The inhibition of Typhonium flagelliforme Lodd. Blume leaf extract on COX-2 expression of WiDr colon cancer cells

by Agustina Setiawati

Submission date: 31-Jan-2023 07:30PM (UTC+0700)
Submission ID: 2003271341
File name: Setiawati_et_al-2016-APJTB.pdf (714.76K)
Word count: 3864
Character count: 21198

HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Floral research

http://dx.doi.org/10.1016/j.apjtb.2015.12.012

The inhibition of *Typhonium flagelliforme* Lodd. Blume leaf extract on COX-2 expression of WiDr colon cancer cells

Agustina Setiawati*, Handika Immanuel, Mery Tri Utami

Department of Drug Design and Discovery, Faculty of Pharmacy, Sanata Dharma University, Yogyakarta 55281, Indonesia

ARTICLE INFO

Article history:
Received 19 Oct 2015
Received in revised form 30 Nov 2015
Accepted 7 Dec 2015
Available online 31 Dec 2015

Keywords:
Typhonium flagelliforme
Colon cancer
WiDr
Cyclooxygenase 2

ABSTRACT

Objective: To determine the inhibition activity of *Typhonium flagelliforme* Lodd. Blume (*T. flagelliforme*) leaf extract on cyclooxygenase 2 (COX-2) expression of colon cancer cells.

Methods: *T. flagelliforme* leaf extract was prepared to macerate in ethyl acetate. *In vitro* anticancer activity was assayed by MTT method on WiDr colon cancer cells. This study applied apoptosis induction assay to investigate the mechanism of cell death using double staining method. COX-2 expression was stained by immunocytochemistry.

Results: *T. flagelliforme* showed anticancer activity and induced apoptosis on WiDr cells through inhibition of COX-2 expression with IC_{50} 70 μg/mL.

Conclusions: This study showed that *T. flagelliforme* is a promising chemopreventive agent for colon cancer through COX-2 inhibition.

1. Introduction

Colon cancer is one of the leading causes of cancer related death in developed countries, and it is a frequently diagnosed cancer in both of male and female [1]. It was also ranked as the third most commonly diagnosed cancer worldwide, especially in the Southeast Asian Nations [2,3]. Colon cancer is known as a disease in industrialized countries, but the pattern is economically changing nowadays. Since its incidence rapidly increases [4,5], many attempts are employed to prevent and cure colon cancer. There are many possible clinical managements of colon cancer treatment, such as surgery, chemotherapy, radiotherapy and adjuvant chemotherapy [5,6]. Many studies had been designed to develop colon cancer treatment by attacking specific targets which is a key strategy to cure colon cancer without endangering normal cells [7–9].

*Corresponding author: Agustina Setiawati, Department of Drug Design and Discovery, Faculty of Pharmacy, Sanata Dharma University, Yogyakarta 55281, Indonesia.
Tel: +62 274 883037
Fax: +62 274 886529
E-mail: nina@usd.ac.id

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Colon cancer overexpresses cyclooxygenase 2 (COX-2) in both carcinoma and adenoma [10–12]. COX-2, an enzyme that is responsible for converting arachidonic acid to prostaglandins [13], plays an important role in cell proliferation and apoptosis regulation of colon cancer [14]. Prostaglandin E_2, a type of COX-2 product, promotes angiogenesis and stimulates colon cancer growth by preventing apoptosis [15,16]. Thus, COX-2 inhibitors had shown to successfully prevent colon cancer growth and polyp formation [16–21]. Moreover, COX-2 is a specific molecular target for anticancer screenings on colon cancer.

Many studies investigated a natural product activity on colon cancer [22–25], especially addressing COX-2 as molecular target [10,25]. Curcumin, a yellow pigment isolated from turmeric, successfully inhibited COX-2 on WiDr colon cancer cells [21]. Another promising natural product which potentially exhibits anticancer activity on colon cancer is *Typhonium flagelliforme* Lodd. Blume (*T. flagelliforme*). Its leaves contain glycoside flavonoid, isovitexin, as well as alkaloids [27]. Previous studies showed that *T. flagelliforme* revealed cytotoxic activity on MCF-7 breast cancer cells [28], and induced apoptosis on murine leukemia WEHI-3 cells [29] and lymphocyte CEM-SS [30]. However, the cytotoxic activity of *T. flagelliforme* on colon cancer cells with COX-2 as the molecular target remains delusive. Therefore, this study investigated cytotoxic activity of

T. flagelliforme leaf extract on WiDr colon cancer cells that highly express COX-2.

2. Materials and methods

2.1. Plant materials

The leaves of *T. flagelliforme* were harvested from Malang (East Java, Indonesia) in June 2014, and sun-dried after thorough washing. The plant materials were identified in Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada (Reference No. BF/275/Ident/Det/VI/2014).

2.2. Cytotoxic, apoptosis and immunocytochemistry assay materials

WiDr cells were collected from Parasitology Department, Faculty of Medicine, Universitas Gadjah Mada. The cells were maintained in Roswell Park Memorial Institute medium (Gibco) containing fetal bovine serum 10% (v/v) (Gibco) and penicillin-streptomycin 1% (v/v) (Gibco). Dimethyl sulfoxide was used to dissolve stock solution of extract and celecoxib was purchased from Merck. Celecoxib, as a positive control, was prepared from Celebrex®. Cytotoxic assay was measured by MTT (Sigma) method, while the apoptosis assay was examined by ethidium bromide and acridine orange (Sigma) staining. Immunocytochemistry used COX-2 primary antibody purchased from Thermo Scientific Lab Vision, while the universal secondary antibody was derived from Starr Trek Universal HRP Detection System No.901-STUHRP700-090314. All culture plates used in this study were Iwaki® and all tips and microtubes were supplied by Biologix®.

2.3. Extract preparation

The extract was prepared by using maceration method. Dried leaves of *T. flagelliforme* were ground and soaked in ethyl acetate (1:10) for 24 h until all substances were extracted. The liquid extract was slowly evaporated to discard the residual solvent, until viscous extract was obtained.

2.4. MTT cytotoxic assay

Cytotoxic assay was designed based on several previous studies [31–33]. WiDr colon cancer cells were cultured in culture tissue flask until 70%–80% confluent, and then 5×10^3 cells were seeded into 96-well plate. The plate was incubated at 37 °C and under 5% CO_2 for 24 h. The medium was removed and the cells were rinsed twice by using phosphate buffer solution (PBS). The stock solution of extract and celecoxib were prepared to dissolve them into dimethyl sulfoxide and dilute them with the medium into various concentrations. Each concentration (100 µL/well) was added into 96-well plate and measured in triplicates. Later, the plate was incubated at 37 °C and under 5% CO_2 for 24 h. The medium was then removed and 10% MTT containing medium was added into each well. The reaction between MTT and succinate hydrogenase of cells to form formazan needed 4 h. At the end of incubation time, 100 µL sodium dodecyl sulfate was added to each well to dissolve formazan crystals. The plate was incubated in dark room for 12–24 h and formazan crystals were measured by using ELISA reader at a wavelength of 595 nm.

2.5. Apoptosis induction assay

WiDr cells were cultured into coverslips in 24-well plate (5×10^4/well). The cells were adapted at 37 °C and 5% CO_2 for 24 h. The medium was removed and rinsed twice by using PBS. The extract (100 µg/mL) and celecoxib at IC_{50} concentration (68 µmol/L) were added into the plate and incubated in the same condition as previously described. The medium was removed from the cells and rinsed by using PBS. The coverslips were transferred to object glasses, then acridine orange-ethidium bromide was dropped into the coverslips. The cells were immediately observed under fluorescence microscope.

2.6. Immunocytochemistry assay

WiDr cells were seeded in 6-well plate and incubated under 5% CO_2 and 37°C for 24 h. The extract at 100 µg/mL and celecoxib at IC_{50} 68 µmol/L were added to the cells and incubated for further 24 h. At the end of incubation time, cells were harvested and washed by PBS. The cells were suspended in medium, placed and fixed in object glass for 15 min. Hydrogen peroxidase was dropped into the object glass and incubated at room temperature for 10–15 min. The cells were washed twice with PBS and monoclonal antibody of COX-2 was added into the cells and incubated at least for 1 h at room temperature. The cells were washed three times with PBS and added with secondary antibody, incubated at room temperature for 10 min, and washed four times with PBS. The solution of 3,3′-diaminobenzidine, as chromogen, was added to the cells and incubated for 3–8 min. Finally, the cells were washed with distilled water and added with hematoxylin solution followed by 3–4 min incubation. The expression of COX-2 was observed under inverted microscope.

2.7. Data analysis

Cell viability was calculated from MTT data using equation:

$$\frac{\text{Sample treatment absorbance} - \text{Medium absorbance}}{\text{Untreated cells absorbance} - \text{Medium absorbance}} \times 100\%$$

Cell viability data were analyzed by using linear regressions at four linier points to calculate IC_{50} of extract and celecoxib. Apoptosis induction semiquantitatively counted the number of apoptotic, necrotic and living cells in three different areas of an object glass. Each treatment was performed in triplicate. The number of COX-2 expressing cells was analyzed by *t*-test using Microsoft Excel 2013.

3. Results

This study investigated the cytotoxic effect of *T. flagelliforme* extract on WiDr colon cancer cells by targeting COX-2. Celecoxib, a selective COX-2 inhibitor, was used as a positive control. Dixon *et al.* reported celecoxib was a prospective anticancer on colon cancer [10]. Cytotoxic effect, assessed with

MTT assays, measured the absorbance of formazan complex at 595 nm that equaled to the number of living cells. As presented in Figures 1 and 2, *T. flagelliforme* leaf extract (R^2 = 0.995) and celecoxib (R^2 = 0.954) showed dose dependent cytotoxic activity on WiDr colon cancer cells with IC_{50} 70 µg/mL and 68 µmol/L, respectively.

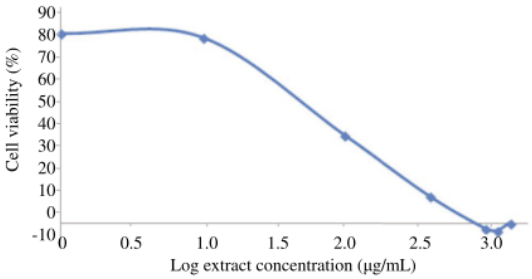

Figure 1. Effect of *T. flagelliforme* leaf extract in various concentrations on WiDr cells viability.

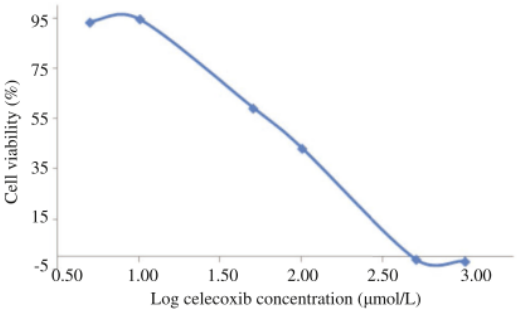

Figure 2. Effect of celecoxib in various concentrations on WiDr cells viability.

Double staining method was employed to determine the mechanism of cells death. *T. flagelliforme* leaf extract induced apoptosis [(65.27 ± 1.27)%] on WiDr cells as well as celecoxib [(88.67 ± 1.23)%] (Table 1). This method also successfully identified apoptosis stage and resulted in insignificant difference to flow cytometry method [34]. In early apoptotic cells, the nuclei showed condensed yellow-green fluorescence by acridine orange. On the other hand, the nuclei of late apoptotic cells showed condensed orange fluorescence by ethidium bromide (Figure 3).

Molecular mechanism of *T. flagelliforme* leaf extract was specifically observed by immunocytochemistry method. COX-2 expressing cells showed brown color while non-expressing cells showed purple color (Figure 4). WiDr cells were stained as

Table 1
Cell distribution after double staining. %.

Treatment	Apoptosis	Necrosis	Living
TLE	65.27 ± 1.27	14.16 ± 0.48	20.94 ± 1.42
Celecoxib	88.67 ± 1.23	2.23 ± 2.59	9.10 ± 0.00
Untreated cells	1.08 ± 0.00	0.00 ± 0.00	98.90 ± 0.00

Data were expressed as mean ± SD. TEL: *T. flagelliforme* leaf extract.

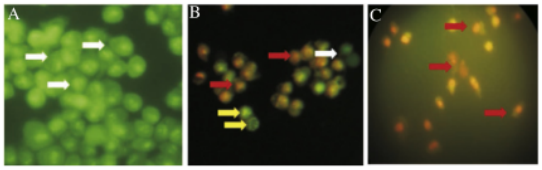

Figure 3. The observation of apoptotic cells on double staining method under fluorescence microscope using 400× magnification.
A: Untreated cells; B: Cells treated 100 µg/mL *T. flagelliforme* leaf extract; C: Cells treated 68 µmol/L celecoxib; White arrow: Living cell; Yellow arrow: Early apoptotic cell; Red arrow: Late apoptotic cell.

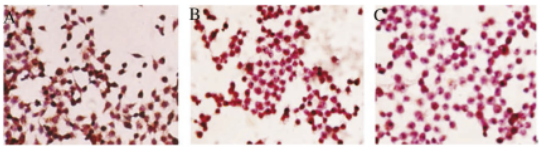

Figure 4. The observation of COX-2 expression cells on immunocytochemistry method under light microscope using 400× magnification.
A: Untreated cells; B: Cells treated 100 µg/mL *T. flagelliforme* leaf extract; C: Cells treated 68 µmol/L celecoxib; Brown color: COX-2 expression.

intense brown color, but *T. flagelliforme* leaf extract and celecoxib treated cells were stained as purple color. This result indicated that WiDr highly expressed COX-2 but *T. flagelliforme* leaf extract and celecoxib inhibited COX-2 expression. Semi-quantitative analysis of this result confirmed that *T. flagelliforme* leaf extract and celecoxib significantly suppressed COX-2 expression on WiDr cells ($P < 0.05$) (Table 2). This data suggested that cytotoxic activity of *T. flagelliforme* leaf extract on WiDr cells was through COX-2 downregulation.

Table 2
Number of cells that expressed COX-2.

Treatment	Mean ± SD
Untreated cells	69.36 ± 10.20
Extract	39.22 ± 3.63[a]
Celecoxib	14.32 ± 1.24[a]

[a]: Referred to significant difference to untreated cells ($P < 0.05$).

4. Discussion

This study evaluated the anticancer activity of *T. flagelliforme* leaf extract on WiDr colon cancer cells through COX-2 expression. COX-2 produced prostaglandin E_2 that increased proliferation and prevented cells from apoptosis [14,15]. Addressing COX-2 is an effective strategy to screen chemopreventive agents on colon cancer.

Our results concluded that *T. flagelliforme* leaf extract, as well as celecoxib, performed anticancer activity on WiDr cells by inhibiting COX-2 expression. These results have also opened interesting question on whether the inhibition of COX-2 expression of *T. flagelliforme* leaf extract was possible due to the presence of antioxidant compounds. It is well known that the colon is prone to oxidative condition and antioxidant deficiency [35]. Under oxidative stress, COX-2 was highly produced to modulate inflammati and induce carcinogenesis [36]. Pro-oxidant stress factor such as cigarette smoking, a diet high in

n-6 polyunsaturated fatty acid, and alcohol consumption may increase genotoxic damage of intestine [37].

On the other hand, the presence of antioxidant suppresses reactive oxygen species (ROS) formation and inhibits cancer cell proliferation [38,39]. A study by La Vecchia *et al.* showed an inverse correlation between antioxidant intakes and the risk of colorectal cancer [40]. Another study reported that low antioxidant status could support cancer development and therefore antioxidant supplement may be beneficial for cancer patient [41].

Isovitexin, isolated compound from ethyl acetate extract of *T. flagelliforme* leaves [26], showed antioxidant activity protecting cells from ROS [42]. This compound was reported to be responsible for the cytotoxic activity and COX-2 inhibition activity of *T. flagelliforme* leaf extract on WiDr cells. Previous studies established that antioxidants reduced the risk of colorectal cancer by COX-2 downregulation [43,44]. However, the mechanism of anticancer activity through antioxidant activity still needs to be further investigated since the role of ROS on carcinogenesis has dual role in either induction or inhibition of carcinogenesis [40,45]. Carcinogenesis can be induced by ROS formation in higher concentration, while ROS could also be possible to induce apoptosis on cells [40,46].

To conclude, *T. flagelliforme* leaf extract offers a new promising chemopreventive agent on colon cancer. Our data showed that *T. flagelliforme* leaf extract inhibits COX-2 expression. Nevertheless, details on molecular mechanism of these benefits remain to be established in the future study.

Anticancer activity of *T. flagelliforme* leaf extract and celecoxib on WiDr colon cancer cells was mediated by COX-2 inhibition, however the molecular mechanism needs to be further investigated.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to Adam Hermawan, PhD., Pharm. and Kholid Nur Alfan, Pharm for helping to prepare this manuscript.

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108.
[2] World Health Organization. *Latest world cancer statistic*. Geneva: World Health Organization; 2013. [Online] Available from: https://www.iarc.fr/en/media-centre/pr/2013/pdfs/pr223_E.pdf [Accessed on 27th August, 2015]
[3] Kimman M, Norman R, Jan S, Kingston D, Woodward M. The burden of cancer in member countries of the Association of Southeast Asian Nations (ASEAN). *Asian Pac J Cancer Prev* 2012; **13**: 411-20.
[4] Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 2010; **19**(8): 1893-907.
[5] Labianca R, Nordinger B, Beretta GD, Brouquet A, Cervantes A, ESMO Guidelines Working Group. Primary colon cancer: ESMO clinical practical guidelines for diagnosis, adjuvant treatment and follow-up. *Ann Oncol* 2010; **21**: v70-7.
[6] The Association of Coloproctology of Great Britain and Ireland. *Guidelines for the management of colorectal cancer*. London: The Association of Coloproctology of Great Britain and Ireland; 2007. [Online] Available from: http://www.uhb.nhs.uk/Downloads/pdf/CancerPbManagementColorectalCancer.pdf [Accessed on 27th August, 2015]
[7] Jiang Y, Zhang C, Chen K, Chen Z, Sun Z, Zhang Z, et al. The clinical significance of DC-SIGN and DC-SIGNR, which are novel markers expressed in human colon cancer. *PLoS One* 2014; **9**: e114748.
[8] Wang XW, Zhang YJ. Targeting mTOR network in colorectal cancer therapy. *World J Gastroenterol* 2014; **20**(15): 4178-88.
[9] Mishra J, Dromund J, Quazi SH, Karanki SS, Shaw JJ, Chen B, et al. Prospective of colon cancer treatments and scope for combinatorial approach to enhanced cancer cell apoptosis. *Crit Rev Oncol Hematol* 2013; **86**(3): 232-50.
[10] Dixon DA, Blanco FF, Bruno A, Patrignani P. Mechanistic aspects of COX-2 expression in colorectal neoplasia. *Recent Results Cancer Res* 2013; **191**: 7-37.
[11] Qi J, Dong Z, Liu J, Zhang JT. EIF3i promotes colon oncogenesis by regulating COX-2 protein synthesis and β-catenin activation. *Oncogene* 2014; **33**(32): 4156-63.
[12] Roelofs HM, Te Morsche RH, van Heumen BW, Nagengast FM, Peters WH. Over-expression of COX-2 mRNA in colorectal cancer. *BMC Gastroenterol* 2014; **14**: 1.
[13] Park J, Conteas CN. Anti-carcinogenic properties of curcumin on colorectal cancer. *World J Gastrointest Oncol* 2010; **2**(4): 169-76.
[14] Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. *Int J Cell Biol* 2010; **2010**: 215158.
[15] Nakanishi M, Rosenberg DW. Multifaceted roles of PGE_2 in inflammation and cancer. *Semin Immunopathol* 2013; **35**(2): 123-37.
[16] Li HJ, Reinhardt F, Herschman HR, Weinberg RA. Cancer-stimulated mesenchymal stem cells create a carcinoma stem-cell niche via prostaglandin E_2 signaling. *Cancer Discov* 2012; **2**(9): 840-55.
[17] Ghosh N, Chaki R, Mandal V, Mandal SC. COX-2 as a target for cancer chemotherapy. *Pharmacol Rep* 2010; **62**: 233-44.
[18] Rahman M, Selvarajan K, Hasan MR, Chan AP, Jin C, Kim J, et al. Inhibition of COX-2 in colon cancer modulate tumor growth and MDR-1 expression to enhance tumor regression in therapy-refractory cancers *in vivo*. *Neoplasia* 2012; **14**(7): 624-33.
[19] Kodela R, Chattopadhyay M, Goswami S, Gan ZY, Rao PP, Nia KV, et al. Positional isomers of aspirin are equally potent in inhibiting colon cancer cell growth: differences in mode of cyclooxygenase inhibition. *J Pharmacol Exp Ther* 2013; **345**: 85-94.
[20] Makar KW, Poole EM, Resler AJ, Seufer B, Curtin K, Kleinstein SE, et al. COX-1 (*PTGS1*) and COX-2 (*PTGS2*) polymorphisms, NSAID interactions, and risk of colon and rectal cancer in two independent populations. *Cancer Causes Control* 2013; **24**(12): 2059-75.
[21] Rosas C, Sinning M, Ferreira A, Fuenzalida M, Lemus D. Celecoxib decreases growth and angiogenesis and promotes apoptosis in a tumor cell line resistant to chemotherapy. *Biol Res* 2014; **47**: 27.
[22] Jaganathan SK, Vellayappan MV, Narasimhan G, Supriyanto E. Role of pomegranate and citrus fruit juices in colon cancer prevention. *World J Gastroenterol* 2014; **20**(16): 4618-25.
[23] Amado NG, Predes D, Moreno MM, Carvalho IO, Mendes FA, Abreu JG. Flavonoids and Wnt/β-catenin signaling: potential role in colorectal cancer therapies. *Int J Mol Sci* 2014; **15**: 12094-106.
[24] Nassar ZD, Aisha AF, Idris N, Khadeer Ahamed MB, Ismail Z, Abu-Salah KM, et al. Koetjapic acid, a natural triterpenoid, induces apoptosis in colon cancer cells. *Oncol Rep* 2012; **27**: 727-33.
[25] Patel VB, Misra S, Patel BB, Majumdar AP. Colorectal cancer: chemopreventive role of curcumin and resveratrol. *Nutr Cancer* 2010; **62**(7): 958-67.

[26] Farida Y, Wahyudi PS, Wahono S, Hanafi M. Flavonoid glycoside from the ethyl acetate extract of keladi tikus *Typhonium flagelliforme* (Lodd) Blume leaves. *Asian J Nat Appl Sci* 2012; **1**(4): 16-21.
[27] Mankaran S, Dinesh K, Deepak S, Gurmeet S. *Typhonium flagelliforme*: a multipurpose plant. *Int Res J Pharm* 2013; **4**(3): 45-8.
[28] Nobakht GM, Kadir MA, Stanslas J, Charng CW. Cytotoxic effect of *Typhonium flagelliforme* extract. *J Med Plant Res* 2014; **8**(31): 1021-4.
[29] Mohan S, Abdul AB, Abdelwahab SI, Al-Zubairi AS, Aspollah Sukarid M, Abdullah R, et al. *Typhonium flagelliforme* inhibits the proliferation of murine leukemia WEHI-3 cells *in vitro* and induces apoptosis *in vivo*. *Leuk Res* 2010; **34**(11): 1483-92.
[30] Mohan S, Bustamam A, Abdelwahab SI, Al Zubairi AS, Aspollah M, Abdullah R, et al. *Typhonium flagelliforme* induces apoptosis in CEMss cells via activation of caspase-9, PARP cleavage and cytochrome c release: its activation coopled with G0/G1 phase cell cycle arrest. *J Ethnopharmacol* 2010; **131**(3): 592-600.
[31] Yuliani SH, Anggraeni CD, Sekarjati W, Panjalu A, Istyastono EP, Setiawati A. Cytotoxic activity of *Anredera cordifolia* leaf extract on hela cervical cancer cells through p53-independent pathway. *Asian J Pharm Clin Res* 2015; **8**(2): 328-31.
[32] Setiawati A, Riswanto FO, Yuliani SH, Istyastono EP. Anticancer activity of mangosteen pericarp dry extract against MCF-7 breast cancer cell line through estrogen receptor-α. *Indonesian J Pharm* 2014; **25**: 119-24.
[33] Astuti P, Wahyono, Nuryastuti T, Purwantini I, Purwanto. Antimicrobial and cytotoxic activities of endophytic fungi isolated from *Artemisia annua* L. *J Appl Pharm Sci* 2014; **4**(10): 47-50.
[34] Liu K, Liu PC, Liu R, Wu X. Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. *Med Sci Monit Basic Res* 2015; **21**: 15-20.
[35] Özgönul A, Aksoy N, Dilmec F, Uzunköy A, Aksoy S. Measurement of total antioxidant response in colorectal cancer using a novel automated method. *Turk J Med Sci* 2009; **39**(4): 503-6.
[36] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* 2010; **49**(11): 1603-16.
[37] Stone WL, Krishnan K, Campbell SE, Palau VE. The role of antioxidants and pro-oxidants in colon cancer. *World J Gastrointest Oncol* 2014; **6**(3): 55-66.
[38] Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. *Toxicol Pathol* 2010; **38**(1): 96-109.
[39] Diaconeasa Z, Leopold L, Rugină D, Ayvaz H, Socaciu C. Antiproliferative and antioxidant properties of anthocyanin rich extracts from blueberry and blackcurrant juice. *Int J Mol Sci* 2015; **16**: 2352-65.
[40] La Vecchia C, Decarli A, Serafini M, Parpinel M, Belloco R, Galeone C, et al. Dietary total antioxidant capacity and colorectal cancer: a large case-control study in Italy. *Int J Cancer* 2013; **133**: 1447-51.
[41] Gupta SC, Hevia D, Patchva S, Park B, Koh W, Aggarwal BB. Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy. *Antioxid Redox Signal* 2012; **16**: 1295-322.
[42] Cao D, Li H, Yi J, Zhang J, Che H, Cao J, et al. Antioxidant properties of the mung bean flavonoids on alleviating heat stress. *PLoS One* 2011; **6**: e21071.
[43] Williams CD, Satia JA, Adair LS, Stevens J, Galanko J, Keku TO, et al. Antioxidant and DNA methylation-related nutrients and risk of distal colorectal cancer. *Cancer Causes Control* 2010; **21**(8): 1171-81.
[44] Wang L, Gao S, Jiang W, Luo C, Xu M, Bohlin L, et al. Antioxidative dietary compounds modulate gene expression associated with apoptosis, DNA repair, inhibition of cell proliferation and migration. *Int J Mol Sci* 2014; **15**: 16226-45.
[45] Afanas'ev I. Reactive oxygen species signaling in cancer: comparison with aging. *Aging Dis* 2011; **2**(3): 219-30.
[46] Vullanueva C, Kross RD. Antioxidant-induced stress. *Int J Mol Sci* 2012; **13**: 2091-109.

The inhibition of Typhonium flagelliforme Lodd. Blume leaf extract on COX-2 expression of WiDr colon cancer cells

ORIGINALITY REPORT

24%	16%	21%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	academicworks.cuny.edu Internet Source	1%
2	Maryam Rassouli. "High prevalence of hemotropic mycoplasmosis among stray cats in Iran", Asian Pacific Journal of Tropical Disease, 2016 Publication	1%
3	jeccr.biomedcentral.com Internet Source	1%
4	www.journal.ugm.ac.id Internet Source	1%
5	www.scitepress.org Internet Source	1%
6	libmast.utm.my Internet Source	1%
7	You-Gang Feng, Bo Liang, Jun Liu, Ming-Dong Jiang, Hong-Jian Liu, Yi-Qiang Huang, Lu Xiao. "Correlation study of podocyte injury and	1%

kidney function in patients with acute kidney injury", Journal of Acute Disease, 2016
Publication

8	Sandip Jadhav, Nitin Sawant. "Comparative pharmacoeconomics and efficacy analysis of a new antibiotic adjuvant entity and piperacillin-tazobactam for the management of intra-abdominal infections: A retrospective study", Asian Pacific Journal of Tropical Disease, 2016 Publication	1 %
9	innspub.net Internet Source	1 %
10	Sandra Ramos-Inza, Ana Carolina Ruberte, Carmen Sanmartín, Arun K. Sharma, Daniel Plano. "NSAIDs: Old Acquaintance in the Pipeline for Cancer Treatment and Prevention—Structural Modulation, Mechanisms of Action, and Bright Future", Journal of Medicinal Chemistry, 2021 Publication	1 %
11	Submitted to Southern Cross University Student Paper	1 %
12	escholarship.org Internet Source	1 %
13	www.koreascience.or.kr Internet Source	1 %

14	Submitted to Management & Science University Student Paper	1 %
15	Shamsuzzaman, , Ayaz Mahmood Dar, Yusuf Khan, and Aamir Sohail. "Synthesis and biological studies of steroidal pyran based derivatives", Journal of Photochemistry and Photobiology B Biology, 2013. Publication	1 %
16	www.karger.com Internet Source	1 %
17	acgpubs.org Internet Source	1 %
18	Systems Biology of Free Radicals and Antioxidants, 2014. Publication	<1 %
19	bmccomplementalternmed.biomedcentral.com Internet Source	<1 %
20	moam.info Internet Source	<1 %
21	repository.stikim.ac.id Internet Source	<1 %
22	www.biorxiv.org Internet Source	<1 %

23	Wen Xiao, Weiyi Zhang, Hai Huang, Yafei Xie et al. "Cancer Targeted Gene Therapy for Inhibition of Melanoma Lung Metastasis with eIF3i shRNA Loaded Liposomes", Molecular Pharmaceutics, 2019 Publication	<1 %
24	journals.biologists.com Internet Source	<1 %
25	Raisch, Jennifer, Nathalie Rolhion, Anaëlle Dubois, Arlette Darfeuille-Michaud, and Marie-Agnès Bringer. "Intracellular colon cancer-associated Escherichia coli promote protumoral activities of human macrophages by inducing sustained COX-2 expression", Laboratory Investigation, 2014. Publication	<1 %
26	bmccomplementmedtherapies.biomedcentral.com Internet Source	<1 %
27	downloads.hindawi.com Internet Source	<1 %
28	Fengkai Xu, Jie Gu, Lin Wang, Ronghua Liu, Yunfeng Yuan, Hao Wang, Jiahao Jiang, Wei Mao, Chunlai Lu, Di Ge. "Up-regulation Of EIF3e Is Associated With The Progression Of Esophageal Squamous Cell Carcinoma And Poor Prognosis In Patients", Journal of Cancer, 2018	<1 %

Publication

29	Noora Amraee, Ahmad Majd, Maryam Bikhof Torbati, Masoud Shaabanzadeh. "Folic Acid-decorated and PEGylated Graphene Quantum Dots as efficient Tamoxifen delivery system against breast cancer cells: in vitro studies", Research Square Platform LLC, 2023 Publication	<1 %
30	Diana Valentina Tudor, Ioana Bâldea, Diana Elena Olteanu, Eva Fischer-Fodor et al. "Celecoxib as a Valuable Adjuvant in Cutaneous Melanoma Treated with Trametinib", International Journal of Molecular Sciences, 2021 Publication	<1 %
31	Fatin Jannus, Marta Medina-O'Donnell, Francisco Rivas, Luis Díaz-Ruiz et al. "A Diamine-PEGylated Oleanolic Acid Derivative Induced Efficient Apoptosis through a Death Receptor and Mitochondrial Apoptotic Pathway in HepG2 Human Hepatoma Cells", Biomolecules, 2020 Publication	<1 %
32	Treda, Cezary, Marta Popeda, Magdalena Ksiazkiewicz, Dawid P. Grzela, Maciej P. Walczak, Mateusz Banaszczyk, Joanna Peciak, Ewelina Stoczynska-Fidelus, and Piotr Rieske. "EGFR Activation Leads to Cell Death	<1 %

	Independent of PI3K/AKT/mTOR in an AD293 Cell Line", PLoS ONE, 2016. Publication	
33	Yu-Jie Wang, Xiao-Ping Niu, Li Yang, Zhen Han, Ying-Jie Ma. "Effects of Celecoxib on Cycle Kinetics of Gastric Cancer Cells and Protein Expression of Cytochrome C and Caspase-9", Asian Pacific Journal of Cancer Prevention, 2013 Publication	<1 %
34	nutritionandmetabolism.biomedcentral.com Internet Source	<1 %
35	patents.glgoo.top Internet Source	<1 %
36	pure.rug.nl Internet Source	<1 %
37	sintadev.ristekdikti.go.id Internet Source	<1 %
38	test.dovepress.com Internet Source	<1 %
39	turkjps.org Internet Source	<1 %
40	www.cureus.com Internet Source	<1 %
41	www.ncbi.nlm.nih.gov Internet Source	<1 %

42 Adeline Péchery, Sylvie Fauconnet, Hugues Bittard, Isabelle Lascombe. "Apoptotic effect of the selective PPARβ/δ agonist GW501516 in invasive bladder cancer cells", Tumor Biology, 2016 <1%
Publication

43 Min-Shan Lu, Yu-Jing Fang, Yu-Ming Chen, Wei-Ping Luo, Zhi-Zhong Pan, Xiao Zhong, Cai-Xia Zhang. "Higher intake of carotenoid is associated with a lower risk of colorectal cancer in Chinese adults: a case–control study", European Journal of Nutrition, 2014 <1%
Publication

44 Norlaily Mohd Ali, Swee-Keong Yeap, Hamidah Mohd Yusof, Boon-Kee Beh et al. "Comparison of free amino acids, antioxidants, soluble phenolic acids, cytotoxicity and immunomodulation of fermented mung bean and soybean", Journal of the Science of Food and Agriculture, 2016 <1%
Publication

45 Agung Endro Nugroho, Fiki Fatihah Akbar, Anggie Wiyani, Sudarsono Sudarsono. "Cytotoxic Effect and Constituent Profile of Alkaloid Fractions from Ethanolic Extract of Ficus septica Burm. f. Leaves on T47D Breast Cancer Cells", Asian Pacific Journal of Cancer Prevention, 2015 <1%

Publication

46	Heshu Sulaiman Rahman, Abdullah Rasedee, Zeenathul Nazariah Binti Allaudin, Max Chartrand et al. "Antileukemic effect of zerumbone-loaded nanostructured lipid carrier in WEHI-3B cell-induced murine leukemia model", International Journal of Nanomedicine, 2015 Publication	<1 %
47	William L Stone, Koyamangalath Krishnan, Sharon E Campbell, Victoria E Palau. "The role of antioxidants and pro-oxidants in colon cancer", World Journal of Gastrointestinal Oncology, 2014 Publication	<1 %
48	Yuan-Chang Chang, Tung-Yuan Lai, Chun-Shu Yu, Hung-Yi Chen et al. " Emodin Induces Apoptotic Death in Murine Myelomonocytic Leukemia WEHI-3 Cells and Enhances Phagocytosis in Leukemia Mice ", Evidence-Based Complementary and Alternative Medicine, 2011 Publication	<1 %

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		