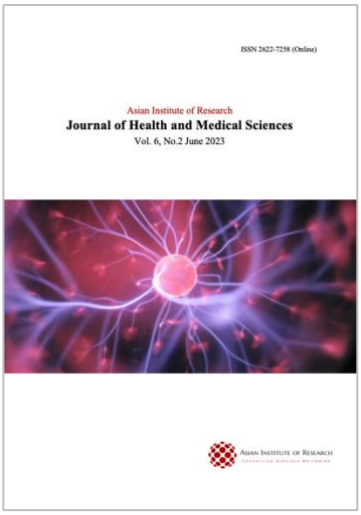

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Ketapang Leaf (*Terminalia Catappa L.*) Metabolite Profiling with Aquadest Fraction Ethanol Extract Using UPLC-MS

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Abstract
Ketapang leaves (*Terminalia catappa L.*) are known for its traditional medicinal function. Ketapang leaves contain saponins, alkaloids, tannins, flavonoids, and triterpenoids. In general, polar solvent can increase the production of Ketapang leaves' active compound. Utilization of a plant as herbal medicine is based on the presence of chemical compounds that have certain pharmacological effects. Therefore, metabolite profiling is needed. This is intended to understand the chemical compounds of Ketapang leaves. The objective of this study is to analyze the metabolite profile of chemical compounds and major compounds contained in the aquadest fraction ethanol extract using the UPLC-MS instrument. The results of the interpretation of the analysis of the compound content using UPLC-MS showed that there are 19 compounds in the ethanol extract of the aquadest fraction of Ketapang leaves. The major compound in the ethanol extract of the aqueous fraction of Ketapang leaves is Pelargonidin 3-O-glucoside with an iFit percentage of 97.56%.

Keywords: Ketapang, Metabolite, Aquadest, UPLC-MS

1. Introduction

Herbal extracts of Ketapang have anti-inflammatory effect on its isoflavones for the periodontal disease. The main compounds that play a role in the blood clotting process are tannins and flavonoids (Marcińczyk et al., 2022). The process happens through their inhibitory effects on inflammatory cytokine production and inhibition of mitogen-activated. (Telrandhe et al., 2021). People in developing countries reaching 80% use traditional medicine for health maintenance (Valizadeh et al., 2021). Medicine made from plants potential healing is also used in periodontal disease, to support the emerging global traditional medicine with antimicrobial resistance (Milovanova-Palmer & Pendry, 2018).

Ketapang leaves (*Terminalia catappa L.*) are known for its nutrient for curing people sickness. However, the current management of periodontal disease problem arises from the antibiotic and antimicrobial resistance (Serwecińska, 2020). The highest flavonoid content is found in Chinese ketapang leaves (*Cassia alata L*) (Chuah & Pisar, 2010). Research on the degumming process and the type of catalyst on the physiochemical and biodiesel properties in tropical almond (*Terminalia catappa*) seed oil was conducted (Punwong et al., 2017). The phytochemical and anthelmintic activities of Katapang leaves were similar to standard drugs meanwhile the lowest inhibition of

95.77% was detected from the methanol extract (Sani et al., 2019).

Ketapang leaves *(Terminalia catappa L.*) contain saponins, alkaloids, tannins, flavonoids, and triterpenoids (Allyn et al., 2018; Praptiwi et al., 2020; Olukotun et al., 2018; Tampemawa et al., 2016), triterpenoids (Dembitsky, 2021; Nugroho et al., 2016), tannins (Ola et al., 2020), alkaloids (Katiki et al., 2017), steroids (Ladele et al., 2016), and fatty acids (Janporn et al., 2015).

Ketapang leaves need to be analyzed for their metabolite content to find out what compounds are contained therein. One of the analysis techniques that can be used is metabolite profiling analysis. The use of metabolite profiles can provide a comparative view of gene function. Metabolite profiles have both the potential to the complex regulatory processes by providing deeper insight and also directly determine the phenotypes. The metabolite profile of a plant can be identified with the help of the UPLC-MS instrument. UPLC-MS is among the analysis techniques from the LC-MS technique which can be used to analyze the metabolite profile of a sample (Attwa et al., 2023). This analytical technique provides several advantages, namely high-resolution, robust, reliable chromatogram results, accurate measurement of mass and structural information, and allows the detection of a wide range of metabolites from plant samples (Zhao & Lin, 2014).

This study is intended to perform the metabolite profile of chemical compounds and major compounds contained in the ethanol extract of the aquadest fraction of Ketapang leaves using the UPLC-MS instrument. Analysis of this profile of metabolite compounds will provide data on what compounds are contained in Ketapang leaves which will then be identified whether these compounds can be used as medicinal ingredients, especially drugs to control bleeding in the teeth.

2. Method

2.1 Instruments and Location of Study

The material used in this research is Ketapang leaves. The solvents used for the extraction and fractionation of the extract are ethanol, aquadest, n-hexane, ethyl acetate. The chemicals used for UPLC-MS testing include methanol (hyper grade for LC-MS), formic acid (ultrapure for UPLC-MS), acetonitrile (hyper grade for LC-MS), and 0.05% water injection for UPLC-MS.

The equipment used in the study was a set of maceration tools and separatory funnel, blender, knife, scissors, sifter, analytical balance, Erlenmeyer Pyrex, Schott beaker, stir bar, IWAKI CTE33 volumetric flask, Pyrex volume pipette, dropper pipette, micro pipette, paper. filter Whatman No.1, Vacuum Rotary Evaporator (Buchi, Sweden). Extract preparation and fractionation were carried out at the Laboratory. Analysis using the UPLC-MS instrument is carried out at the Forensic Laboratory, Research and Criminal Agency of the Republic of Indonesia Police, East Jakarta.

2.2 Research Procedure

2.2.1 Simplicia

Mature dark green Ketapang leaves were chosen as the mature leaves will have effect on the secondary metabolites content. All the leaves were washed under running water. Then the leaves are washed under running water, and chopped into pieces. The following process is drying under 500 °C for 24 hours using oven. After the leaves are dry, ketapang leaf simplicia is made by blending the dry Ketapang leaves. The blended Ketapang leaves are then sieved using a 60-mesh sieve.

2.2.2 Ethanol Extract and Fractionation

Maceration of Ketapang leaves powder with ethanol was performed in a ratio of 1:5 for 2 days (24 hours) at room temperature (20–25) °C. Filtering process was performed using Whatman No.1 filter paper. The drugs obtained were then going through second maceration with 1000 mL of ethanol two times. The filtrates obtained were combined and then evaporated using a vacuum foam evaporator (Buchi, Sweden) at 400C. The result of evaporation was obtained crude extract of ethanol Ketapang leaves.

The ethanol condensed extract of Ketapang leaves was partitioned using distilled water and hexane. A total of 4 mg of crude extract and 200 ml of distilled water and 200 mL of hexane. The process is as follows: First, shaking the mixture in a separatory funnel to make it evenly shaken. Then let it stand for a while until you can see the separation between the aquadest phase and the hexane phase. The two phases were separated and the solvent for each phase was evaporated in a vacuum rotary evaporator to obtain an extract of the distilled water phase. The ethanol extract, the aquadest fraction of ketapang leaves obtained was tested by LCMS.

2.2.3 Metabolite Profiling

Weigh carefully 10.00 mg of the extract sample and then dissolve it with methanol into a 10 ml volumetric flask. Extract in methanol was taken with a microsyringe as much as 5 µl to then be injected into the sample and into the UPLC-MS column. Replication was carried out 4 times. The sample in the form of a liquid will be converted into droplets through a needle that has been given a positive (+) ESI charge. The ions that have been produced by the detector will then be separated by the Q-ToF analyzer. The eluent used was a mixture of (A) water: formic acid (99.9:0.1) and (B) acetonitrile: formic acid (99.9: 0.1) with a gradient elution system as listed in table 1 with a flow rate eluent 0.2 ml/min. The results of polar compounds chromatograms will appear first, followed by compounds with lower polarity. The results of the separation are then read by the QToF-MS detector to produce a chromatogram peak. The interpretation of chromatogram peaks was performed using the Masslynx application.

Table 1: The ratio of solvent used in the gradient elution system

Time (Minute)	Mixture A (%)	Mixture B (%)
0.00	95.0	5.00
2.00	75.0	25.0
3.00	75.0	25.0
14.00	0.00	100.0
15.00	0.00	100.0
19.00	95.0	5.0
23.00	95.0	5.0

3. Results

The chromatogram is processed using the Masslynx 4.1 application so that the molecular formula of each compound can be known and predicted. The chromatogram of the results of the analysis of the metabolite profile of the ethanol extract of the aquades fraction of ketapang leaves can be seen in Figure 1. Each one peak of the chromatogram indicates one compound. Based on the measured mass and calculated mass values in the spectra, it is possible to predict the molecular formula from the spectra. The value of the measured mass and calculated mass must also be reduced by the mass of 1 H atom, namely 1.0078, because when the separation using a column occurs the addition of H atoms comes from the firing of ESI (+) ions. The predicted molecular formula that appears in the data is then chosen, which is the difference between the measured mass and the calculated mass of ± 0.0005. The predicted molecular formula that has been selected is then searched with the help of the chemspider.com website.

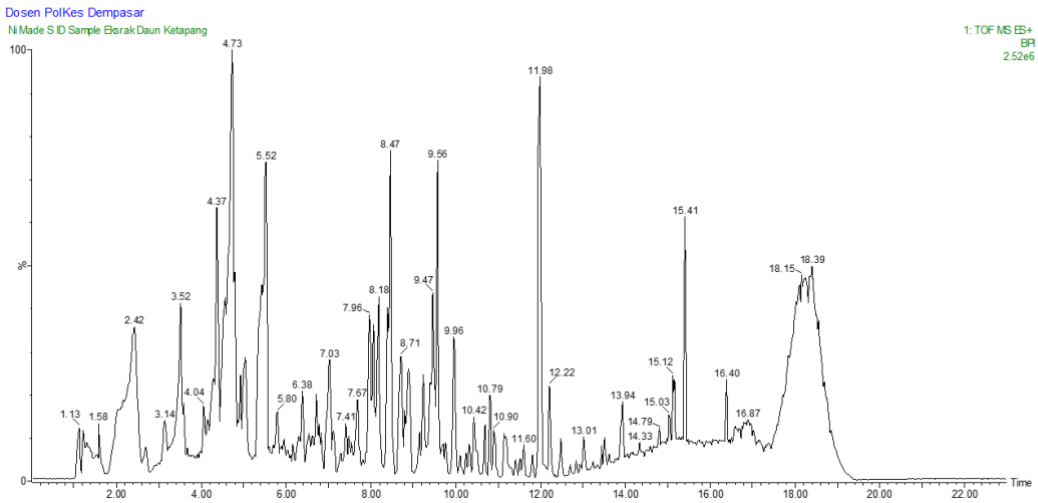

Figure 1: UPLC-MS chromatogram of the ethanol extract of the aquadest fraction of ketapang leaves

The results of the interpretation of the analysis of the compound content using the UPLC-QToFMS showed that there were 19 compounds in the ethanol extract of the aquadest fraction of Ketapang leaves (Table 2).

Table 2: Interpretation of data on metabolite profiling of the ethanol extract of the aquadest fraction of Ketapang leaves

Retention Time	Measured Mass	Calculated Mass	Formula	Compound
1.13	151.0352	151.0395	C_8H7O_3	Mandelate
1.58	130.0873	130.0868	$C_6H_{12}NO_2$	6-Aminohexanoate
2.42	120.0814	120.0813	$C_8H_{10}N$	1-Allylpyridinium

3.14	1102.1033	1102.1029	$C_{40}H_{16}N_{25}O_{14}S$	Unknwon
3.52	188.0720	188.0745	$C_8H_{14}NO_2S$	2-methoxy-1-(2-methyl-4H-thiazol-5-yl)propan-1-ol
4.37	449.1087	449.1084	$C_{21}H_{21}O_{11}$	Cyanidin-3-glucoside
4.73	433.1144	433.1135	$C_{21}H_{21}O_{10}$	**Pelargonidin 3-O-glucoside**
5.52	585.1256	585.1244	$C_{28}H_{25}O_{14}$	Unkown
5.80	197.1178	197.1178	$C_{11}H_{17}O_3$	3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptane-1-carboxylate
6.38	261.1128	261.1127	$C_{15}H_{17}O_4$	7-Hydroxy-4-(methoxycarbonyl)-2-(2-methyl-2-propanyl)chromenium
7.03	309.0872	309.0875	$C_{17}H_{13}N_2O_4$	3-Carbamoyl-1-[2-oxo-2-(2-oxo-2H-chromen-3-yl)ethyl]pyridinium
7.41	570.2218	570.2187	$C_{26}H_{36}NO_{13}$	1-[(4-methoxyphenyl)methyl]-2-methyl-1,2,3,4,5,6,7,8-octahydroisoquinolin-2-ium;(2R,3R)-2,3,4-trihydroxy-4-oxo-butanoate
7.67	648.4308	648.4345	$C_{35}H_{62}N_5O_2S_2$	Unknown
7.96	275.2017	275.2011	$C_{18}H_{27}O_2$	(9E,11E,13E,15E)-9,11,13,15-Octadecatetraenoate
8.18	645.2926	645.2924	$C_{35}H_{41}N_4O_8$	Unknown
8.47	345.0617	345.0610	$C_{17}H_{13}O_8$	5,7-Dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-4-oxo-4H-chromen-3-olate
8.71	181.1230	181.1229	$C_{11}H_{17}O_2$	2-(5-Hexen-1-yl)-5-hydroxy-3,4-dihydropyranium
9.47	343.0454	343.0454	$C_{17}H_{11}O_8$	Unknown
9.56	343.1188	343.1188	$C_{19}H_{19}O_6$	(3R)-3-(2,3-Dihydro-1,4-benzodioxin-6-yl)-3-(3,4-dimethoxyphenyl)propanoate
9.96	345.1337	345.1338	$C_{19}H_{21}O_6$	(1R,2R,5S,8S,9S,10R,11S,12S)-5,12-Dihydroxy-11-methyl-6-methylene-16-oxo-15-oxapentacyclo[9.3.2.$1^{5,8}$.$0^{1,10}$.$0^{2,8}$]heptadec-13-ene-9-carboxylate
10.42	214.2535	214.2535	$C_{14}H_{32}N$	tetradecylammonium
10.79	627.2828	627.2819	$C_{35}H_{39}N_4O_7$	3-{(3S,4S)-5-{2-[(3-Ethyl-5-formyl-4-methyl-1H-pyrrol-2-yl)methyl]-5-(methoxycarbonyl)-3-methyl-4-oxo-1,4-dihydrocyclopenta[b]pyrrol-6-yl}-3-methyl-2-[(3-methyl-5-oxo-4-vinyl-2,5-dihydro-1H-pyrrol-2-y l)methyl]-3,4-dihydro-2H-pyrrol-4-yl}propanoate
10.90	271.1692	271.1692	$C_{18}H_{23}O_2$	(17β)-17-Hydroxyestra-1(10),2,4-trien-3-olate
11.60	277.2166	277.2166	$C_{18}H_{29}O_2$	linolenate
11.98	601.5199	601.5148	$C_{22}H_{65}N_{16}OS$	unknown
12.22	425.3632	425.3632	$C_{27}H_{45}N_4$	unknown
13.01	425.3607	425.3644	$C_{27}H_{45}N_4$	unknown
13.94	423.3973	423.3991	$C_{31}H_{51}$	unknown
14.33	423.3975	423.3991	$C_{31}H_{51}$	unknown
14.79	423.3984	423.3991	$C_{31}H_{51}$	unknown
15.03	419.3139	419.3140	$C_{27}H_{39}N_4$	5-Ethyl-2-methyl-1-[3-({4-[(E)-phenyldiazenyl]-5,6,7,8-tetrahydro-1-naphthalenyl}amino)propyl]piperidinium (Jenis Alkaloid piperidin)
15.12	423.3979	423.3991	$C_{31}H_{51}$	unknown
15.41	423.3986	423.3957	$C_{19}H_{51}N_8S$	unknown
16.40	423.3958	423.3991	$C_{31}H_{51}$	unknown
16.87	423.3968	423.3991	$C_{31}H_{51}$	unknown

Based on the results of the interpretation of the data that has been obtained, several major compounds can be identified, namely compounds that have a higher area percentage compared to other compounds. The major compound in the ethanol extract of the aqueous fraction of Ketapang leaves is Pelargonidin 3-O-glucoside with an iFit percentage of 97.56%. Pelargonidin 3-O-glucoside is a type of anthocyanin belonging to the flavonoin compound (Ergün, 2022). Spectra and chemical structures of the compounds are portrayed in Figure 2.

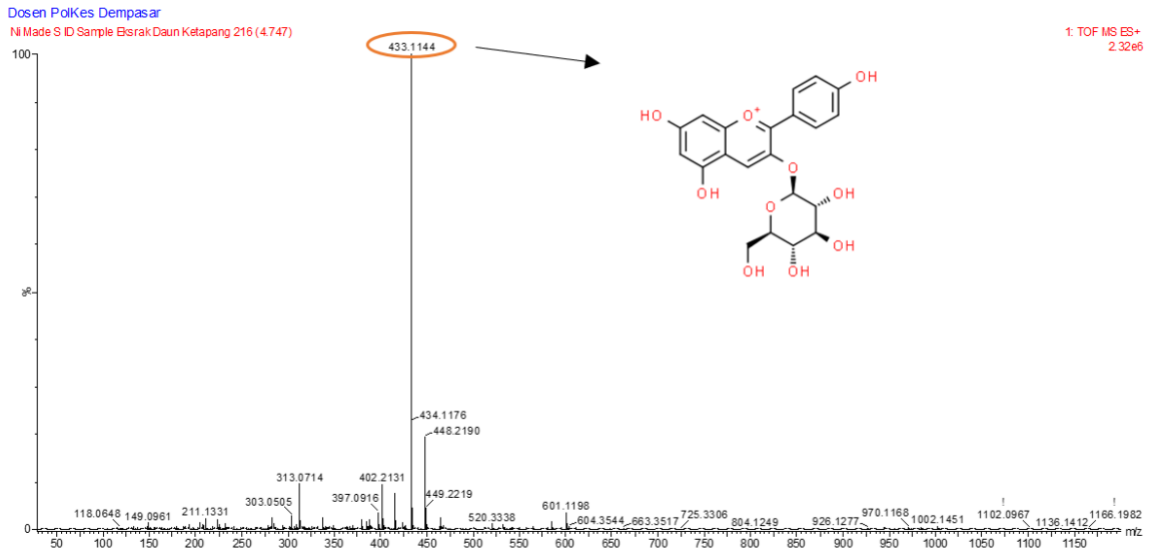

Figure 2: Spectra and chemical structures of major compounds

4. Discussion

Analysis of the metabolite profile of the ethanol extract of the aquades fraction of ketapang leaves in this study used UPLC-MS. UPLC is among the developmental techniques of liquid chromatography which is used for segregation of different components in a mixture with a molecular level of up to two microns of analyte particles. The analytical method using UPLC can reduce the consumption of the mobile phase by up to 80% in a relatively shorter time of about 1.5 minutes than using HPLC. The UPLC-MS used in this study uses an MS detector with an ESI (+) ion source and an MS analyzer in the form of Q-ToF. This instrument has several advantages, namely selective and sensitive with high resolution performance and fast so that the analysis time is faster (Chawla & Ranjan, 2016). Analysis of the metabolite profile of the ethanol extract of the aquades fraction of Ketapang leaves begins with injecting the sample, then the sample will enter the column resulting in the process of separating the metabolite components. In this study the stationary phase used was C18 column or octadecyl silica. The advantage of octadecyl silica as the stationary phase is that this phase is able to separate compounds ranging from low, medium, to high polarity (Dembek & Bocian, 2022).

The ethanol extract of the aquades fraction of ketapang leaves is known to contain many secondary metabolites that are beneficial to health. The ethanol extract of the distilled water fraction is known to contain saponins, alkaloids, tannins, flavonoids, triterpenoids, and phenols based on phytochemical screening tests. The ethanol extract of the distilled water fraction contained saponins of 3787.80 mg/100 g, alkaloid content of 1798.57 mg/100g, tannin content of 53140.72 mg/100g, flavonoid content of 12935.37 mg/100g and phenol content of 29968.05 mg/100g (Muthulakshmi & Neelanarayanan, 2021). According to the test results with UPLC-MS, the ethanol extract of the aquades fraction of ketapang leaves was detected to contain several secondary metabolites such as alkaloids, namely 1-Allylpyridinium and 5-Ethyl-2-methyl-1-[3-({4-[(E)- phenyldiazenyl]-5,6,7,8-tetrahydro-1-naphthalenyl} amino) propyl] piperidinium and the flavonoid group, namely Cyanidin-3-glucoside and Pelargonidin 3-O-glucoside. Therefore, the ethanol extract of the aqudes fraction of ketapang leaves can be used as a drug in controlling bleeding, one of which is bleeding during tooth extraction. In addition to suppression,

the use of topical hemostatic is one step to control bleeding (Milovanova-Palmer & Pendry, 2018). One of the compounds that play a role in the blood clotting process is flavonoids (Ullah et al., 2020). The presence of flavonoids in the ethanol extract of the aquades fraction of ketapang leaves can be used as a solution to reduce bleeding.

The results of the interpretation of the analysis of the compound content using the UPLC-QToFMS showed that there were 19 compounds in the ethanol extract of the aquades fraction of ketapang leaves. The major compound in the ethanol extract of the aqueous fraction of ketapang leaves is Pelargonidin 3-O-glucoside with an iFit percentage of 97.56%. The ethanol extract of the distilled water fraction of ketapang leaves was detected to contain several secondary metabolites such as alkaloids, namely 1-Allylpyridinium and 5-Ethyl-2-methyl-1-[3-({4-[(E)-phenyldiazenyl]-5,6,7) ,8-tetrahydro-1-naphthalenyl} amino) propyl] piperidinium and the flavonoid group, namely Cyanidin-3-glucoside and Pelargonidin 3-O-glucoside.

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