INTRODUCTION
Cancer still becomes a big problem in the world of health. In 2018, 18.1 million people in the world experienced cancer and 9.6 million of them died from the disease, so it cannot be denied that it is responsible for 30% of premature deaths from non-communicable diseases in adults aged 30–69 years. The incidence rate also has the potential to continue to increase to nearly double by 2040, especially in lower-middle-income countries, including Indonesia [1].

One of the types of cancer with the highest prevalence in Indonesia is colorectal cancer which occupies the 2nd and 3rd position, respectively, as the most common cancer in men and women [2]. The incidence rate of colorectal cancer is associated with various risk factors such as unhealthy diet, lack of physical activity, and excess body weight. In most cases, the initial treatment chosen is surgery which can be accompanied by adjuvant therapy in the form of radiotherapy and chemotherapy [3].

The 5-year survival rate and mortality rate depend on the stage and time of diagnosis [4]. For example, patients with metastatic colorectal cancer who do not undergo surgical resection only have 8 months of life expectancy, whereas a combination of chemotherapy and targeted therapy may prolong the patient’s median life expectancy, but long-term therapy can cause long-term adverse effects that reduce the patient’s quality of life [5]. Hence, early primary tumor intervention is crucial to improve the patient’s outcome [4].

Fusobacterium nucleatum microorganism may have a role in colorectal cancer. F. nucleatum has the virulence factors FaA and Fap2 that help to form a bond with intestinal epithelial cells, when reviewed further, Fap2 has a role in suppressing the immune system and increasing inflammatory cells that play a role in the development of neoplasia [6]. This mechanism is suspected to occur through the binding of Fap2 to the T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) domain receptors, causing the growth and proliferation of cancer cells [7]. Thus, targeting TIGIT is a new option in the management of colorectal cancer.

South Sumatra Province is listed as the main producer of coffee in Indonesia with production reaching 193,507 thousand tons (25.59%) [8]. Recent findings in two prospective cohort studies suggested that increased coffee intake after a diagnosis of colorectal cancer was associated with a reduced mortality risk, both related to colorectal cancer and other causes. This was related to various pathways such as active substances that suppressed tumor metastasis, maintained low inflammatory status and high insulin sensitivity, and prevented metastases to the liver, the main contributor to colorectal cancer-related deaths [9].

The molecular docking technique has been frequently used to predict the interaction of proteins with drug molecules. This method is essential to determine the accurate prediction of active sites and test ligand structures based on drug design [10]. Therefore, the purpose of this research is to determine the efficacy of the compounds in robusta coffee against colorectal cancer in silico through the inhibition of the TIGIT protein. This research was useful to provide information for the drug industry and further research in humans, especially in colorectal cancer management.

METHODS
The type of research applied was empirical research using the computational chemistry method (in silico). The methods used in this
Drug-like properties analysis

A drug molecule can be considered ideal if it adheres to the guidelines for physicochemical properties of the Lipinski rule of five to predict the drug similarity of a chemical compound with a particular biological activity designed for the oral route of drug administration. According to these rules, drug-like compounds should have a molecular weight (MW) <500 g/mol, a log p <5 (to describe hydrophobicity), several hydrogen bond donors (HBDs) no more than 5, and several hydrogen bond acceptors (HBA) no more than 10 [13], with the number of violations allowed no more than 1 [14]. The results of the analysis are shown in Table 1.

Given that no more than 1 violation occurred in the results of the analysis in each compound, it meant that all of them could be excellent candidates for further study and manipulation.

Molecular docking analysis

The docking simulation of the test compounds in coffee against the TIGIT protein showed that the best binding energy value was in kahweol (−8.1 kcal/mol) and cafestol (−8.1 kcal/mol) with the comparator drug in the form of 5-fluorouracil (−5.3 kcal/mol). The complete results that had been sorted from the largest binding energy values are shown in Table 2.

The protein-ligand binding occurred when the Gibbs free energy (ΔG) is negative at equilibrium at constant temperature and pressure. ΔG determines the stability of the protein-ligand complex or the binding affinity of the ligand to a particular receptor [15]. A negative ΔG score is significant in determining the drug efficacy [16]. As a result, the compounds in robusta coffee, especially kahweol and cafestol which had the most negative binding energies to TIGIT, were presumed to bind more stable than these compounds.

Furthermore, the visualization of the test compound binds with the TIGIT protein was carried out and images based on the hydrophobic interactions and hydrogen bonds were obtained in Figs. 1-3.

### Table 1: Drug-like properties analysis using Lipinski’s rule of five

| No. | Compounds     | MW (g/mol) | logP | HBA | HBD | Violation |
|-----|---------------|------------|------|-----|-----|-----------|
| 1.  | Caffeine      | 194.19     | 1.03 | 3   | 0   | 0         |
| 2.  | Caffeic acid  | 180.16     | 1.09 | 4   | 3   | 0         |
| 3.  | Chlorogenic acid | 354.31   | 0.75 | 9   | 6   | 1         |
| 4.  | Kahweol       | 314.42     | 3.61 | 3   | 2   | 0         |
| 5.  | Trigonelline  | 137.14     | 1.13 | 2   | 0   | 0         |
| 6.  | OMC           | 800.46     | 4.29 | 3   | 1   | 0         |
| 7.  | Cafestol      | 316.43     | 3.64 | 3   | 2   | 0         |
| 8.  | Furfuryl alcohol | 98.10     | 0.62 | 2   | 1   | 0         |
| 9.  | HMF           | 126.11     | 0.43 | 3   | 1   | 0         |
| 10. | 5-fluorouracil| 130.00     | 0.38 | 3   | 2   | 0         |

### Table 2: Binding energy of the test compounds to the TIGIT protein

| No. | Compounds     | Binding energy (kcal/mol) |
|-----|---------------|---------------------------|
| 1.  | Kahweol       | −8.1                      |
| 2.  | Cafestol      | −8.1                      |
| 3.  | OMC           | −7.9                      |
| 4.  | Chlorogenic acid | −7.8               |
| 5.  | Caffeic acid  | −6.3                      |
| 6.  | Caffeine      | −6.1                      |
| 7.  | Trigonelline  | −5.3                      |
| 8.  | HMF           | −5.1                      |
| 9.  | Furfuryl alcohol | −4.4              |
| 10. | 5-fluorouracil| −5.3                      |
The purpose of visualizing the docking results was to see non-covalent interactions such as hydrogen bonds and hydrophobic interactions between compounds and proteins. This could help in understanding the binding mode, affinity, and orientation of the compounds tethered to the protein active sites [17]. The hydrophobic interaction was a predominant contributor to protein stability so that the hydrophobic bond was the main determinant of the folding configuration balance in most native proteins when compared to the hydrogen bonds [16]. Based on the results in Table 3, various hydrophobic interactions and hydrogen bonds occurred between the test ligands and amino acid residues, including kahweol interacted hydrophobically with amino acid Tyr118 at distances of 4.98 Å and 5.39 Å; cafestol interacted with amino acid Tyr118, Leu73, Ile27, Tyr118, and Tyr118 at distances of 4.60 Å, 5.34 Å, 5.45 Å, 5.05 Å, and 5.41 Å; and 5-fluorouracil interacted with amino acid Leu65, His111, His111, and Ile109 at distances of 3.65 Å, 4.49 Å, 4.46 Å, and 4.76 Å. Accordingly, the most common hydrophobic interaction was found in the amino acid Tyr118 although it was not found in all bonds with the test ligands.

The previous studies had shown that kahweol caused downregulation of D1 cyclin expression in human colorectal cancer cells through proteasomal degradation through Thr26 phosphorylation and transcriptional regulation that might induce anti-cancer effects [18]. In addition, kahweol induced apoptosis through upregulation of activating

| Ligands  | Involved amino acids and the binding distances (Å) | Hydrophobic interactions |
|----------|--------------------------------------------------|--------------------------|
| Kahweol  | His111 (2.62), Ile27 (3.47)                      | Tyr118 (4.98), Tyr118 (5.39) |
| Cafestol | Asp72 (2.57), Ile27 (1.98), Gly120 (3.68)        | Tyr118 (4.60), Leu73 (5.34), Ile27 (5.45), Tyr118 (5.05), Tyr118 (5.41) |
| 5-fluorouracil | Ser78 (2.59), Gln64 (3.71), Ile77 (3.30) | Leu65 (3.65), His111 (4.49), His111 (4.46), Ile109 (4.76) |
transcription factor 3 (ATF3) in human colorectal cancer cells. Increased expression of ERK1/2 or GSK3β-dependent ATF3 occurred through transcriptional regulation and kahweol showed promoter regions between 1.47 and 85 regions. In these regions, fushi tarazu (Ftz) and CREB were reported to be cis-acting elements and kahweol was thought to induce CREB phosphorylation [19].

The anti-carcinogenicness effect has been shown in various studies, especially in renal carcinoma in vitro. Cafestol levels were positively correlated to the inhibition of proliferation and induced apoptosis in Caki cells through the downregulation of anti-apoptotic protein expression, mitochondrial membrane potential levels, cytochrome c release, and inhibition of P53/Akt pathway [20]. Cafestol also sensitized ABT-737-mediated apoptosis through the downregulation of Mcl-1 expression and upregulation of Bim expression in renal Caki cells so that it was presumed to be effective in treating solid tumors [21].

**Toxicity profile analysis**

Toxicity profiles of the compounds are shown in Table 4. From the results of the toxicity test, it was found that all substances did not show the potential carcinogenicity of eye erosion, and Ames mutagenesis. However, caffeic acid, trigonelline, furfuryl alcohol, HMF, and 5-fluorouracil exhibited potential eye irritation. In terms of hepatotoxicity, only caffeine and 5-fluorouracil had this potential. In terms of acute oral toxicity class, the substances were grouped into four categories based on the WHO criteria. Category I consisted of substances with LD₅₀ of ≤50 mg/kg. Category II consisted of substances with LD₅₀ of 50-500 mg/kg. Category III consisted of substances with LD₅₀ of >500 mg/kg. Category II consisted of substances with LD₅₀ of 500-5000 mg/kg [22].

Overall, starting from potential carcinogenicity, eye erosion, eye irritation, Ames mutagenesis, hepatotoxicity and acute oral toxicity, chochogenic acid, kahweol, OMC, and cafestol showed less potency. These results provided basic data regarding the toxicity profile of the test compounds and could assist in determining the appropriate route of administration, form, and dose. Nonetheless, these various probability values indicated that these studies were fundamental and should be confirmed experimentally.

**CONCLUSION**

Kahweol and cafestol were the compounds in robusta coffee that demonstrated the best results in inhibiting the TIGIT protein which played a role in the course of colorectal cancer. Further studies are needed at the in vitro and in vivo levels to strengthen the findings of this research.

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**AUTHORS’ CONTRIBUTIONS**

Desy Agustini, Leo Vernadesly, and Delviana concept the research, collect and analyze the data, and write the manuscript. Theodorus contributes as a supervisor by providing feedback for the research and manuscript.

**CONFLICTS OF INTEREST**

All authors declared that there were no conflicts of interest.

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Table 4: The results of the toxicity profile analysis of the test compounds

| No | Compounds       | Carcinogenicity   | Eye erosion | Eye irritation | Ames mutagenesis | Hepatotoxicity | Acute oral toxicity |
|----|-----------------|-------------------|------------|---------------|------------------|----------------|-------------------|
| 1  | Caffeine        | (0.9429)          | (0.9849)   | (0.9515)      | (0.8800)         | (0.6500)       | II (0.7405)       |
| 2  | Caffeic acid    | (0.8018)          | (0.6303)   | + (1.0000)    | (0.9100)         | (0.6750)       | IV (0.5588)       |
| 3  | Chlorogenic acid| (0.9292)          | (0.9899)   | (0.8966)      | (0.9900)         | (0.5750)       | III (0.7775)      |
| 4  | Kahweol         | (0.8429)          | (0.9897)   | (0.9863)      | (0.7100)         | (0.7750)       | IV (0.5575)       |
| 5  | Trigonelline    | (0.6602)          | (0.8164)   | + (0.9809)    | (0.8200)         | (0.6750)       | IV (0.4690)       |
| 6  | OMC             | (0.8857)          | (0.9880)   | (0.9848)      | (0.7000)         | (0.7000)       | III (0.6023)      |
| 7  | Cafestol        | (0.9000)          | (0.9903)   | (0.9840)      | (0.7500)         | (0.6750)       | III (0.6085)      |
| 8  | Furfuryl alcohol| (0.6286)          | + (0.7252) | + (0.9749)    | (0.8300)         | (0.8750)       | II (0.7493)       |
| 9  | HMF             | (0.8714)          | + (0.7124) | + (0.9899)    | (0.6200)         | (0.7000)       | III (0.7997)      |
| 10 | 5-fluorouracil  | (0.8571)          | (0.9855)   | + (0.6907)    | (0.6400)         | (0.7500)       | II (0.4387)       |

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