Identification and anti-cancer activity of polar isolates leaf of coffee parasite (*Scurulla ferruginea (roxb. ex jack*) danser

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Abstract. *This study aims to determine the potential for polar isolates from the leaves of the coffee parasite scurulla ferruginea (Roxb. Ex Jack) Danser as a natural anticancer. Based on the results of the isolation of leaf polar extracts, it was found that most of them contained flavonoids. Identification of compounds in polar isolates using FT-IR revealed that the flavonoid derivatives found were catechins and quercetin. The isolate activity test against HeLa cervical cancer cells and MCF-7 breast cancer cells resulted in IC_{50} values of 1,366.67 µg/mL and 1.997,50 µg/mL, respectively.*

Keywords : coffee parasite, anticancer, isolation, polar isolates, flavonoid.

Introduction

One of the parasitic plants that have an important role as a medicine is the parasite attached to coffee,which is the *Scurrula ferruginea Danser species*. Researchers reported that the leaf extract of coffee parasite has a high antioxidant content [1,2]. In particular, researcher stated that the extract of the coffee bean parasite showed extraordinary level of antioxidant properties and moderate level of antibacterial activity against gram-positive and gram-negative bacteria. Parasite coffee leaves have been shown to have immunomodulatory properties, anti-inflammatory, antidiabetic, antimicrobial, antihypertensive, antioxidant, antidiarrheal, and hypolipidemic activities [3].

Anti-bioactivity test on coffee parasite leaves has not obtained satisfactory results. Antibacterial activity test result of the disc diffusion method with an extract concentration of 1% against *Staphylococcus aureus, Salmonella typhi, Streptcoccus viridans, Escherichia coli, Streptococcus mutans, and Bacillus aureus bacteria* showed the moderate level of ability to inhibit bacterial growth. Based on the activity test results using the microdilution method, the MIC and MBC values showed that the leaf extract of the coffee parasite plant was only as an inhibitor. The results of the antibacterial activity of the parasite coffee leaf against three bacteria using the disc diffusion

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method for *S. viridians, S. mutans* bacteria were also included in the "moderate" level. Activity test using microdilution method of MIC value also showed only as inhibitory properties [4].

Previous researcher has succeeded in isolating natural flavonols from the ethyl acetate fraction of *Scurrula ferruginea Danser (Loranthaceae*) namely quercetin and quercitrin, flavonol glycosides 4 -O-acetylquercitrin. Flavonoid compounds that have been isolated from coffee parasites are linamarin gallate, walsurside B, catechins, epicatechins, epicatechin 3-O-gallate, epicatechin 3-O-(3-O-methyl) gallate, epicatechin 3-O-(3,5 -O-dimethyl) gallate, epicatechin 3-O-(3,4,5-O-trimethyl) gallate, quercetin 3-O-β-D-glucopyranoside, rutin, peltatoside, -(-) catechin-7-O- rhamnoside, catechin-3-O-rhamnoside and 4'-methoxy-catechin-7-O-rhamnoside [1,5].

Flavonoid compounds are known to have many functions for human health such as antioxidant, anti-inflammatory, antivirus, antibacterial, and others. Flavonoids represent various types of compounds found in nature. Quercetin itself is generally well known in the pharmaceutical world. Some researchers found that quercetin has antimicrobial, antiviral, anticancer, antioxidant, anti-melanogenic and anti-inflammatory [6-9]. For this reason, in this study, the polar fraction of the parasite coffee leaf was isolated, and its bioactivity was tested against cancer cells.

Methodology

Materials

Leaves of the coffee plant parasite *Loranthus Parasiticus (L.) Merr*. from Sidikalang District, Dairi Regency. N-hexane, ethyl acetate, aquadest, silica gel 60 GF254, methanol, ethanol, microtube, tube, T-flask, Cisplatin, Dimethyl sulfoxide (DMSO), Phosphate buffered saline (PBS), 9. PrestoBlue™ Cell Viability Reagent, Roswell Park Memorial Institute Medium (RPMI), Fetal Bovine Serum (FBS), Trypsin-EDTA, Trypan Blue.

Methods

The process of extracting the active compounds from the leaves of the coffee plant parasite is performed with the maceration method using methanol as solvent**.** The thick extract of the plant Loranthus parasiticus L. Merr was separated using Vacuum Liquid Chromatography (VLC). For the isolation of secondary metabolites, the separation by Column Chromatography used silica gel 60 F254 (0.040-0.063 mm as the stationary phase) with N- Hexa:Ethyl Acetate (1:1) and ethyl acetate:methanol (1:1) as eluents.

For the anticancer test, the cell culture to be used was placed in a 96 well plate and then incubated (at 37°C and 5% CO_2 gas until the percentage of cell growth reached 70%). Cells were sampled and then incubated (for 48 hours at 37°C and 5% CO_2). Presto blue reagent was added to the cells. Absorbance measurement using Multimode Reader.

Results and discussion

The separation of the active compounds contained in the parasite leaves was carried out in 2 steps; the phase of fractionation of polar compounds by liquid Vacuum Liquid chromatography (VLC) and isolation using Gravity Column Chromatography (GCC). The methanol extract of the leaves of the coffee plant parasite was eluted with the eluents of N- hexane:ethyl acetate and Ethyl Acetate:Methanol in various ratios. Furthermore, TLC results showed a single stain in the F7 and F15 fractions, then the isolates were identified by FTIR and NMR.

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Identification of the structure of polar isolate compounds using the FT-IR instrument (Figure 1) aims to analyze the types of compounds contained in the isolates based on functional groups. The identification result for the first isolate, it was found a wide absorption in the 3322 cm $^{-1}$ which is thought to be absorption from the OH group. In the area of 2944 cm $^{-1}$ sharp band and weak intensity indicated the presence of aliphatic CH groups. In the area of 1448 cm^{-1} sharp bands indicate the presence of a C=C group (aromatic ring). Moreover, the area of 1113 cm^{-1} shows the absorption of the C-O-C group and in the area of 1020 cm $^{-1}$ shows the absorption of the C-C group. The IR spectrum of the polar isolate of the coffee parasite leaf shows in Figure 1.

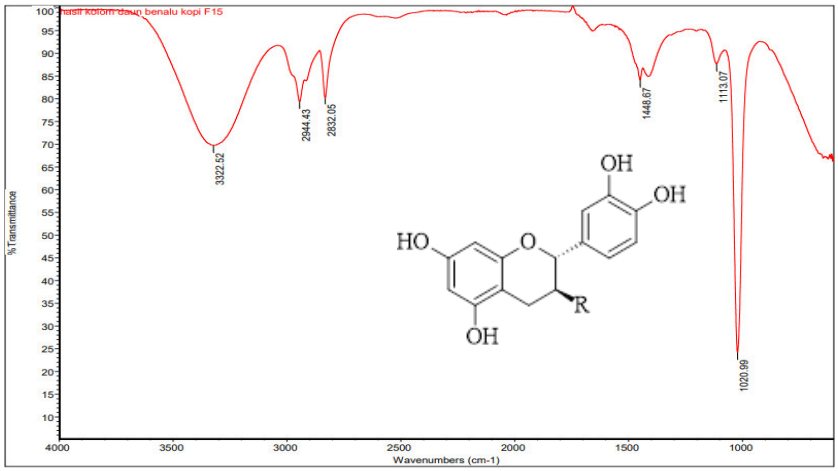

Figure 1. IR Spectrum of polar isolate leaf parasite coffee (*Lornthus parasiticus* (L.) *Merr* .)

Based on spectrum data, it can be concluded that the compounds contained in the polar isolate F_{15} of the leaves of the plant *Loranthus parasiticus* (L.) *Merr.* It is suspected contains catechin-type flavonoid compounds based on previous research [11] which has the same IR spectrum as the results obtained in the coffee parasite leaf as shown in Figure 2 .

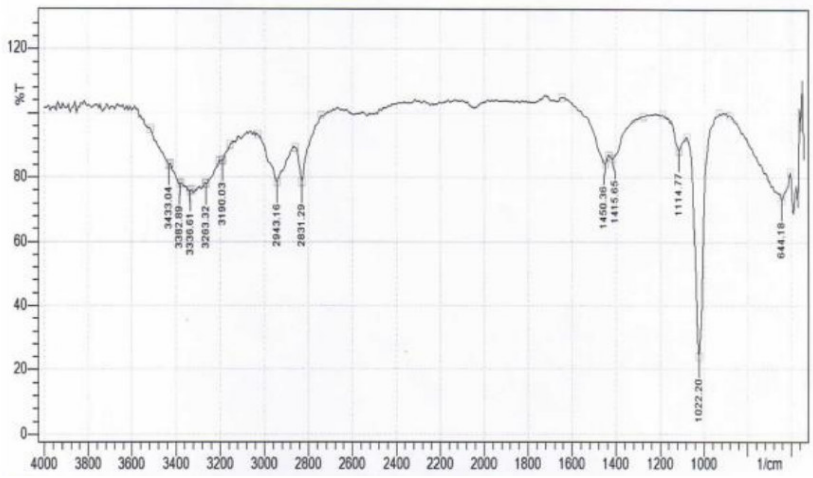

Figure 2. IR Spectrum of the nusa indah bunga ethyl acetate fraction [11].

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The results of the identification for the second isolate formed a wide absorption in the 3331 cm^{-1} area which was thought to be absorption from the O-H group that indicate contained an OH group. There is a C-H bond at 2946 cm $^{-1}$ and absorption at wave number at 1410 cm^{-1} indicating the C-C sp2. Absorption group at a wavelength of 800 cm^{-1} showed the presence of substituted benzene. Absorption at a wavelength of 1648 cm^{-1} indicates the presence of a carbonyl group (C=O). The absorption at 1307 cm^{-1} and the fingerprint region at wave number 1016 cm^{-1} indicate the absorption of the C-O functional group. The IR spectrum of the polar isolate of the coffee parasite leaf can be seen in Figure 3.

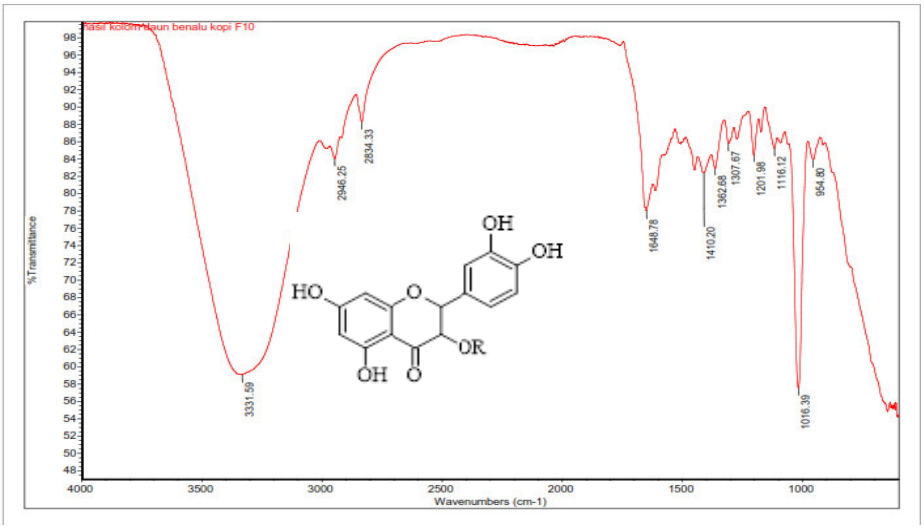

Figure 3. IR Spectrum of parasite coffee leaf isolate *(Loranthus parasiticus (L.) Merr.)*

Based on the spectral data, it can be concluded that the compounds contained in the two polar isolates of the leaves of the plant *Loranthus parasiticus* (L.) *Merr.* it is thought to be a quercetin-type flavonoid compound. The IR spectrum of isolates on the leaves of parasitic flavonoid compounds from the tea parasite plant (*Scurulla arthropurpurea*) has similarities with the IR spectrum of quercetin research that has been carried out by previous research [11].

The presence of catechin and quercetin compounds as flavonoid derivatives in coffee parasite was strengthened through the ^{1}H-NMR spectrum as shown in Figure 4. The presence of a proton peak of C sp2 (=CH-) δH 6.228; 6.411; 6.97; 7.367; 7.381 and 7.9 ppm. Protons at δH 6.228; 6.411 and δH 7.9 are singlet and at δH 7.367; 7.381 ppm is doublet, this indicates that the positions of these protons are meta to each other in ring A, while in ring B there is an ortho position with each other. The presence of a shift of C sp3 (CH_2 -) at a shift of about 1 to 2 ppm indicates that this isolate is closer to the structure of catechins. And peaks at shifts of 3 to 4.4 ppm allow for the presence of a glucose structure. In other words, this ^{1}H-NMR spectrum characterizes the spectrum for a-(-) Catechin-7-O-rhamnoside compound. However, the data were not supported by ^{13}C-NMR, because of the limited number and degree of purity of the isolates, identification could not be continued.

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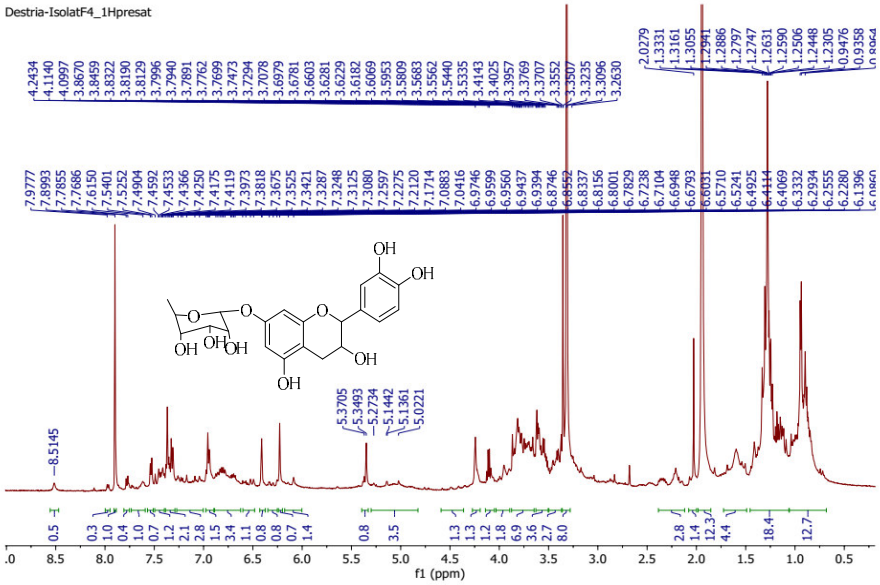

Figure 4. Spectrum of ^{1}H-NMR isolate of leaf parasite coffee *(Loranthus parasiticus (L.) Merr.)*

Extract activity studies were followed by cytotoxicity studies against HeLa cervical cancer cells and MCF-7 breast cancer cells. The IC_{50} value indicates the concentration level that inhibits cell proliferation by 50% and indicates the compound's cytotoxic potential. The higher the IC_{50} value, the less toxic the compound.

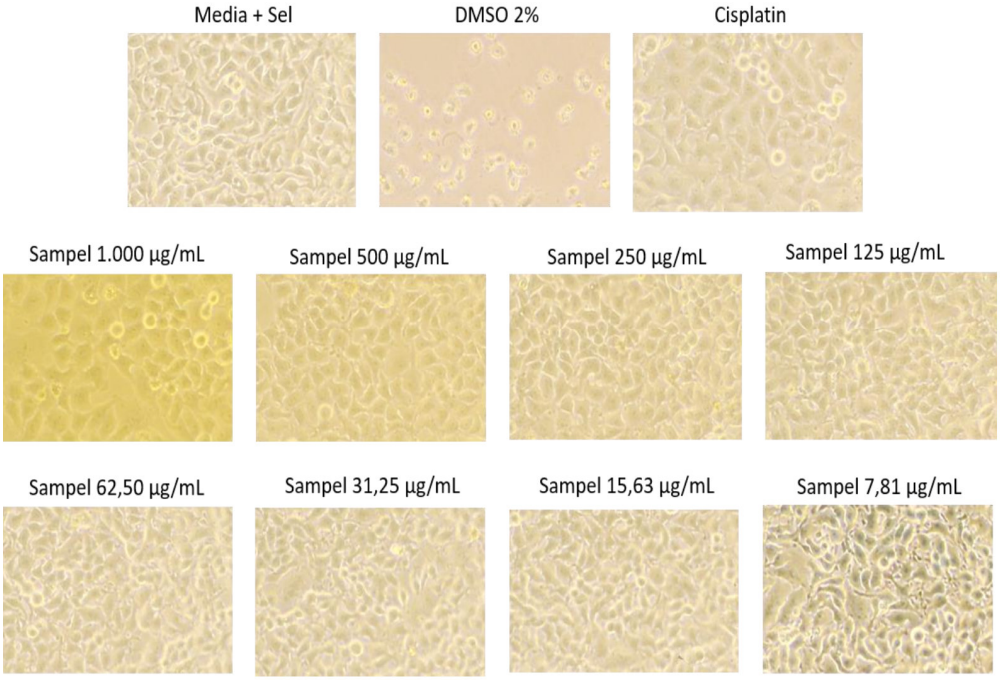

Figure 5. Test result of HeLa cell morphology F14

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Toxicity test of coffee parasite extract on HeLa cervical cancer cells in Figure 5 and MCF-7 breast cancer cells in Figure 6 resulted in IC_{50} of 1,366.67 g/mL and 1,997.50 g / mL, respectively. These results indicate that the isolates of coffee bean parasite at this concentration were able to kill HeLa cells and MCF-7 breast cancer cells by 50% of the initial population. Based on the NCI (National Cancer Institute) standard, a compound is said to have the potential to be further developed as an anticancer if it has an IC_{50} value of 20 g/mL. Thus, it can be concluded that the coffee parasite isolate was able to inhibit the growth of HeLa cells but lacked potential as an anticancer agent. Based on The National Cancer Institute (NCI) the IC_{50} value of this coffee parasite extract is classified as weak or inactive [12].

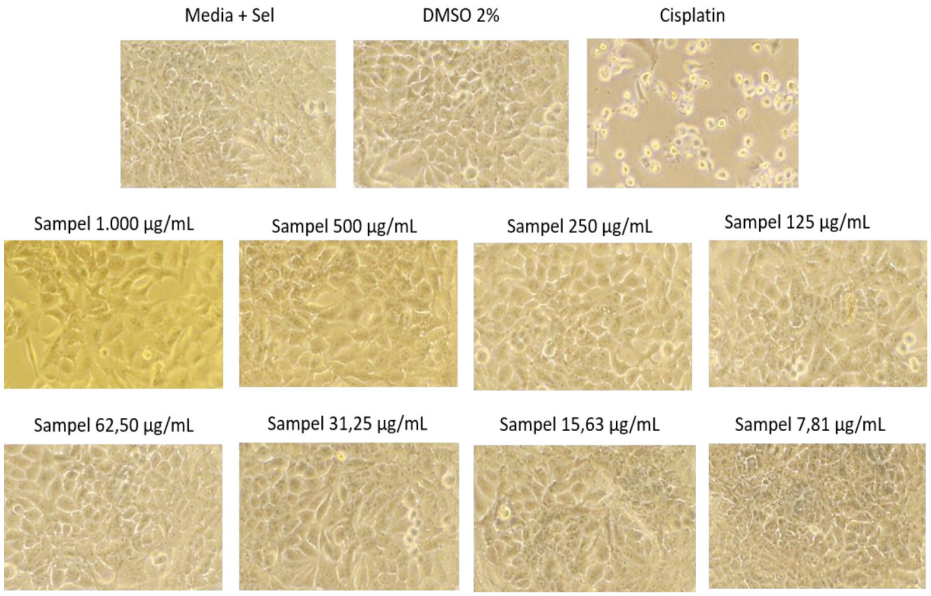

Figure 6. Test result of MCF-7 cell morphology F14

Conclusion

The results of the isolation of secondary metabolites from the identification by IR showed that the isolate was catechin and was strengthened by the ^1H NMR spectrum, indicating that the compound was a spectrum for the compound a-(-) Catechin-7-O-rhamnoside. Moreover, quercetin-type flavonoid compounds were also found, but it could not be confirmed by NMR spectra because there were still a few impurities in the isolate. These two coffee parasite isolates had IC_{50} values of 1.366.67 g/mL and 1.997.50 g/mL, respectively, which means they are not active against HeLa cervical cancer cells and MCF-7 breast cancer cells.

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