFwere analyzed for chemical constituents and tested for cytotoxicity, cell cycle inhibition, apoptosis induction and inhibition of cyclin D1 expression. nHF was found to contain monoterpenes and sesquiterpenes with geranyl acetate (26.34%) as major compound. nHF of *Zanthoxylum acanthopodium* DC. Fruits were found to have IC_{50} 85.41 ± 0.78 µg/mL, cause accumulation in G_0-G_1phase, increased apoptosis and decreased cyclin D1 expression. The results reveal that nHF of *Zanthoxylum acanthopodium* DC. fruits have cytotoxic activities by inhibition cell cycle and induction apoptosis. Our further study is to isolate compounds that responsible for the cytotoxic activity.

Keywords: Cytotoxic, chemical constituents, *Zanthoxylum acanthopodium* DC., n-hexane fraction.

INTRODUCTION

Breast cancer has been known as the leading of cancer in women after cervical cancer. Alteration in lifestyle and diet are the main factor affecting the number of breast cancer patients. The high incidence and the high cost dealing with this cancer which patients should pay for their life, hence there is an urgent need for developing an alternative source of medicine especially from traditional medicine to treat breast cancer patients[1-3].

Zanthoxylum acanthopodium DC. has been used as a tonic and medicate dysentery. Andaliman has been used as a seasoning at North Sumatera[4-6]. *Zanthoxylum* genus has many active compounds such as steroids/triterpenoids, hydroquinones, flavonoids, tannins, glycosides, volatile oils, alkaloids, coumarins, lignans, amides, and terpenes[7-14]. Ethyl acetate fraction was reported active towards breast cancer cell lines and found to have a synergistic effect with doxorubicin. It was showed to have anticancer activity on in vivo study, cardioprotective effect and active on breast cancer resistance cells[15-17]. The purpose of this study was to analysis phytochemical components and determine cytotoxicity activity of n-hexane fraction of *Zanthoxylum acanthopodium* DC. fruits.

EXPERIMENTAL

Plant and Chemical Materials

Fresh fruits were collected from Onan Rungu village, Toba Samosir Regency, Sumatera Utara province, Indonesia. Chemicals used were annexin-V (BioLegend), distilled water, [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma), PI kit (BioLegend), FITC cyclin D1 antibody (Santa Cruz).

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Preparation of n-Hexane Fraction (nHF)
The simplex powder of *Zanthoxylum acanthopodium* DC.(1000 g) was fractionated with n-hexane by maceration based on previous study[17-19].

Analysis of Chemical Constituents by Gas Chromatography
nHF was analyzed with GC-MS Thermo Scientific Trace 1310 (Thermo) gas chromatograph with a fused silica capillary column (TG-5MS, 30 m x 0.25 mm, film 0.25 µm) using helium as a carrier gas with a flow rate of 1.02 mL/minute and with temperature programming from 70°C for 5 minutes to 280°C with increase temperature 5°C/minute. The injector temperature was set at 280°C. The Mass Spectrometer was performed using an interface temperature of 280°C and an electron impact ionization of 70 eV with a scan mass range of 40-500 m/z (sampling rate 1.0 scan/s)[20-21].

Cytotoxicity Assay
The cells were treated with nHF. In this test, T47D cell line was grown in RPMI 1640 medium based on the previous study by Hasibuan, et al (2015) and Harahap, et al (2018)[19,22].

Preparation of Cells for Flow Cytometry Analysis
T47D cells ($7.5x10^5$ cells/well) were seeded into a 6-well plate and incubated for 24 h. After that, the cells were treated with nHF and then incubated for 24 h. Cells were collected and washed, centrifuged at 2500 rpm for 5 min and the sediment was collected[17-18].

Cell Cycle Analysis
Cells were fixed in cold 70% ethanol in PBS at -20°C for 2 h. PI kit (BioLegend) added to sediment and resuspended and incubated at 37°C for 30 min. The samples were analyzed using FACScan flow cytometer. Percentage of cells in each of stage in the cell cycle were calculated using ModFit Lt. 3.0.s[22].

Apoptosis Analysis
Annexin V kit was added to sediment and suspended and incubated at 37°C for 30 min. The samples were analyzed using FACScanflowcytometer[23].

Cyclin D1 Expression
Sediment cells were fixated with ethanol 70% and allowed to stand for 2 h in -20°C and Cyclin D1 antibody was added and incubated at 37°C for 10 min. The samples were analyzed using FACScanflowcytometer[24].

Statistical Analysis
Data were expressed as mean ± SD.

RESULTS AND DISCUSSION

Chemical Constituents of n-Hexane Fraction
Analysis of chemical compounds of n-hexane fraction with gas chromatography with mass spectrometry was resulted in 10 major compounds. Analysis of chemical compounds of nHF with GC-MS is shown in Fig.-1 and Table-1.

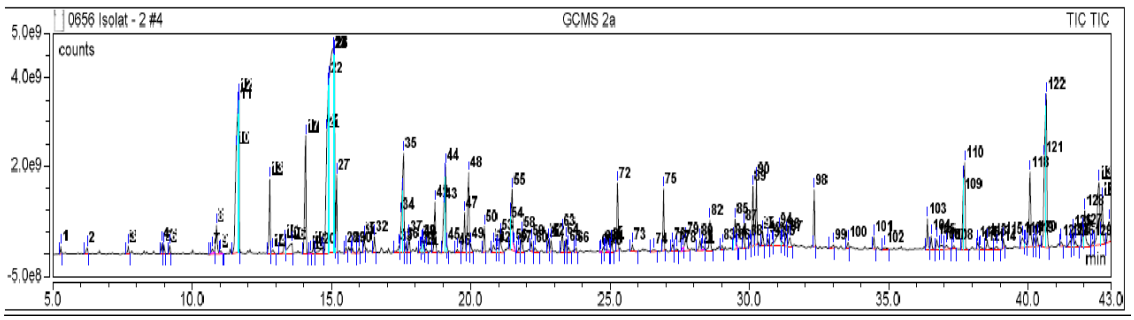

Fig.-1: GC-MS Chromatogram of nHF of *Zanthoxylum acanthopodium* DC.

Inhibitory Concentration 50% (IC_{50})

The IC_{50} score of nHF was 85.41 ± 0.78 µg/mL. The cytotoxicity activity is correlated to the constituent of bioactive compounds in plants including *Zanthoxylum acanthopodium* DC. Essential oils and triterpenoids/steroids estimated as active compounds[25]. Recent studies have reported that monoterpenes exert anticancer activities and as chemopreventive agents[26-28] such as d-limonene, perillyl alcohol, and geraniol[29-34]. Geraniol shows a broad spectrum of bioactivity as antimicrobial, antioxidant, antiulcer, anti-inflammatory, neuroprotective and anti-breast cancer[35-42].

Table-1: Percentage of Compounds in nHF of *Zanthoxylum acanthopodium* DC

No.	Compound	Percentage (%)
1	Geranyl acetate	26.34
2	Geraniol	8.83
3	(E)-3,7-dimethylocta-2,6-diene-1-yl palmitate	6.87
4	2,6-dimethyl-3,5,7-octatriene-2-ol-E,E	4.02
5	(9Z,12Z,15Z),3-7-dimethyloct-6en-1-yl-octadecane-9,12,15- trienoat	3.84
6	Caryophyllene oxide	2.75
7	3,7-dimethyloct-6-an-1-yl palmitate	2.07
8	17-octadecynoic acid	1.33
9	Geranyl palmitoleate	1.19
10	Citronellol	0.98

Cell Cycle Inhibition

To evaluate the effect of nHF to increase cell death by modulating cell cycle was concentrated on it for further studies using the flow cytometry method. The effect of nHF is given in Fig.-2. Treatment with nHF in 42.5 µg/mL caused cell accumulation at G_0-G_1 phase (49.94%) and for control cell (45.37%). This fact was to indicate that nHF can inhibit cell growth at G_0-G_1 phase. In the cell cycle analysis, nHF exhibited higher G_0-G_1 phase accumulation compared to control cells. This analysis also showed that cells underwent apoptosis, indicated by the occurrence of apoptosis during inhibition of cell cycle on the G_0-G_1 phase. Evaluation of cell cycle inhibition was performed using flow cytometry method with propidium iodide as shown in Fig.-2.

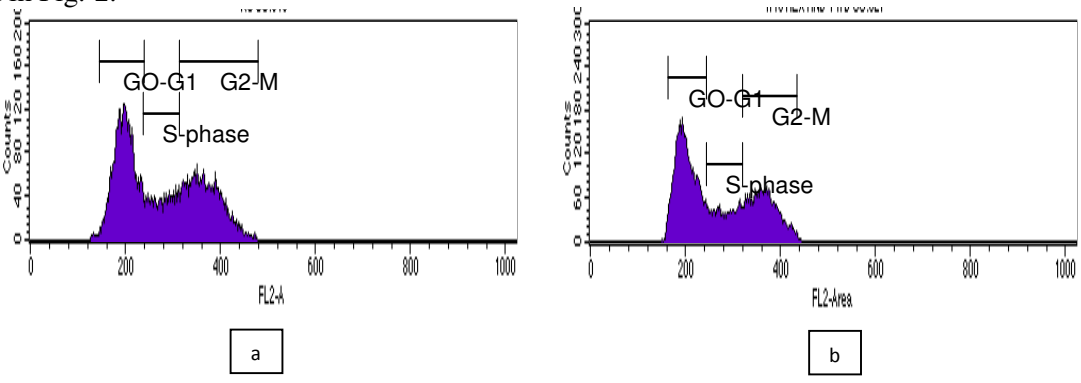

Fig.-2: T47D Cells were treated by nHF for 24h. (a) Control Cells; (b) nHF 42.5 µg/mL. nHF exhibited G_0-G_1 Phase.

Apoptosis Induction

Evaluation of apoptosis initiation was executed using a flow cytometry method with Annexin-V. As shown in Fig.-3, the cells in the upper and lower right quadrants represent late apoptotic/ necrotic and early apoptotic cells, respectively. The percentage of control and nHF in early apoptotic were 5.47% and 33.18%, in late apoptotic/early necrotic are 1.76% and 7.25%. In the apoptotic study, nHF increase the cells undergo apoptosis in early apoptosis and late apoptosis if compared to control T47D cell lines. Geraniol has been shown to be synergistic in combination with chemotherapy drugs.

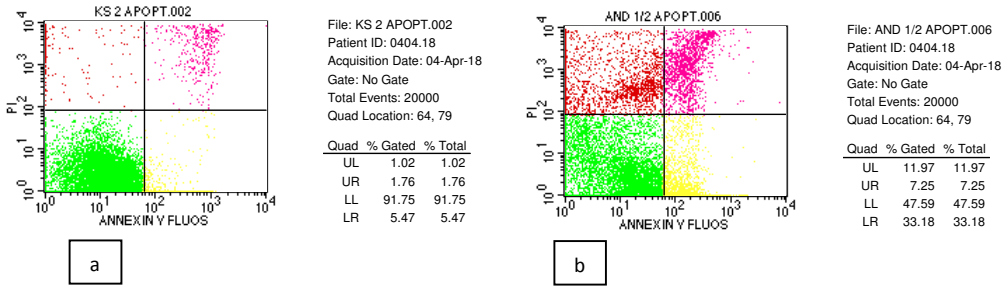

Fig.-3: T47D cells were treated with nHF for 24h (a) Control Cells; (b) nHF 42.5 µg/mL.

Geraniol has been reported to manage various pathways and signaling and therefore the effect in the cell cycle, proliferation, apoptosis, autophagy and metabolism of cancer cells[43-44]. Geraniol suppresses the MCF-7 growth through induction cell cycle arrest[41].

Cyclin D1 Expression

To evaluate the effect of nHF on cyclin D1 expression was conducted by flow cytometry method, and the results were given in Figure 4. Treatment with nHF 42.5 µg/mL caused cell accumulation in the M1 area (22.24%) and for control cell (11.19%). Evaluation of cyclin D1 expressions was performed using flow cytometry method with cyclin D1 antibody as shown in Fig.-4.

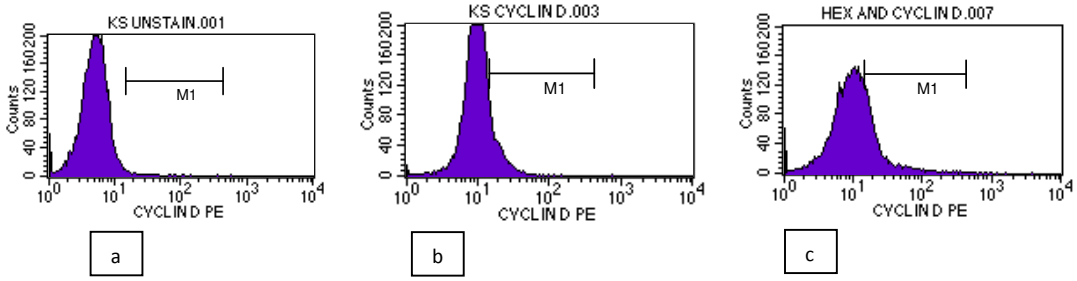

Figure 4. T47D cells were treated withnHF for 24h
(a) control cells unstaining; (b) control cells; (c) nHF 42.5 µg/mL.

CONCLUSION

The results reveal that n-hexane fraction of *Zanthoxylum acanthopodium* DC. fruits contain many bioactive compounds and effective as anticancer towards T47D cell lines by several mechanisms such as cell cycle inhibition, apoptosis induction and down-regulation of cyclin D1 expression.

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REFERENCES

1. A. Montazeri,M. Vahdaninia and I. Harirchi,*Asia Pac Fam Med.*, **7**, 6, (2008), **DOI:** 10.1186/1447-056X-7-6
2. C.H. Yip, 2009 Breast cancer in Asia: Methods in molecular biology. Human Press, Totowa.

3. P.F. Rezaei, S. Fouladdel, C. Silvia, S.M. Ghaffari, G.R. Amin and E. Azizi, *Cytotechnology*, **63**, 503(2011), **DOI:** 10.1007/s10616-011-9373-6
4. E. Suryanto, H. Sastrohamidjojo, S. Raharjoand Tranggongo, *Indonesian Food and Nutrition Progress*,**11(1)**, 15(2004), **DOI:** 10.22146/jifnp.26
5. S.R. Hynniewta and Y. Kumar, *Indian Journal of Traditional Knowledge*, **7(4)**, 581 (2008)
6. M. Sirait, M. Siahaanand Mangkudidjojo.1991, Thesis, Post Graduate School of Pharmacy, Institute Technology of Bandung, Indonesian.
7. A. Parhusip, 2006, Thesis, Post Graduate School of Bogor Agriculture Institute, Indonesian.
8. C.C. Fernandez, P.C. Vieira, V.C. daSilva, E.L. Dall'Oglio, L.E. daSilva and P.T. deSousa, *J. Braz. Chem. Soc*, **20(2)**, 379(2009), **DOI:** 10.1590/S0103-50532009000200025
9. P.A. Yao-Kuassi, C. Caron, H. Ramiarantosa, E. Prost, D. Harakat, E.L. Magrex-Debar, S.C. Gangloff, A.A. Coffy andM. Zeches-Hanrot, *C.R Chimie*,**18**, 891 (2015), **DOI:** 10.1016/j.crci.2015.01.005
10. J. Hu, X. Shi, X. Mao, J. Chen, H. Li, *Chemistry & Biodiversity*, **11**, 970(2014), **DOI:** 10.1002/cbdv.201300381
11. J. Hu, W.D. Zhang, R.H. Liu, C. Zhang, Y.H. Shen, H.L. Li, M.J. Liang andX.K. Xu, *Chemistry & Biodiversity*, **3**, 990(2006), **DOI:** 10.1002/cbdv.200690108
12. Yang, Z.D., Zhang, D., Ren, J., Yang, M, *Med Chem Res*, **21**, 722(2012), **DOI:** 10.1007/s00044-011-9581-9
13. X.G.Cui, Q.J.Zhao, Q.L.Chen, L.Xu, Y.Song, Y.S.JinandD.F. Xu, *Helvetica Chemica Acta*, **91**, 155 (2008), **DOI:** 10.1002/hlca.200890006
14. J.J.Chen, C.K.Yang, Y.H.Kuo, T.L.Hwang, W.L.Kuo, Y.P.Lim, P.J.Sung, T.H.Chang, M.J.Cheng, *Int. J. Mol. Sci*, **16**, 9719(2015), **DOI:** 10.3390/ijms16059719
15. P.A.Z.Hasibuan, U.Harahap, P.Sitorus, D.Satria, *Der. Pharma. Chemica.*, **8(20)**, 172(2016)
16. Y.M.Sihotang, 2015, Thesis, Fakulty of Pharmacy,USU, Indonesian.
17. R.Anggraini, S.Hadisahputra, J.Silalahi, *International Journal of PharmTech Research*, **6(7)**,2032(2014).
18. D.Satria,J.Silalahi,G.Haro,S.Ilyas, P.A.Z.Hasibuan, *Asian Pac. J. Cancer Prev.*, **18(2)**, 399(2017), **DOI:** 10.22034/APJP.2017.18.2.399
19. P.A.Z. Hasibuan,C.Jessy, D. Satria, *Int. J. Pharm. Pharm. Sci.*,**7,** 155(2015).
20. M. Moektiwardoyo, M.Muchtaridi and H.Eli, *Int. J. Pharm. Pharm. Sci.*, **6(2)**, 547(2014).
21. M.Majumder, H.K.Sharma, K.Zaman and W.Lyngdoh, *Int. J. Pharm. Pharm. Sci.*, **6(5)**, 543(2014)
22. U.Harahap, P.A.Z.Hasibuan, P.Sitorus, N.Arfian, D.Satria, *Asian Pac. J. Cancer. Prev.*, **19(2)**, 565 (2018), **DOI:** 10.22034/APJCP.2018.19.2.565
23. A.Dalimunthe,P.A.Z.Hasibuan, D.Satria, *Asian J. Pharm. Clin. Res.*,**10(11)**, 404(2017), **DOI:** 10.22159/ajpcr.2017.v10i11.20204
24. A.Żurynń,L.Anna, S.M.Barbara, K.W.Anna and Maciej G, *Int. J. Onco.*,**48**, 2521(2016), **DOI:** 10.3892/ijo.2016.3444
25. V.R.Yadav, P.Sahdeo, S.Bokyung, K.Ramaswamyand B.A.Bharat, *Toxins*, **2**, 2428(2010), **DOI:** 10.3390/toxins2102428
26. C.E.Elson and S.G.Yu, *J. Nutr.* **124,** 607(1994), **DOI:** 10.1093/jn/124.5.607
27. G.J.Kelloff, C.W.Boone, J.A.Crowell, V.E.Steele, R.A.Lubet, L.A.Doody, W.F.Malone, E.T.Hawk and C.C.Sigman, *J. Cell Biochem.*, **26,** 1(1996), **DOI:** 10.1111/j.1749-6632.1999.tb08718.x
28. P.L.Crowell, *J. Nutr.*,**129,** 775S(1999), **DOI:** 10.1093/jn/129.3.775S
29. Y. Bhalla, V.K.Gupta and V. Jaitak, *J. Sci. Food Agric.*, **93**, 3643(2013), **DOI:** 10.1002/jsfa.6267
30. M. N.Gould, *Environmental Health Perspective* **105 (Suppl 4)**, 977(1997), **DOI:** 10.1289/ehp.97105s4977
31. T.C.Chen, C.O.Fonseca and A.H.Schönthal, *Am. J. Cancer Res.*, **5**, 1580(2015)
32. D.Samaila, B.J.Toy, R.C.Wang and J.A.Elegbede, *Anticancer Res.,* **24**, 3089(2004), **DOI:** 10.1016/j.canlet.2007.07.020
33. S. Bardon, V.Foussard, S.Fournel and A.Loubat, *Cancer Lett.*,**181**, 187(2002), **DOI:** 10.1016/S0304-3835(02)00047-2

34. M.V.Sobral, A.L.Xavier, T.C.Lima and D.P.de Sousa, *Sci. World J.*, (2014), **DOI:** 10.1155/2014/953451
35. F.Solórzano-Santos and M.G.Miranda-Novales, *Curr. Opin. Biotechnol.*, **23**, 136(2012), **DOI:** 10.1016/j.copbio.2011.08.005
36. M.Tiwari and P.Kakkar,*Toxicol In Vitro*, **23**, 295(2009), **DOI:** 10.1016/j.tiv.2008.12.014
37. K.I.de Carvalho, F.Bonamin, R.C.Dos Santos, L.L.Périco, F.P.Beserra, D.P.de Sousa, J.M.Filho, L.R.da Rocha and C.A.Hiruma- Lima, *Naunyn Schmiedebergs Arch Pharmacol*, **387**, 355(2014), **DOI:** 10.1007/s00210-013-0947-z
38. K.R.Rekha, G.P.Selvakumar, S.Sethupathy, K.Santha and R.I.Sivakamasundari, *J. Mol. Neurosci.*,**51,** 851(2013), **DOI:** 10.1007/s12031-013-0074-9.
39. Y. D. Burke, M. J. Stark, S. L. Roach, S. E. Sen and P. L. Crowell, *Lipids*, **32**, 151(1997), **DOI:** 10.1007/s11745-997-0019-y
40. S.Carnesecchi, Y.Schneider, J.Ceraline, B.Duranton, F.Gosse, N.Seiler and F.Raul, *J. Pharmacol. Exp. Ther.*, **298**, 197(2001).
41. R. E. Duncan, D. Lau, A. El-Sohemy and M. C. Archer, *Biochem Pharmacol*, **68**, 1739(2004), **DOI:** 10.1016/j.bcp.2004.06.022
42. T. P. Ong, R. Heidor, A. de Conti, M. L. Dagli and F. S. Moreno, *Carcinogenesis*, **27**, 1194(2006), **DOI:** 10.1093/carcin/bgi291
43. S. H. Kim, H. C.Bae, E.J.Park, C.R.Lee, B.J.Kim, S, H.H.Park, S.J.Kim, I.So, T.W.Kim, *Biochem. Biophys. Res. Commun.*, **407**, 129(2011), **DOI:** 10.1016/j.bbrc.2011.02.124
44. S.Carnesecchi, K.Langley, F.Exinger, F.Gosse and F.Raul, *J. Pharmacol. Exp. Ther.*, **301**, 625(2002), **DOI:** 10.1124/jpet.301.2.625

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