Research Article

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Evaluation of Anticancer and Antioxidant Activities of Coffee Stem Parasite Extract (*Scurrula Ferruginea* (Roxb. Ex Jack) *Danser*) and in Silico Studies of its Isolate

ROZA et al. Coffee Stem Parasite Extract as Anticancer

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ABSTRACT
This research aims to examine the potential of the parasite *Scurrula ferruginea* (Roxb. Ex Jack) *Danser* on coffee stems as a natural anticancer. In-silico and in-vitro studies have been carried out on coffee stem parasite extracts to analyze compounds that have the potential to act as HER2 inhibitors, the antioxidant activity of the extract, and the extract's ability to act as an anticancer agent against HeLa and MCF-7 cells. The research results show that several components in coffee stem parasite extract, including flavonoids and fatty acids, have the potential to act as HER2 inhibitors. The coffee stem parasite extract has strong antioxidant activity with IC50 of 59.736 ppm but is inactive against cancer cells. Characterization using gas chromatography-mass spectrometry showed the presence of bis (2-Ethylhexyl) phthalate ($C_{24}H_{38}O_4$) in coffee stem parasite extract, which is toxic as an anticancer drug.
Key words: anticancer, antioxidant, bis (2-Ethylhexyl) phthalate, coffee parasite, HER2 inhibitors.

INTRODUCTION

The Loranthaceae family includes the coffee parasite stems (*Scurrula ferruginea* (roxb.ex jack) Danser), also known as *Loranthus ferrugineus* is hemiparasitic, whose roots attach to the host plant to access nutrients and water. The community traditionally uses the coffee parasite as a cough medicine for tonsillitis, measles, diabetes, and cancer [1]. Coffee parasite has been shown to exhibit various biological activities, including antioxidant, neuro proactivity, anti-nephrotoxic, antiviral, anti-inflammatory, and antihepatotoxic, anti-inflammatory, antidiabetic, antimicrobial, antihypertensive, antioxidant, antidiarrheal, and anti-inflammatory properties. Immunomodulatory as well as hypolipidemic [2-5]. Based on previous pharmacological studies, coffee parasites are efficacious for treating cancer or cytotoxicity and are vasorelaxants [6-8].

The parasite belonging to the Loranthus family has a general composition of 82% crude fiber, 9% water, 3% crude protein, 2% ash, 1% crude fat, and 3% other substances [2]. The total phenolic content, which includes phenolic acids, anthocyanins, tannins, and flavonoids was also highest in the water fraction [8]. Secondary metabolites in coffee parasites that have been identified include fatty acids: oleic acid, linoleic acid, linolenic acid, octadic-8-10 dinoic acid, (Z)-octade-12-ene-8-10-dioate acid, and octadeca-8-10-12-trinoic acid; quercitrin, quercetin, rutin, icarisid B2, aviculin, (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-O-gallate and (-) epigallocatechin-3-O-gallate, choline, isoleucine, catechins, leucine, sesquiterpenes and chlorogenic acid [9-10]. Three natural flavonol compounds, including quercetin and quercitrin, a flavonol glycoside, have been isolated from the ethyl acetate fraction of coffee parasite stem [8].

The purpose of this study was to examine the antioxidant bioactivity of coffee parasite stem extract and how it affected MCF-7 and HeLa breast cancer cells. The polar and stem fractions of the coffee parasite contained the highest levels of phenolic chemicals and bioactivity [6–8]. Based on this, the research was first conducted by in silico studies to predict compounds of coffee stem parasites that are active as anti-cancer. The study continued with the in vitro method as evidence to see its ability as an antioxidant and anticancer. Isolating secondary metabolites from coffee stem parasites also were carried out.

MATERIALS AND METHODS

In Silico Study

The three-dimensional structure of the HER2 receptor with PDB ID code 3PP0 was prepared by separating the structure from the ligand and water attached to the receptor using the Discovery Studio device. The chemical data on PubChem (https://pubchem.ncbi.nlm.nih.gov) provided all the ligands' chemical structures in the form of bioactive chemicals for coffee parasites. Then the ligands used for molecular docking are parameterized using AutoDock Tools. According to Lipinski's rules, the analysis of Bioavailability and Prediction of Toxicity Ligands were analyzed for their bioavailability by accessing the page http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp. Ligands that have complied with Lipinski's rules are then predicted for their toxicity by accessing the http://lmmd.ecust.edu.cn/admetsar2 page. Molecular docking method validation, tethered ligand 2-{2-[4-({5-chloro-6-[3-(trifluoromethyl)phenoxy]pyridine-3-yl}amino)-5H-pyrrolo[3,2-d] pyrimidine-5-yl]ethoxy}ethanol attached to the chain was separated first and prepared. After that, the AutoDock Tools application carried out directed molecular docking. The grid box has 8 x 16 x10 dimensions with center point x = 16.564, y = 17.282, z = 26.889, and space = 1.00. Molecular docking was performed ten times to obtain the root mean square deviation (RMSD) <2.5Å at least three times. Molecular docking is performed using a command prompt program. The molecular docking results can be seen in the out document with *.pdbqt format opened using the Discovery Studio Visualizer application. A log file is a

document that contains data on affinity energy values (ΔG/binding affinity) in kcal/mol units. 2D visualization is done using the Ligplot+ application.

The plant material used is the stem of *Scurrula ferruginea* (Roxb. Ex Jack) *Danser* obtained by taking it in Sidikalang District, Dairi Regency. Plant identification was carried out at Herbarium Medanese in December 2020. It was conducted to determine whether the taxonomy of the plants used in the study was the same as that in the reference, so the results obtained were more accurate.

The mashed sample, which weighed 2 kg dry, was macerated by being immersed in acetone for three consecutive days. A Buchner funnel was used to filter the resulting macerate before it was evaporated.

Antioxidant Activity Measurement Using the DPPH Method

A standard Roswell Park Memorial Institute Medium (RPMI) liquid culture medium was utilized for the anti-cancer test; it contained 10% fetal bovine serum (FBS) and 50 µL/50 mL of antibiotics. In order to serve as a positive control, cisplatin was introduced. The DMSO solvent, which is not hazardous to cells, was used to dissolve materials at various concentrations of 7.81, 15.63, 31.26, 62.50, 125, 500, and 1000 µg/mL. PrestoBlueTM Cell Viability Reagent is the appropriate working solution to be utilized. HeLa cervical cells and MCF-7 breast cancer cells were cultivated in 96-well plates and incubated at 37°C and 5% CO_2 until 70% of the cells had grown. Presto blue working reagent was applied to the cells, and they were then incubated for 48 hours at a temperature of 37°C and 5% CO_2. The absorbance of the cells was then measured using a multimode reader.

For isolation, the extract was fractionated with vacuum column chromatography (CVC), and thin-layer chromatography (TLC) used to identify each fraction. The resulting fractions on separation were purified with column chromatography (CC) with the appropriate eluent until pure isolates were obtained. Pure isolates are characterized by the presence of one spot in the TLC test with different eluents. Pure isolates were identified using gas chromatography-mass spectrometry (GC-MS).

RESULT AND DISCUSSION

In Silico Test

The molecular docking method was validated first using natural receptors and ligands attached to the structure before molecular docking. The human epidermal growth factor 2 (HER2) 3PP0 receptor has a natural ligand, namely the molecule 2-{2-[4-({5-chloro-6-[3-(trifluoromethyl) phenoxy] pyridine-3-yl} amino)-5H-pyrrolo[3,2-d] pyrimidine-5-yl] ethoxy} ethanol. The natural ligand molecule was redocked ten times to validate the molecular docking. The average of the RMSD values from the ten conformations is 1.17 Å (**Figure 1**).

According to Lipinski's rule, the ligand bioavailability of the coffee parasite's active ingredient was predicted. The Lipinski rule states that the log P value must be less than 5, the relative atomic mass value must be less than 500 Da, the hydrogen bond acceptor value must be less than 10, and the molar refractivity value must be in the range of 40 to 130 [11]. Bioavailability analysis was carried out according to Lipinski's rule. This test is used as a guide to evaluating the design of a drug. Compounds ideal as drugs must be adequately absorbed, distributed, metabolized, and excreted by the body. Compounds with an atomic mass of more than 500 Da can reduce the passive diffusion ability of molecules because large sizes are difficult to penetrate cell membranes and take a long time to be absorbed [11]. Rutin compounds are predicted to have difficulty penetrating cell membranes and being absorbed by the body.

The quantity of hydrogen bond acceptors and donors also has an impact on a compound's capacity to cross the lipid bilayer membrane. Quercetin, rutin, aviculin, (-)-epicatechin-3-O-gallate, and (-) epigallocatechin-3-O-gallate compounds are predicted to require more energy

in the absorption process across the lipid bilayer membrane due to its hydrogen bond. In addition, the high hydrogen capacity will cause more energy needed to carry out the absorption process [11].

The log P value reveals a compound's hydrophobicity and lipophilicity (**Table 2**). A negative log P value denotes a compound's high hydrophilicity, which prevents it from passing through the lipid bilayer. A log P value of more than five indicates high hydrophobicity, so that the compound will be challenging to enter the cell because it is trapped in the lipid bilayer. Drugs will be distributed more widely, increasing their toxicity [11-12]. Hexadecanoic acid, methyl ester; hexanedioic acid, bis(2-Ethylhexyl) ester; oleic acid; linoleic acid; linolenic acid is indicated to be difficult to enter the cell because it is trapped in the lipid bilayer and its toxicity will increase. Rutin compounds are predicted to be unable to pass through the lipid bilayer. Molar refractivity is a value that indicates the distribution of the compound. Values between 40-130 indicate a good level of distribution and absorption [13]. All the active compounds in the coffee parasite showed good distribution and absorption. Rutin compounds violate 4 of 5 Lipinski's rules, so these compounds cannot be continued in the molecular docking process. Rutin compounds show poor bioavailability as drugs. Meanwhile, other compounds except Rutin only violated 1 of 5 Lipinski rules, so the ligand toxicity test was still allowed to continue.

Tested ligands except rutin are continued to see the drug's level of damage or adverse effects when consumed. The parameters used are human Ether-a-go-go-related gene (herG), carcinogenicity, and toxicity. herG is a gene encoding a K+ ion channel that is involved in cardiac repolarization activity. If the drug's toxicity causes blocking of herG, there will be sudden cardiac death due to abnormal heart muscle repolarization [14]. Accordingly, the compound 1,2-benzene-dicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester; aviculin; (-)-epicatechin-3-O-gallate; and (-)epigallocatechin-3-O-gallate was predicted to have an adverse effect on blocking herG (**Table 3**).

Carcinogenicity is a test to determine the potential of a compound in forming tumors or cancer [15]. The carcinogenicity test results showed that the compound 2,6-bis (1,1-dimethyl ethyl)-4-methyl phenol is carcinogenic. This compound is feared to trigger tumors or cancer when consumed.

There are four classifications for acute oral toxicity. Category 1 (LD_{50} 50 mg/kg), Category 2 (LD_{50} 500 mg/kg), Category 3 (LD_{50} 5000 mg/kg), and Category 4 (LD_{50} 5000 mg/kg) are the four different concentration categories. Categories 1 and 2 tend to be toxic, while categories 3 and 4 are non-toxic [16]. Quercetin compounds are included in category two, which tend to be toxic and dangerous when consumed orally. In contrast, other compounds are included in the non-toxic category. Compounds that are not carcinogenic, do not block herG, and are not toxic are continued in the molecular docking process.

The negative value of affinity energy indicates the tendency of a compound to form spontaneous bonds so that the reaction does not require energy or is exothermic [17]. Natural ligands in the molecular docking process show the lowest energy among other ligands. Meanwhile, cyclophosphamide, which is usually used to treat breast cancer, has the greatest energy when interacting with HER-2 receptors. Compared to cyclophosphamide and other active compounds of the coffee parasite, catechins and epicatechins have the most negative energy (**Table 4**).

Several amino acids are associated with catalytic activity, including leu726; val734; lys753; thr862; phe864; leu796; met774; leu785; ser783; gly729; asp863; met801 [18]. These twelve amino acids were detected in all visualized ligands (**Figure 2**). This visualization shows that natural ligands and other ligands bind to the same side to produce the same affinity as natural ligands in inhibiting HER-2 protein [19].

The more hydrophobic bonds, the more negative the affinity energy. The more hydrogen bonds, the greater the energy required to bond [20]. However, hexanedioic acid, bis(2-Ethylhexyl) ester, has the most hydrophobic bonds and no hydrogen bonds; its affinity energy is not the most negative. Likewise, although catechins and epicatechins have the most hydrogen bonds, the energy required is not as large as other ligands. Therefore, there is no relationship between the quantity of hydrogen bonds and the quantity of hydrophobic bonds to affinity energy. In addition, hydrogen bonding also affects the strength of the ligand-receptor interaction. The shorter the hydrogen bond, the stronger the interaction [21]. Although natural ligands have the longest hydrogen bond distance among other ligands, their interactions are still more potent than other ligands. Thus, the hydrogen bond length does not affect the binding affinity.

However, compounds such as 2-Methoxy-4-vinyl phenol; Hexadecanoic acid, methyl ester; Hexanedioic acid, bis(2-Ethylhexyl) ester; Oleic acid; Linoleic acid; Linolenic acid; Octadeca-8-10-dinoic acid; Octadeca-8-10-12-trinoate; Quercitrin; (+)-Catechins; (-)-Epicatechin has potential as an anticancer because of its better binding affinity than commercial therapeutic drugs.

Antioxidant Activity

The investigation continued with an antioxidant test before doing an anticancer test to directly demonstrate its effectiveness. The difference in absorbance between the absorbance of the sample and DPPH is measured using a UV-Visible spectrophotometer. The DPPH technique (2,2-diphenyl-1-picrylhydrazyl) was used to quantitatively measure antioxidant activity. A test method to ascertain the antioxidant activity to fend off free radicals is the DPPH method. The percentage of inhibition of the ethanol extract against DPPH free radicals served as a measure of its antioxidant activity. **Table 5** and **Figure 3** displays the results of the antioxidant activity test performed on the ethanol extract of the parasite coffee stem, revealing that the IC50 value was 59.736 ppm. A linear regression equation (Y= aX + b), where Y is 50, denoting 50%, and X is the IC50 value of the test sample, can be used to get the IC50 value. According to the findings, the ethanolic extract has a strong antioxidant activity based on the antioxidant activity's IC50 value [5].

Anticancer Activity

The extract activity test was continued for cancer testing, namely on HeLa cervical cancer cells and MCF-7 breast cancer cells, based on the coffee parasite stem's substantial IC50 antioxidant strength. The IC50 values of coffee parasite extract against HeLa cervical cancer cells and MCF-7 breast cancer cells were 11825.83 µg/mL and 9084.37 µg/mL, respectively. The IC50 value of this coffee parasite extract is weak or inactive, according to The National Cancer Institute (NCI) [22].

The anticancer activity increases as the IC50 value decreases. Very powerful anticancer substances have an IC50 value of less than 50 ppm, strong anticancer substances have an IC50 value of 50–100 ppm, and adequate anticancer substances have an IC50 value of 100–150 ppm, and a weak anticancer if it is between 151-200 ppm [23]. The majority of the Hela cells and MCF-7 cells were harmed or dead at a dosage of 1000 µg/mL, despite the fact that the ethanol extract of the coffee parasite was weak or ineffective against the two cancer cells (**Figure 5**)

Isolation of Secondary Metabolite

The isolation of the extract coffee stem parasite on the polar fraction, it was found that the compound was classified as pure, which was shown as one spot in the TLC test. Identification using the GC-MS instrument (**Figure 6** and **Table 6**) contained one main peak. At a retention time (RT) of 16.073 minutes, it contained the highest peak from the analytical spectrum with the highest 100% abundance.

The identification results showed that the compound was a bis (2-Ethylhexyl) phthalate compound with a relative molecular mass (m/z) of 149 and a molecular formula of $C_{24}H_{38}O_4$. The peak also gave a lib score (similarity) of 94.9%. The fragmentation peak (**Figure 7**) shows that the fragmentation of the bis (2-Ethylhexyl) phthalate compound indicates the presence of a base peak at m /z 149 and is the peak of the molecular ion itself.

The peak at m/z 279 comes from $C_{24}H_{38}O_4^+$ due to the release of $C_8H_{15}\bullet$ (1-Ethylhexyl) from the molecular ion, followed by the release of C_8H_{16} (octene) to form $C_8H_7O_4^+$, which is shown in m/z 167. As a result, the ion releases H_2O and creates a base peak at m/z 149. The breakdown of two esters, which involves the rearrangement of two H atoms (McLafferty rearrangement) and the release of H_2O, results in the classic phthalate peak at m/z 149 [24]. The molecular ion releases $C_{16}H_{25}O_4$ to generate $C_8H_{17}^+$, which is visible at m/z 113, and then releases C_2H_6 to form $C_6H_{11}^+$, which is visible at m/z 83. This fragmentation also happens in the CO ester bond. The release of C_2H_2 from C_6H_{11}, followed by the release of CH_2 to generate $C_3H_7^+$ as observed in m/z 43, causes $C_4H_9^+$ to reach its peak at m/z 57. **Figure 8** provides a clearer illustration of the bis (2-Ethylhexyl) phthalate compound's fragmentation.

Bis (2-Ethylhexyl) phthalate itself is a secondary metabolite compound that belongs to the fatty acid group. Bis (2-Ethylhexyl) phthalate compound is cytotoxic, whereas cytotoxic is a compound that can damage cancer cells and normal cells [24].
However, it is still necessary to predict the toxicity and bioavailability of Bis(2-Ethylhexyl) phthalate when used as an anticancer. The results of the bioavailability test show a log P value of Bis(2-Ethylhexyl) is 6.4330, which more than five indicates high hydrophobicity so that the compound will be challenging to enter the cell because it is trapped in the lipid bilayer. Drugs will be distributed more widely, increasing their toxicity. The Bis(2-Ethylhexyl) toxicity was included in the strong inhibitor category with a 0.8276 score. It causes blocking of herG; there will be sudden cardiac death due to abnormal heart muscle repolarization.

CONCLUSION

An in-silico study of coffee stem parasite (*Scurrula ferruginea* (roxb. ex jack) *Danser*) showed that several flavonoids and fatty acid compounds had better potential as HER2 inhibitors than drug therapy Cyclophosphamide. The in vitro test results showed that the coffee stem parasite extract has potent antioxidant activity with IC50 value 59.7359 ppm. However, it is not active against HeLa cervical cancer cells and MCF-7 breast cancer cells. Isolation of secondary metabolites in the extract of coffee stem parasite was identified to contain bis (2-Ethylhexyl) phthalate compounds that this pure compound is toxic if used as anticancer drugs. Coffee stem parasite extract does not function as an anti-cancer agent, but its antioxidant activity has potential for other applications.

Conflicts of interest

The authors declares that there is no conflict of interest'.

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25. **Table 1.** Coffee stem parasite classification	
Kingdom	Plantae
Division	Spermatophyta
Class	Dicotyledonea
Order	Santalales
Family	Loranthaceae
Genus	Scurrula
Botanical Name	*Scurrula ferruginea* (Roxb. Ex Jack) *Danser*
Synonym	*Loranthus ferrugineus*
Common Name	Coffee stem parasite
Herbarium Voucher	RG4664

Table 2. Prediction of ligand bioavailability

Ligand Name	Atomic Mass	Hydrogen Bond Donor	Hydrogen Bond Acceptor	logP	Molar refractivity
2-Methoxy-4-vinylphenol	150	1	2	2.044	44.750
2,6-bis(1,1-dimethylethyl)-4-methyl phenol	220	1	1	4.296	70.244
Hexadecanoic acid, methyl ester	270	0	2	5.641	82.328
1,2-benzene-dicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester	336	0	6	3.144	87.782
Hexanedioic acid, bis(2-ethylhexyl) ester	370	0	4	6.066	106.998
Oleic acid	282	1	2	6.109	87.088
Linoleic acid	280	1	2	5.885	86.994
Linolenic acid	280	1	2	5.885	86.994
Octadeca-8-10-dinoic acid	276	1	2	4.779	84.266
Octadeca-8-10-12-trinoate	272	1	2	4.002	82.808
Quercitrin	448	7	11	0.297	104.862
Quercetin	302	5	7	2.011	74.050
Rutin	610	10	16	-1.879	137.496
Aviculin	506	6	10	0.640	126.305
(+)- Catechin	290	5	6	1.546	72.623
(-)- Epicatechin	290	5	6	1.546	72.623
(-)- Epicatechin-3-O-gallate	442	7	10	2.528	107.256
(-) Epigallocatechin-3-O-gallate	458	8	11	2.233	108.921
Description: Lipinski rules violation					

Table 3. Prediction of ligand toxicity

Ligand Name	herG		Carcinogenicity		Acute Oral Toxicity	
	Category	Score	Category	Score	Category	Score
2-Methoxy-4-vinylphenol	Weak Inhibitor	0.719	Non carcinogenic	0.630	III	0.860
2,6-bis(1,1-dimethylethyl)-4-methyl phenol	Weak Inhibitor	0.749	Carcinogenic	0.629	III	0.827
Hexadecanoic acid, methyl ester	Weak Inhibitor	0.408	Non carcinogenic	0.600	III	0.859
1,2-benzene-dicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester	Strong Inhibitor	0.785	Non carcinogenic	0.729	IV	0.792
Hexanedioic acid, bis(2-ethylhexyl) ester	Weak Inhibitor	0.621	Non carcinogenic	0.671	IV	0.772
Oleic acid	Weak Inhibitor	0.394	Non carcinogenic	0.671	IV	0.829
Linoleic acid	Weak Inhibitor	0.461	Non carcinogenic	0.671	IV	0.829
Linolenic acid	Weak Inhibitor	0.360	Non carcinogenic	0.671	IV	0.639
Octadeca-8-10-dinoic acid	Weak Inhibitor	0.580	Non carcinogenic	0.671	IV	0.448
Octadeca-8-10-12-trinoate	Weak Inhibitor	0.689	Non carcinogenic	0.671	IV	0.448
Quercitrin	Weak Inhibitor	0.635	Non carcinogenic	0.986	III	0.518
Quercetin	Weak Inhibitor	0.841	Non carcinogenic	1.000	II	0.735
Aviculin	Strong Inhibitor	0.726	Non carcinogenic	0.971	III	0.618
(+)-Catechins	Weak Inhibitor	0.468	Non carcinogenic	0.929	IV	0.643
(-)-Epicatechin	Weak Inhibitor	0.468	Non carcinogenic	0.929	IV	0.643
(-)-Epicatechin-3-O-gallate	Strong Inhibitor	0.855	Non carcinogenic	0.986	IV	0.376
(-)Epigalocatechin-3-O-gallate	Strong Inhibitor	0.892	Non carcinogenic	0.986	IV	0.376

Description: Lipinski rules violation

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Hexanedioic acid, bis(2-ethylhexyl) ester	Weak Inhibitor	0.621	Non carcinogenic	0.671	IV	0.772
Oleic acid	Weak Inhibitor	0.394	Non carcinogenic	0.671	IV	0.829
Linoleic acid	Weak Inhibitor	0.461	Non carcinogenic	0.671	IV	0.829
Linolenic acid	Weak Inhibitor	0.360	Non carcinogenic	0.671	IV	0.639
Octadeca-8-10-dinoic acid	Weak Inhibitor	0.580	Non carcinogenic	0.671	IV	0.448
Octadeca-8-10-12-trinoate	Weak Inhibitor	0.689	Non carcinogenic	0.671	IV	0.448
Quercitrin	Weak Inhibitor	0.635	Non carcinogenic	0.986	III	0.518
Quercetin	Weak Inhibitor	0.841	Non carcinogenic	1.000	II	0.735
Aviculin	Strong Inhibitor	0.726	Non carcinogenic	0.971	III	0.618
(+)-Catechins	Weak Inhibitor	0.468	Non carcinogenic	0.929	IV	0.643
(-)-Epicatechin	Weak Inhibitor	0.468	Non carcinogenic	0.929	IV	0.643
(-)-Epicatechin-3-O-gallate	Strong Inhibitor	0.855	Non carcinogenic	0.986	IV	0.376
(-)Epigalocatechin-3-O-gallate	Strong Inhibitor	0.892	Non carcinogenic	0.986	IV	0.376

Description: Lipinski rules violation

Table 4. Molecular Docking Results					
Ligand Name	Energy Affinity (kcal/mol)	Amino Acid Residue	Number of Hydrophobic Bonds	Number of Hydrogen Bonds	Hydrogen Bond Length
Natural Ligand	-11.4	leu800; gly804; leu726; leu852; ala751; cys805; ser728; val734; lys753; thr862; phe864; leu796; met774; leu785; ser783; gly729; asp863; thr729; met801; gln799	18	2	Met801 3.03; Asp863 3.28
Cyclophosphamide (breast cancer therapy drug)	-5.4	met801; leu726; leu852; ala751; cys805; ser728; val734; thr862; gly729; gly727	10	0	
2-Methoxy-4-vinylphenol	-7.1	thr798; ser783; lys753; thr862; phe864; leu796; met774; leu785; asp863; ala 771	7	3	asp863 3.22; ser783 2.70; thr862 2.97
Hexadecanoic acid, methyl ester	-6.8	thr798; asp863; lys753; thr863; phe864; leu796; met774; leu785; ser783; arg784; ala771	8	3	asp863 3.24; thr863 2.93; ser783 2.71
Hexanedioic acid, bis(2-Ethylhexyl) ester	-7.6	leu726; leu852; ala751; cys805; ser728; val734; lys753; thr862; phe864; leu796; met774; leu785; ser783, asp863; thr798; met801; arg784; ile752; glu770; ala771	20	0	

Oleic acid	-7.2	leu800; leu726; leu852; ala751; val734; lys753; thr862; phe864; leu796; met774; leu785; ser783; asp863; thr798; met801; glu770; ala751	16	1	met801 2.79
Linoleic acid	-7.6	leu800; leu726; leu852; ala751; val734; lys753; thr862; phe864; leu796; met774; leu785; asp863; thr798; met801; glu770; ala751	15	2	met801 2.79 dan 3.04
Linolenic acid	-7.6	leu800; gly804; leu726; leu852; val734; lys753; thr862; phe864; leu796; met774; leu785; asp863; thr798; met801; ala771	14	2	met801 2.79 dan 2.92
Octadeca-8-10-dinoic acid	-7.5	leu800; leu726; leu852; ala751; val734; lys753; thr862; phe864; leu796; leu785; asp863; thr798; met801; ala771; glu770	14	2	met801 2.92 dan 2.97
Octadeca-8-10-12-trinoate	-7.5	met801; leu800; gly804; leu726; leu852; ala751; val734; lys753; thr862; phe864; leu796; met774; leu785; asp863; glu770; ala771	16	0	
Quercitrin	-8.1	leu800; gly804; leu852; ala751; cys805; leu726; ser728; val734; lys753; thr862;	13	3	met801 2.54; asp863 3.21; leu726 2.86

		leu796; asp863; gly729, thr798; met801; gly727			
(+)-Catechins	-9.3	thr798; ala751; leu852; cys805; val734; asn850; lys753; thr862; phe864; leu796; leu785; asp863; arg849; val797	11	4	asp863 3.22; asn850 2.54; arg849 2.92 dan 3.01
(-)-Epicatechin	-9.3	thr798; ala751; leu852; cys805; val734; asn850; lys753; thr862; phe864; leu796; leu785; asp863; arg849; val797	11	4	asp863 3.24;asn850 2.54; arg849 2.92 dan 3.01
Description: Amino acids on the binding site					

Table 5. Antioxidant activity test data (DPPH) sample of ethanol extract of parasite coffee stem

Concentration (ppm)	Absorbance		% Inhibition	
	1st Repetition	2nd Repetition	1st Repetition	2nd Repetition
0	0.873	0.873	0,000	0,000
15	0.770	0.797	11.798	8.740
30	0.632	0.676	27.629	22.520
45	0.578	0.559	33.837	35.956
60	0.396	0.455	54.685	47.846
75	0.333	0.300	61.856	65.601

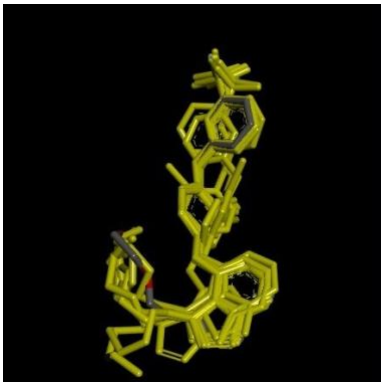
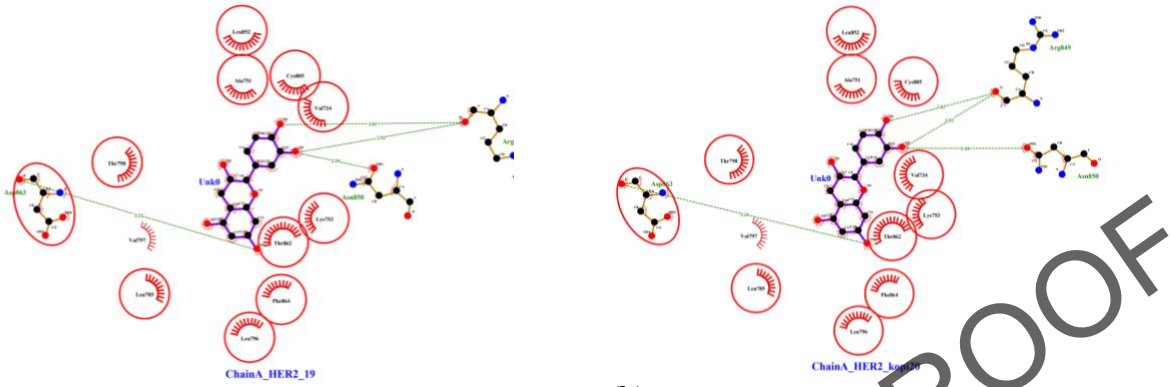


Figure 1. Molecular docking validation results. The average RMSD value is 1.17 Å



(a) (b)

Figure 2. 2D Visualization of (a) catechins and (b) epicatechins against receptors. The red circle indicates hydrophobic interactions between amino acids and ligands that interact on the same side of the receptor as the natural ligand. The dashed green line indicates hydrogen interactions between amino acids and ligands that interact on the same side of the receptor as the natural ligand

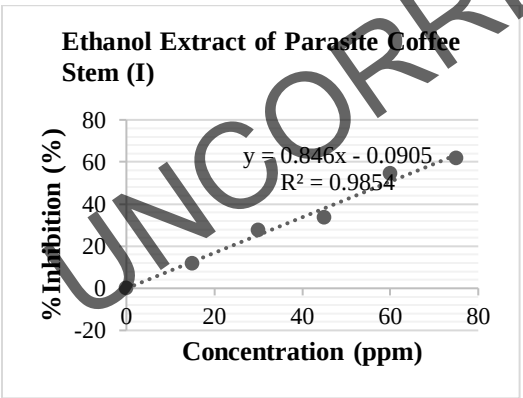
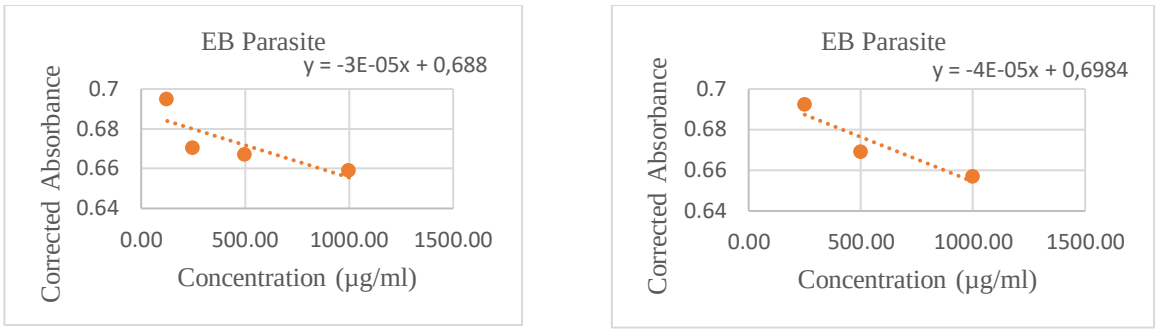


Figure 3. Diagram of antioxidant activity (DPPH) sample of ethanol extract of parasite coffee stem. The IC50 value is 59.736 ppm indicating strong antioxidant activity



(a) (b)

Figure 4. Parasite extract test results curve against (a) Hela cells and (b) MCF-7 cells. The IC50 value of this coffee parasite extract against cancer cells is weak or inactive.

(a) (b)

Figure 5. Documentation of Morphology of Parasite Extract Test Results (a) Hela cells (b) MCF-7 cells

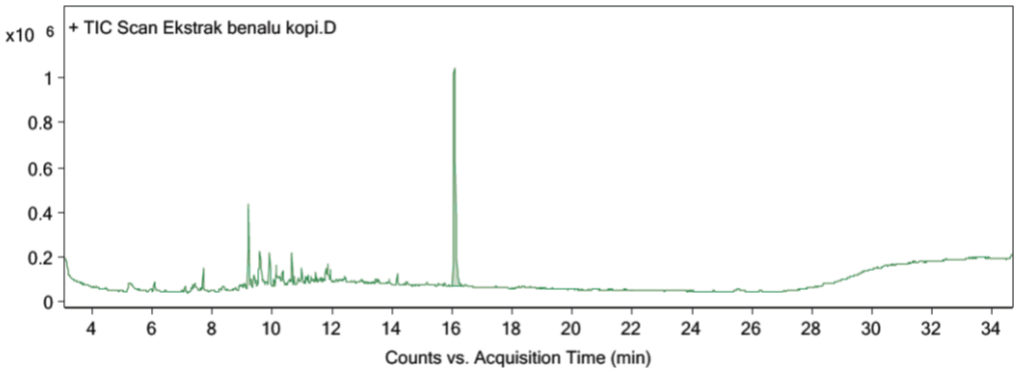


Figure 6. Spectrum of separation in GC-MS analysis showing retention time (RT) of 16.073 minutes indicating 100% of Bis (2- Ethylhexyl) phthalat

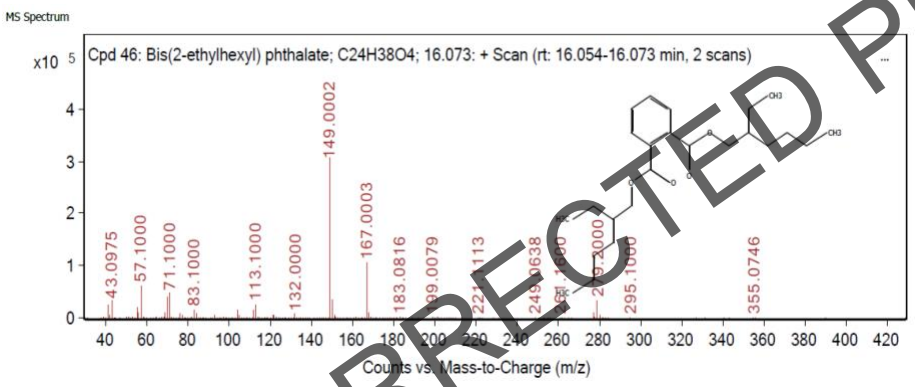


Figure 7. Peak fragmentation of compounds in GC-MS analysis showing the fragmentation of the bis (2-Ethylhexyl) phthalate

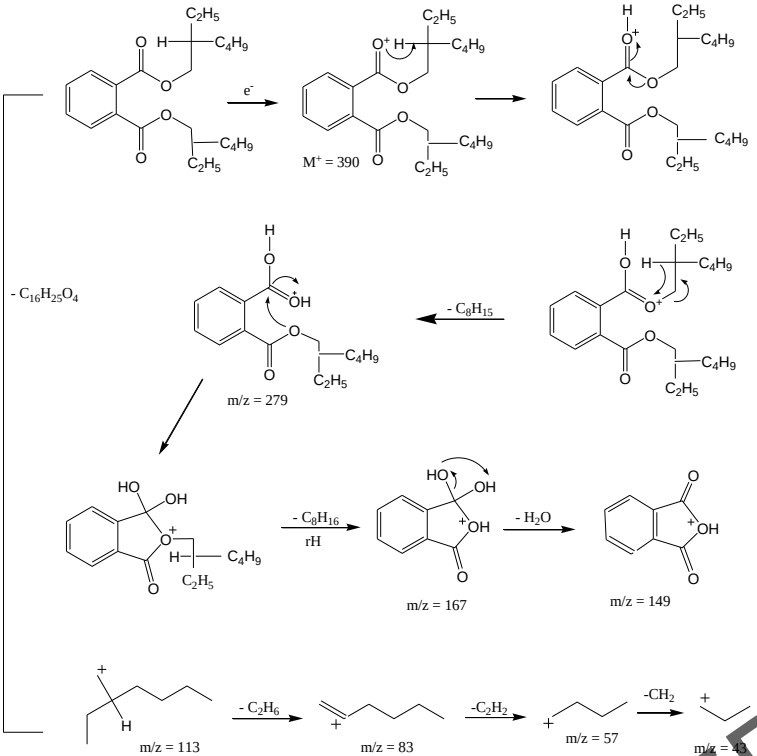


Figure 8. Fragmentation of bis(2-Ethylhexyl) phthalate compound