ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 15, Issue, 09, pp.25933-25938, September, 2023
DOI: https://doi.org/10.24941/ijcr.45909.09.2023

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PHYTOCHEMISTRY TEST AND CYTOTOXIC EFFECT OF SARANG SEMUT (MYRMECODIAPENDANS) EXTRACT TOWARDS COLON WIDR CELL LINE

Ananda Tony Satya Sekarmaji[1] and Ade Arsianti[2,3,*]

[1]Medical student, Faculty of Medicine, University of Indonesia, Jalan Salemba Raya No. 4 Jakarta 10430, Indonesia; [2]Medical Chemistry Department, Faculty of Medicine, University of Indonesia, Jalan Salemba Raya No. 4 Jakarta 10430, Indonesia; [3]Drug Development Research Center (DDRC), Indonesia Medical Education and Research Institute (IMERI), Faculty of Medicine, University of Indonesia, Jalan Salemba Raya No.6 Jakarta 10430, Indonesia

ARTICLE INFO

Article History:
Received 20th June, 2023
Received in revised form
28th July, 2023
Accepted 15th August, 2023
Published online 27th September, 2023

Key words:

Myrmecodiapendans, TLC,
Phytochemistry,
Cytotoxicity,
WiDr cells.

Corresponding Author:
Ade Arsianti

ABSTRACT

Background: In Indonesia, Colorectal cancer is the second most common cancer in males and third most common in females. Like other types of cancer, the main treatment is a series of chemotherapies. On the other hand, it is known that a proportion of "low-class" Indonesians, those who earns less than 1 million rupiah per month, in the economical aspect (58%) prefers the use of herbal medicine instead of conventional treatments. Hence, the research and development of cancer drugs from herbal materials such as Sarang Semut (*Myrmecodiapendans*) is vital. **Objective:** To analyze phytochemical component and cytotoxicity of Sarang Semut (*Myrmecodiapendans*)towards colon Wi Drcell line. **Methods:** Sarang Semut (*Myrmecodiapendans*) was macerated by solvents N-Hexane, Ethyl Acetate, and Ethanol and analyzed through phytochemical tests and thin layer chromatography (TLC). In addition, the cytotoxicity effect of all three extracts were analyzed towards WiDr colon cancer cell using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay. **Results:** Sarang Semut (*Myrmecodiapendans*) contains secondary metabolites Saponin, Flavonoid, Tannin, Triterpenoid, and Alkaloid. TLC analysis revealed that all three extracts of Myrmecodiapendans had six chemical compounds. MTT Assay revealed ethylacetate and ethanol extracts of Myrmecodiapendans had no cytotoxic effect towards WiDr colon cancer cells, whereas n-hexane extract of Sarang Semut (*Myrmecodiapendans*) had strong cytotoxic effect towards WiDr colon cancer cells. **Conclusion:** N-hexane extract of Sarang Semut (*Myrmecodiapendans*) is a promising candidate for development of new anti-colon cancer drug.

Citation: ***Ananda Tony Satya Sekarmaji and Ade Arsianti.*** **2023.** "Phytochemistry Test and Cytotoxic Effect of Sarang Semut (Myrmecodiapendans) Extract towards Colon WiDr Cell Line*". International Journal of Current Research*, 15, (09), 25933-25938.

INTRODUCTION

Cancer is a genetic disorder caused by multiple factors, such as environmental exposures and spontaneous DNA mutation. Due to the multifactorial exposures, a healthy cell can transform into a cancer cell, one of which include colorectal cancer. According to WHO's 2014 cancer profile in Indonesia, colorectal cancer is the second most common cancer in males and third most common in females (1). in 2018, according to Global Cancer Observatory data, the number of deaths by cancer has yet to drop as it plunges into 348,809 new cases and 30,107 deaths are caused by colorectal cancer (2). Colorectal cancer is defined as the growth of abnormal epithelial cells of the colon. The epithelial cells of the colon at risk are the cells which house the mutation of a tumor-suppressing protein, Adenomatous Polyposis Coli (APC). With the mutation of APC, it is unable to degrade β-catenin which in return translocates into the nucleus. The nucleus responds to β-catenin by activating MYC and cyclin-D1 gene, promoting proliferation. Other supporting factors include mutation of KRAS gene which prevents cell apoptosis and mutations of other tumor suppressing genes which include SMAD2 and SMAD4 (3).

Management of colorectal cancer is performed appropriately to the severity of the condition, seen by the staging of cancer. The staging of cancer includes stage I at which the tumor has not invade beyond the muscular layer of the colorectal region, stage II in which tumour penetration occurs towards the serous layer and the surrounding peritoneal region, stage III which begins to metastase and reach lymph nodes, and lastly stage IV tumor meaning it has invaded distant organs from the colorectum region such as liver. According to the National Institute for Health and Clinical Excellence (NICE) 2020 guideline for managing colorectal patients, for colorectal patients who were presented to have resectable tumors, then it is recommended to undergo surgical resection. Meanwhile, higher stages of the colorectal cancer would be an indication for a combination of chemoradiotherapy and surgery (4). Other options at hand, according to NCCN Guideline 2018, include targeted therapy which insert drugs and acts more specific towards the cancer cells rather than the systemic chemotherapy, immunotherapy which increases the patient's immune system in the defensive activities against the cancer cells, and the less commonly used method which uses heat waves to "burn" the cancer cells (5).

With the use of conventional anticancer drugs, several detrimental side-effects are known to be occurring often. These side-effects include nausea, vomiting, and hair loss. Therefore, an alternative drug should be researched which aims to treat cancer as effective but with less side-effects. One drug that is proposed is Sarang Semut which is an herbal medicine originating from Papua (6). In Wamena, Papua, a plant called *Myrmecodiapendans* (Sarang Semut plant) was discovered to be extracted by the locals and was used for medicinal remedies, although it can also be found around the Malay Peninsula, the Philippines, Cambodia, and the Sumatra Island of Indonesia (7). The local tribes use the plant by boiling it into a water and drank it to increase their immune system.

As a supporting proof for the local tribe usage of the Sarang Semut plant, there are evidences which associate the plant with the inhibition of human cancers originating from the brain, blood, cervix, and colon (8). In a study conducted to see the cytotoxic effect sarangsemut extract on HeLa cancer cells, it was found in the phytochemistry test that sarangsemut extract contains major bioactive compounds of flavonoid, tannin, and polyphenol, those which are associated with cancer inhibitory properties (7). Other analysis such as Thin Layer Chromatography also supports the fact that flavonoid is found in sarangsemut extract (9).

The emergence of herbal medicine to thrive in the pharmaceutical industries, especially in Indonesia, can be a crucial factor in increasing patient compliance. Popular beliefs among Indonesian citizens regarding the safer use of herbal medicine compared to pharmaceutical “synthetic” drugs can impact the compliance of drug consumptions. To give an example, a study stated that 58% of Indonesians with low income accept herbs as their medicine, meanwhile Indonesians with a high income manage to score significantly lower in their preference towards herbs as a medicine (10). It is very important for Indonesia’s population, despite the income, to receive adequate treatment. Herbs as a cheaper alternative can solve the problem.

Hence, the research towards sarangsemut plants as a part of herbal medicine is necessary to be looked in depth, whilst looking back at a larger picture to provide an alternative solution on economic problems which hinders adequate medical treatment. In this research, sarangsemut plant originated from Papua, Indonesia, will be macerated and extracted by three different polarity of organic solvents, namely n-hexane (nonpolar), Ethyl acetate (semi polar) and ethanol (polar) to give the corresponding n-hexane extract, ethyl acetate extract and ethanol extract of Sarang semut. Subsequently, these three sarangsemut extracts will be analyzed by thin layer chromatography and phytochemistry test for its phytochemical compounds, as well as to be evaluated its cytotoxic effect towards colon WiDr cancer cells by MTT (3-(4,5-dimethylthiazol-2yl)-2-5-diphenyltetrazolium bromide) assay.

The high prevalence of cancer in Indonesia remains a concern up until this year. As the problem arises, newer solutions are found by scientists in hopes to reduce the mortality of cancer patients. Colorectal cancer patients diagnosed in early stages are provided with surgical therapy as the primary solution, meanwhile patient diagnosis of later stages should undergo chemotherapy with the purpose of palliative care. However, the successful rate of these therapies can be increased by providing newer innovations for managing colorectal cancer. Due to the common detrimental side-effects such as nausea, vomiting and hair loss, an alternative treatment is needed which reduces the probability of having such side-effects. Hence, further studies regarding cancer medicines from the herbal section which includes sarangsemut plant (*Myrmecodiapendans*) should not fall below the cruciality of other therapeutic studies, since the plant itself is known to have anticancer properties. The purpose of this research is to analyze the phytochemical compounds and cytotoxic effects of sarangsemut (*Myrmecodiapendans*) extracts towards colon WiDr cell line.

MATERIAL AND METHODS

Materials: *Myrmecodiapendans* also known as Sarang Semut plant (Figure 1)were derived from Wamena, Papua, Indonesia (11).WiDr cancer cells are obtained from Department of Medical Chemistry, Faculty of Medicine, University of Indonesia. Table 1 shows the taxonomy of *Myrmecodiapendans*(12).

Figure 1. *Myrmecodiapendans*

Table 1. Taxonomy of *Myrmecodiapendans*

Taxonomy of *Myrmecodiapendanns*	
Division	*Tracheophyta*
Class	*Magnoliopsida*
Subclass	*Lamiidae*
Order	*Rubiales*
Family	*Rubiaceae*
Genus	*Myrmecodia*

Methods

Thin layer chromatography (TLC): TLC analysis was used to determine the number of chemical components contained in the extract of the sample. The adsorbent as stationary phase was a silica gel plate. One silica gel with the size of 5x1 cm was drawn using a pencil to create a line on each side which is 0,5 cm from both edges. The extract was dripped on the marked dot located on the starting line. Then, the silica gel was inserted into an eluent chamber without the starting line touching the eluent compound. There was a waiting period until the mobile phase ascended to the other line on top. The plate was transported out of the eluent bottle to be dried out. Then, the plate was observed using 254 nm and 366 nm ultraviolet light devices. Lastly, the retention factor (Rf) was counted using the equation below.

Rf=distance traveled by sample / distance traveled by solvent

Phytochemical screening: Alkaloid, flavonoids, tannins, and glycosides are the secondary metabolites found in *M. pendans,* however as suggested by a study the test had consisted of all examination of the secondary metabolites(13,14).

Alkaloid Test

The amount of *M. pendans* extract used was 2 mL, which was then inserted into a porcelain container, where it got evaporated. After that, the tube was inserted with 5 ml of HCL 2N solution and was divided into 3 test tubes: the first tube is a blank tube, the second tube with the addition of 3 drops of Dragendorff reagent, and the third tube with the addition of 3 drops of Meyer reagent. The presence of Alkaloid would result in yellowish color on the 2nd and 3rd tube.

Flavonoid Test: Amount of1 mL of *M. pendans* extract was put into a test tube.

It was then given 0,5 mL of hydrochloric Acid (HCl) and 4 cm of Magnesium ribbon, in which a red, green, or yellow color would appear, indicating a positive result.

Tannin Test: Firstly, 1 mL of *M. pendans* extract was placed on a test tube. Then, the following step was the addition of 1 mL 10% FeCl3 solution. A positive result will come up if the result becomes greenish-black or dark blue.

Glycoside Test: Amount of 0.1 mL of *M. pendans* extract was put on a test tube, then evaporated above a boiling water container. 5 mL of Acetic anhydride was added right away. Then, the next step was inserting 10 drops of HCl. The result, should it become positive, will change color into blue or green.

Saponin Test: One gram of *M. pendans* extract was inserted into a test tube, followed by 10 mL aquadest. The test tube was shaken vertically for 10 seconds and left stationary for another 10 seconds. An early indication of saponin presence was the formation of foam as tall as 1-10 cm which stayed in a stable form for 10 minutes. To further confirm, 1 drop of HCl 2N solution is mixed to the previous mixture.

Steroid and Triterpenoid Test: Two mL of *M. pendans* extract was put into a porcelain bottle and evaporated above a boiling water. The result of the evaporation was dissolved with 0,5 mL each of chloroform and acetic anhydrase. Following the step, sulfuric acid was inserted in the amount of 2 mL throughout the wall of the bottle. If the sample contains triterpenoid, there will be a ring formation with the color brown or violet. Meanwhile, if the sample contains steroids, a blue-greenish ring will form.

MTT Assay: WiDr cells were inoculated into a 96-well plate for 24 hours with the purpose of reaching 70% confluence. Afterwards, the various concentration extracts of *M. pendans* were inserted to the same plate. The MTT solution was prepared next. The preparation consisted of mixing the MTT solution itself with Dulbecco's Phosphate Buffer Solution. The Result of the MTT mixture underwent filtration and inserted into a container. The sterile container was shielded from light exposure and placed in a 4 °C environment. This was called the incubation period. After the first incubation, 25 µL of the MTT mixture was inserted to the well plate and a second incubation was done in a 37 °C environment for 4 hours. The next step which followed was the solubilization method of DMSO for an amount of 100 µL. Next, the measurement was done through its absorbance (15,16). The toxicity dose of the given extracts was seen by the IC50 value. To ensure that the MTT assay is performed correctly, Doxorubicin which is an adjuvant for chemotherapies was used as a positive control, as other studies had used previously (11,12). Here below is the equation used for determining the half maximal inhibitory concentration (IC50) value.
Cell cytotoxicity (%)=100-((Abs Control-Abs solution tested)/(Abs Control) x 100)

Research flow: The flow of the research began with the extraction of sample *Myrmecodiapendans* using solvents which had included n-hexane, ethyl acetate, and ethanol. The extract sample was then analyzed by Thin Layer Chromatography and Phytochemical test to assess the containing chemical components in the extract. Afterwards, the cell viability test of the extract sample was performed towards the WiDr colon cancer cell line using the MTT assay.

RESULTS

Thin layer chromatography analysis of Myrmecodiapendans: Three extracts of *Myrmecodiapendans*, namely n-hexane extract (NHE), ethyl acetate extract (EAE) and ethanol extract (ETE) were analyzed by TLC on silica plates. There were two plates selected out of several others due to its clearest band visibility of each extracts. As seen on Figure 2, plates were seen under 254 nm UV lamps, although observations were done by both at 254 nm and 366 nm. When performing trial and error on the ratio of hexane-ethyl acetate as the mobile phase, it was learned that plates showed bands presenting most prominent spots when the ratio was 5:1, whilst other ratios (1:1, 2:1, 3:1, 4:1, and 10:1) showed either a clear, straight band with no evident "spots" or no band, through both direct and UV lamp observations. Identical finding was seen when finding the ratio of chloroform-methanol for the ethanol extract (ETE). A total of 6 chemical components were found in all three extracts of *Myrmecodiapendans*. Each of these compounds are differentiated by the retention factors. Table 2 summarizes the chemical compounds on *Myrmecodiapendans* extracts with the retention factors.

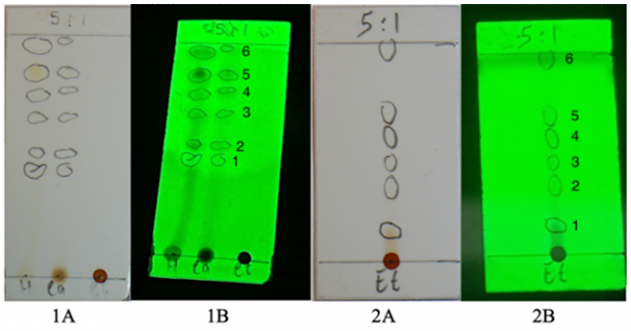

Figure 2. TLC analysis of *Myrmecodiapendans* extracts, NHE, EAE, and ETE, with the mobile phase of n-hexane-ethyl acetate with the ratio of 5:1 for NHE and EAE; and chloroform-methanol with the ratio of 5:1 for ETE. The plates are seen under direct light (1A and 2A) and under 254 nm of UV lamp (1B and 2B)

Table 2. Chemical compounds on the *M. pendans*extract with the retention factors: As seen from Table 2, NHE and EAE extracts had three, insignificant differences on the retention factor of compound 4 and 6, whilst the rest displayed the same distance travelled on the plate. However, ETE had more distinctive retention factors even though the number of compounds persisted.

Table 2. Chemical compounds on the *M. pendans*extract with the retention factors.

Compound	Rf (Retention factor)		
	NHE of *M. pendans*	EAE of *M. pendans*	ETE of *M. pendans*
1	0.5	0.5	0.133
2	0.565	0.565	0.333
3	0.696	0.696	0.467
4	0.783	0.804	0.567
5	0.87	0.87	0.7
6	0.957	0.978	0.967

NHE: n-hexane extract, EAE: ethyl acetate extract, and ETE: ethanol extract of *M. pendans*

Phytochemical analysis: Qualitative phytochemical findings on *Myrmecodiapendans* extracts are seen below on Table 3. As noticed, NHE was reactive towards the phytochemical testing as it only showed positive results on triterpenoid, flavonoid and tannin, and showed negative results on saponin, and glycoside tests, whereas ETE demonstrated otherwise. Also, another essential aspect was the continuous presence of triterpenoid. All three extracts gave reaction towards the alkaloid test, nonetheless only EAE was able to indicate the presence of alkaloid by instigating precipitation with both reagents, Mayer and Dragendorff.

Table 3. The summary of phytochemical test results on three extracts of *M. pendans*

	Saponin	Flavonoid	Tannin	Glycoside	Triterpenoid/ Steroid	Alkaloid
NHE	-	+	+	-	Triterpenoid	-*
EAE	-	+	+	-	Triterpenoid	+
ETE	+	+	+	-	Triterpenoid	-*

*only displayed precipitation on Mayer reagent. NHE: n-hexane extract, EAE: ethyl acetate extract, and ETE: ethanol extract of *M. pendans*

Table 4. IC50 value (µg/mL) of *Myrmecodiapendans* extracts towards WIDR cells

Extract of *Myrmecodiapendans*	IC_{50} (µg/mL) on WiDRcells
n-Hexane	4.74
Ethyl acetate	3065.49
Ethanol	5311.30
Doxorubicin (positive control)	36.86

Table 5. Classification of cytotoxicity based on IC50 value provided by Atjanasuppat et al.[29]

IC_{50} value	Classification
< 20 µg/mL	active
20-100 µg/mL	moderate
100-1000 µg/mL	weak
> 1000 µg/mL	inactive

Grading cytotoxicity of M. pendans extracts on colon WiDr cells by MTT assay: The result of MTT assay of n-hexane extract (NHE), ethyl acetate extract (EAE) and ethanol extract (ETE) of *Myrmecodiapendans*in colon WiDr cells were extracted to Microsoft Excel. The absorbance values of each extracts and positive control of doxorubicin are taken by Visible Spectrophotometer at 630 nm. The data was sorted into a scatterplot with log concentration in x axis versus percentage of inhibition in y axis to give a linear regression model. Table 4 displays the IC50 values of *Myrmecodiapendans* extracts towards colon WIDR cancer cell line. The IC50 values represent the half maximal inhibitory concentration which was obtained using the linear regression method. By means of linear regression equation, the number 50 was put as the "x" value to represent the IC50. As shown on Table 4, n-hexane extract (NHE) of *M. pendans* had the least mean value of IC50 (4.74 µg/mL), whilst ethyl acetate extract (EAE) and ethanol extract (ETE) *of M. pendans* both have drastically higher of IC50 values (>1000 µg/mL). To compare with the positive control, doxorubicin had an average IC50 value of 36.86 µg/mL.

DISCUSSION

Determination of chemical components in *Myrmecodiapendans*by TLC analysis: Separation of compounds by TLC method is considered to be one of the simplest methods for separating a mixture. However, the result of this study may indicate several flaws. As seen on Table 1, all three extracts of *Myrmecodiapendans* had the same number of compounds. Moreover, n-hexane extract (NHE) and ethyl acetate extract (EAE) showed similar retention factors aside from compound 4 and 6, which both had a surprisingly same difference of 0.021. The average distance between retention factors of both extracts also had a small disparity, indicating that the compounds within are the same. On the other hand, compounds in ethanol extract (ETE) travelled relatively slower, as opposed to other extracts. Thus, this study showed that the individual compounds within NHE and EAE are the same, but not entirely for ETE. The varying results of retention factors are due to multifactorial reasons. As stated in Santiago *et al*.(17), the application of extract sample using micropapillary tube should be in the range of 1-5 µl on the line drawn previously, however our preference was "spotting" the extract sample once without using approximate measurement and instead used intuition from trial and errors. The spotting frequency may affect the retention factors as mentioned by Oktaviantari *et al*., alongside other factors for instance temperature and humidity inside the chamber which had the mobile phase (18). In addition, the nature of compounds found from our TLC does not indicate a bioactive characteristic. In a study by Arundina *et al*. (19), *Artemisia vulgaris* were macerated and underwent TLC to confirm whether the visible spot on the plate was terpenoid or other metabolites. Their method of TLC had an addition of terpenoid itself as a standard on the same plate next to the unknown sample. The retention factors between the two compounds' standard terpenoid and unknown spot were the same, which implied that the unknown spot is a terpenoid.

Phytochemical properties of *Myrmecodiapendans* extracts: To truly identify bioactive phytochemical compounds in Myrmecodiapendans extracts, a number of phytochemical tests were accomplished. As reflected on Table 2, n-hexane extract of *Myrmecodiapendans* (NHE) contained triterpenoid and showed precipitation formation on Mayer's reagent of alkaloid test. Triterpenoid does have a role in suppressing colorectal cancer cell proliferation, as supported by Wang *et al*. Colorectal cancer cells in particular are at an energy deficit state and hence require energy produced from glycolysis. The bioactive compound could take a role in down-regulating proteins ASIC2 and calcineurin that contribute to such energy production and eventually water down the proliferation (18,20). Another study by Park *et al*. showed that primisterin, a natural triterpenoid extracted from plants in the family Celastraceae, not only regulate AKT/FOXO3a signaling pathway in colon cancer tissue, but also induce apoptosis (21). For the case of alkaloid, the precipitation only occurring on our Mayer's reagent, instead of dual precipitation on both Mayer's and Dragendorff's reagents, may indicate other compounds such as coumarin, α-pyrone, hydroxyflavone, and tannin (22). Uncertainties regarding the effectiveness of n-hexane extract towards colorectal cancer may be reduced as Bashari *et al*. reported that n-Hexane extract of *Myrmecodiapendans* gave strong cytotoxicity effect towards other cell lines HCT-116 and Caco-2, in which both resulted a positive outcome with IC50 value of 33 and 24 µg/mL, respectively (23).

Unlike n-hexane extract (NHE), not only did ethyl acetate extract (EAE) showed presence of triterpenoid, as reported on Table 2, but also tannin and alkaloid. In accordance with Li *et al*.'s study on tannin-containing Chinese herb, the bioactive compound was able to down-regulate several target genes (c-Myc, Dkk1, Survivin, NKD1, & FGF20) and suppressed Wnt/ β-catenin signalling pathway on a different colon cancer cell line, HT-29 (24). Additionally, tannin also had an effect towards an enzyme PKM2 which is needed for rapid proliferation of colorectal cancer cells. the pyruvate kinase activity on the enzyme was restrained which led to reduced cell proliferation (25). However, there were no studies that focused on tannin's particular role towards the examined cell line WiDr in this research. Alkaloid, on the other hand, affected the G1 phase of WiDr cell cycle according to Mutiah *et al*. The cell growth was inhibited through such mechanism as their report revealed a decreased cell viability, albeit quite weakly in comparison to their doxorubicin group (25). To support the benefits of the compound, another analysis mentioned Red Betel Leaves also contained alkaloids which have been proven to inhibit Survivin protein, similar to tannin (26). Compared to n-hexane extract (NHE) and ethyl acetate extract (EAE), ethanol extract (ETE) of *Myrmecodiapendans* contained the most bioactive compounds, which had included saponin, flavonoid, tannin and triterpenoid. Therefore, ETE may give the most variability towards anticancer mechanisms. For one, steroidal saponins from Digitalis trojana showed cytotoxic activities in colon cancer cell line HT-29. On a surprising case, out of seven compounds of steroidal saponins, one compound called zingiberensis saponin displayed an equal cytotoxic effect as doxorubicin on C26 colon carcinoma cell (27). Relating to another composition of ETE, one paper explained that a type of flavonoid was able to reduce the viability of WiDr cells. Moreover, the flavonoid was recommended as an additional therapy accompanying doxorubicin in treating colorectal cancer (28).

Assessing the cytotoxicity of *Myrmecodiapendans* extracts: In accordance with Atjanasuppat *et al*.'s study on Table 5, each of the means of two*Myrmecodiapendans*extracts, ethyl acetate extract (EAE) and ethanol extract (ETE) are classified as having inactive cytotoxic effect with IC50 value over than 1000 µg/mL (see Table 4). Whereas, when analyzing three trial experiments, n-hexane extract (NHE) had positive results with the average of IC50 value of 4.74µg/mL.This indicated that those three trials had an active cytotoxic effect. Even so, to support this evidence, there are little to no external studies which observed cytotoxicity of those three extracts. There is one study in 2013 by Margo *et al*. which observed the cytotoxicity of *Myrmecodiapendans'* ethanol extract, however, the result is exceptionally different to this study. Margo *et al*. reported the

ethanol extract of *Myrmecodiapendans* had a weak cytotoxicity on WiDr colon cancer cells with IC50 value of 121.059 µg/mL, in comparison to IC50 value of ethanol extract of *Myrmecodiapendans* on WIDR cells in this study, which is 5311.30µg/mL, this value signifies an inactive cytotoxicity (29,30).

CONCLUSION

Phytochemical compounds found in *Myrmecodiapendans* by Phytochemistry test resulted in the presence of the following: saponin, flavonoid, tannin, triterpenoid, and alkaloid. N-hexane extract of Myrmecodiapendans has a strong cytotoxic effect towards colon WiDr cell line by MTT assay.

ACKNOWLEDGEMENT

The authors are grateful to DRPM (Directorate of Research and Public Service), University of Indonesia, for the PUTI SAINTEKES research grants.

CONFLICT OF INTEREST: The authors state that there is no conflict of interest.

SOURCE OF FUNDING

This study is supported by PUTI SAINTEKES research funding from DRPM (Directorate of Research and Public Service), University of Indonesia, during the 2020–2021 fiscal year.

Glossary of Abbreviations

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
TLC: Thin layer chromatography
IC50: half maximal inhibitory concentration
WIDr: human colon cancer carcinoma cell line
DMEM: Dulbecco's modified Eagle's medium
DNA: Dinucleotide Nucleic Acid
NADP (H): Nicotinamide adenine dinucleotide phosphate
PBS: Phosphate Buffered Saline

REFERENCES

World Health Organization. Cancer country profiles – Indonesia [Internet]. Geneva, Switzerland: World Health Organization; 2014 [cited 2020 Jan 31]. 2 p. Available from: https://www.who.int/cancer/country-profiles/idn_en.pdf.

The Global Cancer Observatory. Indonesia [Internet]. Lyon, France: World Health Organization; 2018 [cited 2020 Jan 31]. 2 p. Available from: https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf

Kumar V, Abbas AK, Aster JC. Robbins basic pathology. 10th ed. Philadelphia, PA: Elsevier; 2018. p. 631-2.

National Institute for Health and Care Excellence. Colorectal cancer. United Kingdom: NICE; 2020. p. 8-17.

Shead DA, Hanisch LJ, Vidic E, Corrigan A, Clarke R. Colon cancer. Pennsylvania: NCCN; 2018. p. 23-33.

Nurgali K, Jagoe RJ, Abalo R. Editorial: Adverse effects of cancer chemotherapy: anything new to improve tolerance and reduce sequelae? Front Pharmacol. 2018; 9: 245.

Soeksmanto A, Subroto MA, Wijaya H, Simanjuntak P. Anticancer activity test for extracts of sarangsemut plant (myrmecodya pendens) to hela and mcm-b2 cells. Pak J Biol Sci. 2010;13(3):148-51.

Supriatno J. Antitumor activity of papua'smyrmecodiapendans in human oral tongue squamous cell carcinoma cell line through induction of cyclin-dependent kinase inhibitor p27kip1 and suppression of cyclin e. J Cancer Res Ther. 2014;2(3):48-53.

Muna LN, Ernawati. Perbandinganpenghambatanaktivitas xanthine oxidase oleh ekstraketanolsarangsemut (myrmecodiapendans) dan fraksi butanol herbaceplukan (physalis angulata l) secara in vitro. Medisains. 2017;15(2):108-17.

Andriati, Wahjudi RMT. Society's acceptance level of herb as alternative to modern medicine for lower, middle, and upper class group. Universitas Airlangga. 2016;29(3):133-45.

Sari GA, Permatasari M, IGAA Carlita, Argadanah AF, Munasir M. Potensiantikankerekstraksarangsemutputihpapua (myrmecodiapendans) terhadapsel mcf-7 secara in vitro. InstitutPertanian Bogor. 2014: 1-23. Figure 1, Sarang semut plant; p. 14.

Sitorus O. Identifikasisarangsemutmenggunakan wavelet dan backpropagation momentum [thesis]. Yogyakarta: Universitas Atma Jaya Yogyakarta; 2017. p. 8.

Fatmawati D, Puspitasari PK, Yusuf I. Efeksitotoksikekstraketanolsarangsemut (myrmecodia pendens) pada sel line kankerservikshela: uji eksperimental in vitro. Sains Medika. 2011;3(2):112–20.

Sudiono J, Oka CT, Trisfilha P. The scientific base of myrmecodiapendans as herbal remedies. BJMMR. 2015;8(3):230-7.

Arora S, Tandon S. Mushroom extracts induce human colon cancer cell death by triggering the mitochondrial apoptosis pathway and g0/g1- phase cell cycle arrest. Arch Iran Med. 2015 May;18(5):284-95.

Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, *et al*. Cell viability assays. 2013 May 1 [Updated 2016 Jul 1]. In: Sittampalam GS, Grossman A, Brimacombe K, *et al*., editors. Assay guidance manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004. Available from: https://www.ncbi.nlm.nih.gov/books/NBK144065/

Arudina I, Budhy TI, Luthfi M, Indrawati R. Identifikasikromatografi lapis tipis sudamala (artemisia vulgaris l.). Maj Ked Gi Ind. 2015;1(2):167-71.

Wang G, Wang YZ, Yu Y, Wang JJ. Inhibitory asic2-mediated calcineurin/nfat against colorectal cancer by triterpenoids extracted from rhus chinensis mill. J. Ethnopharmacol. Elsevier B. V. 2019 May;235:255-67.

Park JH, Kim JK. Pristimerin, a naturally occurring triterpenoid, attenuates tumorigenesis in experimental colitis-associated colon cancer. Phytomedicine. 2018;42:164-71.

Ergina, Nuryanti S, Pursitasari ID. Uji kualitatifsenyawametabolitsekunder pada daunpalado (agave angustifolia) yang diekstraksidenganpelarut air dan etanol. J. Akad. Kim. 2014 Aug;3(3):165-72.

Bashari MH, Hidayat S, Ruswandi YAR, Putri T, Qomarilla N, Dwiwina RG, Kurnia D, *et al*. The n-hexane fraction of myrmecodiapendans inhibits cell survival and proliferation in colon cancer cell line. Int J Pharm Pharm Sci. 2018;10(1):108-12.

Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A, *et al*. In vitro screening for anthelmintic and antitumour activity of ethnomedicinal plants from thailand. J.Ethnopharmacol. 2009;123:475–82.

Li W, Yang CJ, Wang LQ, Wu J, Dai C, Yuan YM, *et al*. A tannin compound from sanguisorba officinalis blocks wnt/β-catenin signalling pathway and induces apoptosis of colorectal cancer cells. Chi Med. 2019;14:1-13.

Mutiah R, Kirana FO, Annisa R, Rahmawati A, Sandra F. Extract of yellow root (arcangelisia flava (l.) merr.) from several regions in kalimantan: alkaloid content and cytotoxicity towards widr colorectal cancer cells. Indones. J. Cancer Chemoprevent. 2020;11(2):84-9.

Ikawati M, Armandari I, Khumaira A, Ertanto Y. Effects of peel extract from citrus reticulata and hesperidin, a citrus flavonoid, on macrophage cell line. Indonesian J Pharm. 2019;30(4):260-268.

Wulandari N, Meiftasari A, Fadliyah H, Jenie RI. Red betel leaves methanolic extract (piper crocatumruiz& pav.) increases cytotoxic effect of doxorubicin on widr colon cancer cells through apoptosis induction. Indones. J. Cancer Chemoprevent. 2018;9(1):1-8.

Tunjung WAS, Sayekti PR. Apoptosis induction on human breast cancer t47d cell line by extracts of ancorina sp. F1000research, 07 Feb 2019, 8:168.

Lalitha LJ, Sales TJ, Clarance PP, Agastian P, Kim YO, Mahmoud AH, *et al*. In-vitro phytopharmacological and anticancer activity of loranthuslongiflorusdesv. var. falcatuskurz against the human lung cancer cells. J. King Saud Univ. Sci. 2020;32(1):1246-1253.

Yang P, Ding GB, Liu W, Fu R, Sajid A, Li Z. Tannic acid directly targets pyruvate kinase isoenzyme m2 to attenuate colon cancer cell proliferation. Food Func. 2018 Nov;9(11):5547-59.

Margo W, Ratnawati H, Herdiman H. Efeksitotoksikekst raketanoltanamansarangsemut (myrmecodiapendansmerr& perry) terhadapkarsinomakolon pada kultur selwidr. FakultasKedokteran Universitas Kristen Maranatha. 2013.

****