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Moringa oleifera Leaves Ethanol Extract Inhibits HT-29 Cells and COX-2 Expression Predictably Through PPARγ Activation

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ABSTRACT

Colorectal cancer is the second leading cause of death among all cancer cases worldwide. Cancer cells often exhibit overexpression of cyclooxygenase-2 (COX-2), producing prostaglandin E2 (PEG2) and subsequent inflammation and neoplasia. *Moringa oleifera* is rich in bioactive compounds such as polyphenols, flavonoids, and saponins, known for their anti-inflammatory and antioxidant properties. This study aimed to investigate the inhibitory effects of *M. oleifera* leaves ethanol extract on COX-2 expression in HT-29 cells. Dried *M. oleifera* leaves (5 g) were ethanol-macerated for 24 hours, yielding a 10 mg ethanol extract. MTT inhibition is used for immunocytochemistry evaluation of COX-2 expression. Molecular docking of phenolic compounds from the extract on PPARγ indicated an agonistic potential. The ethanol extract of *M. oleifera* leaves demonstrated anticancer activity with an IC50 value of 114.8 µg/ml, with a significant reduction in COX-2 expression observed at a dose of 100 ppm, resulting in an H-score of 111.83 ± 2.21. Peroxisome proliferator-activated receptor-gamma (PPARγ) activity is thought to be the first step in suppressing COX-2 expression. Three phenolic compounds found in *M. oleifera* are predicted to be PPARγ agonists: rutin, naringin, and hesperidin, according to the molecular docking simulations.
Keywords: colorectal cancer; COX-2 expression; *Moringa oleifera*; PPARγ agonists

INTRODUCTION

Recently, colorectal cancer become the second most frequent cause of cancer-related death globally and become a major health problem. In 2020, the global incidence of colorectal cancer reached approximately 1.9 million cases, with a concerning mortality rate reaching up to 935,000 individuals worldwide (Sung. H., et al., 2021). In Indonesia, there were 369,914 reported cases of colorectal cancer in the same year (Global Cancer Observatory: Cancer Today. Indonesia Fact Sheet. Lyon, France: International Agency for Research on Cancer; 2020). Colorectal cancer primarily emerges from genetic anomalies disrupting the balance of cellular growth and division within the colon and rectum.

COX-2 expression has been investigated in several studies of colorectal cancer (CRC). In one study by Ibrahim et al., it is reported that high COX-2 expression was seen in the cytoplasm of epithelial cells in adenocarcinomas and stromal cells in adenomas, whereas low expression was seen in adenocarcinoma stromal cells and adenoma epithelial cells. In adenocarcinomas, COX-2 overexpression was substantially related to lymphovascular invasion, deeper invasion, positive nodal metastasis, and mild differentiation (Ibrahim et al., 2023).

The expression of cyclooxygenase-2 (COX-2) is associated with colorectal cancer progression and tumorigenesis and is important in inflammation and neoplasia. Cancer cells tend to overexpress COX-2, triggered by the activation of the NF-kB pathway. The catalytic output of COX-2 produces prostaglandin E2 (PGE2), a primary instigator of inflammation and carcinogenesis (Hashemi Goradel et al., 2019; Sheng et al., 2020). Notably, striking COX-2 overexpression is a recurring finding in adenocarcinomas of colorectal cancer and cancer-associated fibroblasts (CAFs), emphasizing its clinical significance as a therapeutic target (Desai S. J. et al., 2018).

Several studies have demonstrated the utility of COX2 inhibitors in colorectal cancer. Celecoxib, a selective COX-2 inhibitor, has been shown to inhibit the activity of HT-29 colorectal cancer cells without increasing the COX-2 mRNA

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expression (Lin et al., 2019; Zhang et al., 2021). Furthermore, celecoxib treatment for seven days prior to surgical resection reduced the expression levels of genes involved in cellular lipid and glutathione metabolism in individuals with primary colorectal adenocarcinoma, suggesting a decrease in cellular proliferation (Kadhum et al., 2022).

Moreover, treatment of HT-29 cells with ciglitazone, a peroxisome proliferator-activated receptor-gamma (PPARγ) agonist, also reduced COX-2 protein expression. Similar effects were observed in HT-29 cells treated with thymoquinone (Venkataraman et al., 2021), a compound found in the oil of *Nigella sativa.* (Pottoo et al., 2022). Activation of PPARγ inhibits the growth of colorectal cancer cells. Pioglitazone, a PPARγ agonist, reduces c-MYC protein expression in a PPARγ-dependent manner, leading to decreased tumor immune escape (Xu et al., 2023).

Several phenolic compounds isolated from ethanol extracts of *Glycyrrhiza glabra* roots also exhibited PPAR-γ binding activity in vitro, with some compounds at a sample concentration of 10μg/ml is showing a binding power that is three times stronger than troglitazone at 0.5μM (Kuroda et al., 2010). Among the rich sources of phenolic compounds is *M. oleifera*, a widely distributed plant in Indonesia. The bioactive compound richness of this plant includes polyphenols, flavonoids, and saponins, renowned for their antioxidant and anti-inflammatory properties (Kusmiyati et al., 2018). As we know, molecular docking has been extensively used in drug discovery for several reasons: prediction of binding poses and affinities between ligands and targets, aiding in the identification of potential drug candidates (Muhammed & Aki-Yalcin, 2024). Moreover, molecular docking is a cost-effective and time-saving approach compared to other traditional experimental methods, reducing the failure rate in preclinical trials (Arya & Kaur, 2022).

This research aims to explore the impact of ethanol extract from *M. oleifera* leaves on COX-2 expression in HT-29 cells, representing a model of colorectal cancer cells. Through this study, efforts to unveil the anticancer potential of this plant extract are anticipated to provide insights into its role in suppressing COX-2 expression and its potential to inhibit the development of colorectal cancer. Molecular docking simulations between the compounds present in *M. oleifera* ethanol extract and PPARγ will be conducted to ascertain whether the reduction in COX-2 expression is due to PPARγ activation.

MATERIALS AND METHODS

Materials

The leaves of *M. oleifera* was obtained from the *Balai Penelitian Tanaman Rempah* & *Obat* (now become: *Balai Pengujian Standar Instrumen Tanaman Rempah, Obat dan Aromatik* or the *Standard Instrument Testing Center for Spice, Medicinal and Aromatic Plant*) at Bogor region in West Java, Indonesia. Dried samples of *M. oleifera* leaves were used to make the extract. The cell line of colorectal cancer cell HT-29 was obtained from the Department of Pathological Anatomy, Faculty of Medicine, Universitas Indonesia.

Methods

Extract preparation

Five grams of dried *M. oleifera* leaves were macerated for twenty-four hours, once a day, in fifty milliliters of ethanol solvent. To gather the filtrates, the maceration procedure was carried out three times. By utilizing a rotary evaporator to evaporate the solvent at 40°C for six hours, the filtrates were concentrated. A total of 1000 μL of DMSO was used to dissolve 10 mg of the *M. oleifera* leaves ethanol extract.

MTT cytotoxic assay (Chelliah & Oh, 2022)

HT-29 colorectal cancer cells were cultured in Gibco DMEM supplemented with 1% penicillin-streptomycin and 10% FBS. The cell culture was maintained at 37°C with 5% CO2 in a humidified environment for 24 hours. HT-29 cells were incubated with these extract concentrations for 24 hours at 37°C with 5% CO2. After incubation, the cells were washed with phosphate buffer saline (PBS) solutions. Colorectal cancer HT-29 cells were incubated with 100 μL of 5 mg/ml MTT solution in Costar well cell culture plates for 24 hours. Next, the mixture was incubated for 4 hours, followed by centrifugation. Afterward, dimethyl sulfoxide (DMSO, 100 μL) was added to form a blue-violet deposit. Absorbance at 590 nm was measured using a microplate reader (Model 550, Bio-Rad, USA). Then, the inhibition percentage was calculated. The IC50 value was determined using Microsoft Office 365 Excel, with a log concentration (μg/ml) from the extract on the x-axis and inhibition percentage (%) on the y-axis.

Immunocytochemistry assay (Hanifah et al., 2019)

The immunocytochemistry test is conducted by incubating the cells in a Petri dish with two different dose variations based on the MTT assay (determination of IC50). The incubation is carried out on a cell suspension placed in a Petri dish containing sterilized cover slips for 24 hours at a temperature of 37°C.

Next, the cells underwent PBS solution washing following the incubation. The sterile object glass was used to fix the cells, and they were left there for ten minutes after absolute methanol was dropped on its surface. One way to remove endogen peroxidase was to drop three percent of hydrogen peroxidase (H2O2) to the surface. Next, normal serum was added to the cells after they had been dipped in 0.01 M citrate buffer (pH = 6). Using buffer phosphate solution, the cells were incubated with anti-COX-2 antibody for one night. Afterward, they were incubated for a further ten minutes with a secondary antibody and for three to eight minutes with HRP-conjugated streptavidin. Following a distilled water wash, hematoxylin-harris solution was added to the cells.

Interpretation of the Immunocytochemistry (ICC) results on HT-29 cell culture cytoplasm and membrane was carried out using a light microscope at 400 x magnification where the camera is directly connected to the eyepiece lens. Assessment of COX-2 protein expression is based on an H-score, guided by varying the cell staining intensities: 0 for negative or no staining, +1 for weak staining, +2 for moderate staining, and +3 for strong staining (Parris et al., 2014). The applications employed to assess this staining are Image J and IHC profiler (Varghese et al., 2014).

H-score = (% of cells with color intensity 0 x 1) + (% of cells with color intensity 1 x 2) + (% of cells with color intensity 2 x 3)

The result of the H-score ranges from 0 to 300, where the value 300 means there are 100% strongly colored cells (+2). A high expression of COX-2 is indicated by H-score > 200, while H-score < 200 indicates a low expression of COX-2.

Molecular docking

The 3D structure or conformation of phenolic compounds obtained from *M. oleifera* leaves ethanol extract (Osman & El-Sobki, 2019) underwent a search in the PubChem database (Kim et al., 2016). The 3D structure energy minimization was performed using Datawarrior (Sander et al., 2015) with the aim of finding the most stable conformation or the one with the lowest energy (Merck Molecular Force Field, MMFF94 value). The crystal structure of PPARγ (PDB ID: 2PRG) was obtained from the RSCB PDB database (www.pdb.org). Molecular docking of phenolic compounds as PPARγ agonists was conducted using Molegro Virtual Docker/MVD (Free trial) (Bitencourt-Ferreira & de Azevedo, 2019). The computer specifications used were Windows 11 Pro, Intel(R) Core (TM) i7-8665U CPU @ 1.90 GHz -2.11 GHz, with 16.0 GB RAM. Prior to molecular docking of phenolic compounds against PPARγ, a re-docking process of the 3D thiazolidinedione molecule (BRL) as the native ligand to chain A of PPARγ (2PRG [A]) was conducted within the protein's binding site area to validate the docking method used. A Root Mean Square Deviation (RMSD) value < 2 Å was set as the criterion for method validity. Subsequently, molecular docking was performed on the 3D structure of phenolic compounds against the crystal structure of the protein. Parameters measured during the docking process included the involved energy values, such as MolDock Score, Rerank Score, and Hbond. Rerank Score is often used to measure the ligand-receptor protein binding strength (Molegro ApS, 2011).

RESULTS

The effect of ethanol extract of *M. oleifera* leaves on HT-29 cell inhibition can be seen in Figure 1. The log concentration of the extract was used to assess the anticancer activity through the percentage of growth inhibition. The extract concentrations used were 1.563, 3.125, 6.250, 100, and 200 µg/ml, wherein the HT-29 cells growth inhibition obtained was 13.85%, 24.60%, 30.46%, 42.90%, and 58.26%, respectively. The value of R2 was 0.941, indicating a high correlation between the log concentration of extract and the inhibition of HT-29 cell growth. The ethanol extract of *M. oleifera* leaves exhibited cytotoxicity against HT-29 cells with IC50 value at 114,8 µg/ml (ppm). It is categorized as moderate cytotoxicity (100-500 µg/ml) according to a previous study (Weerapreeyakul et al., 2012). Based on the IC50 value, the established doses for immunocytochemistry testing are 50 and 100 µg/ml.

Assessment of COX-2 protein expression was carried out through immunocytochemistry assay by examining the intensity of resulting cell color. A darker brown color indicates a high COX-2 expression, whereas a lighter color indicates a low COX-2 expression. COX-2 was located in the cytoplasmic membrane, cytoplasm, and nucleus of the cancer cells (Thanan et al., 2012). The results of the immunocytochemistry assay with 400 x magnification can be seen in Figure 2. The image presentation has been adjusted (partially bleached) to avoid reading COX-2 expressions in the wrong part. At a concentration of 50 µg/ml, a brown color appeared with a higher intensity than the concentrations of 100 µg/ml.

Furthermore, the expression of COX-2 was semi-quantitatively measured by H-score, with a minimum score of 0 and a maximum of 400. The H-

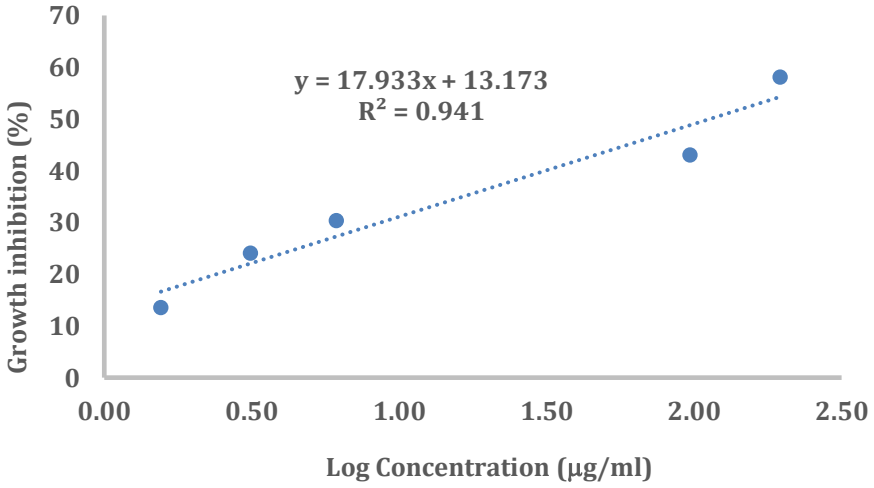

Figure 1. Effect of Moringa oleifera leaves ethanol extract on HT-24 cells inhibition

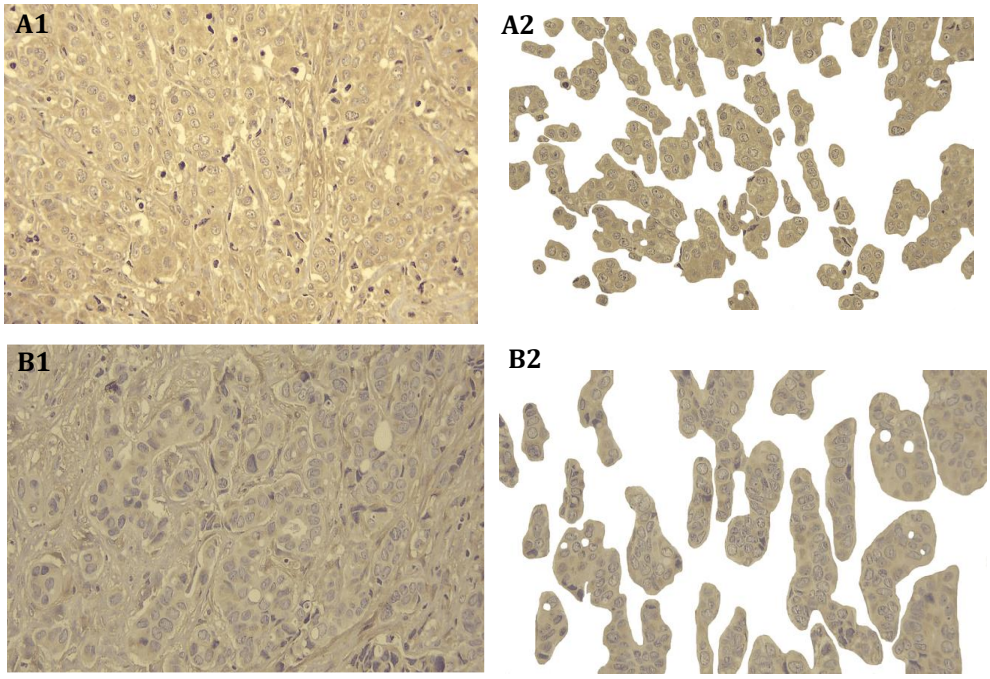

Figure 2. COX-2 expression on HT-29 cells from immunochemistry assay. Images snapped using microscope, and analyzed using ImageJ software. COX-2 expression before treatment (A1, B1) and after (A2, B2) cropping/ partially bleached after annotation of region of interest. A: 50 µg/ml, and B: 100 µg/ml

score for each concentration group can be seen in Table I. In this study, the H-score was <200 in the concentration groups of 100 µg/ml indicating a low COX-2 expression. Therefore, the H-score of the 50 µg/ml concentration group was still >200, indicating a high COX-2 expression. T-test showed p <0.01, indicating at least there was a significant mean difference between the two groups (50 vs 100 µg/ml). The results have shown a significant mean difference between the groups of 50 and 100 µg/ml concentration (p = 0.001). It can be concluded that there is a relationship between the administration of *M. oleifera* leaves extract and the decrease in COX-2 expression in HT-29 cells. Therefore, the ethanol extract of *M. oleifera* leaves has the potential as an agent that reduces the growth of HT-29 cells through the COX-2 pathway.

In the molecular docking, the validation results of the PPARγ interaction (PDB ID: 2PRG) with its native ligand (thiazolidinedione, BRL) can be observed in Figure 3 and Table II. The ligand BRL is situated within one of the cavities in chain A

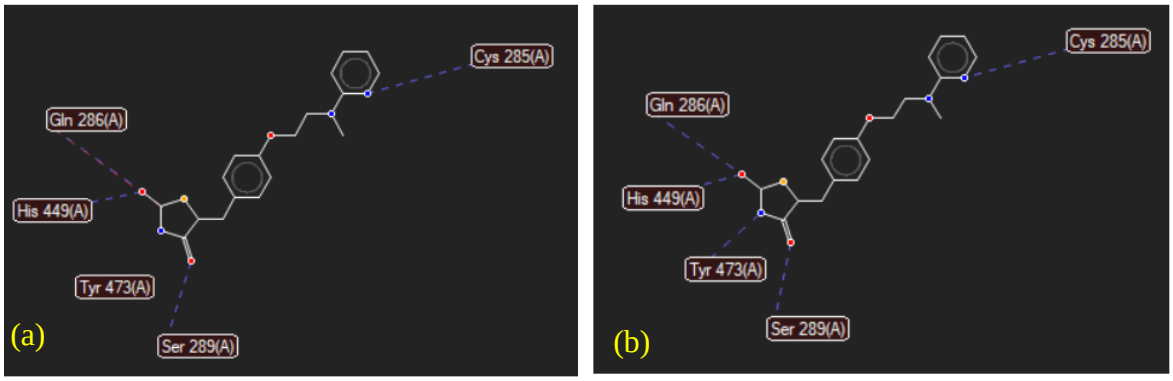

Figure 3. Interaction of BRL with protein 2PRG [A] in its crystal structure (a) and redocking results (b). The blue dashed lines represent hydrogen bonds, and the red dashed lines represent steric-electrostatic interactions

Table I. The result of T-test between extracts concentration

Group concentration	Number of subject	H-score (Mean ± SD)	Sig.
50 µg/ml	3	214.09 ± 4.06	0.001
100 µg/ml	3	111.83 ± 2.21	

Table II. Docking score and RMSD resulting from the ligand-protein interaction of the redocking process of the native ligand (thiazolidinedione)

2PRG [A] Ligand Name	MolDock Score	Rerank Score	RMSD	HBond
BRL	-141.392	-117.046	1.61436	-8.15303

of the 2PRG protein. Based on the redocking process of BRL onto protein 2PRG [A], hydrogen bonding interactions and steric-electrostatic interactions are observed at the same amino acid residues. Moreover, the RMSD values resulting from the redocking process show values below 2 Å. Therefore, the molecular docking method to be employed is deemed valid, as shown in Table II. After validating the molecular docking method, the subsequent step involved molecular docking between phenolic compounds (Osman & El-Sobki, 2019) and protein 2PRG [A]. The results of the molecular docking can be observed in Table III and Figure 4.

M. oleifera is widely recognized for its medicinal properties, which encompass a broad spectrum of physiological benefits, including antioxidant, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, and neuroprotective properties (Kou et al., 2018; Vergara-Jimenez et al., 2017). The findings from previous studies are in line with our investigation. According to Fard et al. (2015), COX-2 significantly decreased after treatment with *M. oleifera* hydroethanolic extracts through LPS-induced RAW264.7 cells. *M. oleifera* and its extract also induced the down-regulation of COX2 gene expression from gastric tissue of rats induced with gastric ulcers (Mabrok & Mohamed, 2019).

Osman & El-Sobki (2019) analyzed the 70% ethanol extract of *M. oleifera* leaves and found 35 phenolic compounds with varying concentrations. Through a molecular docking of these 35 compounds with the PPARγ protein (2PRG [A]), hesperidin, naringin, and rutin displayed more negative Rerank Scores compared to thiazolidinedione (BRL), which is its native ligand (Table III). MVD recommends the utilization of Rerank Score for evaluating molecular docking results. The Rerank Score measures binding affinity, comprising a linear combination of energy released from ligand-protein interactions (Einter) and the ligand's internal or intramolecular energy (Eintra). (Molegro ApS, 2011) Incorporating the ligand's internal energy (bond torsion, sp2-sp2 bonding, hydrogen bonding, Van der Waals interactions, and electrostatic interactions) enhances the accuracy of ligand pose selection. The more negative the Rerank Score, the more stable the bond formed between the ligand and the receptor. In Figure 4, the native ligand (BRL) and phenolic compounds from the ethanol extract of

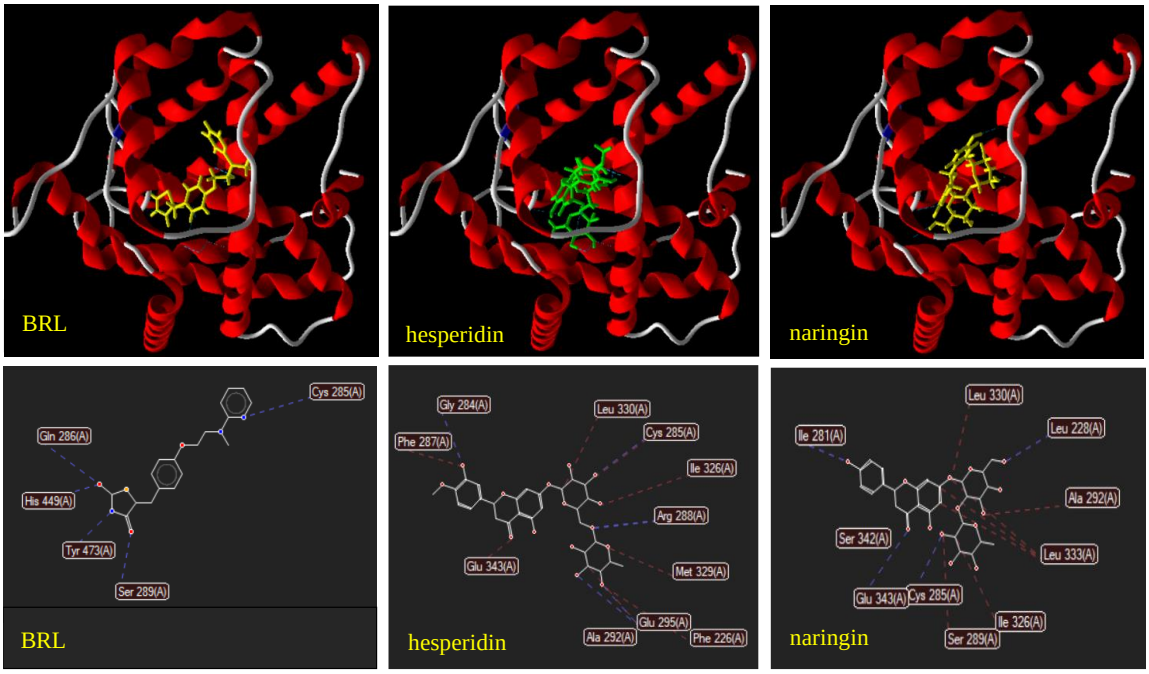

Figure 4. Interaction of BRL, hesperidin, and naringin with protein 2PRG [A] binding site. The blue dashed lines represent hydrogen bonds, and the red dashed lines represent steric-electrostatic interactions

Table III. The results of molecular docking simulation between phenolics from *M. oleifera* with 2PRG [A]

Ligand Name	MolDock Score	Rerank Score	HBond
hesperidin	-165.02	-152.715	-13.7073
naringin	-157.872	-132.162	-6.74579
rutin	-138.587	-129.375	-11.7836
BRL	-148.101	-121.001	-2.5

M. oleifera leaves (hesperidin and naringin) can be observed binding to 2PRG within the same or adjacent binding site residues.

This indicates that both compounds are predicted to operate through a similar mechanism as the native ligand, targeting as agonists for PPARγ. Based on the Rerank Score and similarity of the binding site, hesperidin, naringin, and rutin are predicted to be better PPARγ agonists than thiazolidinedione. Thiazolidinedione (TZD) drugs are oral insulin sensitizers used in type 2 diabetes mellitus as agonists targeting PPARγ (Arnold et al., 2019).

CONCLUSION

Anticancer properties of the ethanol extract of *Moringa oleifera* leaves mediated by inhibition of COX-2 expression in HT-29 cells. The ethanol extract of *M. oleifera* leaves showed anticancer activity with an IC50 value of 114.8 ppm and a significant decrease in COX-2 expression occurred at a dose of 100 ppm with an H-score of 111.83 ± 2.21. Based on the results of molecular docking simulations, it is suggested that hesperidin, naringin, and rutin, phenolic compounds found in moringa oleifera, are predicted to play a role in inhibiting COX-2 expression through their activity as PPAR agonists.

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REFERENCES

Arnold, S. V, Inzucchi, S. E., Echouffo-Tcheugui, J. B., Tang, F., Lam, C. S. P., Sperling, L. S., & Kosiborod, M. (2019). Understanding Contemporary Use of Thiazolidinediones. *Circulation. Heart Failure*, *12*(6), e005855. https://doi.org/10.1161/CIRCHEARTFAILURE.118.005855

Arya, N., & Kaur, Dr. A. (2022). Molecular Docking: A Review Paper. *International Journal of Innovative Research in Engineering & Management*, 140–146. https://doi.org/10.55524/ijirem.2022.9.1.25

Bitencourt-Ferreira, G., & de Azevedo, W. F. (2019). Molegro Virtual Docker for Docking. *Methods in Molecular Biology (Clifton, N.J.)*, *2053*, 149–167. https://doi.org/10.1007/978-1-4939-9752-7_10

Chelliah, R., & Oh, D.-H. (2022). *Screening for Anticancer Activity: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium (MTT) Assay* (pp. 423–425). https://doi.org/10.1007/978-1-0716-1728-1_54

Desai, S. J., Prickril, B., & Rasooly, A. (2018). Mechanisms of Phytonutrient Modulation of Cyclooxygenase-2 (COX-2) and Inflammation Related to Cancer. *Nutrition and Cancer*, *70*(3), 350–375. https://doi.org/10.1080/01635581.2018.1446091

Global Cancer Observatory: Cancer Today. Indonesia Fact Sheet. Lyon, France: International Agency for Research on Cancer; 2020. (n.d.). Retrieved November 17, 2023, from https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf

Hanifah, R., Novitarani, N., Harmen, F., Tedjo, A., Azizah, N., Putrianingsih, R., Fachri, W., & Kusmardi, K. (2019). The Inhibition of Ethanol Extract of Phaleria macrocarpa Stem Bark on COX-2 Expression of HCT116 Colorectal Cancer Cell Line. *Research Journal of Pharmacy and Technology*, *12*, 2902. https://doi.org/10.5958/0974-360X.2019.00489.X

Hashemi Goradel, N., Najafi, M., Salehi, E., Farhood, B., & Mortezaee, K. (2019). Cyclooxygenase-2 in cancer: A review. *Journal of Cellular Physiology*, *234*(5), 5683–5699. https://doi.org/10.1002/jcp.27411

Ibrahim, N. I., Shamsudin, S. H., Lee, S. K., & T. Sharif, S. E. (2023). Prognostic significance of Cyclooxygenase-2 expression in colorectal adenoma and Adenocarcinoma: A clinicopathologic study. *Human Pathology Reports*, *31*, 300692. https://doi.org/10.1016/j.hpr.2023.300692

Kadhum, R., Abbas, M. K., & Madlum, K. N. (2022). Aspirin and celecoxib revealed anticancer and immunomodulatory effects on colon cancer cells. *International Journal of Health Sciences*, 13527–13535. https://doi.org/10.53730/ijhs.v6nS1.8380

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B. A., Wang, J., Yu, B., Zhang, J., & Bryant, S. H. (2016). PubChem Substance and Compound databases. *Nucleic Acids Research*, *44*(D1), D1202-13. https://doi.org/10.1093/nar/gkv951

Kou, X., Li, B., Olayanju, J. B., Drake, J. M., & Chen, N. (2018). Nutraceutical or Pharmacological Potential of Moringa oleifera Lam. *Nutrients*, *10*(3). https://doi.org/10.3390/nu10030343

Kuroda, M., Mimaki, Y., Honda, S., Tanaka, H., Yokota, S., & Mae, T. (2010). Phenolics from Glycyrrhiza glabra roots and their PPAR-gamma ligand-binding activity. *Bioorganic & Medicinal Chemistry*, *18*(2), 962–70. https://doi.org/10.1016/j.bmc.2009.11.027

Kusmiyati, K., Keman, S., Amin, M., Suwarno, S., & Rahmawati, I. (2018). Phytochemical Compounds Found In Moringa (Moringa Oleifera) Leaves Ethanol Extract From Kupang, East Nusa Tenggara. *Proceeding 1st. International Conference Health Polytechnic of Kupang*, 184–189. https://proceeding.poltekeskupang.ac.id/index.php/ichpk/article/view/48

Lin, Y., Lu, C., Hsiang, Y., Pi, S., Chen, C., Cheng, K., Pan, H., Chien, P., & Chen, Y. (2019). c-Met inhibition is required for the celecoxib-attenuated stemness property of human colorectal cancer cells. *Journal of Cellular Physiology*, *234*(7), 10336–10344. https://doi.org/10.1002/jcp.27701

Molegro ApS. (2011). *Molegro Virtual Docker User Manual*. Høegh-Guldbergs Gade 10, Building 1090 DK-8000 Aarhus C Denmark.

Muhammed, M. T., & Aki-Yalcin, E. (2024). Molecular Docking: Principles, Advances, and Its Applications in Drug Discovery. *Letters in Drug Design & Discovery*, *21*(3), 480–495. https://doi.org/10.2174/1570180819666220922103109

Osman, A., & El-Sobki, A. (2019). Insecticidal Activity and Chemical Composition of Moringa oleifera Extract Against The Leguminous Aphid, Aphis craccivora Koch on Broad Bean Plants. *Journal of Plant Protection and Pathology*, *10*, 567–571. https://doi.org/10.21608/jppp.2019.77958

Parris, T. Z., Aziz, L., Kovács, A., Hajizadeh, S., Nemes, S., Semaan, M., Chen, C. Y., Karlsson,

P., & Helou, K. (2014). Clinical relevance of breast cancer-related genes as potential biomarkers for oral squamous cell carcinoma. *BMC Cancer*, *14*, 324. https://doi.org/10.1186/1471-2407-14-324

Pottoo, F. H., Ibrahim, A. M., Alammar, A., Alsinan, R., Aleid, M., Alshehhi, A., Alshehri, M., Mishra, S., & Alhajri, N. (2022). Thymoquinone: Review of Its Potential in the Treatment of Neurological Diseases. *Pharmaceuticals (Basel, Switzerland)*, *15*(4). https://doi.org/10.3390/ph15040408

Sander, T., Freyss, J., von Korff, M., & Rufener, C. (2015). DataWarrior: An Open-Source Program For Chemistry Aware Data Visualization And Analysis. *Journal of Chemical Information and Modeling*, *55*(2), 460–473. https://doi.org/10.1021/ci500588j

Sheng, J., Sun, H., Yu, F.-B., Li, B., Zhang, Y., & Zhu, Y.-T. (2020). The Role of Cyclooxygenase-2 in Colorectal Cancer. *International Journal of Medical Sciences*, *17*(8), 1095–1101. https://doi.org/10.7150/ijms.44439

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Thanan, R., Murata, M., Ma, N., Hammam, O., Wishahi, M., El Leithy, T., Hiraku, Y., Oikawa, S., & Kawanishi, S. (2012). Nuclear localization of COX-2 in relation to the expression of stemness markers in urinary bladder cancer. *Mediators of Inflammation*, *2012*, 165879. https://doi.org/10.1155/2012/165879

Varghese, F., Bukhari, A. B., Malhotra, R., & De, A. (2014). IHC Profiler: An Open Source Plugin for the Quantitative Evaluation and Automated Scoring of Immunohistochemistry Images of Human Tissue Samples. *PLoS ONE*, *9*(5), e96801. https://doi.org/10.1371/journal.pone.0096801

Venkataraman, B., Almarzooqi, S., Raj, V., Alhassani, A. T., Alhassani, A. S., Ahmed, K. J., Subramanian, V. S., Ojha, S. K., Attoub, S., Adrian, T. E., & Subramanya, S. B. (2021). Thymoquinone, a Dietary Bioactive Compound, Exerts Anti-Inflammatory Effects in Colitis by Stimulating Expression of the Colonic Epithelial PPAR-γ Transcription Factor. *Nutrients*, *13*(4). https://doi.org/10.3390/nu13041343

Vergara-Jimenez, M., Almatrafi, M. M., & Fernandez, M. L. (2017). Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. *Antioxidants (Basel, Switzerland)*, *6*(4). https://doi.org/10.3390/antiox6040091

Xu, L., Che, S., Chen, H., Liu, Q., Shi, J., Jin, J., & Hou, Y. (2023). PPARγ agonist inhibits c-Myc-mediated colorectal cancer tumor immune escape. *Journal of Cellular Biochemistry*, *124*(8), 1145–1154. https://doi.org/10.1002/jcb.30437

Zhang, Z., Ghosh, A., Connolly, P. J., King, P., Wilde, T., Wang, J., Dong, Y., Li, X., Liao, D., Chen, H., Tian, G., Suarez, J., Bonnette, W. G., Pande, V., Diloreto, K. A., Shi, Y., Patel, S., Pietrak, B., Szewczuk, L., … Evans, D. C. (2021). Gut-Restricted Selective Cyclooxygenase-2 (COX-2) Inhibitors for Chemoprevention of Colorectal Cancer. *Journal of Medicinal Chemistry*, *64*(15), 11570–11596. https://doi.org/10.1021/acs.jmedchem.1c00890