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RESEARCH PAPER

Isolation and Identification of Andrographolide Compounds from the Leaves of Sambiloto Plant (*Andrographis paniculata* Ness)

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Abbreviations: TLC: Thin-Layer Chromatography

Abstract: Sambiloto plant (*Andrographis paniculata* Ness) is a plant that has been used as medicine from generation to generation. Bioactive compounds in Sambiloto have pharmacological effects such as immunostimulants (increase immunity), antibiotic diuretics (facilitate urine), antipyretics, anti-inflammatory (anti-inflammatory), hepatoprotective, hypotensive, hypoglycemic, antibacterial, anti-inflammatory, respiratory tract, and heart and lung meridians - lungs. The bioactive compound in Sambiloto, which is mostly found in the leaves, is Andrographolide. In this study, the isolation of Andrographolide from the leaves of the sambiloto plant (*Andrographis paniculata* Ness) was carried out using purification and crystallization methods to obtain pure Andrographolide isolates more efficiently and identifying the results of Andrographolide isolates. The results showed that the isolates obtained using the purification and crystallization methods obtained 0.47%. In the qualitative test of Andrographolide isolates using eluent and acetate: n-hexane (3: 2), the Rf value was 0.38. The results obtained from Andrographolide isolates using infrared spectroscopy (FT-IR) were identical to the literature on Andrographolide. The absorption peaks at the wavenumbers obtained includes 3400,41 cm^{-1}, 1979,68 cm^{-1}, 1959,46 cm^{-1}, 2928,22 cm^{-1}, 1727,56 cm^{-1}, 1646,98 cm^{-1}, and 907,53 cm^{-1}.

Keywords: Andrographolide, *Andrographis paniculata* Ness, isolation, identification, purification-crystallization method

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INTRODUCTION

Sambiloto plant (*Andrographis paniculata* Ness) is a plant that has been used for generations as medicine [1]. This plant is believed and used by the community as a medicine for various diseases. It is because the bioactive compounds in Sambiloto have pharmacological effects such as immunostimulants (enhancing immunity), antibiotic diuretics (smoothing urine), antipyretics (reducing heat and fever), anti-inflammatory (anti-inflammatory), hepatoprotective, hypotensive, hypoglycemic, antibacterial, anti-inflammation of the airways, as well as the heart and lung meridians [2].

Sambiloto plants contain terpenes, lactones, and flavonoids. Four lactone compounds were identified in sambiloto leaves, namely deoxyandrographolide, andrographolide, neoandrographolide, and 14-deoxy-11, 12-didehydroandrographolide [1]. Many flavonoid compounds are found in the roots and found in the leaves [3]. The roots of the bitter plant contain flavonoids in the form of polymethoxyflavone andrographine, picoline, alkane, ketones, aldehydes, potassium, calcium, sodium, kersik acid, monomethylwithin, and apigenin-7,4-dimethyl ether [4]. The branches and leaves of the sambiloto plant contain alkane, ketone, and aldehyde compounds [3].

All parts of the *Andrographis paniculata* plant, such as the leaves, stems, flowers, and roots have a very bitter taste. The parts of the plant often used in medicine are all parts of the plant above the ground (herbs). In general, *Andrographis paniculata* Ness contains diterpenes lactones and flavonoids [1]. Andrographolide

is included in the unsaturated trihydroxy lactone group with the molecular formula C20H30O5. Andrographolide is soluble in methanol, ethanol, pyridine, acetic acid, and acetone but less soluble in ether and water. The melting point of Andrographolide is 228– 2300C [5]. The maximum wavelength of Andrographolide is 230-235 nm [6-8].

Andrographolide in sambiloto is mostly found in the leaves and stems. Andrographolide content in the leaves of sambiloto is 2.5 to 4.8% of the dry weight [9-10]. In comparison, the lowest was found in seeds [11]. Meanwhile, the lactone content of diterpenes isolated from sambiloto leaves ranged from 0.1-2% [12]. The maximum content of Andrographolide and other terpenes is found in ripe leaves. Andrographolide content in stems 0.2%, 0.13% seeds, 0.44% roots and 2.39% leaves. Andrographolide content varies depending on the harvest season. Andrographolide content in leaves is more than 2% before flowering plants, after which the content is reduced to less than 0.5% [13].

MATERIALS AND METHODS

The research was conducted at the Laboratory of Chemistry, FKIP, the University of Mataram.

Tools

The tools used in this study include glass jars, blenders, ordinary scales, analytical scales, hot plates, hairdryers, thermometers, funnels, Buchner funnels, vials, beakers, states & clamps, spatulas, stirring rods, dropper pipettes, micropipettes, volume pipettes, rubber bumps, TLC with a stationary phase of silica gel 60 F254, UV254 lamps, and a set of FTIR tools.

Materials

Sambiloto herbs, 96% ethanol solvent, n-hexane, ethyl-acetate, water (aquadest), chloroform, methanol p.a, filter paper, and clear plastic.

Procedure

Sample Preparation

The part of the bitter plant used is the leaves of the plant. Before being used as raw material, sambiloto leaves are given a pre-treatment, namely drying and grinding. Drying is done to reduce the water content in the plants so that the enzymatic reactions can be stopped, so they are not easily damaged. Drying is carried out by drying it in the room until dry Simplicia is obtained with a simplicia water content <10%. The drying process is carried out for 6 days. The dried sambiloto leaves are mashed in a blender until they become powder.

Extraction

Simplicia powder (leaf) is added with 96% ethanol in a ratio of 1: 5, then stirred and left to stand in a closed glass container protected from sunlight for 4 days. The mixture is filtered with competitive paper and the first macerate (filtrate 1) is obtained. The residue was re-macerated 2 times with 96% ethanol (1: 5) for 1 day each. Macerate 1,2 and 3 are combined and deposited, then stored for concentration. Concentration is done by transferring the macerate to a beaker on a hot plate and followed by a blow dryer to let it dry to dry so that a thick extract is obtained.

Purification

The thick extract obtained was washed by adding n-hexane solvent (1: 1), then vortexed for 5 minutes. The solvent will turn a thick green color. The procedure was repeated 19 times or until the green color faded (disappeared). The insoluble fraction of n-hexane was purified with the addition of ethyl-acetate 10 times the residual weight. The mixture is vortexed for 5 minutes, and the solvent will turn dark brown. The procedure was repeated 4 times or until the brown color faded (disappeared). The ethyl-acetate insoluble fraction was purified by adding hot water 10 times the residual weight. A hot water-insoluble fraction is evaporated to dryness.

Crystallization - Recrystallization

The thick purified extract was dissolved with methanol p.a little by little then heated at 78°C until dissolved. The solution was filtered hot and the filtrate cooled slowly. The crystals formed are dissolved using methanol then heated until dissolved. The solution is hot filtered and the resulting filtrate is recrystallized to obtain pure Andrographolide crystals.

Compound Qualitative Test

The qualitative test of pure Andrographolide compounds using thin-layer chromatography (TLC) [16-17]. Qualitative testing with TLC was carried out by preparing a 10 mg/ml test solution in ethanol. Meanwhile, the comparison of andrographolide was 0.1 mg/ml in ethanol as the mobile phase is Ethyl Acetate: N-Hexane (3: 2). The stationary phase used a TLC plate of silica gel 60 F_{254}. The bottling volume of the test and comparison solution was 5 µL—observation of blemishes on UV_{254}.

Identification of Compound Structures

In the identification of the structure of the compound used the infrared spectroscopy test or (FT-IR). Testing was carried out through FT-IR. Pure Andrographolide crystals were weighed and put into the tool. Install the DRS-8000A appliance. Sample test by including sample and KBr, the number of samples is about 5% -10% compared to the number of KBr. Operations are carried out with the aid of a computer. If the resulting spectrum is relatively short, it means that the number of samples mixed is small, whereas if the resulting spectrum is relatively long it means that there are many mixed samples.

RESULTS AND DISCUSSION

The large milky white crystal of Andrographolide was successfully isolated. It yields 1.4146 grams (0.47 %) (Figure 1). In the observation under UV_{254} presented in Figure 2. one spot is visible purple.

Figure 1. Isolated Andrographolide Crystal

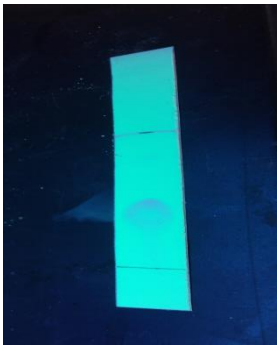

Figure 2. Results of Observation of Andrographolide Isolate Samples at UV_{254}

The results of the observation of the Andrographolide infra-red spectrum of the isolate are shown in **Figure 3**.

Table 1. Wave Numbers of Andrographolide Isolates Compared with Library Andrographolide

Bonds and Types of Compounds	Andrographolide Frequency Area (cm^{-1}) Experiment	Andrographolide Frequency Area (cm^{-1}) References
Hydroxyl groups with hydrogen bonds (stretching vibration of O–H)	3400,41	3399
Alkane bond groups (stretching vibration of C-H)	2979,68 2959,46 2928,22	2980 2958 2927
Groups of γ--lactones	1727,56	1728
Unsaturated α, β groups (stretching vibration of C = C bond)	1646,98	1647
Exometylene double bond groups (stretching vibration of =CH)	907,53	906,6

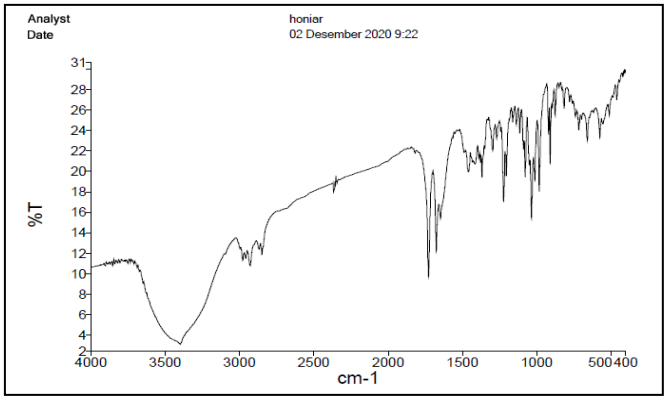

Figure 3. Results of the IR Spectrum of Andrographolide isolated

Isolation of Andrographolide from the leaves of *Andrographis paniculata* Ness

From the extraction of 300 grams of sambiloto leaf simplicia powder (*Andrographis paniculata* Ness) with the re-maceration method, 1900 ml of ethanol filtrate was obtained, after concentrating it obtained 42 grams of thick extract in solid form. After purification and recrystallization were carried out, 1.4146 grams of Andrographolide Crystals were obtained, so that the level obtained was 0.47%.

Extraction is a means of separating a mixture of several substances into separate components [18]. The first stage of extraction was carried out by the remaceration method. Remaceration is an extraction method that occurs with repeated addition of solvent after the first

filtering of macerate, and so on [16]. The choice of remaceration method in Andrographolide isolation is considered because it can extract the active substance completely. The extraction using the remaceration method has the highest yield value ranging from 9.9 - 11.9% compared to the maceration, percolation, and repercolation methods [19]. It is because, in the remaceration method, the long contact time between the solvent and the simplicia allows the solvent to more easily enter the cell and draw the compounds maximally without fear of washing out or washing out. The existence of shaking is also beneficial for the solvent in dissolving these compounds. In this extraction, the solvent used is 96% ethanol. The choice of solvent is based on several reasons, including polarity, toxicity, and previous studies.

The second stage of extraction is the evaporation or concentration method. The imagery results obtained were concentrated with a hot plate and hairdryer to get a thick extract. The concentration process is carried out quite carefully so that there is no emptying of the fiber to be thickened. By thickening the extract first before moving to the next stage, it aims to reduce the solvent used.

Furthermore, isolation is carried out with the first stage, namely the purification method. The viscous extract was purified using n-hexane, ethyl acetate, and hot aquadest as a solvent. The addition of n-hexane aims to attract non-polar fats and chlorophyll in the extract. The purification process with n-hexane was repeated 19 times until the green color in the solution was reduced (lost). Ethyl acetate attracts other impurities present in the extract, which is more polar than chlorophyll and is not dissolved in n-hexane. The purification process with ethyl acetate was carried out 4 times until the color of the solvent turned light brown. Finally, adding hot aquadest (H2O) is used to remove impurities that are very polar in the extract. The extract is evaporated again until thick. Purification can remove impurities that can interfere with insulation compared to using liquid-liquid extraction. Besides, this purification method is also considered cheaper and more efficient because it does not require other materials such as silica gel as in the use of slow column chromatography [20].

Then the isolation stage was continued with the crystallization-recrystallization method using methanol p.a. The purified extract was added with methanol p.a little by little, then heated until it dissolved. Methanol p.a solvent is used because Andrographolide is soluble in methanol, so less volume is needed to saturate Andrographolide [21]. Besides, based on the principle of recrystallization, andrographolide dissolves in methanol at hot temperatures but at low temperatures will precipitate to form crystals again. The supersaturation condition is needed to facilitate crystal formation after the cooling process. The solution is filtered hot and then cooled slowly to get a large crystal form because if the temperature decreases rapidly, the growth rate of the crystal nucleus is faster than the growth rate of the crystal so that the resulting crystals are small brittle, amorphous [22].

Andrographolide Qualitative Test Using TLC

The working principle of TLC is the transfer of a substance based on the polarity difference between the sample and the solvent. This test usually uses the stationary phase of the silica gel plate, and the mobile phase is adjusted to the sample being tested. The solution or a mixture of solutions used as the mobile phase is called an eluent. The closer the polarity between the sample and the eluent, the more the sample will be carried away by the mobile phase. The isolated compound is declared pure if a spot is obtained on the TLC plate where the spot distance must be the same as the desired compound spot distance. So it takes a certain calculation to find out that the spots have the same distance. The value of the calculation is called Rf. It states the degree of retention of a component in the stationary phase. The value of Rf can be used as evidence in identifying a compound. If the identification of the Rf value with the Rf value of the compound is the same, then the compound can be identified as having the same or similar characteristics, and vice versa. The formula can determine the price of Rf:

$$\text{Rf} = \frac{the\ distance\ covered\ by\ the\ substrate}{the\ distance\ traveled\ by\ the\ solvent}$$

TLC test results with ethyl acetate: n-hexane (3 : 2) eluent showed a single spot with an Rf value of 0.38. The Rf price obtained in this study is following the Andrographolide Rf price in the literature, namely 0.38 [6,23].

Based on the results of the calculation, it shows that the percent yield for isolated compounds is 0.47%. The content of the Andrographolide in the dry weight of the simplicia (leaf) of sambiloto using the purification-recrystallization method was 0.47%. The content of Andrographolide in leaves was 2.39% [13], other research shows 0.48% yields [20]. The difference in Andrographolide content obtained can be caused by several factors, including season, plant age, place of growth, plant parts, and analysis method [24]. In this research, the raw material is the flowering sambiloto simplicia is used, including the mature sambiloto plant.

Andrographolide Identification Using IR Spectroscopy

The wave numbers read in table 1 show the presence of a hydroxyl group with hydrogen bonds, alkane bond groups, groups of γ--lactones, unsaturated α, β groups, and exometylene double bond groups. The experimental Andrographolide wave number value is suitable with the literature as shown in table 1, so it can be concluded that the experimental results obtained have identical characteristics to the chemical structure of Andrographolide literature [23, 25-26].

The results of identification with infrared against Andrographolide crystals in the experiment showed the peak absorption at wavenumbers 3400,41 cm^{-1}, 1979,68 cm^{-1}, 1959,46 cm^{-1}, 2928,22 cm^{-1}, 1727,56 cm^{-1}, 1646,98 cm^{-1}, and 907,53 cm^{-1}. The absorption at wave number 3400.41 cm^{-1} is characteristic of the stretching vibration of the hydroxyl groups with hydrogen bonds. Wavenumber 1979.68 cm^{-1}; 1959.46 cm^{-1} and 2928.22 cm^{-1} are alkane bond groups. Wave numbers 1727.56 cm^{-1} and 1646.98 cm^{-1} indicate the functional groups of γ--lactones and α, β groups are unsaturated, while wave numbers 907.53 cm-1 provide information on the presence of exo-methylene double bond group. These functional groups are a specific characteristic of the terpene lactone compound, Andrographolide in *Andrographis paniculata* Ness. According to literature [23, 25-26] the IR spectrum of the Andrographolide compound shows absorption at wavenumbers 3399 cm^{-1}, 2980 cm^{-1}, 2958 cm^{-1}, 2927 cm^{-1}, 1728 cm^{-1}, 1647 cm^{-1}, 906,6 cm^{-1}.

CONCLUSION

Based on our findings, it can be concluded as follows:

1) Extraction and isolation from 300 grams of dried leaves of a bitter plant (*Andrographis paniculata* Ness) using purification and recrystallization methods obtained 1.4146 grams of Andrographolide crystals (0.47%).
2) The eluent used in the qualitative test of isolated Andrographolide compounds was ethyl acetate: n-hexane (3: 2), and the Rf value was 0.38.
3) The results of the identification of Andrographolide compounds in sambiloto plants using infrared spectroscopy (FT-IR) are identical to the Andrographolide literature. The absorption peak at the wave number includes 3400,41 cm^{-1}, 1979,68 cm^{-1}, 1959,46 cm^{-1}, 2928,22 cm^{-1}, 1727,56 cm^{-1}, 1646,98 cm^{-1}, dan 907,53 cm^{-1}.

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