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Identification of the Active Compound of Ethyl Acetate Fraction of Yellow Root Extract (Fatoua pilosa Gaudich)

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ABSTRACT

Yellow root *(Fatoua pilosa Gaudich*) is a weed plant that comes from the morceae family. This plant can grow up to 60 cm tall and can survive in dry soil conditions. Empirically, the yellow root plant is used by the people of East Flores Regency as an anti-diabetic drug. When using it, 2 grams of yellow root is boiled in 400 mL of water, then filtered and the water is drunk. This study aims to identify the active compounds found in yellow roots (Fatoua pilosa Gaudich) from East Flores Regency. The research was conducted in December 2024 at the Fisheries Product Technology Laboratory, Larantuka Teacher Training and Technology Institute and continued at the Central Jakarta Basic Health Laboratory. The main material used in this study was yellow root (Faoua pilosa Gaudich) which was taken from East Flores Regency. Sample extraction was carried out using the maceration method with a ratio of 1:10 between yellow root and 70% ethanol. The ethanol extract was then fractionated with ethyl acetate solvent, then the compounds were identified using LC-MS/MS. The research results show that the ethyl acetate extract of yellow root contains 8 active compounds with varying activities. These 8 compounds include 2,4-Pentadiynylbenzene, Propionaldehyde, beta-Lysylmethanediamine, Phellodenol-A, N-(1-adamantyl)-3-[16-[3-(1-adamantylamino)-3-oxopropanoyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadec-7-yl]-3 oxopropanamide, Ethyl 2-acetylheptanoate, Lupeol, Deferoxamine.

Keywords: yellow root, fatoua pilosa, LC-MS/MS

1. INTRODUCTION

Yellow root (Fatoua pilosa Gaudich) is a weed plant that comes from the Moraceae family. This plant grows up to 60 cm tall and can survive in dry soil conditions. Yellow root is found in the Philippines, Maluku, East Nusa Tenggara, Papua New Guinea, and Taiwan. According to (Dianto et al., 2015) this garden root can cure liver disease. Empirically, the yellow root plant is used by the people of East Flores Regency as an antidiabetic drug. To use it, boil 2 grams of yellow root with 400 mL of water, then strain and drink the water. The results of research conducted by (Nona et al., 2023) on yellow root samples showed that yellow root has antioxidant activity and is able to inhibit the activity of the α-glucosidase enzyme in vitro.

The many benefits of yellow root (Fatoua pilosa Gaudich) for health because it is believed that yellow root (Fatoua pilosa Gaudich) contains active compounds of secondary metabolites that act as traditional medicine. Based on research conducted by (Chiang et al., 2010) on F. Pilosa root samples, it is known to contain several secondary metabolite compounds classified as coumarin and triterpenoid compounds that have the potential to be antibacterial. against Mycobacterium

tuberculosis H37Rv. This study aims to identify the active compounds found in yellow root (Fatoua pilosa Gaudich) from East Flores Regency.

2. RESEARCH METHOD

This research was conducted in March 2026 at the IKTL Fisheries Product Technology laboratory and continued at the Central Jakarta Basic Health laboratory. The materials used in this study include, Yellow root (Faoua pilosa Gaudich) taken from East Flores Regency, 70% Ethanol, ethyl acetate, distilled water, cotton, filter paper, and aluminum foil. The tools used in this study include, analytical scales, Erlenmeyer flasks, grinders, shakers, separating funnels, funnels, micropipettes, evaporators, watch glasses, LC-MS/MS.

Sample Preparation.

The yellow roots were washed with running water and dried in an oven at 50°C for 24 hours. The dried yellow roots were ground into a 60-mesh powder using a grinder.

Sample extraction.

Sample extraction was carried out using the maceration method with a ratio of yellow root powder and solvent of 1:10. Maceration was carried out for 24 hours by soaking the yellow root powder in 70% ethanol solvent and shaking it at 150 rpm at room temperature. The mixture was separated and the filtrate obtained was concentrated using an evaporator to obtain a 70% ethanol extract.

Fractionation.

The 70% ethanol extract was fractionated by liquid-liquid extraction using ethyl acetate solvent. A total of 500 mg of the extract was dissolved in 100 mL of water, stirred until dilute and homogeneous, then put into a separating funnel and fractionated with 100 mL of ethyl acetate solvent. The mixture was shaken every 10 minutes for 3 times, then left for approximately 30 minutes to reach saturation conditions, so that the n-ethyl acetate and water layers were obtained. After the ethyl acetate layer was separated, the layer formed from the fractionation results was concentrated with an evaporator.

Identification of Active Compounds of Ethyl Acetate Fraction of Yellow Root Extract.

The fractionated ethyl acetate extract was analyzed using LC-MS/MS with the QMicro QAA 842 type. The liquid sample was inserted into the mobile phase in the LC column with a flow rate of 0.2 mL/min. The column temperature used was 40^0C and the end time was 35 minutes. Separation of chemical compounds occurred in the column with the help of a pump. The results of the analysis with LC were continued to MS to identify the chemical components. The type of mass analysis used was quadrupole with a scan mode for 5 seconds. The spectrum obtained showed the mass to charge ratio (m/z).

3. RESULTS AND ANALYSIS

Identification of active compounds of ethyl acetate extract of yellow root was analyzed using LC-MS/MS. The results of the identification of the ethyl acetate fraction of yellow root using LC-MS/MS in Figure 1 show a number of chromatograms with different retention times as characteristics of a compound. Based on the search for compounds from the ethyl acetate fraction chromatogram using the chemical compound data bank "Pubchem", data on 8 active compounds were obtained. (Table 1).

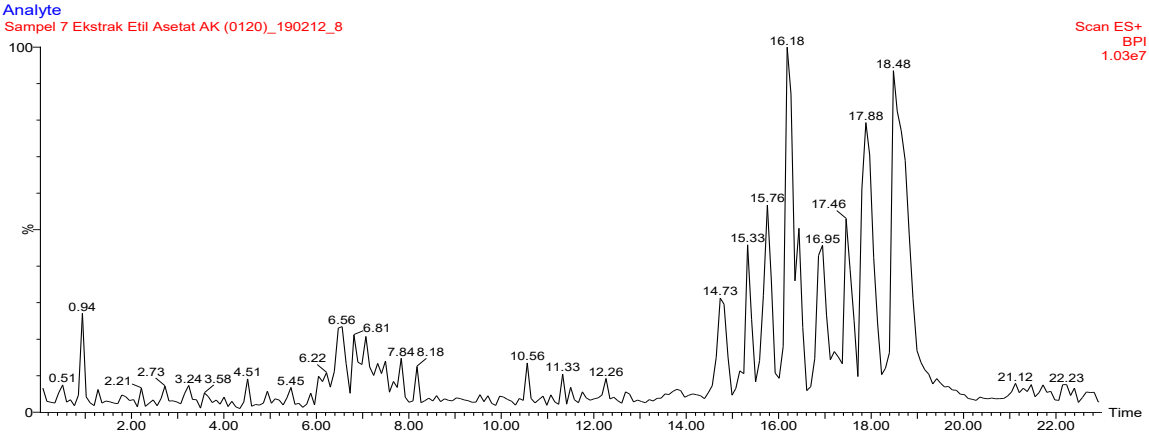

Figure 1. LC-MS/MS chromatography of the ethyl acetate fraction of yellow root

Table 1. Active compound content of the ethyl acetate fraction of yellow root

Peak	Retention Time (minutes)	Molecular Weight	Compound Name	Relative Abundance (%)
1	3.58	141.0704	2,4-Pentadiynylbenzene	8
2	5.45	59.0497	Propionaldehyde	10
3	6.22	175.1559	beta-Lysylmethanediamine	16
4	16.18	561.3612	Phellodenol-A	100
5	16.95	701.4489	N-(1-adamantyl)-3-[16-[3-(1-adamantylamino)-3-oxopropanoyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadec-7-yl]-3-oxopropanamide	48
6	17.46	201.1491	Ethyl 2-acetylheptanoate	54
7	17.88	426.72	Lupeol	85
8	18.48	187.1083	Deferoxamine	94

2,4-Pentadiynylbenzene

2,4-Pentadiynylbenzene is a compound that appears at a retention time of 3.58 minutes with a relative abundance of 8%. This compound has the molecular formula $C_{11}H_8$ and a molecular weight of 141.0704 g/mol.

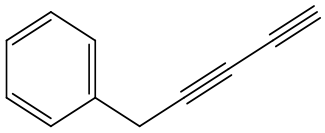

Figure 2. 2,4-Pentadiene Benzene compound

Propionaldehyde

This compound appeared at a retention time of 5.45 minutes with a relative abundance of 10%. The molecular formula of Propionaldehyde is C_3H_6O and its molecular weight is 59.0497 g/mol. According to (Michael et al., 2024), Propionaldehyde is found in natural smoothies and has antioxidant activity and can reduce blood sugar levels and pro-inflammatory gene expression in streptozotocin-induced diabetic rats.

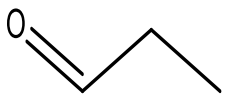

Figure 3. Propionaldehyde Compound

Beta-Lysylmethanediamine

Beta-Lysylmethanediamine (Bellenamine) is a compound that appears at a retention time of 6.22 minutes with a relative abundance of 6.22%. Beta-Lysylmethanediamine has a molecular formula of $C_7H_{18}N_4O$ and a molecular weight of 175.1559 g/mol. According to (De Clercq, 2000), beta-Lysylmethanediamine (Bellenamine) is an antibiotic that has antibacterial activity. This compound can inhibit Human Immunodeficiency Virus I (HIV I) infection with an IC50 value of 0.62 mg/mL. Beta-Lysylmethanediamine inhibits the secondary spread of HIV, although, unlike glycosylation inhibitors, bellenamine does not have a clear inhibitory effect on the glycosylation process. The structure of the beta-Lysylmethanediamine (Bellenamine) compound can be seen in Figure 4.

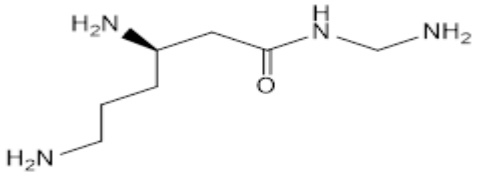

Figure 4. Beta-Lysylmethanediamine (Bellenamine) compound

Phellodenol-A

Phellodenol-A appeared at a retention time of 16.18 minutes with a relative abundance of 100% and was the most abundant compound in yellow root. This compound has a molecular formula of $C_{11}H_{10}O_4$ and a molecular weight of 561.3612 g/mol. Phellodenol-A belongs to the coumarin group. (Raj et al., 2011) stated that administering an extract containing flavonoids, alkaloids, and coumarins can improve insulin secretion in pancreatic β cells in mice. Coumarin is a type of phenolic compound that has antioxidant activity.

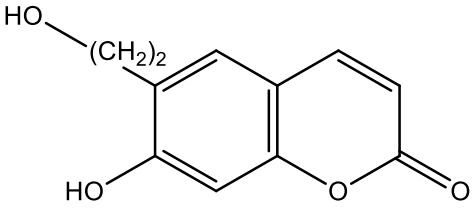

Figure 5. Phellodenol-A compound

N-(1-adamantyl)-3-[16-[3-(1-adamantylamino)-3-oxopropanoyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadec-7-yl]-3-

The compound N-(1-adamantyl)-3-[16-[3-(1-adamantylamino)-3-oxopropanoyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadec- 7-yl]-3- is a compound that appears at a retention time of 16.95% with a relative abundance of 48%. This compound has a molecular formula of $C_{38}H_{60}N_4O_8$ and a molecular weight of 701.4489 g/mol.

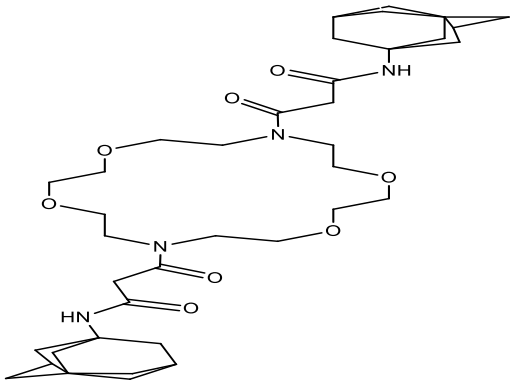

Figure 6. Compound N-(1-adamantyl)-3-[16-[3-(1-adamantylamino)-3-oxopropanoyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadec-7-yl]-3-

Ethyl 2-acetylheptanoate

Ethyl 2-acetylheptanoate is a compound that appears at a retention time of 17.46 minutes with a relative abundance of 54%. This compound has the molecular formula $C_{11}H_{20}O_3$ and a molecular weight of 201.1491 g/mol.

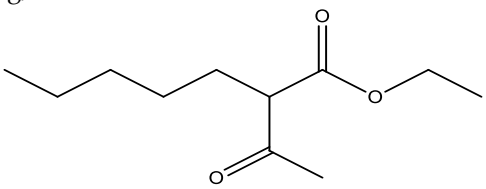

Figure 7. Ethyl 2-acetylheptanoate compound

Lupeol

The lupeol compound appeared at a retention time of 17.88 minutes with a relative abundance of 85%. This compound has a molecular weight of 426.72 g/mol and a molecular formula of $C_{30}H_{50}O$. Lupeol is a pentacyclic triterpenoid compound commonly found in the plant kingdom and is found in edible fruits and vegetables. This compound belongs to the natural triterpene group used to reduce inflammatory responses and also has immunomodulatory properties. Lupeol and its derivatives have great potential to act as anti-inflammatory, anti-microbial, anti-proliferative, anti-invasive, anti-angiogenic, antiprotozoal, and cholesterol-lowering agents (Sharma et al., 2020). The structure of the lupeol compound can be seen in Figure 8.

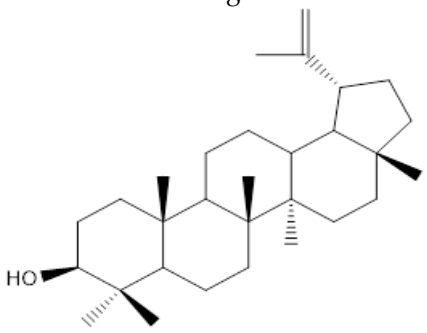

Figure 8. Structure of the Lupeol Compound.

Deferoxamine

Deferoxamine compound appeared at a retention time of 18.48 minutes with a relative abundance of 94%. This compound has a molecular weight of 560.7g/mol and a molecular formula of C25H48N6O8. This compound is used to overcome excess iron and aluminum in the body by binding and excreting excess iron in the body through urine and feces. According to research (Wang et al., 2025)the addition of iron-binding ligands such as deferoxamine (DFO) compounds strongly increases the formation of microbial superoxide. In addition, it was found that this iron-binding

ligand plays an important role in converting superoxide into hydroxyl radicals by modulating the rate of electron transfer between Fe (III)/ Fe (II) and superoxide.

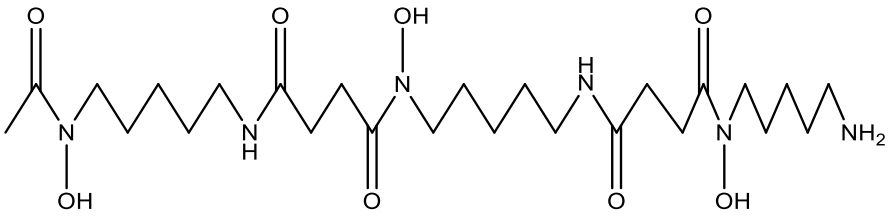

Figure 9. Structure of deferoxamine.

4. CONCLUSION

The results of the study showed that the yellow root ethyl acetate extract contains 8 active compounds with varying activities. These 8 compounds include 2,4-Pentadiynylbenzene, Propionaldehyde, beta-Lysylmethanediamine, Phellodenol-A, N-(1-adamantyl)-3-[16-[3-(1-adamantylamino)-3-oxopropanoyl]-1,4,10,13-tetraoxa-7,16-diazacyclooctadec-7-yl]-3 oxopropanamide, Ethyl 2-acetylheptanoate, Lupeol, Deferoxamine.

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