

# Artikel 6

*by* Madyawati Latief

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## Isolation of active compounds from the leaf extract of patah kemudi (*Abroma augusta* L.) and its anti-inflammatory activity

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**Abstract** . Patah kemudi (*Abroma augusta* L.) is one of the shrubs that live on the edge of a river. *A. augusta* contains several secondary metabolite compounds including alkaloids, triterpenoids, steroids and flavonoids. The aim of this study was to isolate the compounds contained in the leaves of *Abroma augusta* and determine anti-inflammatory activity. Extraction and fractionation were performed by multilevel maceration. Isolation of compounds was carried out by column chromatography. The anti-inflammatory activity test was done using the Winter method. The n-hexane extract was separated by vacuum column chromatography and continued with gravity column chromatography to obtain isolate 8.1, which was then characterized using UV-Vis and IR spectrophotometer. The results of UV-Vis spectrophotometer on isolate 8.1 showed absorption at a maximum wavelength of 268 indicating a conjugate double bond which may be an aromatic originating from steroid compounds. While, the results of IR spectrophotometer characterization from isolate 8.1 manifested the presence of steroid compounds. The results of the activity test of isolate 8.1 with a dose of 10 mg presented the best anti-inflammatory activity compared to the positive control of diclofenac sodium. From the results of the GCMS shows that isolates contain  $\gamma$ -sitosterol compounds with a value of m/z 414.

### 1. Introduction

Isolation of chemical compounds from natural substances is an effort to separate mixed compounds so that we can produce a single, pure compound. Usually the process of isolation of compounds from natural substances is focused on secondary metabolites, because secondary metabolites have been studied and it believed can offer benefits to human life [1].

Inflammation is a complex response in vascular tissue that results from exogenous and endogenous stimuli. Inflammation is a normal response, protection against tissue injury caused by trauma, chemical or microbiological hazards which are defense of the body. Inflammation occurs to inactive or destroy foreign organisms, eliminating irritation is the first stage of tissue repair. The inflammatory process can usually be cured in the treatment process, but it can also be worse into severe inflammation which could be fatal [2].

Many drugs are currently developed that can treat inflammation, but inflammatory drugs are known to have various kinds of side effects that are quite harmful to patients. Therefore, the community is



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trying to choose alternatives by consuming natural ingredients. One natural ingredient that is known to have anti-inflammatory effects is a *patah kemudi* (*Abroma augusta* L) .

Based on the ethno-botanical survey in the land area of Muaro Jambi district, *A. augusta* plant was used as a traditional medicine by the community. The plant part which is efficacious as a medicine is from the bark mucus which serves to treat inflammation in the joints and also for fractures. Another part of the plant is the boiled steering leaf which serves to facilitate urine and also treat dysmenorrhea in menstruating women. A *A. augusta* plant is usually called a ghost or kapasan cotton plant. According to [3] secondary metabolite compounds contained in all parts of the *A. augusta* plant are alkaloids, steroids, triterpenoids and flavonoids. Meanwhile, on the leaf, the steering wheel contains taraxerol,  $\beta$ -sitosterol, acetate, octacosanol and aliphatic alcohol. For the distribution of the broken-wheeled plants it is spread naturally in India, Southeast Asia to Australia. In this study the characterization of the active compounds contained in the *A. augusta* n-hexane extract using UV-Vis spectrophotometer, IR spectrophotometer, and NMR spectrophotometer.

Previous research explained that this *A. augusta* plant had anti-inflammatory bioactivity. Where in the study, methanol extract of *A. augusta* showed the best anti-inflammatory activity than the standard drug, sodium diclofenac (Das et al, 2012) [4]. While the research of [5] about the methanol extract of *Abroma augusta* leaves are used as anti-hyperglycemic which was tested in Swiss albino glucose mice with the results obtained at doses of 100, 250 and 500 mg/kg body weight significantly decreased serum glucose levels by 36.9%.

Saikot, et al (2012) [6] also conducted research on the leaf extract of *Abroma augusta* used as antibacterial and cytotoxic with the results of the extract showed significant antibacterial activity tested on three gram positive (*Bacillus subtilis*, *Bacillus megaterium* and *Staphylococcus aureus*) and four gram negative (*Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei* and *Salmonella typhi*) bacteria. Determination, LC50 extract of saltwater shrimp nauplii was 7.06  $\mu$ g / ml which means it showed activity as cytotoxic.

Based on this studies showed that methanol extract of all parts of *Abroma augusta* plant has anti-inflammatory activity, while for the leaf section not only has anti-inflammatory activity but also has anti-hyperglycemic, anti-microbial and cytotoxic activity. But in the leaf section there is no specific research as anti-inflammatory. The purpose of this study was to isolate the compounds found in *Abroma augusta* leaves and determine anti-inflammatory activity.

## 2. Materials And Methods

### 2.1. Plant materials and chemicals

*Patah kemudi* leaves (*Abroma augusta* L), n-hexane, ethyl acetate, methanol, ethanol, 2N H<sub>2</sub>SO<sub>4</sub>, Dragendorff reagents, Wagner reagents, Mayer reagents, ethanol pa, Mg powder, 2 N HCl, concentrated HCl, chloroform pa, acetic anhydrous , FeCl<sub>3</sub>, sodium Dichlofenac, carrageenan, NaCl and distilled water.

### 2.2. Extraction and isolation

The thick n-hexane extract of *A. augusta* leaves was isolated by column chromatography. Chromatographic columns are prepared by making silica slurry which is inserted into the chromatography column slowly. The impregnated samples above the silica gel are inserted into the column, the impregnation is preadsorption of the sample in the stationary phase. The purpose of impregnation is to flatten the sample so that the elution is fast, the separation is good and the results of the separation are evenly distributed. The solvents used to elute are n-hexane, ethyl acetate and methanol. Where is done with multilevel polarity with the aim that what comes out first is a non-polar compound to the most polar compounds. Isolates are accommodated in vials that have previously been weighed and numbered. then thin layer chromatography (TLC) is carried out in order to classify the compounds obtained, where similar compounds can be combined to further separate until pure isolates are obtained.

### 2.3. Characterization and Identification of Isolated Compounds

Compound characterization was carried out using a UV-Vis spectrophotometer (*Biochrom Libra S70*) with wavelength of 200-400 nm and FT-IR spectrophotometer (Pelkin Elmer) at a wave number of 4000-400  $\text{cm}^{-1}$ . Furthermore, isolation compounds were identified using spectroscopic methods GCMS.

### 2.4. Antiinflammatory activity test

Mice were fastened from food for  $\pm 18$  hours before the experiment, while still providing drinking water. Mice were grouped into 5 groups randomly, each group consisting of 5 mice, then weighed and coded with markers on the tail. The initial volume of the foot of the mouse is measured before being treated and expressed as the base foot volume ( $V_0$ ). Then all mice were injected with a 1% carrageenan suspension on the soles of the mice as much as 0.3 ml. Submerged carrageenan injections. Before injecting the feet of mice which cleaned with 70% ethanol before. After 30 minutes of carrageenan injection, the volume of the feet of the mice was measured using songkor. After that it was injected with the prepared isolates, the negative control group was not given any treatment, the positive control group was given a dose of 0.14 mg / kgBB sodium diclofenac and the other three groups were given extracts and n-hexane isolates according to the doses planned subplantar. Then, the feet of the mouse are measured every 30 minutes for 90 minutes of observation and expressed as the final volume ( $V_t$ ) [7].

## 3. Results and Discussions

### 3.1. Phytochemistry test of each extract

Identification of compounds in each extract was done by phytochemical screening observed by color change. Phytochemical test gave concentrated n-hexane extract of *Abroma augusta* leaf was positive contained secondary metabolite compounds of steroid group, meanwhile the other secondary metabolite compounds were not detected. Generally, fraction of n-hexane was contained by nonpolar compounds such as alkaloids, steroids and terpenoids.

### 3.2. Isolation of *Abroma augusta* Leaf

Isolation was done by using column chromatography, that initiated by vacuum column chromatography to separated and simplified compounds in extract. Fraction 8 of vacuum column chromatography then further separated by gravity column chromatography and obtained 4 sub-fractions. Fraction 8.1 had most dominant spot pattern of TLC. Isolate 8.1 was tested by phytochemical assay and gave result that secondary metabolite was positive a steroid compound.

### 3.3. Characterization by UV-Vis spektrophotometer

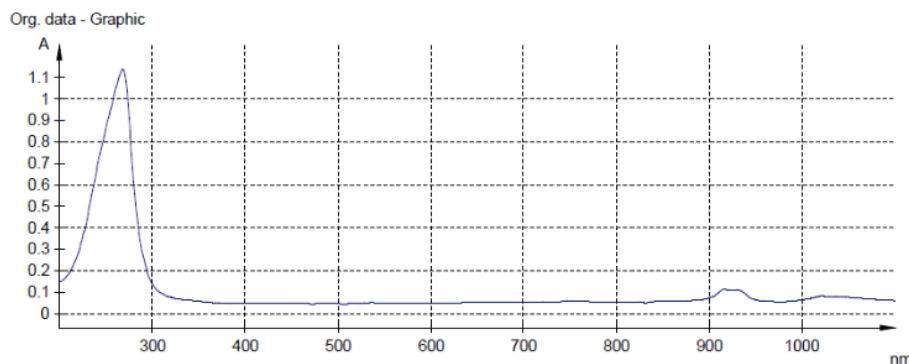
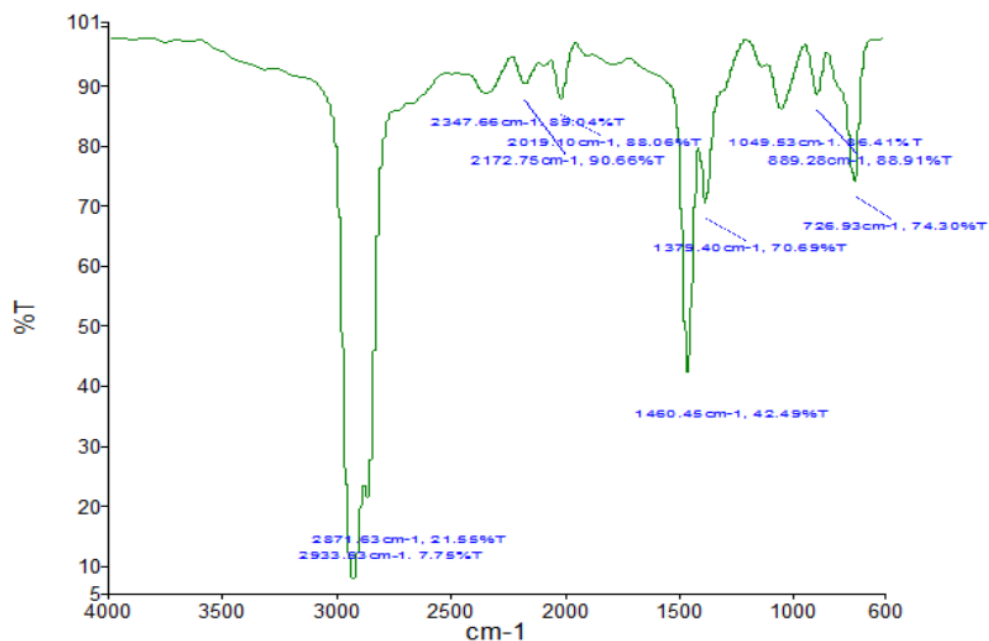


Figure 1. UV Spectrum of Isolate 8.1

Figure 1 showed one uniform maximum absorption band for compound in isolate 8.1 at 268 nm region with absorption 1.1401. Based on research done by Maharani et al (2016) [8], wavelength 268,40 nm is due electron transition from  $\pi \rightarrow \pi^*$  of double bond.

### 3.4. Characterization by FT-IR spektrophotometer

Analysis of Sepctrum data revealed absorption <sup>20</sup>wavenumber 2933  $\text{cm}^{-1}$  and 2871  $\text{cm}^{-1}$ , strong intensity form showed existence of C-H bond and stretching vibration. Symmetric stretching vibration of C-H was from  $-\text{CH}_3$  and  $-\text{CH}_2-$  gave absorption peak realtively close one each other. Besides, medium obsorption at 1460  $\text{cm}^{-1}$  and 1379  $\text{cm}^{-1}$  showed existence of C-H bond and bending vibration. Bending vibration also indicated by appearance of absorption at 726  $\text{cm}^{-1}$  from  $\text{CH}_2$  group [9].

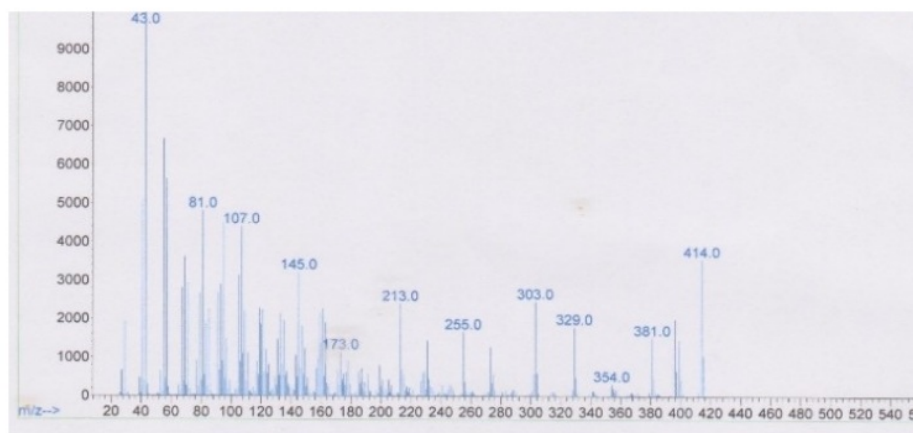


**Figure 2.** FT-IR Spectrum of Isolate 8.1

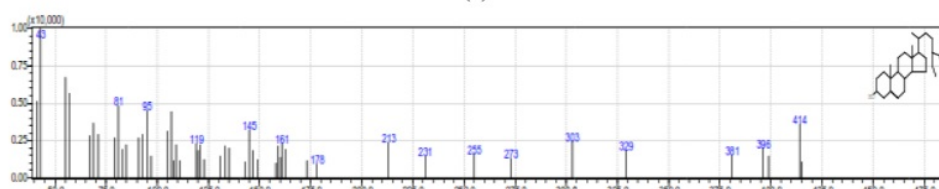
### 3.5. Characterization by GC-MS Spektrophotometer

Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample [10]. Characterization by spektrophotometer GC-MS intended to observed molecular weight of obtained compound. Isolate characterization showed fragmentation pattern data MS with molecular ion price  $m/z$  414,4 that had similarity with MS data from  $\gamma$ -sitosterol compound obtained from literature [11]. Fragmentation pattern of isolate and literature can be seen in figure 3.





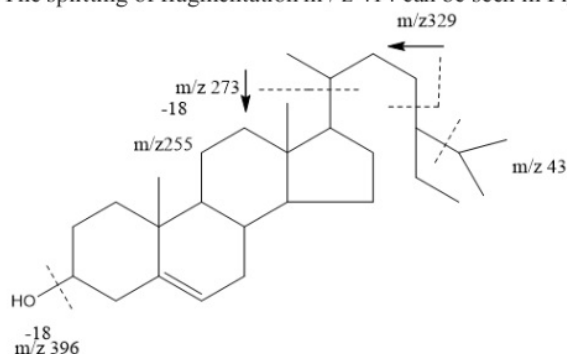
(a)



(b)

**Figure 3.** (a) GC-MS Spectra of Isolat 8.1, (b) GC-MS Spectra of  $\gamma$ -sitosterol

Spectra MS above showed similarity  $m/z$  price at fragment 414, 381, 329, 303, 255, 213, 145, 81, and 43. Can be concluded that isolate from n-hexane fraction plant patah kemudi leaf (*Abroma augusta L*) contained  $\gamma$ -sitosterol. The splitting of fragmentation  $m/z$  414 can be seen in Figure 4.

**Figure 4.** Splitting of fragmentation GC-MS Spectra

### 3.6. Anti-inflammatory activity

Antiinflammation test was carried out by winter method. One of most common technique used based on ability of given agent to inhibited production of edeme at mice mencit after injected by inflammtion agent and inflammtion volume was measured. Edema volume was measured before and after treatment by substances tested. Some iritan used as edema inductor e.g. formaline, caoline, yeast, and dextrane. Some common irritant used and had high sensitivity is karagen [12]. Measurement was done by using caliper at mice leg after 15 minutes of karagenan injection. Edema diameter after injection can be seen on table 1.

**Table 1.** Mice leg diameter after injection by karageenan

Testing animal	Diameter (mm)
S1 (positive control)	0,46
S2 (negatif control)	0,46
S3 Extract (5 mg)	0,52
S4 Extract (10 mg)	0,48
S5 Extract (15 mg)	0,49
S6 Isolat (5 mg)	0,42
S7 Isolat (10 mg)	0,46
S8 Isolat (15 mg)	0,44

Ability of anti-inflammatory activity can be seen from percent edema of mice leg, the result can be seen on table 2.

**Table 2.** % edema calculation of mice leg

Testing animal	Time of measurement (minute)		
	30	60	90
S1 (positive control)	61,53%	50%	46,15%
S2 (negatif control)	76%	68%	64%
S3 Extract (5 mg)	71,42%	60,71%	53,57%
S4 Extract (10 mg)	77,78%	70,37%	51,85%
S5 Extract (15 mg)	76%	72%	60%
S6 Isolat (5 mg)	57,69%	34,62%	28,57%
S7 Isolat (10 mg)	44%	24%	20%
S8 Isolat (15 mg)	65,38%	57,69%	50%

Table 2 showed that isolate with dose 10 mg/kg BW had best antiinflammation activity, even beyond of the positive control i.e. sodium dichlofenac.

#### 4. Conclusion

Based on phytochemical screening test, N-hexane extract of *Abroma augusta* contained steroid compound. Results of UV-vis, FT-IR and LC-MS characterization of isolat 8.1 from *Abroma augusta* revealed that obtained compound was  $\gamma$ -sitosterol, it can be seen from UV-vis absorbtion at 268 nm and m/z price at 414. Isolat 8.1 of n-hexane extract from *Abroma augusta* was active as anti-inflammatory agent, that isolat 8.1 with dose 10 mg/kg BW was able to decreased size of edema in mice leg after 90 minutes measurement, activity level is said very well the result obtained was better than positive control i.e. sodium dichlofenac.

#### Acknowledgment

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