AIP Conference Proceedings

RESEARCH ARTICLE | APRIL 11 2024

Phytochemical screening and cytotoxicity of *Melastoma malabathricum* L. leaves extracts against MCF-7, HeLa, A549, B16, and HT29 cells

Muddatstsir Idris; Adi Setyo Purnomo; Fahimah Martak; Sri Fatmawati

AIP Conf. Proc. 3071, 020025 (2024)
https://doi.org/10.1063/5.0207722

AIP Publishing

Phytochemical Screening and Cytotoxicity of *Melastoma malabathricum* L. Leaves Extracts Against MCF-7, HeLa, A549, B16, and HT29 Cells

Muddatstsir Idris[1,a)], Adi Setyo Purnomo[2)], Fahimah Martak[2)], Sri Fatmawati[2,b)]

[1] *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat, A. Yani KM. 35.8 Banjarbaru 70714, South Kalimantan, Indonesia*
[2] *Department of Chemistry, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Jalan Arif Rahman Hakim, Sukolilo, Surabaya 60111, East Java, Indonesia*

[b)] *Corresponding Author: fatma@chem.its.ac.id*
[a)]*mu_idris74@yahoo.com*

Abstract. A medicinal plant widely distributed in South Kalimantan, Indonesia, is *Melastoma malabathricum*. Therefore, this research aimed to assess the phytochemicals (terpenoid, steroid, flavonoid, and alkaloid contents) and cytotoxicity of *M. malabathricum* leaves extracts in order to investigate *M. malabathricum* as a medicinal plant for anticancer agents. Using phytochemical screening techniques, the terpenoid, steroid, alkaloid, and flavonoid contents were examined. A cytotoxicity test was conducted *in vitro* on several cancer cell lines, such as MCF-7, HeLa, and A549. B16, and HT29. *M. malabathricum* leaves extract contains terpenoids, steroids, alkaloids, and flavonoids. MCF-7, HeLa, A549, B16, and HT29 cancer cell lines were used to demonstrate the cytotoxicity of the methanol extract, with IC_{50} values of 327.37±0.67, 327.05±0.48, 304.46±1.93, 319.21±0.67, and 1.43±0.19 µg/mL, respectively. These findings suggest that the plant could serve as a promising source of anticancer agents.

INTRODUCTION

Cancer is a menacing and lethal disease for which the discovery of effective drugs that do not cause harmful side effects is still being sought to date. It is characterized by uncontrolled cell growth invading healthy tissue [1]. Numerous plant species have been used in the treatment and prevention of cancer, with approximately 3,350 species used in ethnomedicine [2].

As an herbal medicine, *M. malabathricum* has been traditionally used for a long time. In Malaysia, the leaves and roots have been utilized for treating wounds, postnatal care, and preventing scars from smallpox infections, as well as for the treatment of stomach ulcers, dysentery, and diarrhea. The Melastomataceae family, to which *M. malabathricum* belongs, exhibits various bioactivities such as antioxidant, anticancer, cytotoxic, anti-hypertensive, and anti-nociceptive, antipyretic, antiviral, anti-inflammatory, antibacterial, as well as antifungal properties [3]. The leaves of *M. malabathricum* contain tannins, saponins, triterpenes, flavonoids, and steroids [4].

The IC_{50} value for the DPPH antioxidant activity demonstrated by the *M. malabathricum* flower methanol extract was 17.23 µg/mL [5]. Furthermore, methanol and chloroform flower extracts showed anticancer activity against the MCF-7 cell line with 45.76 and 33.63 µg/mL IC_{50} after 72 hours of testing, respectively [3]. Compound isolates, kaempferol-3-O-(2",6"-di-O-p-trans-coumaroyl)-β-glucoside as well as naringenin, acquired from the

5th International Seminar on Chemistry
AIP Conf. Proc. 3071, 020025-1–020025-11; https://doi.org/10.1063/5.0207722
Published under an exclusive license by AIP Publishing. 978-0-7354-4926-8/$30.00

flower ethyl acetate extract exhibited cytotoxicity against the MCF-7 cell line, with respective 1.3 µM and 0.28 µM IC_{50} values, as stated in reference [6]. The administration of methanol extract from *M. malabatricum* leaves does not cause death, behavioral irregularities, weight changes, and changes in food and water intake. The results suggest that *M. malabatricum* leaves can be safely consumed orally at both subacute and subchronic levels. Moreover, the extract may have cytotoxic effects against HT29 colon cancer cells through apoptosis [7]. Previously, *M. malabathricum* leaves were investigated because of their antioxidant properties (DPPH, ABTS, as well as FRAP) and phenolic and flavonoid content using various extracts, including methanol, *n*-hexane, dichloromethane, and ethyl acetate. The highest total phenol content was found in the methanol extract, which measured 183.71±0.11 mg GAE/g extract. The highest total flavonoid concentration was found in the ethyl acetate extract, measuring 24.10±0.04 mg QE/g extract. Methanol extract demonstrated the strongest ABTS, DPPH, and FRAP activity, with IC_{50} values of 4.59 ± 0.03 µg/mL, 8.58± 0.03 µg/mL, and 51.150.10 M Fe^{+2}/g, respectively. Furthermore, the strongest anti-α-glucosidase and anti-α-amylase activities were found in the anti-diabetic test *in vitro* from methanol extract, with IC_{50} values of 75.25±1.60 and 52.38±1.32 µg/mL, respectively. According to the *in vivo* antidiabetic test, administering 200 mg/Kg body weight of methanol extract from *M. malabathricum* leaves resulted in a reduction of glucose and serum levels in rat, with values of 51% and 37.82%, respectively, according to reference [8]. Therefore, this research aimed to explore medicinal plants in South Kalimantan, Indonesia, and as such to evaluate the content of terpenoids, steroids, alkaloids, and flavonoids using *M. malabathricum* leaves extracts phytochemical screening as well as to ascertain the cytotoxicity of methanol extract on MCF-7, Hela, A549, B16, and HT29 cells.

MATERIAL AND METHODS

Plant Material

The leaves of *M. malabathricum* were collected in June 2019 at Banjarbaru, South Kalimantan, Indonesia, and identified by Mr Edi Suroto, a botanist at Purwodadi Botanical Garden, Pasuruan, Indonesia. The voucher specimen was deposited at the Institut Teknologi Sepuluh Nopember's Laboratory of Natural Products and Synthetic Chemistry, with the reference number 013/VI/HT-KIBAS/2019.

Cell Lines

The cell lines used were B16-F10 (ATCC CRL-6475) a skin melanoma, A549 (ATCC CCL-185) a lung carcinoma, Breast adenocarcinoma MCF-7 (ATCC HTB-22), cervical adenocarcinoma HeLa (ATCC CCL-2), and colon HT29 (ATCC HTB-38). The Central Laboratory of Pajajaran University in Bandung, Indonesia provided the MCF-7, Hela, A549, and B16 cells. HT-29 was obtained from the Biomedical Central Laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia

Extraction

The dried leaves of *M. malabathricum* dried leaves extraction involved the use of 20 g of plant material and 250 ml each of ethyl acetate (EA), *n*-hexane (HX), methylene chloride (MC), as well as methanol (MeOH), with an extraction duration of 24 hours. The resultant extract was concentrated on a rotary evaporator and then filtered using filter paper.

Flavonoid Test

The liquid filtrate of the extract received approximately 5 mL of liquid ammonia and then had 1 mL of concentrated sulfuric acid added to it. The appearance of a yellow color, as reported in the reference, indicated the existence of flavonoids [9].

Alkaloid Test

Two test tubes were labeled A and B, and 1 mL of extract was added to each tube. To tube A, 0.5 mL of 2% v/v hydrochloric acid was added and shaken until homogenous. Subsequently, 2-3 drops of Meyer reagent were added to tube A. On the other hand,Wagner reagent was put to tube B in 2-3 drops. The white precipitate formation in tube A and a brown precipitate in tube B indicated the presence of alkaloids in the extract, according to reference [10].

Terpenoid Test

2 mL of 98% chloroform were added to a reaction tube after 1 mL of extract, and the mixture was agitated. The chloroform layer was isolated, and a few drops were put on a drip plate and left to dry. Next, 5 drops of 98% anhydrous acetic acid and 3 drops of 98% sulfuric acid were added to the extract. As described in the reference, the appearance of a red, orange, or yellow color signaled the existence of terpenoids, while a green color formed in the extract indicated the occurrence of steroid [11].

In vitro Cytotoxicity Assay

The *in vitro* cytotoxicity of *M. malabathricum* extracts against various cancer cell lines was assessed using the PrestoBlue (PB) method, including the HeLa (human cervix adenocarcinoma), A549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), B16 (murine melanoma), as well as HT29 [12]. A full RPMI (Roswell Park Memorial Institute) medium with antibiotics in 50 µL/50 mL and 10% (v/v) FBS (Fetal Bovine Serum) were used to maintain the cells. Moreover, cell cultures were cultured in 96-well plates at a density of 170,000 cells/mL of medium and incubated for 24 hours at 37°C with 5% CO_2. The samples were then transferred to a brand-new medium and cultured for an additional 48 hours at concentrations ranging from 2.34 to 300 µg/mL. The medium was then mixed with PB reagent from Thermo Fisher Scientific in Uppsala, Sweden, and the mixture's 570 nm absorbance was measured using a multimode reader. As a positive control, cisplatin was utilized.

RESULTS AND DISCUSSION

Phytochemical Test

Table 1 displays the results of phytochemical tests conducted on extracts of *M. malabathricum* leaves obtained through extraction with methanol (MeOH), *n*-hexane (HX), methylene chloride (MC), as well as ethyl acetate (EA).

TABLE 1. Phytochemical test of *M. malabathricum* leaf extracts

Test	***M. malabathricum* Leaves Extract**			
	HX	**MC**	**EA**	**MeOH**
Terpenoid	+	-	-	+
Steroid	-	+	+	-
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+

Secondary metabolites are present in positive (+) but are absent in negative (-).

M. malabathricum leaves extract contains secondary metabolites (alkaloids, flavonoids, terpenoids, as well as steroids) are shown in the Table 1. Subsequently, terpenoids are mostly found in non-polar but also in polar (methanol extract) because methanol is a polar solvent, hence, can extract both polar and nonpolar compounds. In the *M. malabathricum* leaves methanol extract, terpenoid compounds (*β*-sitosterol as well as *β*-sitosterol-3-O-*β*-D-glucopyranoside) were discovered, according to Jofry et al. (2012) [13]. In addition, polar, semi-polar, and non-polar extracts all contain alkaloids and flavonoids. A flavonoid known as quercetin was sequestered from the *M. malabathricum* leaves using *n*-hexane extract [13]. Previous research conveyed that the *M. malabathricum* leaves extract contains flavonoids as well as terpenoids. The leaf extracts of *n*-hexane, methylene chloride, ethyl acetate, as well as methanol had flavonoid contents of 16.77±0.05, 22.69±0.04, 24.10±0.04, and 23.18±0.05, (mg QE/g Extract)

respectively. Meanwhile, the amounts of phenols in the leaves extract of *n*-hexane, methanol, methylene chloride, as well as ethyl acetate were 26.97±0.19, 183.71±0.11, 27.21±0.17, and 78.87±0.14 (mg GAE/g Extract), respectively [8].

The terpenoid test is shown in Figure 1a, the yellow color of the *n*-hexane and MeOH extracts indicates the presence of terpenoids, while the green color in the methylene chloride and ethyl acetate extracts indicates that they contain steroids. In Figure 1b, the brown color indicates the extract contains alkaloids. Based on the test results, *M. malabathricum* leaves contain alkaloids in the extracts of MeOH, methylene chloride, *n*-hexane, as well as ethyl acetate. Meanwhile, Figure 1c shows that MeOH, methylene chloride, *n*-hexane, as well as ethyl acetate extracts contain flavonoids. The yellow color on the flavonoid test indicates that the extract contains flavonoids.

Jofry et. al., (2012) reported that *M. malabathricum* leaves extract contains flavonoids, triterpenoids, tannins, saponins, steroids, alkaloids, and phenolics [13]. Plants have bioactivity because they have secondary metabolites. *M. malabathricum* displays a range of bioactivities, including antiviral, cytotoxic, antioxidant, cancer-fighting, antihypertensive, antinociceptive, anti-inflammatory, antipyretic, antibacterial, and antifungal properties [3].

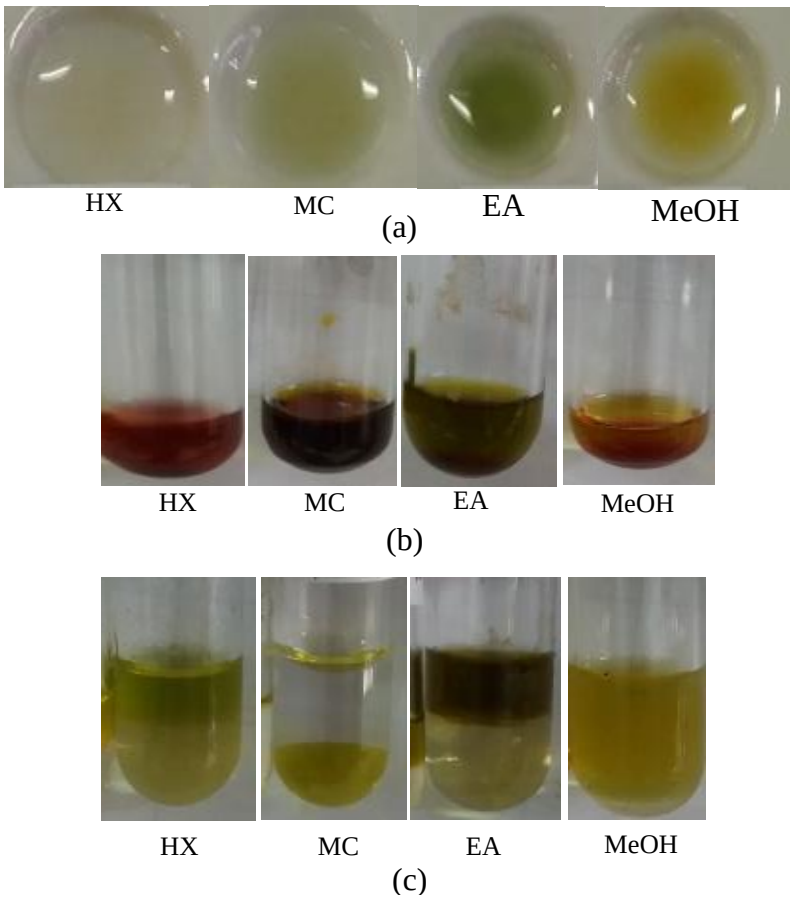

FIGURE 1. Terpenoid (a), alkaloid (b), and flavonoid (c) test results on extracts of *n*-hexane (HX), methylene chloride (MC), ethyl acetate (EA), and methanol (MeOH).

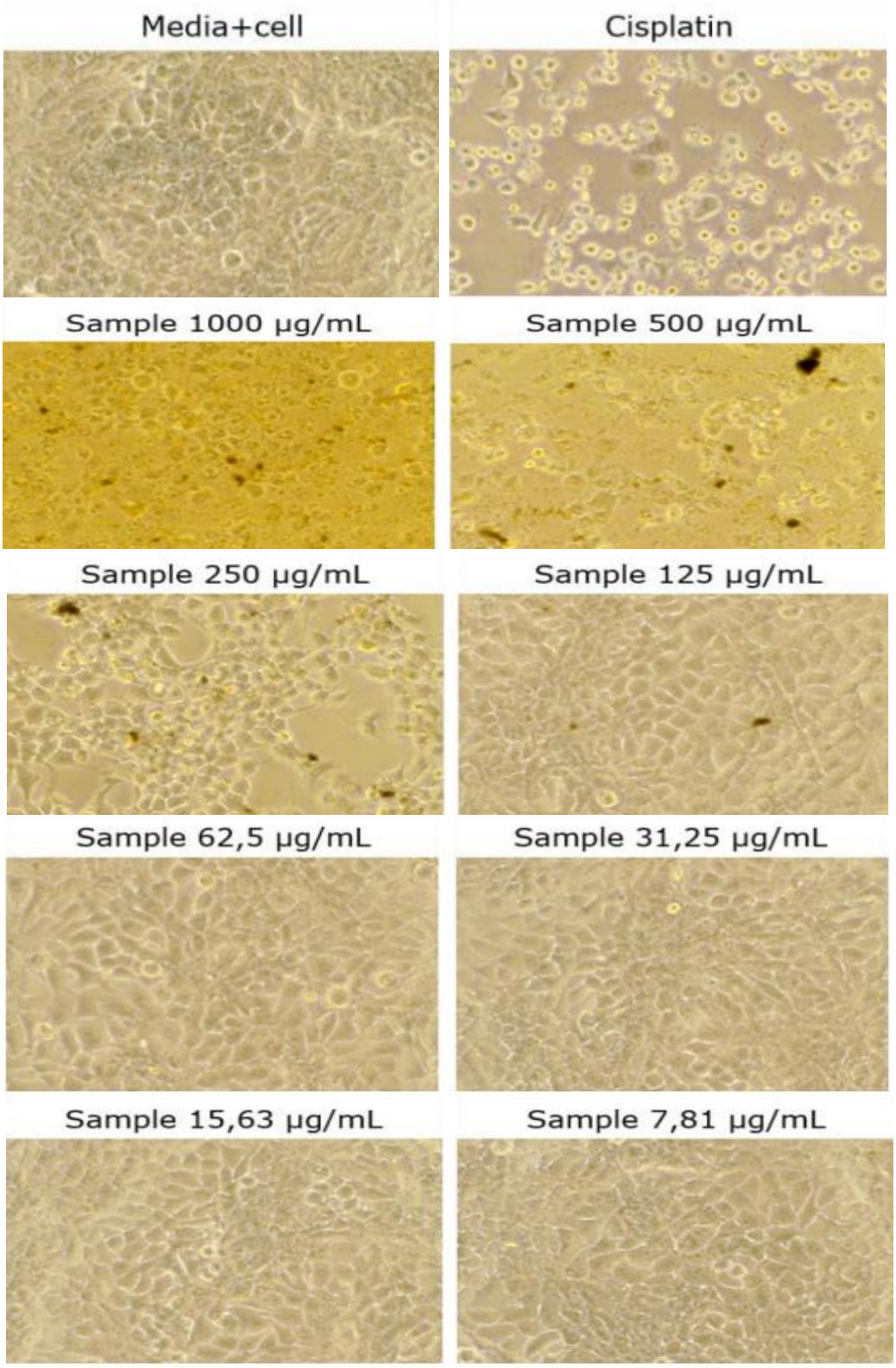

FIGURE 2. The MCF-7 cells morphology was treated with different doses of *M. malabathricum* leaf methanol extract.

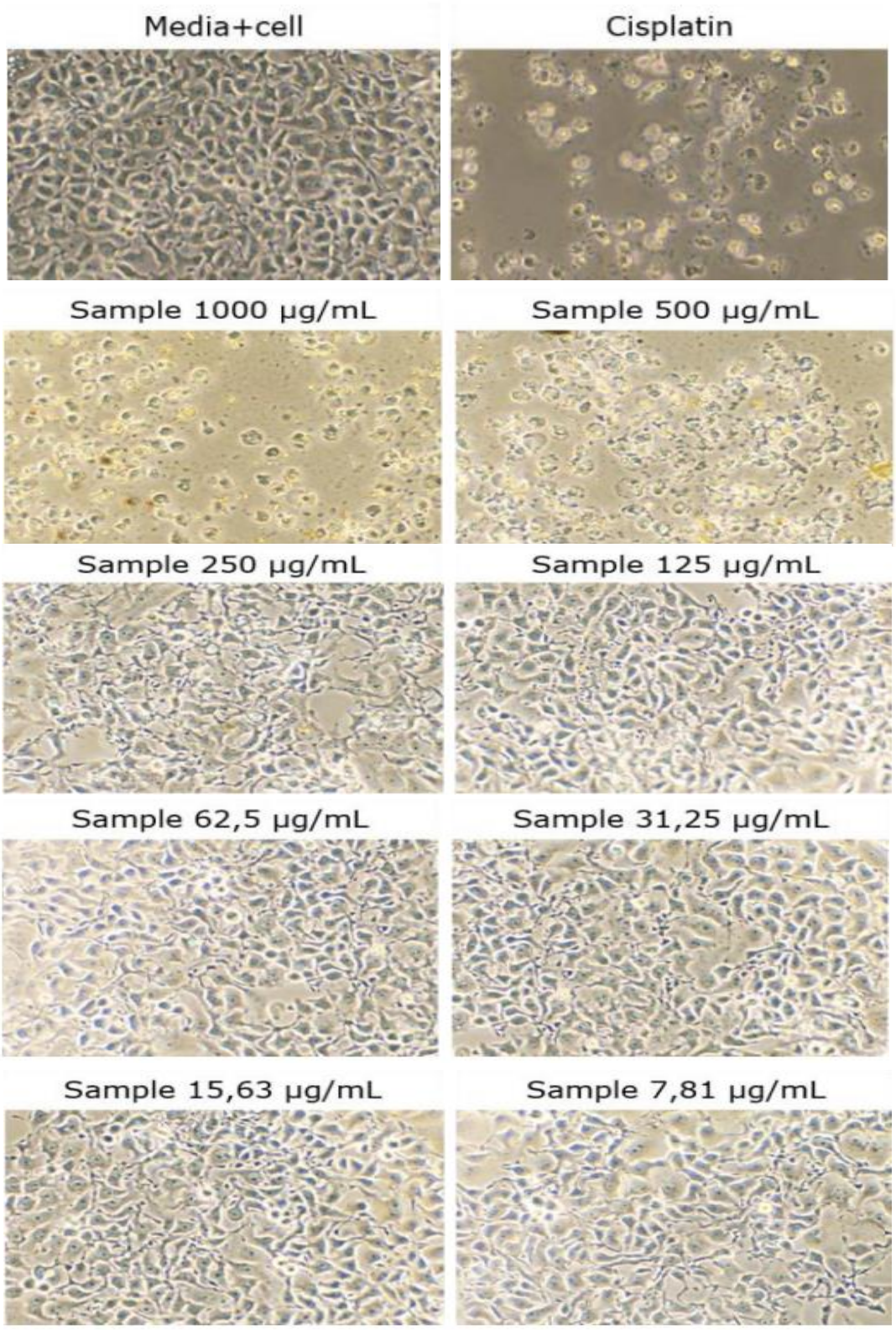

FIGURE 3. The HeLa cells' morphology was treated with various amounts of methanol extract from *M. malabathricum* leaves.

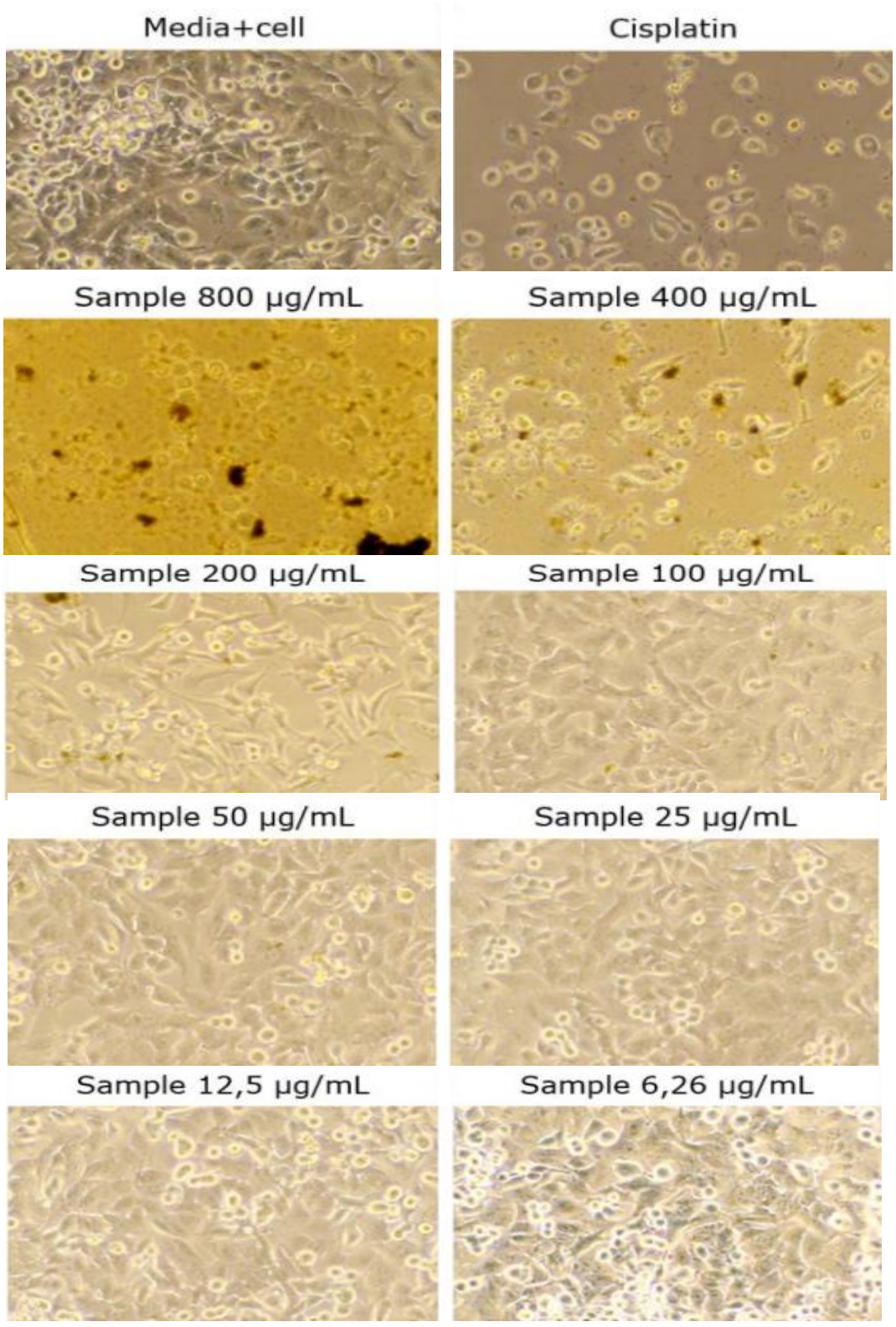

FIGURE 4. The A549 cells morphology was treated with various doses of *M. malabathricum* leaves.methanol extract.

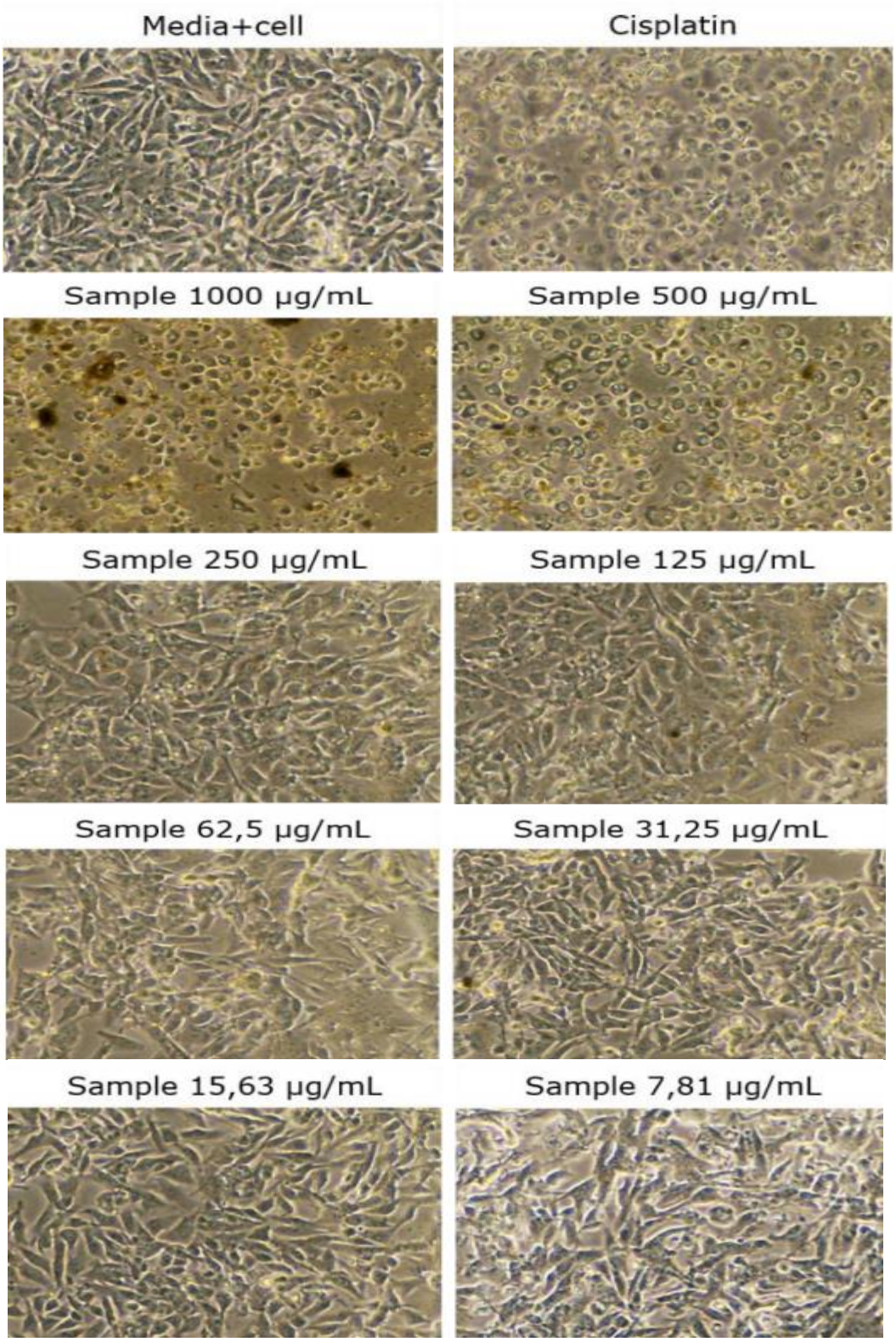

FIGURE 5. The B16 cells morphology was treated with various doses of *M. malabathricum* leaves.methanol extract.

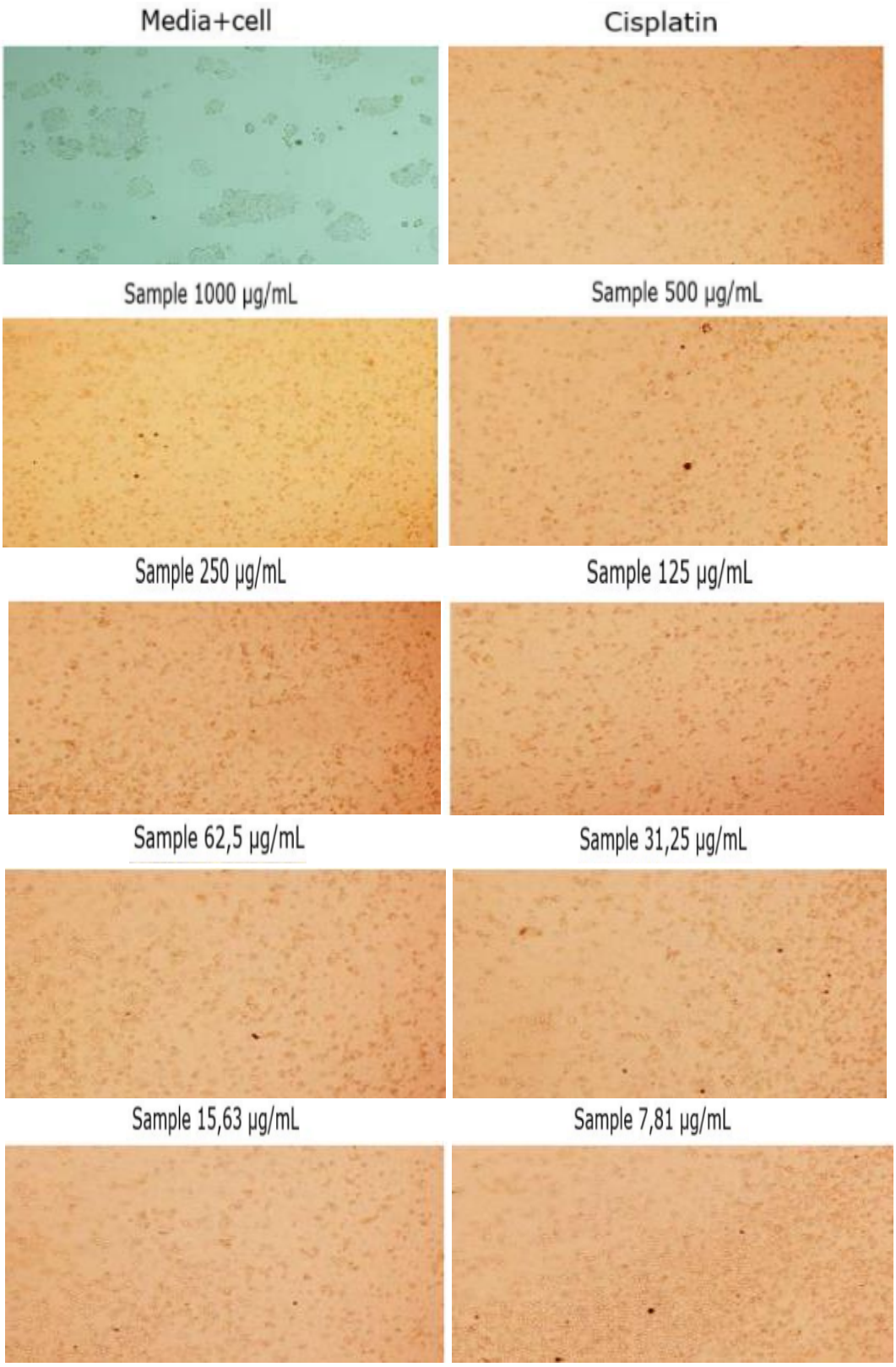

FIGURE 6. The HT29 cells morpphology was treated with various doses of *M. malabathricum* leaves methanol extract.

In vitro Cytotoxic Assay

Using the PrestoBlue technique, the methanol extract's *in vitro* cytotoxicity toward the MCF-7, HeLa, A549, B16, and HT29 cancer cell lines was evaluated. With IC_{50} values ranging from 1.43 to 327.37 µg/mL, Table 2 demonstrates that the methanol extract is cytotoxic against all five human cancer cell lines.

TABLE 2. Methanol extract's cytotoxicity *in vitro* against five cancer cell lines

Samples	IC_{50} (µg/mL)				
	MCF-7	**HeLa**	**A549**	**B16**	**HT29**
MeOH extract	327.37±0.67	327.05±0.48	304.46±1.93	319.21±0.67	1.43±0.19
Cisplatin	15.96±0.13	5.72±0.50	150.58±0.27	12.97±0.11	2.98±0.48

The data describe the mean ± SD of two experiments.

Figures 2–6 show the morphology of MCF-7, HeLa, A549, B16, and HT29 cells after treatment with the methanol extract. The cytotoxicity effect of the extract depends on the concentration, where the cytotoxicity increases with increasing the concentration of the extract. The appearance of cells resembled needles closely packed together at low concentrations. At the same time, the cells' morphology at high concentrations of the extract was spherical and floating, this indicates that many cells died. The anticancer properties observed in this investigation regarding the *M. malabathricum* extract could be explained by the presence of combination of polyphenols including tannins, flavonoids, glycosides, and terpenoids as well as other substances, all of which are found in *M. malabathricum* [14]. In previous research, the methanol extract contained relatively high total phenolics and flavonoids with values of 183.71±0.11 (mg GAE/g extract), 23.18±0.05 TFC (mg QE/g extract) respectively [8], hence, the methanol extract used for cytotoxicity test. The extract demonstrated noteworthy activity against HT29 cancer cells, as indicated by its IC_{50} values being twice as strong as those of cisplatin, the positive control. Due to the presence of flavonoids in the methanol extract, it was discovered to have the greatest cytotoxicity against the HT29 cell line [7]. A flavonoid (apigenin) which was incubated for 72 hours at a dose of 90 µM showed an apoptotic percentage of 24.9% against HT29 cells [15]. Meanwhile, flavonoids from apple extract showed inhibition of HT29 cell growth [16]. The flavonoid compound, naringenin, at a concentration of 0.71-2.85 mmol inhibits HT29 cell proliferation [17]. Antioxidants, antidiabetic, and anticancer characteristics are only a few of the bioactivities that flavonoid and as well as phenolic compounds have been linked to [18]. It has been demonstrated that certain flavonoids serve as antiproliferative agents in cancer cells and induce apoptosis in human tumor cells [6]. With their chemically diverse structures, bioactive compounds from plants hold the promise to treat cancer with less adverse effects than current treatments [18]. The methanol extract has the potential to be used in the HT29 cells treatment, according to the results of the cytotoxicity tests.

CONCLUSION

The *M. malabathricum* leaves extract phytochemical screening revealed that the extract contained secondary metabolites (terpenoids, steroids, alkaloids, and flavonoids). With IC_{50} values ranging from 1.43 to 327.37 µg/mL, the methanol extract of *M. malabathricum* leaves showed cytotoxic action against a number of cancer cell lines, including MCF-7, HeLa, A549, B16, and HT29. In particular, the extract demonstrated a 2-fold stronger IC_{50} value against HT29 cells compared to cisplatin, indicating its potent activity against HT29 cancer cells. These findings imply that *M. malabathricum* leaf methanolic extract has the potential to be an important source of anticancer drugs.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Directorate of Research and Community Service and Directorate General of Strengthening Research and Development, Ministry of Research, Technology and Higher Education of Republic Indonesia for the financial support for Doctoral Dissertation Research with contract number 828/PKS/ITS/2021.

REFERENCES

1. D. Buyinza, L. J. Yang, S. Derese, A. Ndakala, P. Coghi, M. Heydenreich, V. K. W. Wong, H. M. Möller, and A. Yenesew, Nat. Prod. Res. 35, 2744-2747 (2019).
2. R. Fang, P. J. Houghton, and P. J. Hylands, J. Ethnopharmacol. 118, 257–263 (2008).
3. N. A. Roslen, N. A. M. Alewi, H. Ahamada, and M. S. B. A. Rasad, Asian Pac. J. Trop. Biomed. 4(7), 545-548 (2014).
4. Z. A. Zakaria, R. N. S. R. M. Nor, G. H. Kumar, Z. D. F. A. Ghani, M. R. Sulaiman, G. R. Devi, A. M. M. Jais, M. N. Somchit, and C. A. Fatimah, Can. J. Physiol. Pharmacol. 84, 1291-1299 (2006).
5. D. P. Giri and M. Rajbhandari, J. Institute Sci. Technol. 23, 18-25 (2018).
6. D. Susanti, H. M. Sirat, F. Ahmad, R. M. Ali, N. Aimi, and M. Kitajima, Food Chem. 103, 710-716 (2007).
7. N. E. Kamsani, Z. A. Zakaria, N. L. Md Nasir, N. Mohtarrudin, and N. B. M. Alitheen, Evidence-Based Complement. Altern. Med. 2019, 5207958 (2019).
8. M. Idris, A. S. Purnomo, F. Martak, and S. Fatmawati, Journal of Hunan University Natural Sciences. 49 (2022).
9. G. A. Ayoola, H. A. B. Coker, S. A. Adesegun, A. A. Adepoju-Bello, K. Obaweya, E. C. Ezennia, and T. O. Atangbayila, Trop. J. Pharm. Res. 7(3), 1019-1024 (2008).
10. N. Kancherla, A. Dhakshinamoothi, K. Chitra, and R. B. Komaram, Maedica (Bucur), 14(4), 350-356 (2019).
11. K. Ikhiri, D. Boureima, and D. D. Dan-Koulodo. Int. J. Pharmacog. 30(4), 251-262 (1992).
12. Nurlelasari, I. Rahmayanti, S. Salam, A. Safari, D. Harneti, R. Maharani, A. T. Hidayat, M. Tanjung, R. Retnowati, Y. Shiono, and U. Supratman, Appl. Biol. Chem. 64, 35 (2021).
13. S. M. Joffry, N. J. Yob, M. S. Rofiee, M. M. R. M. M. Affandi, Z. Suhaili, F. Othman, A. M. Akim, M. N M. Desa, and Z. A. Zakaria, Evidence-Based Complement. Altern. Med. 2012, 48 (2012).
14. N. Elumalai, P. Ayyakkannu, S. Palanivelu and S. Panchanadham, RSC Adv. 5, 23125–23133 (2015).
15. M. Turktekin, E. Konac, H. I. Onen, E. Alp, A. Yilmaz, and S. Menevse, J. Med. Food. 14(10), 1107-1117 (2011).
16. S. Veeriah, T. Kautenburger, N. Habermann, J. Sauer, H. Dietrich, F. Will, and B. L. Pool-Zobel, Molecular Carcinogenesis, 45, 164-174 (2006).
17. H. R. Frydoonfar, D. R. McGrath and A. D. Spigelman, Colorectal Dis. 5(2), 149-152 (2003).
18. M. Idris, E. R. Sukandar, A. S. Purnomo, F. Martak, and S. Fatmawati, RSC Adv. 12, 25697 (2022).