2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce) | 978-1-6654-4181-0/21/$31.00 ©2021 IEEE | DOI: 10.1109/InHeNce52833.2021.9537186

Effect of Andaliman Fruit Extract on Cervical Cancer Rat's Histology

Rostime Hermayerni Simanullang
Biology Department
Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara
Medan, Indonesia
hermayerni@gmail.com

Syafruddin Ilyas
Biology Department
Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara
Medan, Indonesia
syafruddin6@usu.ac.id

Salomo Hutahaean
Biology Department
Faculty of Mathematics and Natural Sciences, Sumatera Utara
Medan, Indonesia
salomo@usu.ac.id

Rosidah
Pharmacy Department
Faculty of Pharmacy
Universitas Sumatera Utara
Medan, Indonesia
rosidah_noersal@yahoo.com

Risma Dumiri Manurung
Poltekkes Kemenkes RI
Sumatera Utara
Medan, Indonesia
rismadumiri@poltekkes-medan.ac.id

Putri Cahaya Situmorang
Biology Department
Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara
Medan, Indonesia
uthiecahaya@gmail.com

***Abstract*— Andaliman (Zanthoxylum acanthopodium DC) is one of the endemic plants from North Sumatra, Indonesia which has high antioxidants. In this study, the effect of andaliman on the histological appearance of rat cervical cancer cells with AgNOR staining was evaluated. In the experiment, rats as a subject were previously benzopyrene-induced with cancer. Subjects used in the experiment were divided into 5 groups; K-: control, K+: rat model of cancer, P1: dose of 100 mg/BW/day, P2: dose of 200 mg/BW/day, and P3: andaliman dose of 400 mg/kg BW/day for 30 days. On the day of 31, surgery was performed on the subjects. Based on the Kruskal Wallis test, a significant difference in the histology of benzopyrene-induced cervical cancer cells by AgNOR staining was achieved p value <0.05 (p 0.000). Andaliman is able to repair cell damage caused by cancer and can be described as a cancer drug in the future.**

Keywords*— *Andaliman, Zanthoxylum acanthopodium, rat's, benzoapyren, cervical, cancer, AgNOR

I. INTRODUCTION

Cervical cancer is a malignant tumor that grows in the lower neck of the uterus that attaches to a branch at the top of the vagina. Cervical cancer is generally caused by infection with Human Papilloma Virus (HPV) in more than 99% of cases [1]. The use of traditional medicine has been entrenched in Indonesian society. In connection with this, it is necessary to conduct further research on the benefits and how the healing process affects human organs, especially in disease (cervical cancer is the second highest cause of death for women in the world) [2]. The most common cancers in men are lung, prostate, colorectal, stomach and liver cancers. While cancers that attack women are breast, cervical, colorectal, lung, brain, and thyroid. From previous research, it was found that the increase in cervical cancer cases in women was caused by a lack of women's knowledge regarding related diseases and the Pap smear as early detection of cervical cancer is still low [3]. About 70% of cancer deaths occur in low- and middle-income countries. One third of deaths are caused by five (5) risk factors related to behavior and a strict diet such as: body mass index, low fruit and vegetable intake, lack of physical activity, tobacco and alcohol use [4]. There are still many potential local plants that are beneficial to health that have not been studied and some of the plants that have been studied, one of which is the andaliman plant (Zanthoxylum acanthopodium DC.) both fruit and leaves. Andaliman is a special seasoning from local people such as "carp arsik, natinombur, and saksang". Generally, dishes with andaliman spices tend to be more durable [5]. Andaliman fruit has a chemical group of alkaloid compounds, flavonoids, tannins, glycosides, steroids, terpenoids [6] as well as antioxidant compounds such as flavonoids, antioxidant activity, and inhibits carbonyl reductase, ascorbic acid (vitamin c) [7]–[10]. Zanthoxylum fruit has previously been widely used to treat pain, vomiting, diarrhea, ascariase, and eczema treatment, topical anesthetic, stomach medicine, and itching in China [11]. The characteristic taste of andaliman is dominated by the aroma of citrus limonene and citronellol (the essential oil content contained in it is mostly a terpenoid group, namely geranyl acetate). Other components are -myrcene, -ocimene, linalool and E-1-decenal [12]. Andaliman extract contains alkaloids, steroids, and terpenoids that have antimicrobial activity against insects, bronchitis, dyspepsia, antiviral, anticonvulsant, antifungal, analgesic, antibiotic, hepatoprotective, cancer and preeclampsia agents [13]–[18]. Andaliman compounds used as antioxidants can be considered as inhibitors of cancer cell growth and repair of kidney tubules, hepatocyte cells and rat parenchymal degeneration [19]. There have been many studies on bioactive compounds such as stems, leaves, roots and bark of Zanthoxylum which are useful as anti-cancer [20]–[22]. The content of Zanthoxylum lupeol is cytotoxic to tumour's [23], [24] and flavonoids, alkaloids and a group of glycosides have the potential as strong antioxidant activity in vitro [25] and isolated from Zanthoxylum (cyclohexapeptides A and B) have antiproliferative activity in cancer cells [26]–[28].

The use of traditional medicine in human medicine has become a community culture because it is proven to be able to cure diseases. Therefore, traditional medicines of plant origin in Indonesia still need to be explored, researched, developed and inventoried. Traditional medicines derived from plants generally have no side effects and the price is relatively cheaper when compared to synthetic drugs [29]–[35].

The use of herbal medicine is growing and advancing rapidly, mainly because of the bioactive substance andaliman which is recommended as a treatment for cancer and other diseases [36], [37]. In general, several studies have been conducted regarding the benefits of Zanthoxylum's bioactive content against cancer. In this study, the effect of andaliman extract on cancer cells was

tested. The novelty of this study was to analyze the effect of andaliman methanol extract on benzopyrene-induced rat cervical cancer with AgNOR staining for further use as a cancer drug in the future.

II. METHOD

A. Research Location

Research location: determination of body weight and cervix was carried out at the Laboratory of Anatomical and Animal Pathology, University of North Sumatra. Histological observations of cancer cells supporting AgNOR were carried out at the Haji Adam Malik Anatomical Pathology Laboratory, Medan, North Sumatra. This research project was conducted from May 2019 to April 2020.

B. Materials and tools

The used material in this research: 20 Kg of andaliman fruit, 20 L of Methanol 99%, Na-CMC 0,5 %, ethanol (50%, 70%, 95% and 100%), Xylene, nathrium citrate buffer, gelatine 2%, phormiate acid 1%, nitrate silver 25%, paraffin block set, EDTA powder, aquades, corn oil, and ketamine hydrochloride 50 mg. The applied tools consist of: Blender, beaker glass 250 ml, measuring cylinder 50 ml, electric scale, syringe 5 ml, vacuum tube 2 ml, scalpel set, glass object, coverslip, cooler incubator, microscope, optic lab, oven (heater), and rotary evaporator.

C. Andaliman methanol extract.

The Andaliman fruit used in the study came from the Gibeon Hills of Sibisa Parapat, North Sumatra Regency. Initially, the andaliman fruit is separated from the twigs and leaves, then cleaned with running water, drained, and weighed to get the wet weight. After that, the manufacture of andaliman extract with the following three steps:

1. Drying: andaliman fruit is dried in an oven at a temperature of 30ºC - 40ºC until crumbly, weighed, and mashed with a blender.
2. Preparation of andaliman extract: 800 grams of andaliman fruit powder was macerated with 6000 ml of 96% methanol in a tightly closed container. Left for 5 days in a place protected from sunlight, stirring frequently, squeezed, then sifted. The maceration dregs were soaked again with 96% methanol as needed to obtain 1600 ml. Then, transfer to a closed container, leave in a cool place (protected from light) for 2 days. The obtained macerate was concentrated using a rotary evaporator at a temperature of 40°C to obtain a thick extract. Then evaporated over a water heater with a temperature of 40-50°C to get a thick extract. The results of maceration and percolation are combined to reach a clear liquid.
3. Preparation of pharmaceutical suspension: considering that the andaliman extract is partially insoluble in water, to obtain a homogeneous mixture, the suspending agent Na-CMC 0.5% is used as much as 1.0% or 1 ml in 150 ml of distilled water. The drug was washed with 96% methanol solvent, then transferred in a closed container and left in a cool place for 2 days [19].

Animal. This study used 30 female rats (Ratus norvegicus) from The Development Animal and Research Centre of North Sumatra with the average weight of 200-250 grams. The use of animals as part of the experiment is carried out in accordance with the requirements of the applicable code of ethics. Rats were divided into 5 groups: negative or normal control (only given standard food) (K-), positive control was cervical cancer rat model (K+) and 3 treatment groups were given andaliman methanol extract at a dose of 100 mg/kg/BW (P1) , 200 mg/kg/BW (P2), and 400 mg/Kg/BW/ (P3), respectively, where one group contained 6 rats as replicates. To get a mouse model of Cancer, animals were induced with benzopyrene 50mg/Kg/BW/0.5 corn oil, then left for 3 (three) months to grow cancer [31], [38]. To ensure that the cancer cells have grown then a pap smear is done. Furthermore, the three treatment groups were given andaliman methanol extract at a dose of 100 mg/kg/BW (P1), 200 mg/kg/BW (P2) and 400 mg/kg/BW (P3), respectively, for 30 days [39]. The design used in this study was an experimental study with a completely randomized design. The parameters of the study included body weight and cervix of rats, as well as histological observations of cancer cells by counting AgNOR items.

D. Ethical consideration

Ethical approval of this research was obtained from The Research Ethics Commission, Faculty of Mathematis and Natural Sciences, Universitas Sumatera Utara Medan, Indonesia, with approval number: 0199/KEPH-FMIPA/2019.

E. Data Analysis

The measurement data in this study were analyzed using IBM SPSS Statistics 24 software, with the one way Anova test followed by the Post Hoc test. If the data is not normal, then the data will be analyzed by the Kruskal-Wallis test and followed by the Mann-Whitney test.

III. RESULTS AND DISCUSSION

Based on the results of the study, it was found that benzopyrene induction could significantly affect cervical histology, although the difference in body weight and cervix was not significant, but the average value indicated that an increase in body weight of rats was found. Differences in body weight between treatment groups with a dose of andaliman extract were found in the group with a dose of 400 mg/kg/BW which showed a lower weight difference than the other groups (Table 1). This shows that the highest dose of andaliman methanol extract can reduce body weight in mice cancer patients although it is not statistically significant. Based on cervical weight data (Table 1), after administration of andaliman methanol extract there was no significant difference between the treatment groups. Based on the average value, the lowest cervical weight was in the negative control group, while the highest cervical weight was in the positive control group (cervical cancer model). Based on the average value of andaliman fruit methanol extract can reduce body weight in mice model of cervical cancer, although not significantly different.

Alkaloids and saponins are commonly used in prescription drugs. Alkaloids are toxic, while saponins are used to reduce the tension of body fluids and can cause hemolysis of blood cells [8]. Almost all alkaloids found in nature have physiological activity, some are very toxic and some are very useful in medicine [40], [41]. The ethanolic

extract of Zanthoxylum pipperitum DC fruit has an antiobesity effect in mice by inhibiting adipogenesis through the regulation of genes involved in adipogenesis pathway [42]. The possibility of weight loss and the cervix is caused by the high alkaloid and steroid compounds in the andaliman fruit extract.

Based on statistical analysis using the Kruskal-Wallis test for cervical cancer cells with AgNOR induced benzoapyrene staining, there was a significant difference at $p < 0.000$. Then the Mann-Whitney test was performed to see the difference between the two groups. There was a significant difference between the negative control group and the andaliman dose of 400 mg/kg (P3). It was suspected that the methanol extract of andaliman fruit could improve rat cervical cancer cells, especially at the highest dose (400 mg/kg). kg/BW). This indicates that the highest dose of andaliman fruit methanol extract has the potential to improve cervical cancer tissue stained with AgNOR or almost the same as the negative control group (K-) Table 2 and Figure 1).

TABLE I. THE AVERAGE OF RATS BODY WEIGHT

Group	Body Weight (g)	
	Before (g)	**After (g)**
K-	200,50 ± 7,01	247,33 ± 16,77
K+	207,33 ± 11.43	266,00 ± 10,19
P1	221,00 ± 25,89	276,67 ± 16,48
P2	228,83 ± 27,03	277,17 ± 18,18
P3	233,33 ± 27,75	275,67 ± 19,02

* K(-): Negative control group (normal rats). K(+): positive control (rats cancer model BaP 50 mg/KgBW/0,5 ml corn oil). P1: extract methanol of andaliman 100 mg/KgBW. P2: 200 mg/KgBW. P3: 400 mg/KgBW.

TABLE II. THE AVERAGE OF RATS CERVIX WEIGHT

Group	**Cervix Weight (g) & SDV**
K-	0,37 ± 0,16
K+	1,60 ± 1,21
P1	1,00 ± 0,99
P2	0,78 ± 0,72
P3	0,38 ± 0,13

* K(-): Negative control group (normal rats). K(+): positive control (rats cancer model BaP 50 mg/KgBW/0,5 ml corn oil). P1: extract methanol of andaliman 100 mg/KgBW. P2: 200 mg/KgBW. P3: 400 mg/KgBW. SDV: Standard Deviation

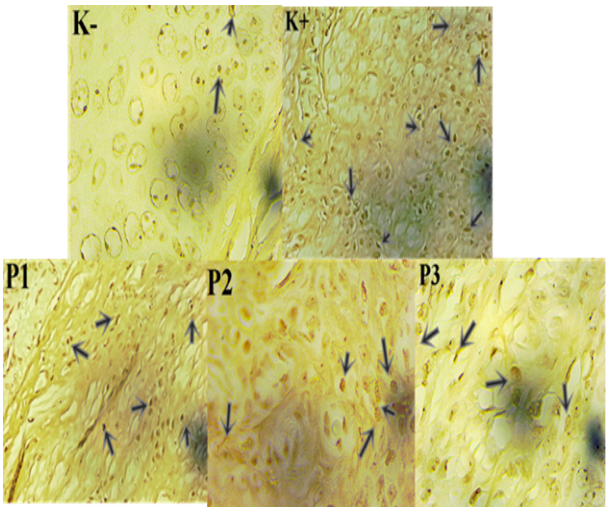
TABLE III. ANALYSIS OF KRUSKAL-WALLIS AND MANN-WHITNEY WITH SUSTAINING AGNOR

Group	***Mean Rank***	***Kruskal-Wallis***	***Mann-Whitney***				
			K-	**K+**	**P1**	**P2**	**P3**
K-	4,10	0,000		0.009[b]	0.009[c]	0.009[d]	0,142
K+	22,60				0.028[c]	0.009[d]	0.009[e]
P1	18,40					0.009[d]	0.009[e]
P2	13,00						0.009[e]
P3	6,90						

* K(-): Negative control group (normal rats). K(+): positive control (rats cancer model BaP 50 mg/KgBW/0,5 ml corn oil). P1: extract methanol of andaliman 100 mg/KgBW. P2: 200 mg/KgBW. P3: 400 mg/KgBW. Numbers followed by superscript are different on the same line shows appearing the difference significant, p= 0.000 (p<0.05).

Sustaining AgNOR is a diagnostic tool that is highly recommended to see changes in tissue morphological characteristics that can distinguish benign or malignant tumors from breast cancer. The AgNOR is a NOR stain associated with proteins, according to the cell type [43]. Based on the number of the AgNOR grains per cyst, it can be a marker of the onset of cell proliferation in potentially malignant aggressive cyst lesions [44] and directly increase the AgNOR grains progressively compared to the increase in cell proliferative activity. cells: normal cervix and inflammatory cervix without dysplasia [45].

FIGURE I. THE HISTOLOGY OF SERVICAL CANCER CELL BY STAINING OF AgNOR



*The histology of the cancer cells of the cervix of rats on the staining of AgNOR induced by benzopyrene. K(-): Negative control group (normal rats). K(+): positive control (rats cancer model BaP 50 mg/ mg/KgBW /0,5 ml corn oil). P1: extract methanol of *andaliman* 100 mg/KgBW. P2: 200 mg/KgBW. P3: 400 mg/KgBW. The arrows indicate the presence of AgNOR grains in cervical cancer cells.

Counting AgNOR grains is very helpful as a marker of cell proliferation, which can help diagnose through a pap smear to stage cervical lesions [46] and breast cancer and the correlation between high mean AgNOR items and HPV positivity [47]. The AgNOR staining is also used to see changes from benign to precancerous and malignant tumours [48].

Extracts of the Zanthoxylum family can reduce and potentially inhibit microorganisms and cancer cells [42], [49]. Zanthoxylum family in silver dye can be an anti-cancer agent of economic value in the future. The Zanthoxylum family can reduce the inflammatory process in endothelial cells and repair cell lesions that will become cancerous [50]. Based on this, it is suspected that the content of andaliman which is a member of the Zanthoxylum family can repair lesion cells and prevent the growth of cancer cells.

IV. CONCLUSION

There was a significant effect of Andaliman methanol extract on rat cervical cancer cells induced by benzopyrene with AgNOR staining (p=0.000). This is because Andaliman has the ability to repair benzopyrene-induced rat cervical cell damage. Giving andaliman as an antioxidant and alkaloid is

one way to repair the damage. Andaliman can be recommended as a drug to repair cervical cell damage caused by cancer. However, the dose of andaliman needs to be analyzed in further research to find out a safe dose for the body and it is necessary to continue about the biomolecular pathway.

ACKNOWLEDGMENT

My great thanks to the Funding Agencies of Education (LPDP) for Indonesia who has funded part of this research.

REFERENCES

[1] M. Li, Y. He, C. Peng, X. Xie, and G. Hu, "Erianin inhibits human cervical cancer cell through regulation of tumor protein p53 via the extracellular signal - regulated kinase signaling pathway," pp. 5006–5012, 2018, doi: 10.3892/ol.2018.9267.

[2] R. H. Simanullang, "Impact of Health Education Intervention on Knowledge of Cervical Cancer Prevention Among Women in Bahorok's Village, North Sumatra Indonesia," Belitung Nurs. Journ al, vol. 4, no. 6, pp. 591–595, 2018.

[3] R. H. Simanullang and S. D. Sitopu, "Effect of Health Education on Women's Knowledge Level about Pap Smear's Early Detection of Cervical Cancer Prevention," Asian J. Oncol., 2020, doi: 10.1055/s-0040-1709365.

[4] T. D. Singh, H. T. Meitei, A. L. Sharma, A. Robinson, L. S. Singh, and T. R. Singh, "Anticancer properties and enhancement of therapeutic potential of cisplatin by leaf extract of Zanthoxylum armatum DC," Biol. Res., 2015, doi: 10.1186/s40659-015-0037-4.

[5] C. Suriani, E. Prasetya, T. Harsono, and D. Handayani, "Habitat characteristics of Andaliman (Zanthoxylumacanthopodium DC) in North Sumatra using a GIS (Geographical Information System) approach," J. Phys. Conf. Ser., vol. 1317, no. 1, pp. 0–7, 2019, doi: 10.1088/1742-6596/1317/1/012097.

[6] Fauzi, "Anticancer Activity of Ethanol Extract From Andaliman Fruit (Zanthoxylum acanthopodium DC.) on Cervical Cancer Cells," North Sumatra Of University, 2015.

[7] K. Sayuti and R. Yenrina, Antioksidan, Alami dan Sintetik. Padang. Andalas University Press, 2015.

[8] Rosidah, P. A. Z. Hasibuan, G. Haro, P. Masri, and D. Satria, "Antioxidant activity of alkaloid fractions of zanthoxylum acanthopodium dc. Fruits with 1,1-diphenyl-2-picrylhydrazyl assay," Asian J. Pharm. Clin. Res., 2018, doi: 10.22159/ajpcr.2018.v11s1.26560.

[9] D. M. Syari, R. Rosidah, P. A. Z. Hasibuan, G. Haro, and D. Satria, "Evaluation of cytotoxic activity alkaloid fractions of zanthoxylum acanthopodium DC. Fruits," Open Access Maced. J. Med. Sci., 2019, doi: 10.3889/oamjms.2019.495.

[10] R. Yam, S. A, and M. Z. Asmawi, "Antioxidant Potential of Gynura Procumbens," Pharm. Biol., vol. 46, no. 9, p. 618, 2008.

[11] L. Xiang et al., "The Chemical and Genetic Characteristics of Szechuan Pepper (Zanthoxylum bungeanum and Z. armatum) Cultivars and Their Suitable Habitat," Front. Plant Sci., vol. 7, no. April, 2016, doi: 10.3389/fpls.2016.00467.

[12] G. Katzer, "Sichuan Pepper and Others (Zantoxylum , piperitum, stimulan, bungeanum, rhetsa, acanthopodium)," -, 2012.

[13] D. Satria, J. Silalahi, G. Haro, S. Ilyas, and P. A. Z. Hasibuan, "Chemical analysis and cytotoxic activity of N-hexane fraction of Zanthoxylum acanthopodium DC. fruits," Rasayan J. Chem., 2019, doi: 10.31788/RJC.2019.1225180.

[14] A. Abdel-Moneim, O. M. Ahmed, H. I. Fahim, and E. E. Mohamed, "The Preventive Effects of Avocado Fruit and Seed Extracts on Cardio-nephrotoxicity Induced by Diethylnitrosamine/2-acetylaminoflurine in Wistar Rats," Basic Sci. Med., vol. 6, no. 1, pp. 4–13, 2017, doi: 10.5923/j.medicine.20170601.02.

[15] C. I. Ribeiro, A. M. Gomes, R. Carmo, and A. Luís, "Membranous nephropathy with light chain restriction," J. Nephropathol., vol. 10, no. 1, pp. 1–2, 2020, doi: 10.34172/jnp.2021.03.

[16] P. C. Situmorang, S. Ilyas, S. Hutahaean, and R. Rosidah, "Effect of nanoherbal andaliman (zanthoxylum acanthopodium) and extra virgin olive oil combination on preeclamptic rats liver histology," Open Access Maced. J. Med. Sci., vol. 7, no. 14, pp. 2226–2231, 2019, doi: 10.3889/oamjms.2019.651.

[17] P. C. Situmorang, S. Ilyas, and S. Hutahaean, "Effect of Combination of Nano Herbal Andaliman (Zanthoxylum acanthopodium DC.) and Extra Virgin Olive Oil (EVOO) to Kidney Histology of Preeclampsia Rats," 2019, doi: 10.1088/1755-1315/305/1/012081.

[18] P. C. Situmorang, S. Ilyas, and S. Hutahaean, "Study of combination of nanoherbal andaliman (zanthoxylum acanthopodium) and extra virgin olive oil (evoo) effects in the expression of malondialdehyde (mda), heat shock protein-70 (hsp70) and placental histology of preeclamptic rats," Pharm. Sci., 2019, doi: 10.15171/PS.2019.37.

[19] R. H. Simanullang, S. Ilyas, S. Hutahaean, and Rosidah, "Effect of andaliman (Zanthoxylum acanthopodium dc.) methanol extract on rat's kidney and liver histology induced by benzopyrene," Pakistan J. Biol. Sci., vol. 24, no. 2, pp. 274–281, 2021, doi: 10.3923/pjbs.2021.274.281.

[20] F. I. Eze, X. Siwe-Noundou, M. Isaacs, S. Patnala, P. O. Osadebe, and R. W. M. Krause, "Anti-cancer and anti-trypanosomal properties of alkaloids from the root bark of Zanthoxylum leprieurii Guill and Perr," Trop. J. Pharm. Res., vol. 19, no. 11, pp. 2377–2383, 2020, doi: 10.4314/tjpr.v19i11.19.

[21] M. Andima et al., "Antiproliferative Activity of Secondary Metabolites from Zanthoxylum zanthoxyloides Lam: In vitro and in silico Studies," Pharmacogn. Commun., vol. 10, no. 1, pp. 44–51, 2019, doi: 10.5530/pc.2020.1.8.

[22] W. Pang et al., "Anticancer activities of Zanthoxylum bungeanum seed oil on malignant melanoma," J. Ethnopharmacol., vol. 229, no. January, pp. 180–189, 2019, doi: 10.1016/j.jep.2018.10.012.

[23] O. H. Nyaboke et al., "Cytotoxicity of Lupeol from the Stem Bark of Zanthoxylum gilletii against Multi-factorial Drug Resistant Cancer Cell Lines," Investig. Med. Chem. Pharmacol., vol. 1, no. 1, p. 10, 2018, [Online]. Available: https://investchempharm.com/.

[24] N. T. H. Van et al., "Alkaloid glycosides and their cytotoxic constituents from Zanthoxylum nitidum," Phytochem. Lett., vol. 32, no. August, pp. 47–51, 2019, doi: 10.1016/j.phytol.2019.04.022.

[25] R. Sahu et al., "LC-MS characterized methanolic extract of zanthoxylum armatum possess anti-breast cancer activity through Nrf2-Keap1 pathway: An in-silico, in-vitro and in-vivo evaluation," J. Ethnopharmacol., vol. 269, 2021, doi: 10.1016/j.jep.2020.113758.

[26] F. Qin et al., "Nitidumpeptins A and B, Cyclohexapeptides Isolated from Zanthoxylum nitidum var. tomentosum: Structural Elucidation, Total Synthesis, and Antiproliferative Activity in Cancer Cells," J. Org. Chem., vol. 86, no. 2, pp. 1462–1470, 2021, doi: 10.1021/acs.joc.0c02057.

[27] M. T. Sibero et al., "Antibacterial, cytotoxicity and metabolite profiling of crude methanolic extract from andaliman (Zanthoxylum acanthopodium) fruit," Biodiversitas, 2020, doi: 10.13057/biodiv/d210928.

[28] Y. Asbur and Khariunnisyah, "Pemanfatan andaliman (Zanthoxylum acanthopodium DC) sebagai tanaman penghasil minyak atsiri Utilization andaliman (Zanthoxylum acanthopodium DC) for producing plant essential oils," vol. 17, no. 1, pp. 537–543, 2018.

[29] M. S. Batubara, E. Sabri, and M. Tanjung, "Pengaruh Pemberian Ekstrak Etanol Daun Andaliman (Zanthoxylum acanthopodium DC .) Terhadap Gambaran Morfologi Ovarium Mencit (Mus musculus L .)," vol. 1, no. 1, pp. 5–10, 2017.

[30] Barkatullah, M. Ibrar, and N. Muhammad, "Evaluation of zanthoxylum armatum DC for in-vitro and in-vivo pharmacological screening," African J. Pharm. Pharmacol., 2011, doi: 10.5897/AJPP11.405.

[31] Rosidah, P. A. Z. Hasibuan, G. Haro, and D. Satria, "Cytotoxicity Activity of Ethanol Extract of Andaliman Fruits (Zanthoxylum acanthopodium DC .) towards 4T1 Breast Cancer Cells," Indones. J. Pharm. Clin. Res., vol. 02, no. 2, pp. 31–35, 2019.

[32] S. Sikdar et al., "Post-cancer Treatment with Condurango 30C Shows Amelioration of Benzo[a]pyrene-induced Lung Cancer in Rats Through the Molecular Pathway of Caspase-3-mediated Apoptosis Induction -Anti-lung cancer potential of Condurango 30C in rats-," J. Pharmacopuncture, 2013, doi: 10.3831/kpi.2013.16.021.

[33] L. I. Sirait, M. N. Massi, M. Hatta, and Prihantono, "The effects of extract andaliman fruit (Zanthoxylum acanthopodium dc) to CAMP mRNA expression and bacterial load in mice balb-C after gardnerella vaginal infection," Indian J. Public Heal. Res. Dev., vol. 9, no. 11, pp. 607–611, 2018, doi: 10.5958/0976-5506.2018.01525.5.

[34] R. Supabphol and J. Tangjitjareonkun, “Chemical constituents and biological activities of Zanthoxylum limonella (Rutaceae): A review,” Trop. J. Pharm. Res., vol. 13, no. 12, pp. 2119–2130, 2014, doi: 10.4314/tjpr.v13i12.25.

[35] E. Suryanto, H. Sastrohamidjojo, S. Raharjo, and Tranggono, “Singlet Oxygen Quenching Effect of Andaliman (Zanthoxylum acanthopodium DC.) Extracts in Light-Induced Lipid Oxidation,” Indones. Food Nutr. Prog., vol. 11, no. 2, pp. 48–55, 2004.

[36] A. K. Yadav and V. Tangpu, “Therapeutic efficacy of zanthoxylum rhetsa DC extract against experimental hymenolepis diminuta (Cestoda) infections in rats,” J. Parasit. Dis., 2009, doi: 10.1007/s12639-009-0007-2.

[37] A. M. Emmanuel, B. A. Nadia, C. F. Amadou, and K. Koffi, “Evaluation of Acute and Subacute Induced by Methanol Extract Ammarathus viridus Leaves in Wistar Rats (Rattus novergicus),” Pharma Innov. J., vol. 7, no. 7, pp. 625–639, 2018.

[38] P. C. Situmorang, S. Ilyas, S. Hutahaean, Rosidah, and R. D. Manurung, “Acute toxicity test and histological description of organs after giving nano herbal andaliman (Zanthoxylum acanthopodium),” Rasayan J. Chem., vol. 13, no. 2, pp. 780–788, 2020, doi: 10.31788/RJC.2020.1325621.

[39] S. Y. Gwon, J. Y. Ahn, T. W. Kim, and T. Y. Ha, “Zanthoxylum piperitum DC ethanol extract suppresses fat accumulation in adipocytes and high fat diet-induced obese mice by regulating adipogenesis,” J. Nutr. Sci. Vitaminol. (Tokyo)., 2012, doi: 10.3177/jnsv.58.393.

[40] L. Ranawat, J. Bhatt, and J. Patel, “Hepatoprotective activity of ethanolic extracts of bark of Zanthoxylum armatum DC in CCl4 induced hepatic damage in rats,” J. Ethnopharmacol., 2010, doi: 10.1016/j.jep.2009.10.019.

[41] K. Tyagi et al., “AgNOR as an effective diagnostic tool for determining the proliferative nature of different types of odontogenic cysts,” J. Fam. Med. Prim. Care, vol. 9, no. 1, p. 125, 2020, doi: 10.4103/jfmpc.jfmpc_617_19.

[42] R. Mahobia, M. Khandare, R. Nayak, and B. S. Yadav, “Value of Argyrophilic Nucleolar Organizer regions in Benign , Premalignant , and Malignant Lesions of Cervix Uteri,” vol. 5, no. 12, pp. 121–125, 2018, doi: 10.17354/ijss/2018/97.

[43] S. Shukla et al., “A study on the nucleolar organizer regions-as a tumour proliferative marker in cervical smears,” J. Clin. Diagnostic Res., 2013, doi: 10.7860/JCDR/2013/4808.2746.

[44] C. T. F. Oshima and N. M. Forones, “AgNOR in cancer of the stomach,” Arq. Gastroenterol., vol. 38, no. 2, pp. 89–93, 2001, doi: 10.1590/s0004-28032001000200003.

[45] H. G. Ahmed, M. A. Al-Adhraei, and I. M. Ashankyty, “Association between AgNORs and Immunohistochemical Expression of ER, PR, HER2/neu, and p53 in Breast Carcinoma,” Patholog. Res. Int., vol. 2011, pp. 1–6, 2011, doi: 10.4061/2011/237217.

[46] W. K. Darkwah et al., “Assessment of proliferative index in different grades of breast cancers using AgNOR (Agyrophilic Nuclear Organizer Region) expression,” Beni-Suef Univ. J. Basic Appl. Sci., vol. 7, no. 4, pp. 587–592, 2018, doi: 10.1016/j.bjbas.2018.06.011.

[47] A. N. Srivastava, J. S. Misra, U. Singh, M. Khan, and S. Raza, “AgNOR Pleomorphic Count as a Tumor Marker in Cervical Carcinogenesis and Feasibility of Its Introduction in Cervical Cancer Screening Programs to Discriminate High-Risk Cases of Squamous Intraepithelial Lesions of the Cervix,” Acta Cytol., 2019, doi: 10.1159/000498978.

[48] S. R. KC and O. Talwar, “Value of Nucleolar Organizer Regions count in cervical pathology,” J. Pathol. Nepal, vol. 2, no. 3, pp. 180–185, 1970, doi: 10.3126/jpn.v2i3.6017.

[49] S. Nayaka, B. Chakraborty, S. Pallavi, M. P. Bhat, K. Shashiraj, and B. Ghasti, “Synthesis of biogenic silver nanoparticles using zanthoxylum rhetsa (Roxb.) dc seed coat extract as reducing agent and in-vitro assessment of anticancer effect on a549 lung cancer cell line,” Int. J. Pharm. Res., vol. 12, no. 3, pp. 302–314, 2020, doi: 10.31838/ijpr/2020.12.03.024.

[50] S. T. Chou, H. H. Chan, H. Y. Peng, M. J. Liou, and T. S. Wu, “Isolation of substances with antiproliferative and apoptosis-inducing activities against leukemia cells from the leaves of Zanthoxylum ailanthoides Sieb. & Zucc,” Phytomedicine, 2011, doi: 10.1016/j.phymed.2010.08.018.