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Research Paper

Isolation and Identification of Ethanol Extract Andrographis Paniculata Nees and Moringa Oleifera Lam

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***ABSTRACT**: Sambiloto and Kelor had been used frequently to decrease disease simptoms and pain. It will be correlated with the composition of some secondary metabolite compound and other chemicals in Sambiloto and Kelor such as alkaloid, flavonoid and terpenoid. This study was to isolate and identified the secondary metabolite of ethanol extract Andrographis paniculata Ness and Moringa oleifera Lam. The objective of this study was to get substrate as alternative of nature material which easy to obtain, rationally and economics. Sambiloto and Kelor extract prepared by maceration used ethanol 70%. Sambiloto and Kelor ethanol extract (EES and EDK) then separated with thin layer chromatography (TLC) and identified with UV lamp. Then activity test using GCMS. The most peak of Sambiloto in testing with GCMS was Phytol (C20H40O) which was diterpen alcohol compound acyclic hydrogenated while in Kelor it was 9, 12, 15-Octadecatrienoic acid ($C_{18}H_{30}O_2$) which was linolenic acid. Targeted output of this study was a manuscript published at accredited international journal. Technology Readiness Level in this study was in second level and after this study become third level by doing some activities such as extraction, isolation, identification and activity test.*
***KEYWORDS**: Sambiloto, Kelor, TLC, GCMS.*

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I. INTRODUCTION

Sambiloto (*Andrographis paniculata* Nees) was one of plant used as traditional medicine came from India and can grow up at lowland or highland. Sambiloto have properties in treating some diseases and common used by people. Sambiloto leaves used as medicines since 1919. Active compound in sambiloto such as andrographolid make sambiloto become one of medicines component. Part of sambiloto used as medicine for snake or insect bite, fever, dysentery, rheumatic, tuberculosis, digest infection, and also for inflammation, infection, breathless and to improve heart function. It also caused by the composition in sambiloto such as andrographolid, saponin, flavonoid, alkaloid and tannin. These compounds were very useful for human body [1].

Kelor (*Moringa oleifera* Lam) have been known for many years as a multi function plant, full of nutrition and have medication effect. Kelor was known consist of more than 90 items of nutrition such as essential vitamin, mineral, amino acid, anti aging, and ant iinflamation. Kelor consists of 539 compounds which was known in African and Indian traditional medicine and have been used in traditional medicine to protect more than 300 diseases. Some parts of kelor were worked as heart stimulant and blood circulation, have function as anti tumour, antipyretic, anti epileptic, anti inflammation, anti ulcer, diuretic, anti hypertension, decrease cholesterol, antioxidant, anti diabetic, antimicrobial and anti fungi [2].

Pain was common in human being and one of the very often reason to visit the doctor because pain can raise inconvenience and disturb social function and quality of life. Inflammation was the manifestation of tissue damage signed by presence of pain [3]. *The U.S. Centre for Health Statistic* study for 8 years said that 32% of American people suffer chronic pain. Study of WHO which involved more than 25.000 patients from 14 countries said 22% patients suffer from pain at least for 6 months. Pain will be accompanied stress response such as decrease of anxiety, heart rate, blood pressure and breath rate. Continued pain or not adequate handling can rise long stress response which decrease body resistance by decrease immune function, hasten tissue damage, metabolic rate, blood coagulation and urine retention and at the end decrease health quality [4].

Based on above conditions, we had done the screening to ethanol extract of Andrographis paniculata Nees (sambiloto) and Moringa oleifera Lam (kelor), and formulated combination of ethanol extract of Andrographis paniculata Nees (sambiloto) and Moringa oleifera Lam (kelor). Then the effectivity of the extract

was examinated as analgesic to male white mice using chemist method by giving acetic acid injection intraperitoneally and use diclofenac sodium as controlled.

II. METHODE

The design of this study was experimental study. Variation of concentration sambiloto and kelor ethanol extract formulated as independent variable, and some parameter examination tests as dependent variable. This study consists of some steps such as sample preparation, chemical preparation, extraction, Thin Layer Chromatography (TLC) test and Gas Chromatography Mass Spectra (GCMS) test.

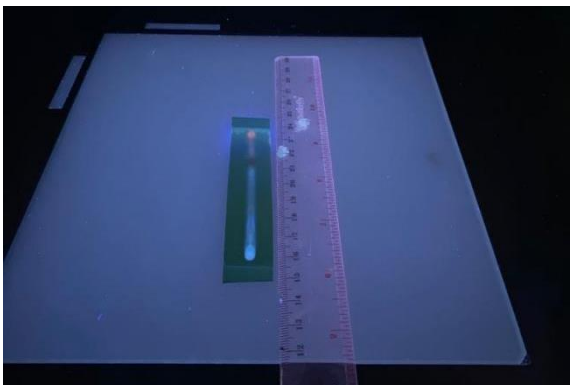
Sambiloto dan kelor fresh leaves collected and cleaned from garbage by washing with clean water, drained, and dried in cabinet. The sample was dried if it was broken when crushed. Then the sample was delicated by using blender, so we got simplicia, and stored in container.

About 3 kilograms of sambiloto/kelor leaves was wet sortationed to disappear contaminant by washing used running water 3 times and then dried under sunshine in a few minutes and passed the wind. Sambiloto/kelor cleaned and dried leaves delicated by using blender. Then about 200 grams sambiloto/kelor simplicia put in closed container and add ethanol 70% about 1500 ml to submersed, then stir for a few minutes, and stored for 5 days. Stored in protected from sunshine place. During that the container stir for a few times everyday. After 5 days filtered with flannel cloth and put in extraction bottle. Residu was macerated by 500 ml ethanol 70% and stored for 2 days. During that the container stir for a few times everyday. Filtered and the result combined to extraction bottle. Then the macerate distilled in low pressure at not more than 60 ^{0} C using rotary evaporator until thick extract obtained. Dried by *freeze dryer* for about 24 hours until sambiloto/kelor extract obtained, called extract etanol sambiloto (EES) and extract daun kelor (EDK) [5].

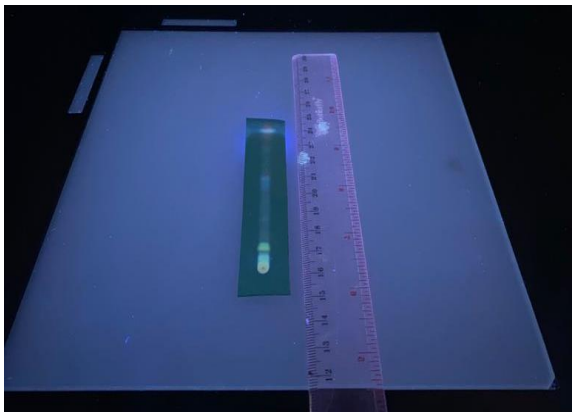
Thin Layer Chromatography (TLC) process aimed to look for chromatogram pattern of compound component in sambiloto and kelor ethanol extract. The process in TLC was trial and error principe to look for eluent which give good segregation. Ethanol extract was spotted on TLC plate 1X5 cm and eluent with the eluent. The eluent was chloroform, methanol and ammonia (84 : 15 : 1) and chloroform and methanol (8 : 2). The extract spotted TLC plate eluent with prepared eluent, radiance with UV to observe the spot. Then look for amount of stain and the distance between the stain. Gas Chromatography Mass Spectra (GCMS) test aimed to know functional groups from the isolates .

III. RESULT

From Thin Layer Chromatography (TLC) test for Sambiloto extract using Chloroform : Methanol : Ammonia (84:15:1) as eluent and Dragendorf as detector obtained the estimation of Alkaloid compound at 0.98 Rf with orange node, while using Chloroform : methanol (8 : 2) as eluent and $AlCl_3$ as detector obtained the estimation of Flavonoid compound.

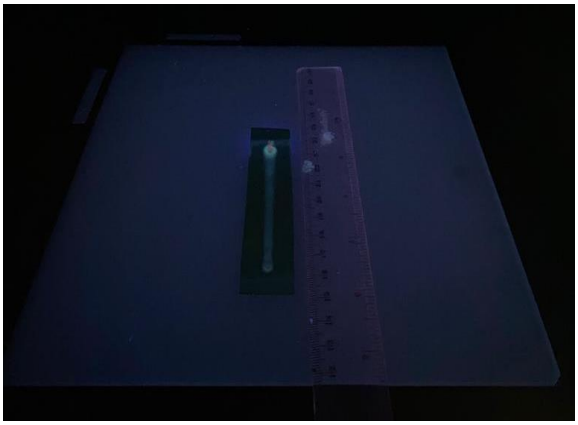


Pic. 1. TLC chromatogram profile of Sambiloto using silica gel 60 F254 and Chloroform : Methanol : Ammonia (84:15:1) as eluent and Dragendorf as detector

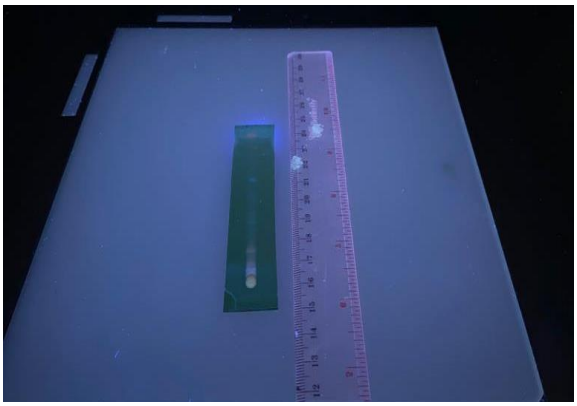


Pic. 2. TLC chromatogram profile of Sambiloto using silica gel 60 F254 and Chloroform : Methanol (8:2) as eluent and $AlCl_3$ as detector

From Thin Layer Chromatography (TLC) test for Kelor extract using Chloroform : methanol : ammonia (84:15:1) as eluent and Dragendorf as detector did not obtained the estimation of Alkaloid compounds, while using Chloroform : methanol (8 : 2) as eluent and $AlCl_3$ as detector obtained the estimation of Flavonoid compound.

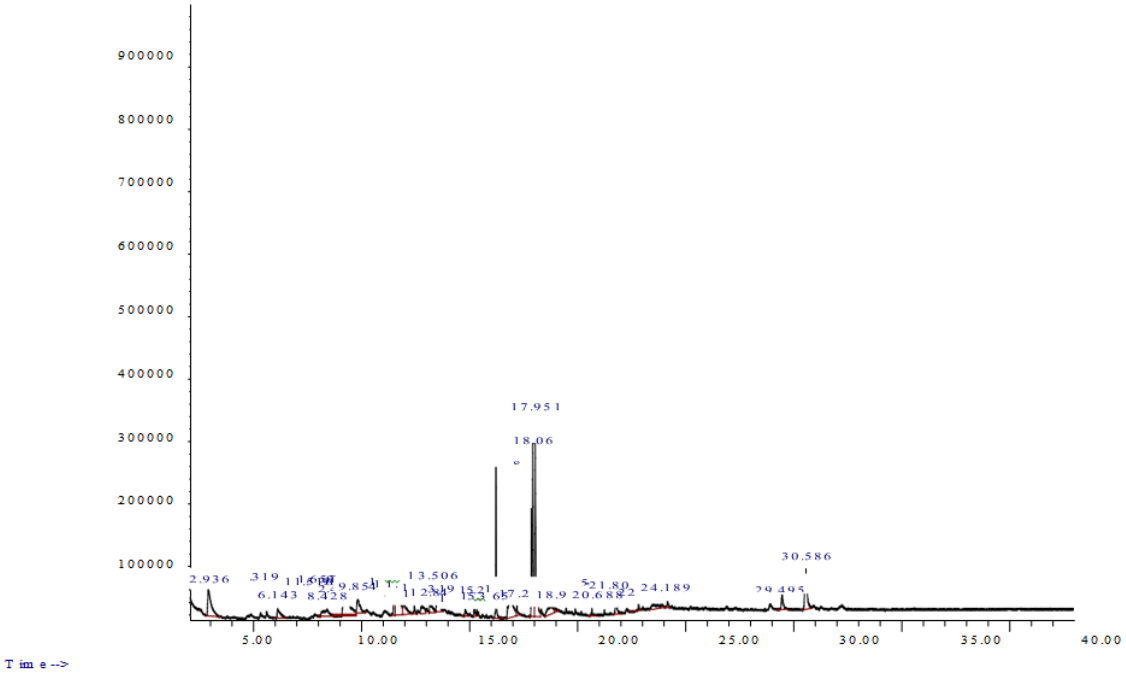


Pic. 3. TLC chromatogram profile of Kelor using silica gel 60 F254 and Chloroform : Methanol : Ammonia (84:15:1) as eluent and Dragendorf as detector

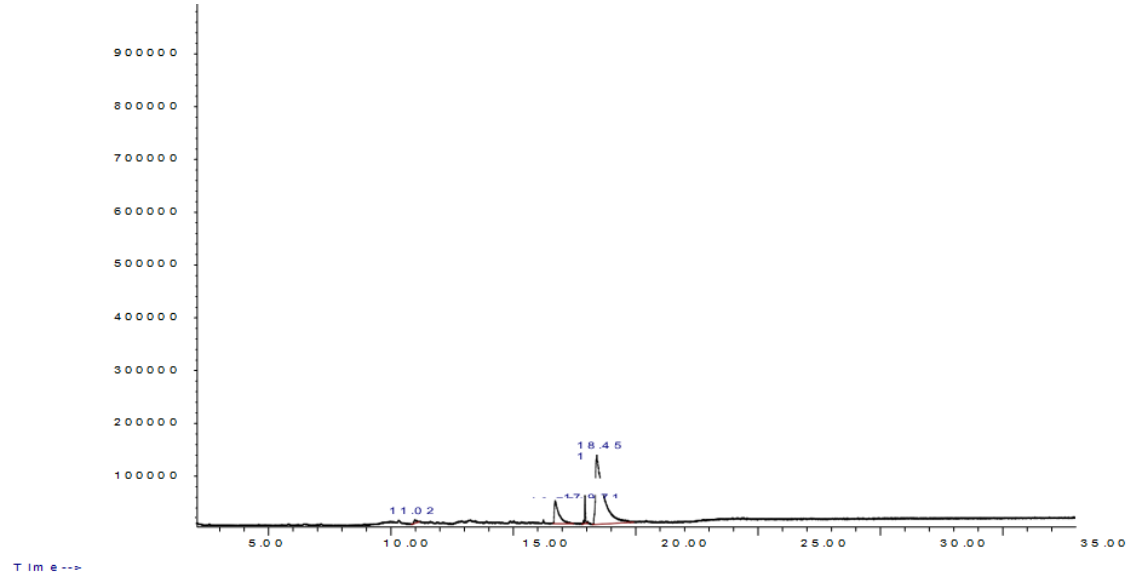


Pic. 4. TLC chromatogram profile of Kelor using silica gel 60 F254 and Chloroform : Methanol (8:2) as eluent and $AlCl_3$ as detector

From Gas Chromatography Mass Spectra (GCMS) test for Sambiloto extract showed some compound with the tallest peak at 18,068 Rf was Phytol compound while for Kelor extract showed a few compounds with the tallest at 18,451 Rf was 9, 12, 15-Octadecatrienoic acid.



Pic. 5. Spectrum GCMS Spectrum of Sambiloto



Pic. 6. Spectrum GCMS Spectrum of Kelor

IV. CONCLUTION

1. Ethanol extract of Andrographis paniculata Nees (sambiloto) and Moringa oleifera Lam (kelor) consisted of secondary metabolite such as alkaloid and flavonoid.
2. The tallest peak of Ethanol extract of Andrographis paniculata Nees (sambiloto) at 18,068 Rf was Pytol
3. The tallest peak of Ethanol extract of Moringa oleifera Lam (kelor) at 18,451 Rf was 9, 12, 15-Octadecatrienoic acid

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