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Screening the Activity of Epigallocatechin Gallate as a Colorectal Anticancer Agent: *In Silico* and ADMET/Pharmacokinetic Studies

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ABSTRACT
Introduction - Colorectal cancer is one of the third ranked cancers in Indonesia and has a high level of malignancy. The bioactive compound *Epigallocatechin Gallate* (EGCG) is known to have antioxidant and cytotoxic activity, so it has the potential to be developed as an anticancer agent. **Purpose** - This study aims to determine the potential of EGCG compounds as anticancer *in silico*. **Methodology/Approach** - The *in-silico* tests used are molecular docking and ADMET/Pharmacokinetic profiles using *biosig pkcsm*.**Finding** - The results of the respective binding affinities formed from EGCG compounds with the target protein affinities of COX-2 and *Caspace-3* are -9.2 and -5.4 which are better links between EGCG and *Caspace*. The Lipinski rule show that the epigallocatecin compound has strength to absorb is 47%. This indicates that EGCG has the potential as a candidate for colorectal anticancer drugs with poor absorption. **Originality/Value/Implications** – *In Silico*, many studies have been carried out, but it is still rare to combine molecular docking methods with pKCSM to determine the solubility properties of compounds. The prospect of this research can become a basic or key for developing ECGC compounds into preparations because they have low bioavailability and can be continued in in vitro and in vivo research.

Keywords: Colorectal Cancer, Epigallocatechin Gallate, molecular docking, ADMET

INTRODUCTION

Cancer is a high prevalence cause of death which is characterized by cell growth that is faster than its normal cycle inducing damage to healthy segments and can invade other segments of the body or metastasize (Depkes RI, 2009). This cancer ranks third and is the second deadliest in the world. Based on research by Xi and Xu, (2021), colorectal cancer has 1.9 million cases in the world and causes 900 thousand deaths in 2020 (Xi & Xu, 2021). Colorectal cancer is a tumor that grows uncontrollably in the mucosal lining of the intestine to the rectum which specifically represents a condition of malignancy of the tumor (Sayuti & Nouva, 2019). Treatment of cancer that is often found in general is with chemotherapy instruments, radiation, and surgery. Treatment through chemotherapy is defined as an instrument of eradicating cancer cells with drugs in certain categories. Chemotherapy has several disadvantages, such as the emergence of undesirable toxic effects that invade healthy segments in the body. Nausea and vomiting are an effect of chemotherapy. Current therapy regimens need to be approached with side effects due to chemotherapy, not all of them have good effects. Chemotherapy has many effects that lead discomfort to patients (Nurgali, *et al.*, 2018). Thus, it is necessary to develop new treatments with minimal side effects and maximum therapeutic effects.

Trend 'Back to Nature' is currently a new pattern for the world community in medicine because it is considered to have minimal side effects compared to conventional drugs. Green tea (*Camelia sinensis*) is a commodity that is consumed in various parts of the world and widely cultivated in the highlands of Indonesia. According to research, *Camellia sinensis* can be used to support antibacterial, antidiabetic, antiviral, antifungal, and anticancer therapy because contain of the seconder metabolites such as *catechin*. *Epigallocatechin Gallate* (EGCG) contains the most abundant concentration in *catechins* (Amelia, 2019). EGCG has anticancer effects with several pathways such as modulation, inhibition of proliferative activity, tumorigenesis, and inducing cell death (Min & Kwon, 2014).

In this paper, we will discuss the content of EGCG which has the potential as an anticancer using *in silico* studies. This research was conducted using molecular docking and *pkcsm* so that in the future it can be developed as an insight into knowledge and preparation innovation. Previous research discussed the activity of *Epigallocatechin* and *Epicatechin* as antivirals for SARS Cov-2 (Naufa, et al., 2022) and the effects of *quercetin* on COX-2 and *Caspase-3* cells (Putri, et al., 2019). This study aims to determine the anticancer activity and pharmacokinetic profile of EGCG linked to COX-2 and *Caspase-3* cells. The research results are expected to be a scientific trace of the potential of the EGCG compound as a colorectal anticancer agent based on molecular docking and to develop its pharmacokinetic profile based on *pKCSM*.

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LITERATURE REVIEW

Colorectal Cancer

Colorectal cancer is an event that is associated with epithelial changes in the normal intestine into a small precancerous wound that widens into invasive carcinoma and somatically or inherited genetic mutations with a span of 10-15 years. Supporting conditions run from three molecular pathways, namely chromosomal instability, mismatch repair, and hypermethylation (Lotfollahzadeh, et al., 2022). Based on Saraiva, et al. (2023), risk factors for colorectal cancer are classified as modifiable and unmodifiable. Modifiable factors include:

- Overweight
- Diabetes mellitus
- Use of alcohol and tobacco
- Fiber-poor foods
- Dyslipidemia
- Low amount of normal flora

Factors that cannot be modified, such as:

- Genetic factor
- Lynch syndrome
- Familial adenomatous polyposis
- Inflammatory bowel disease (IBD)

Symptoms that are a sign of the appearance of colorectal cancer are changes in bowel movements, such as changes in stool volume and colour, diarrhoea or constipation, a full stomach, and blood in the stool (Sayuti & Nouva, 2019).

EGCG Compound

ECGC is one of the main polyphenolic compounds in the composition of green tea secondary metabolites. EGCG (Figure 1) is the only interesting compound to study in medicinal chemistry because of its very high antioxidant effect (Legeay, et al., 2015). In addition, EGCG has several pharmacological effects, such as increasing insulin receptor sensitization, inhibiting carcinogenesis, tumorigenesis, and mutagenesis, preventing metastatic cancer, protecting against cardiovascular disease and neurodegenerative diseases (Shiyan, 2021).

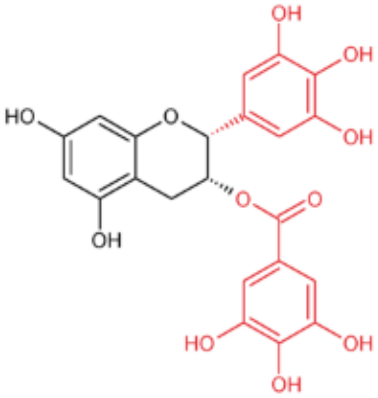

Figure 1. ECGC Molecular Structure (Legeay, *et.al.*, 2015).

Based on previous research conducted by Febriansah, et al. (2021) regarding EGCG and *Acetogenin* targeting the *bcl-xl* protein having a docking score of -8.1 kcal/mol and -6.7 kcal/mol. ECGC can be supposed to work to inhibit cell life and trigger apoptosis through the emergence of cancer cells. In addition, research by Fitriyani, et al. (2020), found that the *catechin* compounds like EGCG, EGC, and ECG can interact with the *HER-2* receptor even though the free energy formed is higher than the original ligand. In addition, *catechins* are also known to interact with *ASP863*.

METHOD

Tools

The instruments used in this study were Visualizer Discovery Studio software, Marvin Sketch, Autodock Tool, Autodock Vina, and the website https://biosig.lab.uq.edu.au/pkcsm/prediction accessed on 23 March 2023.

Materials

The materials used in this study are inflammatory proteins COX-2 *(5F19)* and executioner *Caspase-3 (2XZT)* which can be downloaded via https://www.rscb.org/ and the structure of the EGCG compound can be downloaded from https://pubchem.ncbi.nlm.nih.gov/ accessed on 23 March 2023.

RESEARCH PROCEDURE

Molecular Docking Methods

Should be downloaded several supporting applications in this method such as *Autodock Vina*, Discovery Studio Visualizer, and supporting software. Protein targets are obtained by downloading them to the *RSCB* in Protein Data Bank format. Native ligands were obtained from absorbed target proteins and stored in *PDBQT* Autodock Tools format. After obtaining the file in *PDBQT* form, it can be docked with *Autodock Vina* to get the *RSMD* value. The ideal *RSMD* value is determined by less than 2 Å. Docking visualization was carried out to see the bonds between proteins and ligands in the Discovery Studio test as a visualization application (Saputri, et al., 2022).

Pharmacokinetics Profile

To analyze ECGC compounds including absorption, distribution, metabolism, excretion, and toxicity properties with *pkcsm*. The data will be entered by entering *smiles* from *pubchem* to be entered and analyzed on the *pkcsm* website (Fajriaty, et al., 2023).

RESULT AND DISCUSSIONTest *In Silico* Molecular Docking

Molecular docking is part of a computational method with directions to describe the interaction events between compounds and their target receptors (Motiejunas & Wade, 2006). This computational method has a role in predicting the binding strength of two molecules (Mukesh & Rakesh, 2011). Another function is to relate the interactions of active substances and protein targets with

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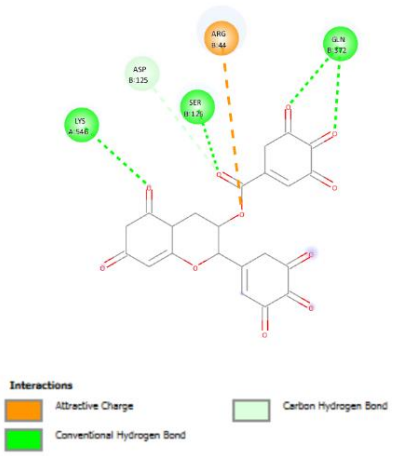
the specific aim of obtaining data that can be used in conducting *in vitro* tests. This research is devoted to linking ECGC compounds to proteins that cause inflammation and execute apoptosis in colorectal cancer cells. Molecular docking results show an interaction between ECGC and COX-2 and *Caspase-3*.

Table 1. Observation results of docking EGCG with cancer cell proteins.

Active Compound	Protein	RSMD (Å)	Docking Score
Native Ligand	COX-2	1.902	-8.0
EGCG		1.943	-9.2
Native Ligand	*Caspase-3*	1.020	-3.1
EGCG		1.970	-5.4

Based on table 1, the results of the docking of the link between EGCG and COX-2 and *Caspase-3* showed a higher affinity value compared to the native ligand of each receptor. The resulting affinity value between EGCG and COX-2 is -9.2 and EGCG with *Caspase-3* is -5.4. This shows good affinity compared to the native value of the ligand. Apart from that, research conducted by Febriansah et al. (2020), the binding between EGCG and the BCL-xl receptor showed the highest docking score compared to other compounds such as acetogenin, doxorubicin, and 5-Flourouracil (Febriansah, *et al.*, 2021). In previous studies, EGCG had good binding affinity for *bcl-xl* apoptosis-inducing compounds. In more detail, EGCG binds to amino acid residues in the COX-2 protein, namely *ARG44, ASP125, SER126, GLN372* and in EGCG bonds with *Caspase-3* protein binds to amino acid residues, namely *HIS277, SER36, ARG238, TYR226*.



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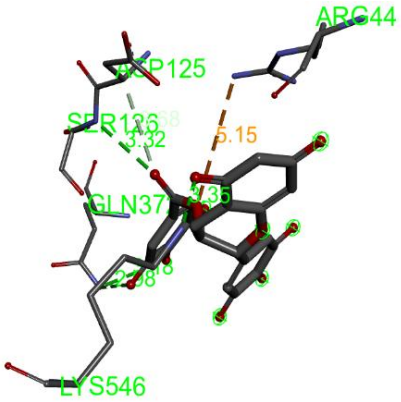

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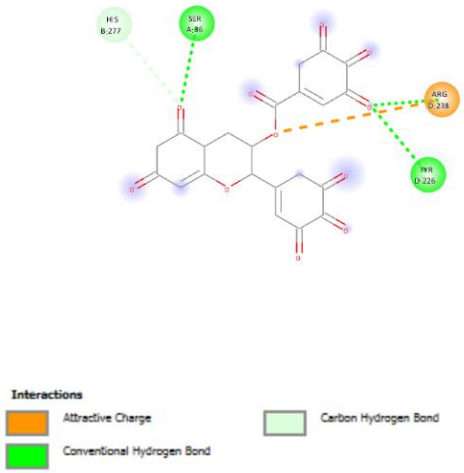

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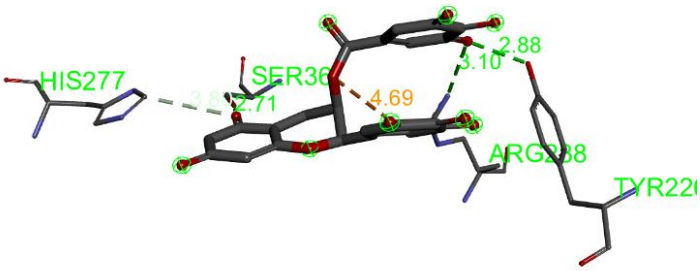

(4)

Figure 2. 2D (1) and 3D (2) interactions between EGCG and *COX-2* protein; 2D (3) and 3D (4) interaction between EGCG and *Caspase-3* protein.

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Pharmacokinetic Profile Test

Table 2. Pharmacokinetic Prediction of ADMET on EGCG compounds

ADMET Indicator	Result
Intestinal Absorption (%)	47.395
Distribution Volume (Log L/kg)	0.806
Metabolism (Log ml/min/kg)	CYP3A4 Inhibitor
Total Clearance (Log ml/min/kg)	0.292
Oral Rat Toxicity (mol/kg)	2.522
Hepatotoxic	No

Table 2 describes the ADMET profile (Absorption, Distribution, Metabolism, Excretion, and Toxicity) of EGCG compounds by looking at indicators of water solubility, gastrointestinal absorption, volume of distribution in blood, metabolism, total clearance, LD_{50}, and hepatotoxicity. The results showed that the EGCG compound had a BM of 458 (<500 Da), a log P value of 2.2332 (<5) and had a donor-acceptor value of 11;8 (maximum 5;10) respectively, so that is uncomplied Lipinski's rules it was categorized as having poor absorption. This compound has an absorption value of 47% so it is still in the intermediate range when given orally, has a high VD (volume of distribution) value, is metabolized in CYP3A4 inhibitors, and does not cause hepatotoxicity based on *biosig* laboratories. This test can become data for the development of the EGCG compound.

CONCLUSION AND RECOMMENDATION

Based on the linkage with molecular docking, the EGCG compound has better binding affinity than the native ligand in COX-2 and *Caspase-3* proteins by giving respective scores of -9.2 and -5.4 kcal/mol. A review of the ADMET profile using the *pKCSM biosig* lab found that the EGCG compound cannot be absorbed properly when given orally, has a high VD and metabolized in CYP3A4 inhibitors. This research can be continued to support the best profile of EGCG uptake with various possible modifications. Furthermore, to strengthen this compound, *in vitro* and *in vivo* tests can be carried out, thus it can be used as an alternative cancer treatment.

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