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In-Vitro Anticancer Activity Combination of Eugenol and Simple Aromatic Benzoate Compounds against Human Colon HCT-116 cells and WiDr Cells

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Abstract:

Colon cancer is the third most common malignancy around the world. Surgery, chemotherapy, and radiotherapy are generally used to treat colon cancer, but no effective therapy for advanced colon carcinoma is available. The purpose of this research work is to evaluation of the anticancer activity of combination simple aromatic benzoate (SAB) compounds and eugenol (EU) against colon cancer cell line HCT 116. In this study cytotoxicity of SP and EU on HCT116 and WiDr cell was evaluated by MTT assay. Inhibitory action on HCT116 cell line in concentration range between 50 mg/ml to 3.125 mg/ml by using MTT assay. IC50 value single compounds of TFBA and Eu on HCT116 cell was 0.35 and 22.3 respectively. IC50 value TFBA and Eu on WiDr cell was 0.29 and 26.7 respectively by MTT assay. IC50 value combined compounds of EU-TFBA on HCT116 and WiDr cell was 20.7 and 20.1 respectively by MTT assay. From the performed assay, TFBA of these simple aromatics compounds shows greater activity on HCT116 and WiDr cell line and combined EU-TFBA shows greater activity on HCT116 and WiDr cell line than other combined EU-SAB, that mean TFBA and EU-TFBA can be used as anticancer activity.

Keywords: Eugenol, simple aromatic benzoate compounds, anticancer activity, HCT 116, WiDr colon cancer cell line

INTRODUCTION

Colon cancer is the third most common type of cancer world-wide and its incidence is increasing in East Asia and Western countries [1, 2]. Current treatment for colon cancer mainly includes surgery and chemotherapy along with radiotherapy[3]. Therefore, there is a need to identify other therapeutic agents against this disease. In-fact, natural products play a major role in cancer prevention and treatment. A considerable number of antitumor agents currently used in the clinic are of natural origin. For instance, over half of all anticancer prescription drugs approved internationally between the 1940s and 2006 were natural products or their derivatives[4, 5]. During the 1960s, the National Cancer Institute (United States) began to screen plant extracts with antitumor activity[6]. Natural compounds isolated from medicinal plants, as rich sources of novel anticancer drugs, have been of increasing interest since then.

Natural compounds are defined as bioactive nonessential nutrients from. They have a variety of human health effects such as possessing putative chemo-preventive properties (anti carcinogenic and anti- mutagenic) and interfering with tumor promotion and progression[7, 8]. Many plant-derived agents have been recognized as potential alternative agents for cancer treatment over the last few years. There is growing interest in natural compounds with anticancer potential and low toxicity such as phenolic compounds [9].

Eugenol (EU) is a natural phytochemical found in cloves (*Syzgium aromaticum* L.) and has analgesic, antibacterial, antiinflamatory and anti-cancerous properties [10, 11]. Eugenol is a compound found in certain plants, such as basil, cinnamon, lemon balm, and nutmeg, but is primarily extracted from clove plants [12]. Cloves are widely grown in Indonesia, Madagascar and also in other countries like India and Sri Lanka. Further, aromatic plants like Cinnamomum tamala, Myristica fragrans, Illicium anisatum and Cinnamomum verum also contain eugenol [13, 14]. Eugenol consist of phenol and allyl structure.

Simple aromatic benzoiate such as phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom. As shown in Fig.1, predominant phenolic acids include hydroxyl benzoic acids (gallic acid, p-hydroxy benzoic acid, amino salicylic acid, salicylic acid, dichloro benzoic acid, trifluoromethyl benzoic acid) [15]. Gallic acid is widely distributed in medicinal herbs, such as Barringtonia racemosa, Cornus officinalis. Other hydroxybenzoic acids are also ubiquitous in medicinal herbs and dietary plants (spices, fruits, vegetables). For example, Dolichos biflorus, Feronia elephantum, and Paeonia lactiflora contain hydroxybenzoic acid. Based on research shows that salicylic acid and its derivatives are used as anticancer colorectal by inducing apoptosis and reduced the growth of colon cancer compounds[16]. While other guides amino salicylic acid is used as a chemo-preventive colon cancer proved safe for use in clinical studies [17]. Research conducted by Yumnam and co-woker that gallic acid can inhibit the growth of colon cancer cell line HCT 15[18].

Several researchers have investigated the e□ect of EU and others phenolic compounds on cancer. This work reports the results of antitumor e□ects of the simple aromatic benzoate compounds and eugenol against colon cancer. This work will determine whether the amount of aromatic benzoate constituent combined with Eugenol will have any effect on the antitumor activity against human colon cancer cell line

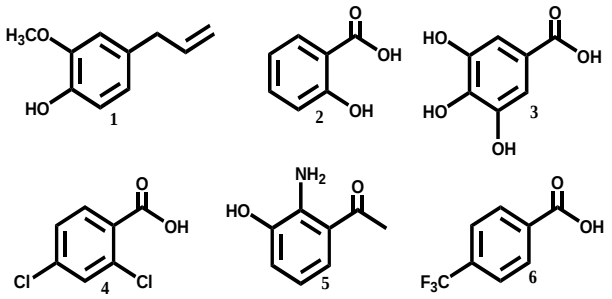


Figure 1: Structure of eugenol and simple aromatic benzoate (1) Eugenol (Eu), (2) salicylic acid (SA), (3) Gallic acid (GA), (4) Gentisic acid (GEA), (5) Aminosalicylic acid (ASA), (6) 4-trifluoromethyl benzoic acid (TFBA)

MATERIALS AND METHODS:

Eugenol and simple aromatic benzoate SAB) such as gallic acid, salicylic acid, amino salicylic acid, gentisic acid, methoxy tri fluoro benzoic acid, were purchased from TCI (Japan). Chemicals and all other standard reagents were purchased from Sigma Chemical Company (St Louis, MO, USA). DMEM, RPMI (Gibco BRL, Life Technologies, USA), Trypsin-EDTA solution and fetal bovine serum from Sigma Chemical Company (St Louis, MO, USA).

Cell culture Colon cancer (HCT116) cells were obtained from Patological Anatomy Faculty of Medicine Universitas Indonesia. Briefly, Cells were maintained in DMEM and RPMI 1640 supplemented with 10% FBS, 100 µg/ml penicillin and 100 µg/ml streptomycin incubated with 5% CO2 at 37oC. Both cell types were treated with various concentrations of EU and EU NE for 24 h.

MTT assay is performed to measure the anti-proliferation effects of eugenol and SAB with single dose and combine eugenol with SAB on the colon cancer HCT-116 cells and WiDr cells. The Eu and SAB are diluted and added to target cells in triplicates. Cell viability assay was determined by the reduction of MTT to formazan crystals. Briefly, cells were seeded at a density of 2×103 cells/mL in a 96-well plate, after 12 h of cell attachment, cells were treated with EU, SAB and combine EU-SAB (50, 25, 12.5, 6.25, 3.125 µg/ml) and incubated for 24 h. Next, 20 µL of MTT solution (5 mg/ml in PBS) was added to each well and the cells further incubated at 37oC for 4 h in a 5% CO2 humidified incubator. The medium was carefully removed, and colored formazan were dissolved in 150 µL dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA), and , the absorbance is determined at 590 nm on a microplate reader (Model 550, Bio-Rad, USA). The percentage growth inhibition was calculated using the formula below: The percentage growth inhibition was calculated using following formula,

Inhibiton rate (%)

= 1 – (absorbance of treatment group/absorbance of control group) x 100%.

The 50% inhibitory concentrations (IC50) of the 48 hours are calculated with Bliss assay.

Data interpretation Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

RESULTS AND DISCUSSION

In vitro cytotoxicity testing conducted as the first step in screening of potential anticancer compounds. This test using a cell line that provides advantages, such as test material needed is less and it requires short time. The anticancer activity is represented by IC50 value. The lower IC50 value, the greater anticancer activity. Before determining the IC50, the important thing is to determine the percentage inhibition of the test compounds. MTT measures cell respiration and the amount of formazan produced is proportional to the number of living cells present in culture. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the drug. IC50 is the concentration of the tested drug able to cause the death of 50% of the cells and can be predictive of the degree of cytotoxic effect. The lower the value, the more cytotoxic is the substance. Table 1 shows the comparison of the IC50 of some Eu, SAB and combined Eu-SAB as chemotherapeutic drugs against Colon cancer cell lines.

Table 1: Anticancer activity of Eugenol and simple aromatic benzoate (IC50 µg/mL) against colorectal HCT-116 and WiDr

Compounds	IC 50	
	WIDR cell line	HCT116 cell line
EU	26.7 ± 2.2	22.3 ± 2.9
GA	3.2 ± 0.4	25.1 ± 3.1
SA	21.8 ± 2.5	26.9 ± 3.5
ASA	3.26 ± 0.4	26.5 ± 3.8
TFBA	0.29 ± 0.032	0.35 ± 0.045
DCBA	1.3 ± 1.4	5.3 ± 3.2

IC_{50} is the 50% half maximal inhibitory activity in µg/mL, expressed in mean value (n=3) ± SD. SD: Standard deviation

Table 2: Anticancer activity of combination Eugenol with simple aromatic benzoate (IC50 µg/mL) against colorectal HCT-116 and WiDr

Compounds	IC 50	
	WIDR cell line	HCT116 cell line
Eu-GA	21.5 ± 2.3	22.6 ± 2.9
Eu-SA	21.8 ± 2.4	23.4 ± 3.1
Eu- ASA	21.1 ± 2.2	22.8 ± 2.8
Eu-DCBA	24.4 ± 2.6	26.9 ± 3.5
Eu-TFBA	20.1 ± 2.2	20.7 ± 2.6

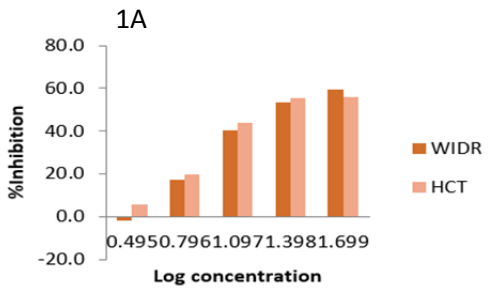
IC_{50} is the 50% half maximal inhibitory activity in µg/mL, expressed in mean value (n=3) ± SD. SD: Standard deviation.

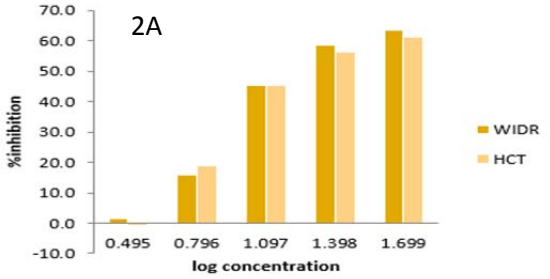
This study demonstrates the in vitro e□ect of the Eu and simple aromatic benzoate (SAB) on HCT 116 cells and WiDr cells. The results of cytotoxicity assay of single compounds are presented in Table 1 and IC50 of combine EU-SAB are presented in Table 2. All compounds were able to inhibit the proliferation of the cancer cells (HCT

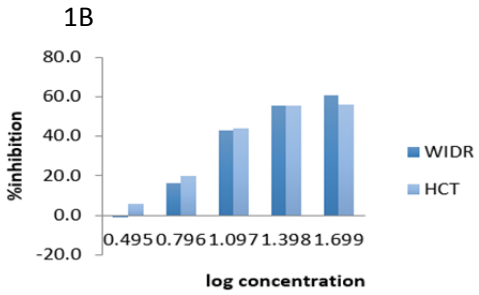
116, WiDr). The American National Cancer Institute guidelines (NCI) set the limit of activity for compounds at 50% inhibition (IC50) of proliferation of less than 30 mg/ml after an exposure time of 24 h 19. MTT assay also shows significant effect on HCT116 cell and WiDr cell. The results of viability shown in graphically represented in Fig. 2 (1A-1F and 2A-2E). It was found that the % growth inhibition increasing with increasing concentration steadily up to 3.125 mg/ml on HCT116 cell line and IC50 value of this assay in ranging 0.35 to 26.9 and R2 value was 0.9447, while in case of WiDr, so that IC50 value in ranging 0.29 to 26.7 and R2 value was 0.9582. Now overall study evaluate that single Eu and SAB and combined EU-SAB has potential activity on HCT116 cell and WiDr cell so these drug has considerable anticancer activity on colorectal cancer

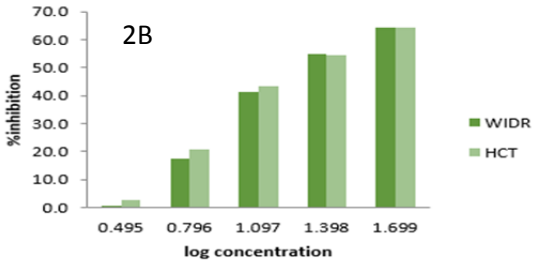
These data shown that the TFBA are more toxic to cancer cells. Cogitating the overall activity of the compounds, it was exhumed that sample two could be considered as potential anticancer drugs. This is in accordance of the preliminary antitumor studies, which previously demonstrated that TFBA and combined EU-TFBA showing anticancer effect. The results showed percentage of inhibition increases by increasing concentrations of the compounds (Fig 2). There is a difference inhibitory activity between single compounds with combined Eu-SAB against HCT-116 and WiDr cell proliferation. Whereas the percentage of inhibition of EU, SAB, and EU-SAB are incresing. Overall, the percentage inhibition of Eugenol and SAB in ranging of 0.3% to 76.6% against HCT116 cells and -1.6% and 75.1% against WiDr cells. The percentage inhibition of combined EU-SAB in ranging of -1.7% to 64.5% against HCT116 cells and -2.9% and 64.5% against WiDr cells.

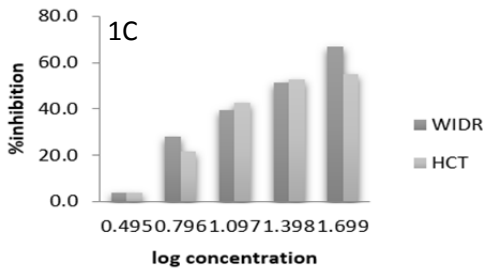
As shown in Table 1, TFBA on HCT116 and WiDr has IC50 value greater than 300µg/mL, is assigned as active compounds. While EU, GA, SA, ASA and DCBA which have IC50 values ranging from 22.3 to 26.9 µg/mL are assigned as compounds SAB high activity in inhibiting the growth of cancer cells HCT116. The best anticancer activity has shown by TFBA which has IC50 value of 0.35 µg/mL and 0.29 µg/mL, respectively. This result indicates that TFBA is potential to be developed as an anti-colorectal cancer drug.

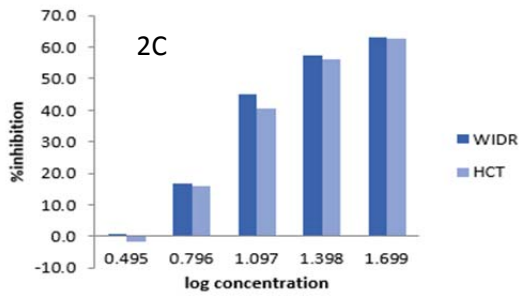












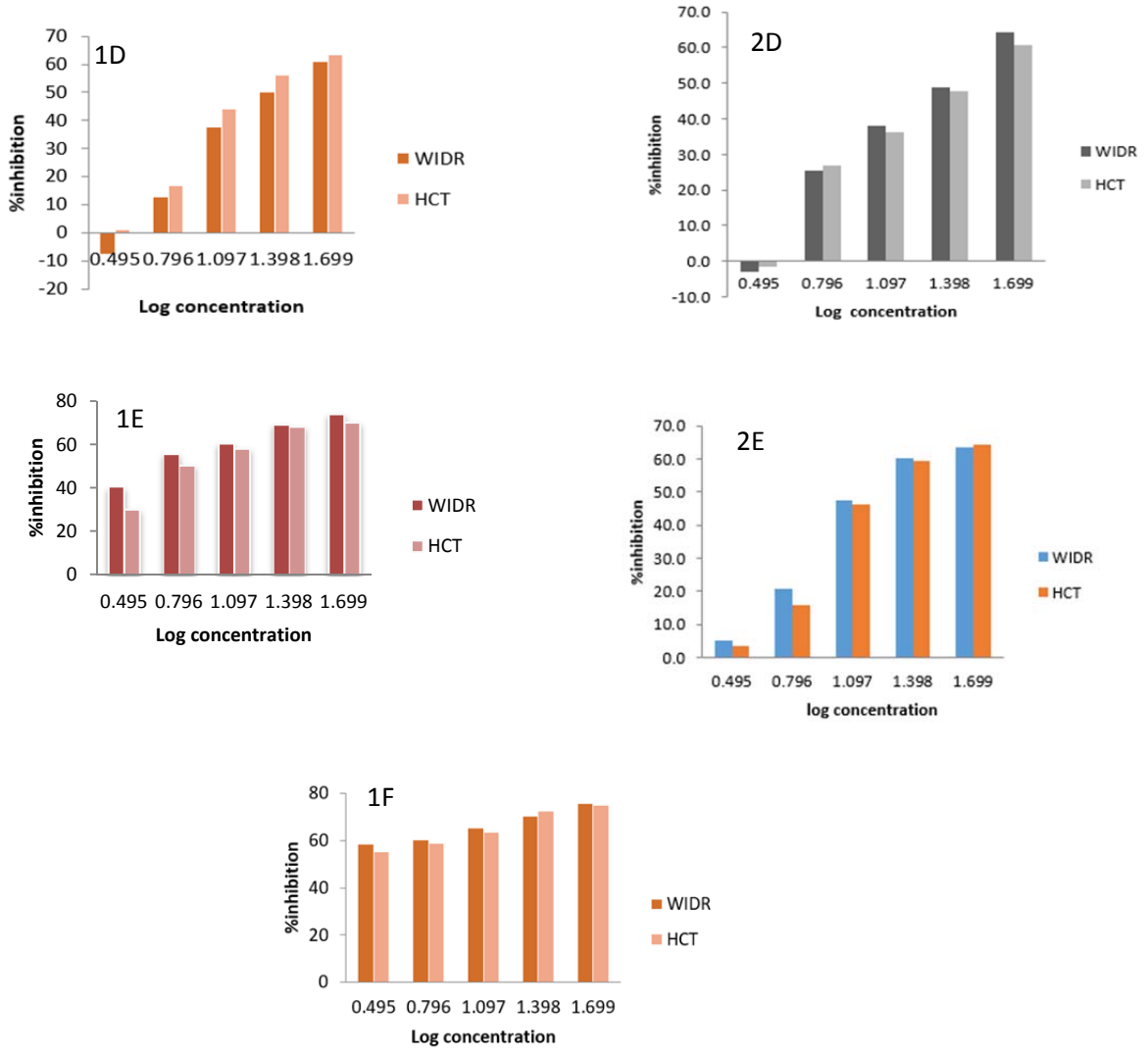


Figure 2: Inhibition value of eugenol and simple aromatic benzoate (SAB) and combination EU-SAB. Inhibition value with single compounds (1A) GA, (1B) ASA, (1C) SA, (1D) Eu, (1E) DCBA, (1F) TFBA. Combined compounds (2A) Eu-GA, (2B) Eu-ASA, (2C) Eu-SA, (2D) Eu-DCBA, and (2E) EU-TFBA

Interest in the pharmacological effects of bioactive compounds on cancer treatments and prevention has increased dramatically over the past twenty years. It has been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells [20]. Eugenol is a member of the phenylpropanoids class and is remarkably versatile molecule incorporated as a functional ingredient in several products and has found applications in the pharmaceutical [21]. It belongs to a class of naturally occurring phenolic monoterpenoids, chemically it is an allyl chain-substituted guaiacol. From results at above shows that the SAB compounds have IC50 greater than combination EU-SAB. SAB compound with halogen group has a lower IC50 value than SAB compound with hydroxyl group. This mean the SAB compound with presence of halogen groups in aromatic chain more active than SAB with hydroxyl group in aromatic chain.

Thus, it is imperative to search for new alternatives to colon cancer prevention agents. The inhibitory effect of Eu and simple aromatic benzoate with halogen group in aromatic chain may be a potential chemotherapeutic or a chemopreventive agent based on its ability to induce apoptosis in cancer cells with relatively low toxicity

CONCLUSION

In conclusion the result of the present study indicated that the Eugenol and SAB as Phenolic compounds are rich in medicinal herbs. Various phenolic compounds contribute to their potent effects on inhibiting carcinogenesis. Extensive research has been conducted in vitro anticancer activities to colorectal cancer cell line

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