

ANTI-COLON CANCER ACTIVITIES OF CHALCONE AND ITS DERIVATIVES ON HCT116 CELL LINES

Kittiyaporn Permpool¹, Atchara Janthong², Kraikrit Utama^{3,4}, Nopawit Khomto¹, Puttinun Meepowpan^{1,2,3}, Padchane Sangthong^{3,4}

¹ Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

² Program in Biotechnology, Multidisciplinary and Interdisciplinary School, Chiang Mai University

³ Office of Research Administration, Chiang Mai University, Chiang Mai 50200, Thailand

⁴ Center of Excellence in Materials Science and Technology, Chiang Mai University, 239 Huay Kaew Road, Chiang Mai 50200, Thailand

ABSTRACT

Colon cancer is the fourth most common cancer in Thailand. Colon cancer is caused by abnormal cell divisions in the intestinal lining (polyps), which subsequently mutate into cancer that can spread to other organs. 5-fluorouracil (5-FU) is a widely used chemotherapeutic drug for colon cancer patients. Despite its potent anti-cancer properties, this drug must be combined with other chemotherapy drugs to effectively treat colon cancer. Currently, natural compounds have been reported to exhibit anti-cancer activity while demonstrating lower toxicity to normal cells compared to chemotherapy drugs. This research focuses on studying natural compounds that contain the inhibition potential of two compounds including 2',4'-dihydroxy-6'-methoxy 3',5'-dimethylchalcone (DMC) and gallic acid (GA). DMC is a phytochemical in the flavonoid group which were found in Ma-Kiang (*Syzygium nervosum* A. Cunn. Ex.DC.), GA is a phenolic compound which is found in garlic (*Allium sativum* L.). The aim of this study is to investigate the biological activity of DMC, GA, and the conjugated compound of DMC and GA (DMC-GA) to enhance the anti-cancer in colon cancer cells (HCT116) The cytotoxicity on normal cells (Vero cells) is compared with 5-FU as positive control using the MTT assay. The results showed that the half-maximal inhibitory concentration (IC₅₀) values for DMC, DMC-GA, GA, and 5-FU were 14.56 ± 0.41, 9.04 ± 1.52, 305.83 ± 3.21, and 28.21 ± 1.98 μM, respectively. The cytotoxicity on Vero cells were 14.32 ± 2.96, 120.5 ± 2.26, 5.49 ± 1.21 and >80 μM, respectively. As the result, DMC-GA demonstrated increased anti-colon cancer effects on colon cancer (HCT116) after structural modification. DMC-GA exhibits three-fold greater than 5 FU. However, DMC-GA also shows increasing in toxicity when compared to normal cells. To investigate the cell death mechanism by acridine orange (AO) and propidium iodide (PI) assay, it was found that DMC and GA were able to induce apoptosis in colon cancer cells. On the other hand, DMC-GA could not induce apoptosis in colon cancer cells. To determine the cell death pathway, cell cycles and apoptosis assay will be further performed.

INTRODUCTION

Currently, natural extracts can be used in the treatment of cancer to reduce the side effects of chemotherapy drugs. Dimethyl chalcone, a flavonoid, has been shown to inhibit the proliferation of colon cancer (HT-29 cell lines) (Khamto *et al.*, 2021) and Gallic acid, a polyphenol, has demonstrated inhibitory effects on the growth of breast cancer (MCF-7 cell lines) (Arsianti *et al.*, 2024). This research aims to investigate the antiproliferative effects of two natural extracts, 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (DMC) from *Syzygium nervosum* A. Cunn. Ex. DC. (ma-kiang) seeds and 3,4,5-trihydroxybenzoic acid (GA) from *Allium sativum* L. (garlic) on colon cancer. Furthermore, structural modification through chemical conjugation of these two compounds, resulting in the DMC-GA. The compound was designed to enhance biological activity against cancer cells.

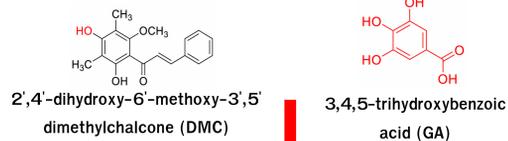
Biological activities	
DMC	GA
Anti-inflammatory	Anti-inflammatory
Antiviral	Antioxidant
Antidiabetic	Antimicrobial
Anti-colon cancer	Anti-breast cancer



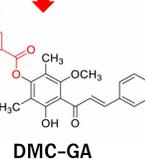
Syzygium nervosum A. Cunn. Ex. DC. (ma-kiang)



Allium sativum L. (garlic)



The chalcone derivatives structure were kind provided by Assoc. Prof. Dr. Puttinun Meepowpan



RESULTS AND DISCUSSIONS

1. Anti-colon cancer activity and cytotoxicity by MTT assay

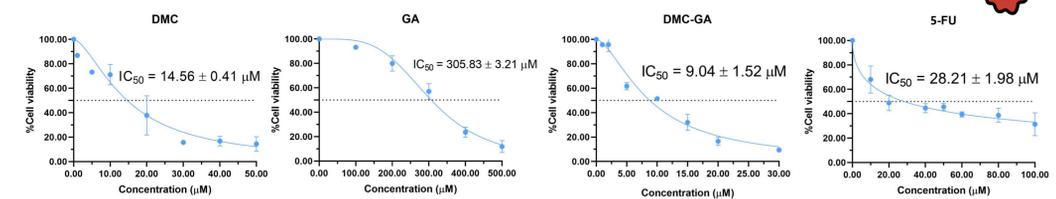


Figure 1 Relationship between drug concentration and %cell viability on HCT116 cells. The anti-proliferative activity of DMC, GA, DMC-GA and 5-FU were expressed as IC₅₀ values.

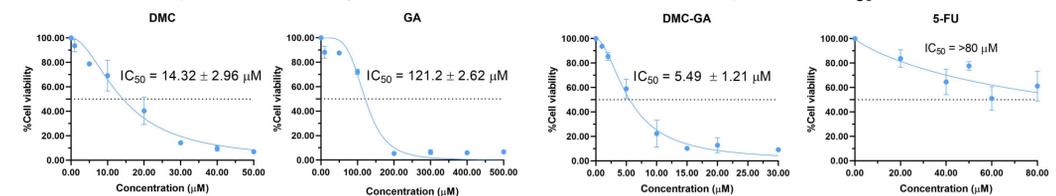


Figure 2 : Relationship between drug concentration and %cell viability on Vero cells. The anti-proliferative activity of DMC, GA, DMC-GA and 5-FU were expressed as IC₅₀ values.

After structural modification by conjugating these two natural extracts, forming DMC-GA the IC₅₀ value decreased to 9.04 μM in HCT116. This indicated that the DMC-GA was improved by 1.6-fold when compared to DMC and 33.8-fold when compared to GA. The anti cancer efficacy of DMC-GA is 3.1-fold greater than 5-FU

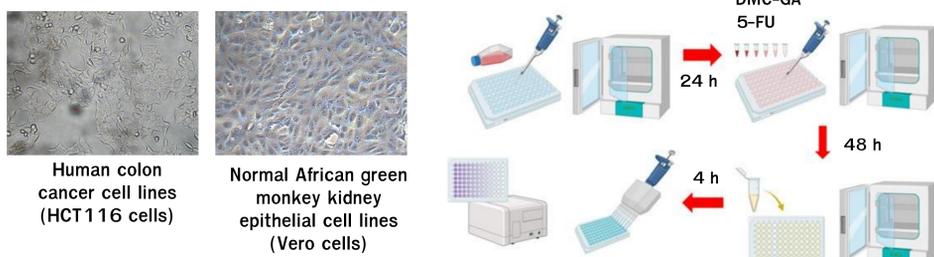
Meanwhile, in Vero cells, DMC-GA revealed the lowest IC₅₀ value, indicating that the structurally modified DMC-GA exhibited the highest toxicity in normal cells.

Table 1. Anti-cancer activity and of chalcone and its derivatives on colon cancer cells (HCT116 cells) and cytotoxicity on normal cells lines (Vero cells).

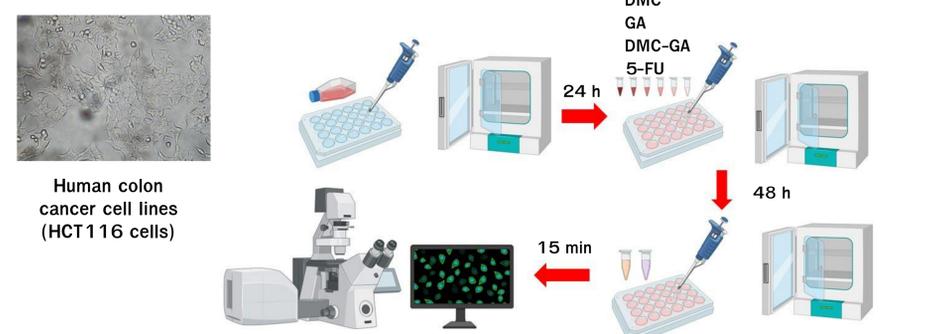
Compounds	IC ₅₀ (μM)	
	HCT116 cells	Vero cells
DMC	14.56 ± 0.41	14.32 ± 2.96
GA	305.83 ± 3.21	121.2 ± 2.62
DMC-GA	9.04 ± 1.52	5.49 ± 1.21
5-FU	28.21 ± 1.98	>80

MATERIALS AND METHODS

1. Anti-colon cancer activity and cytotoxicity by MTT assay

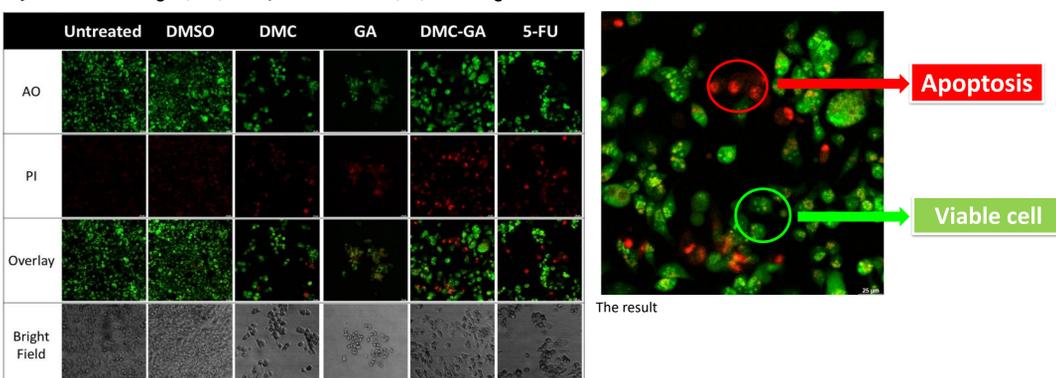


2. Apoptosis Assay with Acridine orange (AO)/Propidium iodide (PI) staining



2. Apoptosis assay with acridine orange (AO)/propidium iodide (PI) staining

Table 2 : Detection of apoptosis after treatment with DMC, GA, and DMC-GA by Acridine orange (AO)/Propidium iodide (PI) staining on HCT116 cells.



CONCLUSIONS

- The conjugated compound (DMC-GA) between dimethylchalcone (DMC) and gallic acid (GA) exhibits enhanced anti-colon cancer activity compared to their parental compounds.
- DMC, DMC-GA, and GA induce apoptosis in treated cells compared to untreated and 1% DMSO-treated cells.
- DMC-GA, a semi-synthetic compound, represents a promising candidate for colon cancer treatment.

ACKNOWLEDGMENTS

- Assoc.Prof.Dr. Padchane Sangthong (Advisor)
- Assoc. Prof. Dr. Puttinun Meepowpan (Co-advisor)
- Dr. Kraikrit Utama (Mentor)
- Ms. Atchara Janthong (Mentor)
- Division of Biochemistry and Biological Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University

REFERENCES

- Kahkeshani N., Farzaei F., Fotouhi M., Alavi S., Bahramsoltani R., Naseri R., Momtaz S., Abbasbadi Z., Rahimi R., Farzaei M., Bishayee A., Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iran J Basic Med Sci* 2019; 22:225-237.
- Utama K., Khamto N., Meepowpan P., Aobchey P., Kantapan J., Srirangam K., Roytrakul S., Sangthong P., P. Effects of 2,4-Dihydroxy-6-methoxy-3,5 dimethylchalcone from *Syzygium nervosum* seeds on Antiproliferative, DNA damage, cell cycle arrest, and apoptosis in Human Cervical Cancer Cell Lines. *Molecules* 2022, 27, 1154.
- Khamto N., Chaichuang L., Rithchumpon P., Phupong W., Bhoopong P., Tateing S., Pompimon W., Semakul N., Chomsri., Meepowpan P. Synthesis, cytotoxicity evaluation and molecular docking studies on 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone derivatives. *RSC Adv* 2021, 11, 31433