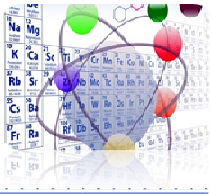
**Indonesian Journal of Chemical Science and Technology (IJCST)**

State University of Medan, https://jurnal.unimed.ac.id/2012/index.php/aromatika

IJCST-UNIMED 2023, Vol. 07, No. 2 Page; 175 – 181

Received : May 13th, 2024 Accepted : July 28th, 2024 Web Published : July 31st, 2024



# FLAVONOID ISOLATION AND TOXICITY TEST USING THE BSLT (*Brine Shrimp Lethality TestI)* METHOD FROM PARASITE FLOWER COFFE EXTRACT (*Loranthus ferrugineus* Roxb.)

Destria Roza*, Sedianta Girsang

*Department of Chemistry, Faculty of Mathematics and Natural Sciences, State University of medan, Jl. Williem Iskandar Pasar V Medan Estate, Medan 20221, Indonesia*

**Corresponding author : destriaroza@unimed.ac.id*

**ABSTRACT**

*This research aims to isolate compounds from extracts of coffee parasite flowers (Loranthus ferrugineus Roxb.) and determine their toxicity activity using the BSLT (Brine Shrimp Lethality Test) method. The results of the identification of secondary metabolite compounds show that the coffee parasite flower extract contains alkaloids, saponins, tannins and flavonoids. The results of the toxicity activity of the coffee parasite flower extract using the BSLT method show that the extract has high bioactivity and has an $LC_{50}$ value of 93.4266 µg/mL. Meanwhile, isolation of flavonoid compounds from coffee parasite flower extract (Loranthus ferrugineus Roxb.) based on infrared spectroscopy (IR) analysis showed the presence of vibrations of the O-H, C-H, C-O, C-C, and C-O groups which indicated the presence of flavonoid group compounds.*

Keywords **:** Coffee parasite Flower (*Loranthus ferrugineus* Roxb.), Flavonoid, Toxicity

## 1. INTRODUCTION

Plants have secondary metabolite compounds that are made up of specific small molecules and have a varied structure with different functions and roles in each type.[1] Secondary metabolites are organic compounds synthesized by plants and are a source of medicinal compounds, classified as alkaloids, terpenoids, steroids, phenolics, flavonoids and saponins.[2] The benefits of the content of this secondary metabolite compound have the potential to be antioxidant, anticancer, anti-inflammatory, antimicrobial, antidiabetic and antitripanosoma.[3] Phytochemical screening tests on samples of coffee leaf (*Loranthus ferrugineus* Roxb.) showed that the ethanol extract of coffee leaf contained secondary metabolite compounds of alkaloids, flavonoids, tannins and steroids.[4] Phytochemical screening tests on coffee bean leaf extract samples (Scurrula Parasitica L.) in the Gayo highlands showed that coffee bean leaves were positive for alkaloids, terpenoids, steroids, saponins, tannins and phenols.[5]



e-ISSN : 2622-4968, p-ISSN : 2622-1349 

Coffee parasite has several bioactivities so it is interesting to be developed into medicine. Coffee parasite is a parasitic plant on the coffee host that can damage the host plant. The chemical content of parasite includes flavonoids, alkaloids, saponins, phenols, and tannins.[6] Flavanoids are polyphenol compounds that are widely found in the epidermis of leaves, leaves, and skin of fruits and have an important role in human life as antioxidants, antimutagenics, antineoplastics and vasodilator activity.[7] Phenolic compounds have structures that can easily contribute hydrogen or electrons to acceptors such as reactive oxygen species or perocyl groups from fats, so they can dampen the activity of oxygen and perocyl radicals.[8] Based on various studies, the compounds in coffee beans that are suspected of having anticancer activity are flavonoids, namely the flavonoid compound quercetin, quercetin has antioxidant activity which is made possible by its highly reactive phenolic components, quercetin will bind free radicals so that it can reduce the reactivity of these free radicals.[4] The content of secondary metabolite compounds contained in coffee stem stem is flavonoids, alkaloids, tannins and terpenoids are the same as the content of secondary metabolites contained in coffee bean leaves. Based on previous research that has been conducted on the stems and leaves of coffee beans, further research is needed on the flowers of coffee beans (*Loranthus ferrugineus* Roxb.).

## 2. EXPERIMENTAL

### *2.1. Tools and Material*

The tools used are laboratory glass equipment, blenders, analytical balances, KLT chambers, UV lamps, rotary evaporators, Bunch funnels, Erlenmeyer bunchers, vacuum pumps, a set of liquid vacuum column chromatography equipment, a set of column chromatography equipment, hot plates, incandescent lamps and aerators. The ingredients used are coffee flowers (*Loranthus ferrugineus* Roxb) obtained from Sidikalang, Dairi Regency, North Sumatra. The process of extracting active compounds from coffee flowers uses methanol solvents. The isolation of flavonoids of condensed extract of parasite coffee flowers is used solvents n-hexane, ethyl acetate, methanol, ethanol, aquaades, silica gel 60 $F_{254}$, and silica merck 60 $F_{254}$. The process of identifying secondary metabolite components of coffee parasite flower extract uses Hydrochloric Acid, Sulfuric Acid, Anhydrotic Acetate, Chloride Ferry, and Magnesium powder. The toxicity test process of coffee parasite flower extract used artemia eggs, salina leach and yeast.

### *2.2. Research Procedure*

Extraction was carried out by the maceration method. A total of 1 Kg of coffee bean flowers (*Loranthus ferrugineus* Roxb.) that have been dried and mashed, macerated with methanol solvent. The methanol extracts that have been macerated are combined and filtered. The resulting filtrate is evaporated using a rotary evaporator so that a thick extract is obtained.

**Identification of secondary metabolit compounds**

1. Alkaloid

Coffee parasite flower extract *(Loranthus ferrugineus* Roxb.) 2 ml, then added with 2 drops of reagent/mayer reagent. Positive for alkaloids when white deposits form[9]

2. Uji steroid/triterpenoid

A total of 3 ml of coffee parasite flower extract (*Loranthus ferrugineus* Roxb.) is added 2-3 drops of anhydrous acetic acid, then stirred slowly for a while, then added 1-2 drops of $H_2SO_4$ 12M and observe what



e-ISSN : 2622-4968, p-ISSN : 2622-1349 

color arises. Triterpenoid positive when given a red-purple or red-red color while a green-blue color for steroids[10]

3. Tanin

A total of 3 ml of coffee parasite flower extract (*Loranthus ferrugineus* Roxb.) is added 1-2 drops of 1% $FeCl_3$ reagent. The presence of tannins will be indicated by the change in the color of the filtrate to blackish-green[10]

4. Saponin

A total of 3 ml of coffee parasite flower extract (*Loranthus ferrugineus* Roxb.) is added hot $H_2O$, cooled and then shaken for 10 seconds. After that, the changes that occurred were observed. Then 1 drop of 2N hydrochloric acid was added again and the changes that occurred were observed again. Positive result if stable foam appears for ± 10 minutes[10]

5. Flavonoid

A total of 3 mL of parasite flower extract is added 5 drops of 5% $FeCl_3$ solution and shaken vigorously. The formation of a blackish-green color after the addition of 5% $FeCl_3$ indicates the presence of flavonoid compounds [11]

a. Toxicity Activity Test

The toxicity test was carried out by preparing 1 gram of Artemia salina Leach larvae which were hatched in artificial seawater for 48 hours and given 40-60 watt incandescent lamp lighting. 10 48-day-old Artemia salina Leach larvae were tested with extracts at concentrations of 500, 100, 50 and 25 ppm with 3 replications and negative controls and observed after 24 hours.

b. Isolation of Flavonoid Compounds of Parasite Coffee Flower Extract (*Loranthus ferrugineus* Roxb.) Identification by Thin-Layer Chromatography (KLT)

Coffee parasite flower extract (*Loranthus ferrugineus* Roxb.) was carried out by stain analysis using silica plate as its resting phase and eluene n-hexane, ethyl acetate, and ethanol as its moving phase. The eluene used is made with several variations of n-hexane ratios: ethyl (10:0), (9:1), (8:2), (7:3), (6:4), (5; 5), (4:6), (3:7), (2:8), (1:9), (0:10), and ethyl acetate: ethanol (10:0), (9:1), (8:2), (7:3), (6:4), (5; 5), (4:6),(3:7), (2:8), (1:9), (0:10).

c. Separation by Liquid Vacuum Column Chromatography (KVC)

A total of 20 grams of flower extract of the coffee plant (*Loranthus ferrugineus* Roxb.) was fused with silica gel 60 merck 60 $F_{254}$ as the stationary phase with several types of eluene as its moving phase using N-hexane eluene: Ethyl Acetate with comparative variations (10:0), (9:1), (8:2), (7:3), (6:4), (5; 5), (4:6), (3:7), (2:8), (1:9), (0:10) and Ethyl Acetate: Ethanol with comparative variations (10:0), (9:1), (8:2), (7:3), (6:4), (5; 5), (4:6),(3:7), (2:8), (1:9), (0:10).

d. Separation by Gravitational Column Chromatography (KKG)

The combination of fractions with the same Rf pattern as the KLT result from KVC was further separated using column chromatography as the quiescent phase of silica gel 60 F254 (0.040-0.063 mm) and the moving phase in the form of the best eluent in the separation of the isolate stain used. Eluents dripping every 3 mL of the column were accommodated and each resulting fraction was analyzed by Thin-Layer Chromatography (KLT).



e-ISSN : 2622-4968, p-ISSN : 2622-1349 

## 3. RESULTS AND DISCUSSION

### *3.1. Identification of Secondary Metabolite Compounds*

Phytochemical screening is the initial stage of research that aims to provide an overview of the content of secondary metabolites contained in plants. The secondary metabolite compounds tested are alkaloids, steroids/triterpenoids, tannins, saponins and flavonoids. The results of phytochemical screening can be seen in table 1.

**Tabel 1.** Phytochemistry Test

| No | Parameter | pereaksi | warna | keterangan |
|---|---|---|---|---|
| 1. | Alkaloid | Mayer | White aggregate | + |
| 2. | Steroid/ Triterpenoid | Anhydrous acetic acid and $H_2SO_4$ | Red purple/green blue solution | - |
| 3. | Tanin | $FeCl_3$ 1% | Blackish Green Solution | + |
| 4. | Saponin | HCl 2N | Foamy | + |
| 5. | Flavonoid | FeCl 5% | Blackish Green Solution | + |

### *3.2 Toxicity Activity Test*

The toxicity test method used is the Brine Shrimp Lethality Test (BSLT) which aims to determine the concentration needed in the thick extract of the coffee flower (*Loranthus ferrugineus* Roxb.) in killing half of the initial population of test animals. BSLT is a preliminary test of a compound that has the advantage of faster, cheaper, easy processing because it does not require equipment and relatively few samples are used. The Brine Shrimp Lethality Test method is also useful for detecting the presence of toxic compounds in the process of isolating compounds from natural materials that have cytotoxic effects by determining the LC50 price of the active compound. LC50 is the concentration of a chemical compound in water that can cause 50% mortality in the test animal population. The LC50 value can be used to indicate the level of toxic effects of a compound so that it can also estimate its potential as an anticancer.[12]

Artemia salina L larval death probit from a thick extract sample of coffee parasite flower (*Loranthus ferrugineus* Roxb.), an LC50 value of 93.43 µg/mL was obtained. A compound is said to be toxic if the LC50 value is between 30-1000 µg/mL and a compound is said to be highly toxic if the LC50 value < 30 µg/mL. So the results of the study show that the thick extract of the coffee flower (*Loranthus ferrugineus* Roxb.). are toxic and have high bioactivity because they have a low LC50.

### *3.3 Isolation of Flavonoid Compounds of Parasite Coffee Flower Extract* (*Loranthus ferrugineus* Roxb.)

The isolation and purification of the viscous extract compound of coffee parasite flower (*Loranthus ferrugineus* Roxb.) was carried out by liquid vacuum chromatography (KVC) and gravity column chromatography (KKG) methods. Before the thickening is carried out, it is necessary to do a thin layer chromatography (KLT) first. The plates that have been soiled by the sample are then diluted with 2 mL of eluene in the chamber. Elucidating uses the comparison of n-hexane eluent: ethyl acetate (10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 and 0:10) and ethyl acetate: ethanol (10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 and 0:10). The results of the preliminary KLT of the thick extract of the coffee parasite flower (*Loranthus ferrugineus* Roxb.) with the ratio of n-hexane: ethyl acetate and the ratio of ethyl acetate: ethanol are presented in Figure 1 and Figure 2.



e-ISSN : 2622-4968, p-ISSN : 2622-1349 

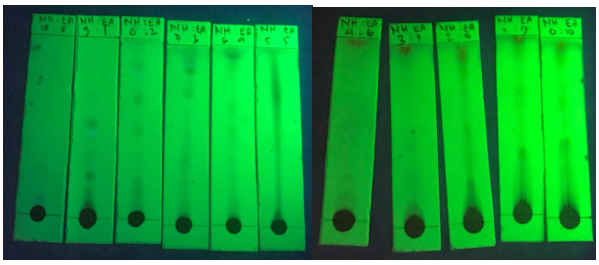


**Figure 1**. Preliminary KLT Results of Concentrated Extract of parasite Coffee Flower (*Loranthus ferrugineus* Roxb.) with N-Hexane: Ethyl Acetate ratio (10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 and 0:10)

**Figure 2.** Preliminary KLT Results of Concentrated Extract of Parasite Coffee Flower (*Loranthus ferrugineus* Roxb.) with Ethyl Acetate : Ethanol (10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 and 0:10)

Further separation was carried out using Liquid Vacuum Chromatogravy (KVC). The principle of this method refers to the separation of a mixture of compounds based on the difference in solubility in the stationary phase and the motion phase. Where the stationary phase will be held on the column while the motion phase flows through the column with the help of a vacuum. Dilution starts from non-polar eluene (n-hexane: ethyl acetate) to polar eluene (ethyl acetate: ethanol). The fractions obtained are grouped into nonpolar fractions and polar fractions. In the nonpolar fraction, namely fractions 1-11, 5 fractions were selected that had a fairly clear stain pattern, namely in the 7-11 fraction which was then identified and monitored using the KLT method in the ratio of eluen n-hexane:ethyl acetate (1:1). KLT stain pattern of KVC isolate of *Loranthus ferrugineus* Roxb flower. presented in figure 3.

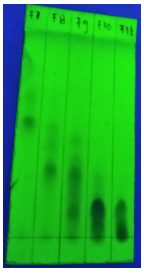


**Figure 3.** KLT stain pattern of KVC isolate of *Loranthus ferrugineus* Roxb flower. In fractions 7-11 using the eluen N-hexane:ethyl acetate ratio (1:1)

The separation of advanced compounds was carried out using the Gravitational Column Chromatogravi (KKG) method. The KKG was chosen because of its purer and more selective nature of separation. Factions 9, 10 and 11 from the results of the KVC were chosen to continue the separation of the



e-ISSN : 2622-4968, p-ISSN : 2622-1349 

KKG. After the inoculation 49 fractions were obtained and then identified with KLT with the eluene hexane n-hexane: ethyl acetate (1:1). The results of the fractional KLT from the column chromatographic separation are presented in figure 4.

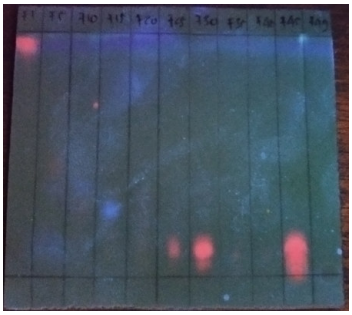


**Figure 4.** Fractional KLT Results from Column Chromatography Separation

From the results of the identification of compounds using the KLT method, fraction 25 was selected to be analyzed using the FTIR (Fourier Transform Infra Red) instrument presented in figure 5.

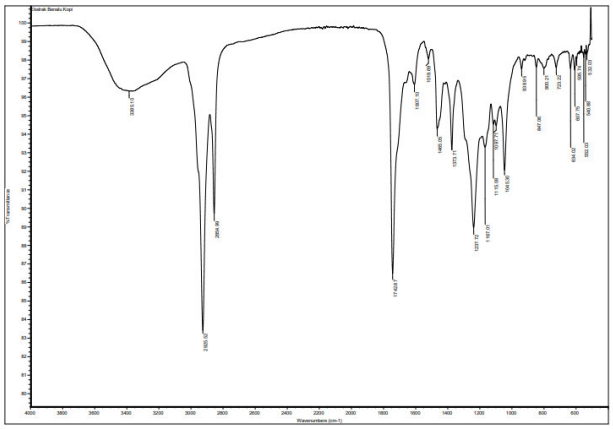


**Figure 5.** Fractional IR Spectrum 25

Based on the results of the infrared spectrum identification, the fractionation compounds in Figure 4.10 show the absorption of several functional groups. In the infrared (IR) spectrum, it can be seen that there is a widening band at the wave number of 3385.10 $cm^{-1}$ which is a vibration of the hydroxy group tendon that can form hydrogen bonds indicating the presence of O-H. At the numbers 2925.52 cm-1 and 2854.99 cm-1 are detected as C-H functional groups of alkanes. At the number 1742.67 cm-1 is detected as the C=O aldehyde functional group. At wave numbers 1607.10 cm-1, 1519.69 cm-1 and 1465.05 are detected as aromatic C=C functional groups. At wave number 1373.71 cm-1 is detected as a C-H group. At wave numbers 1167.01 $cm^{-1}$, 1115.08 $cm^{-1}$, 1097.71 $cm^{-1}$, and 1045.30 $cm^{-1}$ are detected as C-O clusters. The results of infrared spectroscopic analysis of compounds isolated from coffee parasite flower extract (*Loranthus ferrugineus* Roxb.) showed the presence of vibrations of the O-H, C-H, C=O, C=C, and C-O groups which showed the presence of flavonoid group compounds.



e-ISSN : 2622-4968, p-ISSN : 2622-1349 

## 4. CONCLUSION

The results of phytochemical tests from coffee parasite flower extract (*Loranthus ferrugineus* Roxb.) were positive for alkaloids, tannins, saponins and alkaloids. The results of the toxicity test showed that the extract of coffee flower (*Loranthus ferrugineus* Roxb.) was toxic and had high bioactivity and had an LC50 value of 93.4266 µg/mL. The results of isolation from the extract of coffee bean flower (*Loranthus ferrugineus* Roxb.) based on infrared spectroscopic (IR) analysis showed the presence of vibrations of the O-H, C-H, C=O, C=C, and C-O groups which showed the presence of flavonoid group compounds.

## REFERENCES


1. Khafid, A. (**2023**). UJi Kualitatif Metabolit Sekunder pada Beberapa Tanaman yang Berkhasiat sebagai Obat Tradisional. *Buletin Anatomi dan Fisiologi*, 8(1).
2. Saifuddin, A. (**2014**). *Senyawa alam metabolit sekunder*. Yogyakarta: Deepublish.
3. Gunawan., Tatik C., Sobir., & Sulistijorini. (**2016**). Review : Fitokimi genus *Baccaurea* spp. *Bioeksperimen*. 2(2). 96-110.
4. Yulian, M., & Safrijal, S. (2**018**). Uji Aktivitas Antioksidan Daun Benalu Kopi (*Loranthus ferrugineus* Roxb.) dengan Metode Dpph (1, 1–Difenil-2-Pikrilhidrazil). *Lantanida Journal*, 6(2), 192-202.
5. Alviani, S., Adelia, R. F., Amri, Y., & Amna, U. (**2022**) Skrining Fitokimia Ekstrak Daun Benalu Kopi (*Scurrula Parasitica* L.) Dataran Tinggi Gayo. *Jurnal Kimia Sains dan Terapan*, 4(1), 9-14.
6. Sari, E.R., & Vera. F. (**2016**). Isolasi Dan Karakteristik Senyawa Flavonoid Tumbuhan Benalu Mangga (*Dendropthoe petandra* Miq). *Jurnal Ilmiah Bakti Farmasi*. 1(2). 15-24.
7. Piaru, S.P., Mahmud, R., Majid, A.M.S.A. & Nassar, Z.D.M. (**2012**). Antioxidant and antiangiogenic activities of the essential oils of Miristica fragrans and Morinda citrifolia. *Asian Pacific Journal of Tropical Medicine*. 5(4), 294 – 298.
8. Gupta, A.D. & Rajpurohit, D. (**2011**). Antioxidant and Antimicrobial Activity of Nutmeg (*Myristica fragrans*). In Preedy, V.R., Watson, R.R. & Patel, V.B. (eds). Nuts and Seeds in Health and Disease Prevention.831 – 838.
9. Susanti, N., Purba, J., & Simatupang, D. P. (**2021**). Increased Stability of Styrax benzoin Extract and Fraction with the Addition of Cosolvents. Journal of Physics: Conference Series. 1819(1), 1-5.
10. Meigaria, K.M., Mudianta, I.W., Martiningsih, N.W. (**2016**). Skrining Fitokimia Dan Uji Aktivitas Antioksidan Ekstrak Aseton Daun Kelor (*Moringa oleifera*). *Wahana Matematika dan Sains*: *Jurnal Matematika, Sains, dan Pembelajrannya*, 10(2), 1-11.
11. Lumowa, S. V., & Bardin, S. (**2017**). Uji fitokimia kulit pisang kepok (*musa paradisiaca* l.) Bahan alam sebagai pestisida nabati berpotensi menekan serangan serangga hama tanaman umur pendek. *Jurnal sains dan kesehatan*, *1*(9), 465-469.
12. Jelita, S.F., Gita, W.S., Michelle, F., Ade,Z., & Sandra, M. (**2020**). Uji Toksisitas Infusa Acalypha Siamensis dengan Metode *Brine Shrimp Lethality Test* (Bslt). *Farmaka*. 18(1). 14-22.




e-ISSN : 2622-4968, p-ISSN : 2622-1349 