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ORIGINAL ARTICLE

# Evaluation of Anticancer and Antioxidant Activities (*In Vitro* Studies) of Coffee Stem Parasite Extract [*Scurrula ferruginea* (Roxb. ex Jack) Danser] and *In Silico* Studies of its Isolate

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## ABSTRACT

**Objectives:** The coffee parasite *Scurrula ferruginea* (Roxb. ex Jack) Danser has been shown to exhibit various biological activities. Based on previous pharmacological studies, coffee parasites are effective for treating cancer or cytotoxicity and are vasorelaxant. The aim of this study was to examine the potential of the worm *S. ferruginea* (Roxb. ex Jack): danser on coffee stems as a natural anticancer.

**Materials and Methods:** *In silico* and *in vitro* studies have been conducted on coffee stem parasite extracts to analyze compounds that have the potential to act as human epidermal growth factor 2 (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.

**Results:** The results show that several components of the 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 an $IC_{50}$ of 59,736 ppm and it is inactive against cancer cells. Characterization using gas chromatography-mass spectrometry revealed the presence of bis (2-Ethylhexyl) phthalate (C24H38O4) in the coffee stem parasite extract, which is toxic as an anticancer drug.

**Conclusion:** Although coffee stem parasite extract does not function as an anti-cancer agent, its strong antioxidant activity has potential for other applications.

**Keywords:** 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*, which is hemiparasitic, whose roots attach to the host plant to access nutrients and water (Table 1). The community traditionally uses the coffee parasite as cough medicine for tonsillitis, measles, diabetes, and cancer.[1] Coffee parasites have been shown to exhibit various biological activities, including antioxidant, neuroproactivity, anti-nephrotoxic, antiviral, anti-inflammatory, antihepatotoxic, anti-inflammatory, antidiabetic, antimicrobial, antihypertensive, antioxidant, antidiarrheal, and anti-inflammatory properties. Immunomodulatory and hypolipidemic.[2-5] Based on previous pharmacological studies, coffee parasites are effective for treating cancer or cytotoxicity and are vasorelaxant.[6-8]

The parasite belonging to the Loranthus family comprises 82% crude fiber, 9% water, 3% crude protein, 2% ash, 1% crude fat, and 3% other substances.[2] The total phenolic content, which

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includes phenolic acids, anthocyanins, tannins, and flavonoids, was also the 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, icariside B2, aciculin, (+)-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 stems.[8]

The aim of this study was to examine the antioxidant bioactivity of the coffee parasite stem extract and its effect on 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 these findings, this study was first conducted *via in silico* studies to predict compounds of coffee stem parasites that are active as anti-cancer. The study continued with the *in vitro* method to determine its antioxidant and anticancer. Isolation of secondary metabolites from coffee stem parasites was also performed.

## MATERIALS AND METHODS

*In silico study*

The three-dimensional structure of the human epidermal growth factor 2 (HER2) receptor with protein data bank 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 from PubChem (https://pubchem.ncbi.nlm.nih.gov) provided the chemical structures of all the ligands 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 bioavailability and prediction of toxicity ligands were analyzed for their bioavailability by accessing the website 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. Validation of the molecular docking method, the 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. Then, the AutoDock Tools application was used for directed molecular docking. The grid box has 8 x 16 x10 dimensions with center points x= 16,564, y= 17,282, z= 26,889, and space= 1.00. Molecular docking was performed ten times to obtain a root mean square deviation (RMSD) < 2.5 Å at least three times. Molecular docking was performed using a command prompt program. The molecular docking results can be seen in an out document with the *.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 was performed using the Ligplot+ application.

*Sample preparation*

The plant material used was the stem of *S. ferruginea* (Roxb. ex Jack) *Danser* obtained it in Sidikalang District, Dairi Regency. Plant identification was carried out at the Herbarium Medanese in December 2020. This study was conducted to determine whether the taxonomy of the plants used in the study was the same as that in the reference, resulting in more accurate results.

The mashed sample, which weighed 2 kg, was macerated by immersion 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 2,2 diphenyl-1-picrylhydrazyl (DPPH) method*

A standard Roswell Park Memorial Institute Medium liquid culture medium was utilized for the anti-cancer test; it contained 10% fetal bovine serum and 50 µL/50 mL of antibiotics. Cisplatin was introduced as a positive control. A dimethyl sulfoxide 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. HeLa cervical cells and MCF-7 breast cancer cells were cultured in 96-well plates and incubated at 37 ˚C in an atmosphere containing 5% $CO_2$ until 70% of the cells had grown. Presto blue working reagent was applied to the cells, which were then incubated for 48 hours at 37 ˚C in an atmosphere of 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, and thin-layer chromatography (TLC) was used to identify each fraction. The resulting fractions upon separation were purified using column chromatography with an appropriate eluent until pure isolates were obtained. Pure isolates were characterized by the presence of a single spot in the TLC test for different eluents. Pure isolates were identified using gas chromatography-mass spectrometry (GC-MS).

## RESULTS

*Findings of in silico test*

First, the molecular docking method was validated using natural receptors and ligands attached to the structure. The 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 molecules were redocked ten times to validate their molecular docking. The average of the RMSD values from the ten conformations is 1.17 Å (Figure 1).

Accordig to Lipinski's rule, the ligand bioavailability of the coffee parasite's active ingredient was predicted (Table 2). Based on Lipinski's rule, several ligands such as Hexadecanoic acid, methyl ester; hexanedioic acid, bis(2-ethylhexyl) ester; Oleic acid; Linoleic acid; Linolenic acid; Quercitrin; Aviculin; (-)- Epicatechin-3-O-gallate; and (-) Epigallocatechin-3-O-gallate violate one of the five Lipinski rules. While Rutin violates

four of the five Lipinski rules. Further testing was then carried out without using the Rutin ligand because it was predicted to have poor bioavailability.

The study was then continued with toxicity testing. Based on the parameters of human Ether-a-go-go-related gene (herG), carcinogenicity, and toxicity, ligands such as 2,6-bis(1,1-dimethylethyl)-4-methyl phenol; 1,2-benzene-dicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester; Quercetin; Aviculin; (-)-Epicatechin-3-O-gallate; and (-)Epigalocatechin-3-O-gallate are toxic ligands, so they cannot be used for further studies (Table 3).

**Table 1. Coffee stem parasite classification**

| | |
|---|---|
| Kingdom | Plantae |
| Division | Spermatophyta |
| Class | Dicotyledonea |
| Order | Santalales |
| Family | Loranthaceae |
| Genus | Scurrula |
| Botanical name | *Scurrula ferruginea* (Roxb. ex Jackie) Danser |
| Synonym | *Loranthus ferrugineus* |
| Common name | Coffee stem parasites |
| Herbarium voucher | RG4664 |

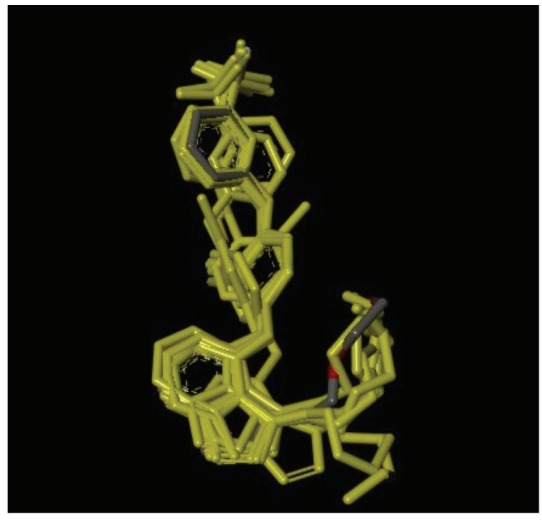

**Figure 1.** Molecular docking validation results. The average RMSD value is 1.17 Å

RMSD: Root mean square deviation

**Table 2. Prediction of ligand bioavailability**

| Ligand name | Atomic mass | Hydrogen bond donor | Hydrogen bond donor | logP | Molar refractivity |
|---|---|---|---|---|---|
| 2-Methoxy-4-vinyl phenol | 150 | 1 | 2 | 2,044 | 44,750 |
| 2,6-bis(1,1-dimethyl ethyl)-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 |

*Lipinski rule violation

Natural ligands in the molecular docking process have the lowest energy than other ligands. Cyclophosphamide, which is commonly used to treat breast cancer, has the greatest energy when interacting with HER2 receptors. Compared with cyclophosphamide and other active compounds of the coffee parasite, catechins and epicatechin have the most negative energy (Table 4). In addition, there are several amino acids related to catalytic activity (yellow highlights in Table 4) detected in all visualized ligands (Figure 2).

*Antioxidant activity*

The investigation continued with an antioxidant test before an anticancer test to directly demonstrate its effectiveness. Figure 3 and Table 5 present the results of the antioxidant activity test performed on the ethanol extract of the parasitic coffee stem, revealing that the $IC_{50}$ value was 59.736 ppm.

*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 $IC_{50}$ antioxidant strength. The $IC_{50}$ values of the 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 (Figure 4). The majority of HeLa 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).

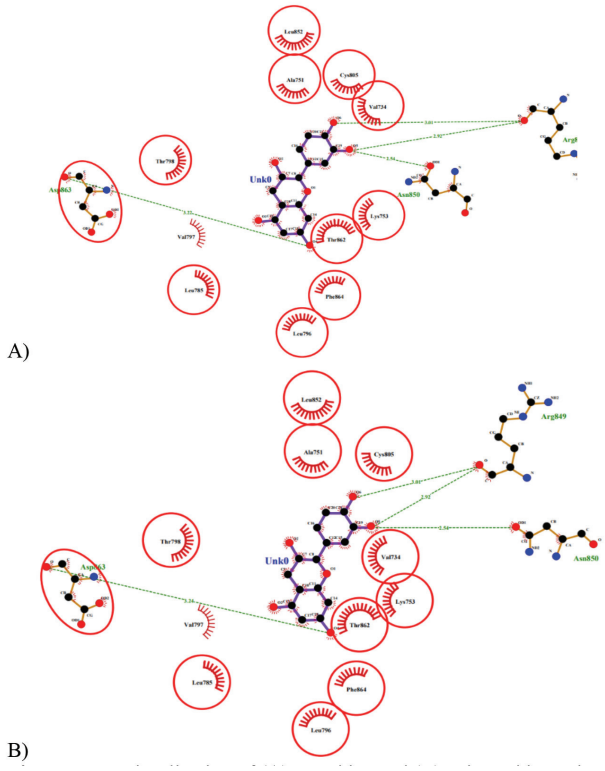


**Figure 2.** 2D visualization of (A) catechins and (B) epicatechin against receptors. The red circle indicates hydrophobic interactions between amino acids and ligands that interact on the same side of the receptor as the natural ligand. The dashed green line indicates hydrogen interactions between amino acids and ligands that interact on the same side of the receptor as that of the natural ligand.

**Table 3. Prediction of ligand toxicity**

| Ligand name | herG | | Carcinogenicity | | Acute oral toxicity | |
|---|---|---|---|---|---|---|
| | Category | Score | Category | Score | Category | Score |
| 2-Methoxy-4-vinyl phenol vinyl phenol | 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 |

*Lipinski rule violation

**Table 4. Molecular docking results**

| Ligand name | Energy affinity (kcal/mol) | Amino acid residues | 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 and 3.04, respectively |
| Linolenic acid | -7.6 | leu800; gly804; **leu726**; leu852; **val734**; **lys753**; **thr862**; **phe864**; **leu796**; **met774**; **leu785**; **asp863**; thr798; **met801**; ala771 | 14 | 2 | met801 2.79 and 2.92, respectively |
| 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 and 2.97, respectively |
| 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**; **leu796**; **asp863**; **gly729**, thr798; **met801**; gly727 | 13 | 3 | met801 2.54; asp863 3.21; leu726 2.86 |
| (+)-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 |

Bold part: Amino acids on the binding site

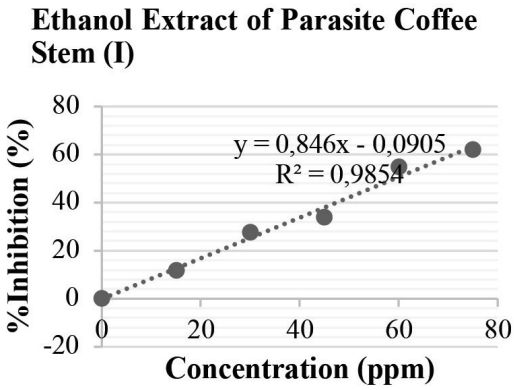


**Figure 3.** Diagram of antioxidant activity (DPPH) of the ethanol extract of parasite coffee stem. The IC50 value of 59,736 ppm indicates strong antioxidant activity

*Isolation of secondary metabolites*

After isolation of the extract of the coffee stem parasite in 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 of 16.073 min, the peak was the highest in the analytical spectrum with the highest 100% abundance.

The identification results indicated 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 provided a lib score (similarity) of 94.9%. The fragmentation peak (Figure 7) indicates that the fragmentation of the bis (2-Ethylhexyl) phthalate compound indicates the presence of a base peak at m/z 149, which is the peak of the molecular ion itself.

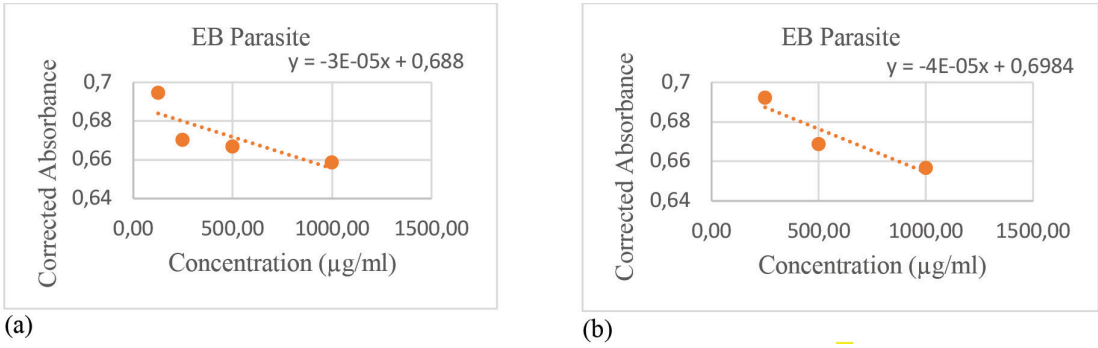


**Figure 4.** Parasite extract test results against (a) HeLa and (b) MCF-7 cells. The IC50 value of this coffee parasite extract against cancer cells was low or inactive

**Table 5. Antioxidant activity test data (DPPH) of the ethanol extract of coffee stem parasites**

| 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 |

DPPH: 2,2 diphenyl-1-picrylhydrazyl

**Table 6. Identification of compounds detected by GC-MS in fraction**

| | |
|---|---|
| Retention time (minute) | 16,073 |
| Area | 4303860.41 |
| Concentration (%) | 100% |
| Probability (%) | 100% |
| Compound Name | Bis (2-Ethylhexyl) phthalate |
| Score lib | 94.9% |

GC-MS: Gas chromatography-mass spectrometry

## DISCUSSION

*Findings of in silico test*

The Lipinski's 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 40-130.[11] Bioavailability analysis was carried out according to Lipinski's rule. This test is used as a guide for evaluating the drug design. Compounds that are ideal drugs must be adequately absorbed, distributed, metabolized, and excreted by the body. Compounds with an atomic mass exceeding 500 Da can reduce the passive diffusion ability of molecules because large molecules are difficult to penetrate cell membranes and take a long time to absorb.[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 affects the capacity of a compound to cross the lipid bilayer membrane. Quercetin, rutin, aviculin, (-)-epicatechin-3-O-gallate, and (-) epigallocatechin-3-O-gallate are predicted to require more energy for absorption across the lipid bilayer membrane due to hydrogen bonding. In addition, the high hydrogen capacity requires more energy for the absorption process.[11]

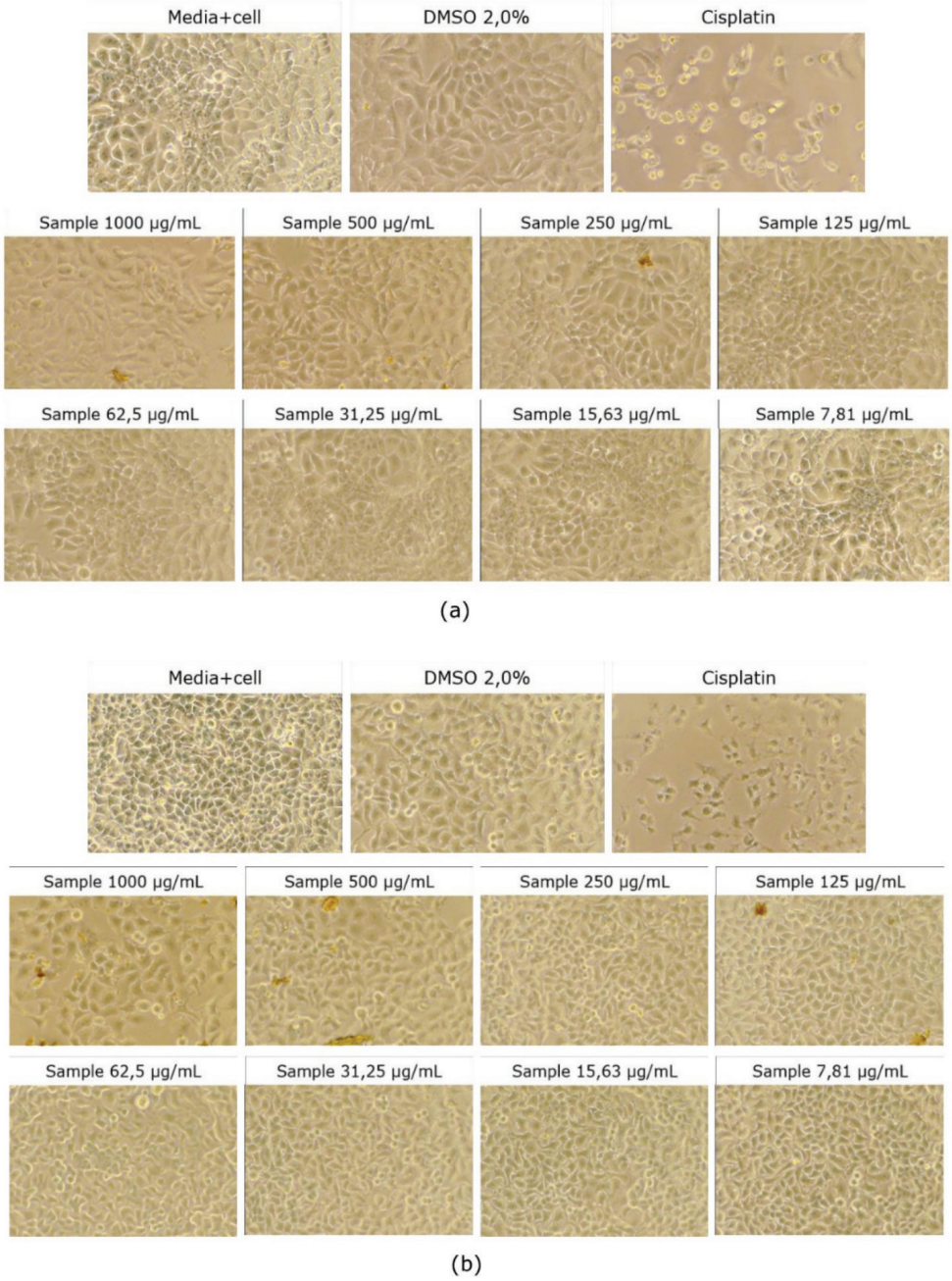


**Figure 5.** Documentation of the morphology of parasite extract test results (a) Hela cells (b) MCF-7 cells

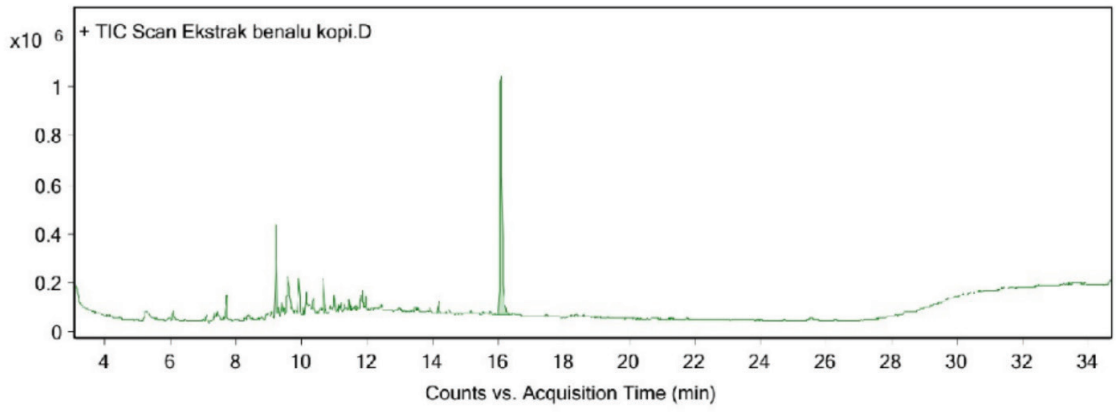


**Figure 6.** The spectrum of separation in GC-MS analysis shows an RT of 16.073 min, indicating 100% of bis (2- Ethylhexyl) phthalate
GC-MS: Gas chromatography-mass spectrometry, RT: Retention time

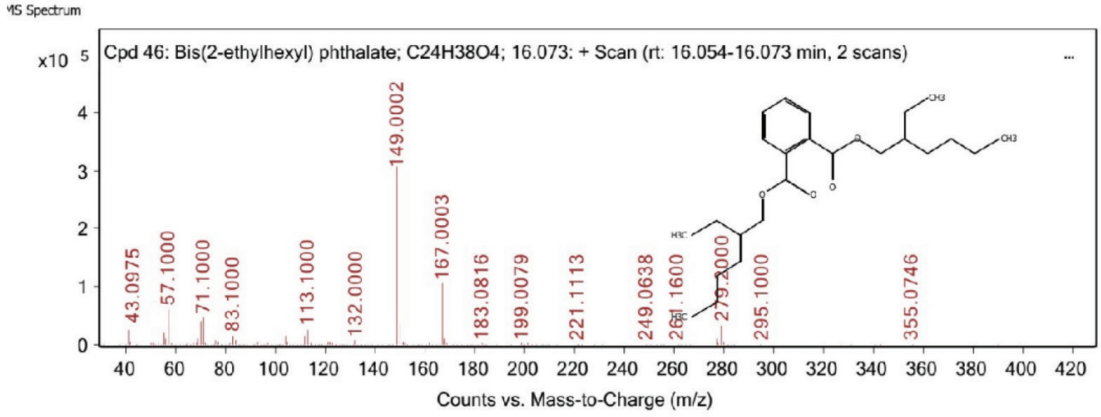


**Figure 7.** Peak fragmentation of compounds in GC-MS analysis showing the fragmentation of the bis (2-Ethylhexyl) phthalate
GC-MS: Gas chromatography-mass spectrometry

The log *p* value reveals a compound's hydrophobicity and lipophilicity. 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; thus, the compound is challenging to enter the cell because it is trapped in the lipid bilayer. Drugs will be distributed more widely, thereby 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.

The molar refractivity value indicates the distribution of the compound. Values between 40 and 130 indicate good distribution and absorption.[13] All 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 have 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 proceed.

Tested ligands except rutin are continued to determine 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 encodes a K+ ion channel that is involved in cardiac repolarization activity. If drug toxicity causes blocking of herG, sudden cardiac death occurs due to abnormal heart muscle repolarization.[14] Accordingly, 1,2-benzenedicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester; aviculin; (-)-epicatechin-3-O-gallate; and (-)epigallocatechin-3-O-gallate were predicted to have adverse effects on herG.

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

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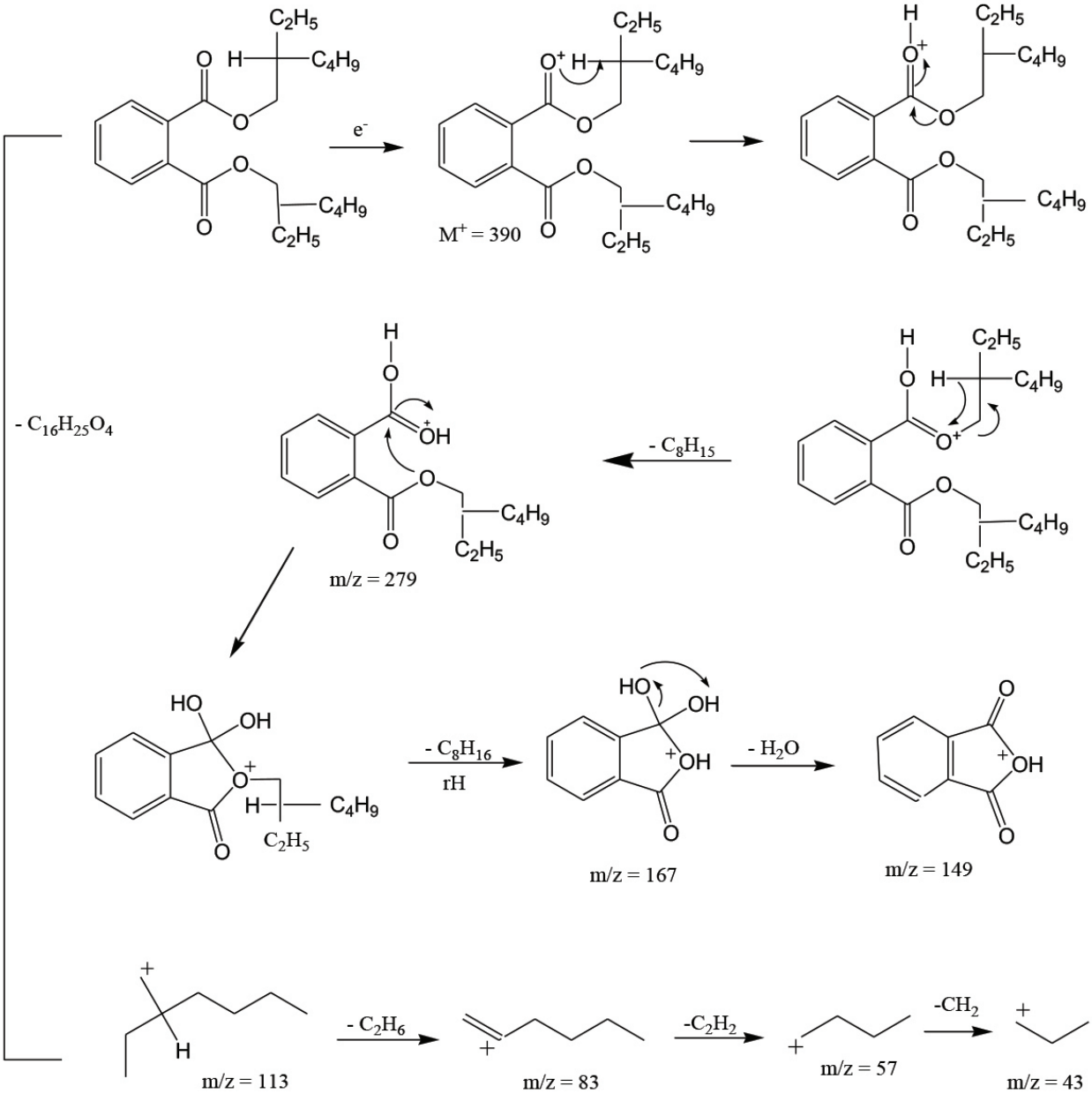


**Figure 8.** Fragmentation of bis (2-Ethylhexyl) phthalate compound

due to abnormal heart muscle repolarization.[14] Accordingly, 1,2-benzenedicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester; aviculin; (-)-epicatechin-3-O-gallate; and (-)epigallocatechin-3-O-gallate were predicted to have adverse effects on herG.

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There are four classifications of 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 tended to be toxic, while Categories 3 and 4 were non-toxic.[16] Quercetin compounds are included in category 2, and they 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.

Negative values of affinity energy indicate the tendency of a compound to form spontaneous bonds so that the reaction does not require energy or is exothermic.[17] Catechins and epicatechin have the most negative energy, this means that these two ligands interact better with the HER2 receptor than breast cancer therapy drugs.

Several amino acids are associated with catalytic activity, including leu726; val734; lys753; thr862; phe864; leu796; met774; leu785; ser783; gly729; asp863; met801.[18] These 12 amino acids were detected in all visualized ligands. This visualization shows that natural and other ligands bind to the same side to produce the same affinity as natural ligands in inhibiting HER2 protein.[19]

The more the number of hydrophobic bonds, the more negative the affinity energy. The greater the number of 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 epicatechin have the most hydrogen bonds, the energy required is not as large as that required for other ligands. Therefore, there is no relationship between the quantity of hydrogen bonds and the quantity of hydrophobic bonds to affinity energy. 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 distances among other ligands, their interactions are still more potent than those of 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 anticancer agents because of their better binding affinity than commercial therapeutic drugs.

*Antioxidant activity*

The difference in absorbance between the absorbance of the sample and that of DPPH was measured using an ultraviolet-vis spectrophotometer. The DPPH technique (2,2-diphenyl-1-picrylhydrazyl) was used to quantitatively measure the antioxidant activity. The DPPH method is a test method to ascertain antioxidant activity to fend off free radicals. The percentage inhibition of DPPH free radicals by ethanol extract served as a measure of its antioxidant activity. A linear regression equation (Y= aX + b), where Y is 50, denoting 50%, and X is the $IC_{50}$ value of the test sample, can be used to obtain the $IC_{50}$ value. According to the $IC_{50}$ value, the ethanolic extract has a strong antioxidant activity.[5]

*Anticancer activity*

According to The National Cancer Institute, the $IC_{50}$ value of this coffee parasite extract is low or inactive.[22] The anticancer activity increased as the $IC_{50}$ value decreased. Very powerful anticancer substances have an $IC_{50}$ value of less than 50 ppm, strong anticancer substances have an $IC_{50}$ value of 50-100 ppm, adequate anticancer substances have an $IC_{50}$ value of 100-150 ppm, and a weak anticancer if it is between 151-200 ppm.[23] The coffee parasite extract is inactive as anticancer because it's $IC_{50}$ value more than 150 ppm.

*Isolation of secondary metabolites*

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 $C_8H_7O_4^+$, as shown at 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 occurs in carbon and oxygen bonding in ester compound. The release of $C_2H_2$ from $C_6H_{11}$, followed by the release of $CH_2$ to generate $C_3H_7^+$, as observed at 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 is a secondary metabolite compound that belongs to the fatty acid group. Bis (2-Ethylhexyl) phthalate is cytotoxic, and it can damage cancer 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 that the log *p* value of bis(2-Ethylhexyl) is 6.4330, which is more than five, indicating high hydrophobicity. Thus, the compound will be challenging to enter the cell because it is trapped in the lipid bilayer. Drugs will be distributed more widely, thereby increasing their toxicity. The bis(2-Ethylhexyl) toxicity was included in the strong inhibitor category with a score of 0.8276. It causes blocking of herG; there will be sudden cardiac death occurs due to abnormal heart muscle repolarization.

## CONCLUSION

An *in silico* study of the coffee stem parasite [*S. ferruginea* (Roxb. ex Jack) Danser] showed that several flavonoids and fatty acid compounds have better potential as HER2 inhibitors than cyclophosphamide. The *in vitro* test results showed that the coffee stem parasite extract has potent antioxidant activity with an $IC_{50}$ value of 59,7359 ppm. However, it is not active against HeLa and MCF-7 breast cancer cells. Isolation of secondary metabolites from the extract of coffee stem parasites revealed that they contained bis (2-Ethylhexyl) phthalate compounds, which are toxic if used as anticancer drugs. Although coffee stem parasite extract does not function as an anti-cancer agent, its antioxidant activity has potential for other applications.

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*Ethics*

**Ethics Committee Approval:** This article does not contain any studies with human or animal subjects.

**Informed Consent:** Informed consent is not applicable.

*Authorship Contributions*

Concept: D.R., G.F., Design: D.R., G.F., T.J., Data Collection or Processing: G.F., E.I., Y.A.W., Analysis or Interpretation: D.R., G.F., T.J., Literature Search: G.F., E.I., Y.A.W., T.J., Writing: D.R., G.F.

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