

Original Research

*Anti-inflammatory activities of squalene compound of methanol extract of *Abroma augusta* L*

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HIGHLIGHTS

- Characterized isolate from methanol extract of *Abroma augusta* then we obtain squalene compound which belongs to triterpene that intermediate in the biosynthesis of steroids.
- The anti-inflammatory activity of isolate F211 has good results with edema values approaching positive control results with a reduction in edema of 28-30%.

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ABSTRACT

Abroma augusta L plant traditionally was used to treat swellings, cuts, sores, and bruises. In the province of Jambi, *A. augusta* is used in folk medicine to treat wounds. This study aims to isolate the steroid compound from the root of *A. augusta* L and determine its anti-inflammatory activities. Extraction and fractionation have been done with graded maceration using solvents with different polarities, which are n-hexane, ethyl acetate, and methanol. The separation was performed by column chromatography, followed by preparative thin-layer chromatography. The characterization of the isolate was carried out using UV-Vis spectrophotometry and infrared spectrophotometry, GC-MS. The anti-inflammatory activities of methanol extract and isolate of *A. augusta* was performed in this study was designed to evaluate the dose-response relationship of the anti-inflammatory activity in rat models of chronic inflammation chromatography to obtain isolate 2.1.1 that characterize and showed maximum absorbance at 265. The result of IR showed the presence of functional groups, -C=C-H, -C=H, -CH, CH₃, CH₂, and -CO belongs to the steroid compound. The results of the GC-MS shows that isolates contain squalene compounds with a value of m/z 410, Isolate and crude extract showed an anti-inflammatory activity that almost approached the positive control of sodium 4-chlorophenolate. It could be concluded that isolate and extract provide good anti-inflammatory activity, that promise for new drug candidate squalene-based *A. augusta*.

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1. INTRODUCTION

Indonesia is a country that has mega biodiversity, which is rich in plants that need to be explored in its potential. In Indonesia's tropical forests it is estimated that there are 30,000 species of plants, and it is estimated that about 9,600 species are known to have medicinal properties and 200 of them are important medicinal plants for the traditional medicine industry as raw materials.¹ The content of medicinal compounds in plants needs to be isolated, to separate active compounds that are mixed to get pure compounds.² The process of isolating compounds from natural materials is targeted at the isolation of secondary metabolites because secondary metabolite compounds can provide benefits for human life. The utilization of secondary metabolites is very much among them as antioxidants, antibiotics, anticancer, anti-inflammatory, as well as environmentally friendly pest control antigens. Some secondary metabolite compounds are alkaloids, terpenoids, flavonoids, steroids, and others. The content of secondary metabolites found in many leaves.³

Traditional medicinal plants are natural ingredients used for treatment based on the experience and diversity of medicinal plants. One of them is *Abroma augusta* L. which is widely used as a fracture drug or inflammatory drug. Inflammation is a response from the body to injury or infection. When had an injury occurs, the body will try to neutralize and eliminate harmful agents from the body and make preparations for tissue repair.⁴ The protective response due to damage to the tissue that serves to destroy, reduce, or localize both the injured agent and the injured tissue. The main signs of acute inflammation include swelling or edema, redness, heat, pain, and changes in function.⁵ Inflammation can occur acute and chronic which causes pathological abnormalities. Other than that, the inflammatory process underlies the pathogenesis of several diseases such as cancer, rheumatoid arthritis, Chronic, Obstructive Pulmonary Disease (COPD), atherosclerosis, and cardiovascular.⁶

Abroma augusta Linn (Family-Malvaceae) is commonly known as the Caterpillar of the genus of large evergreen plants, growing rapidly on shrubs, small trees with velvety branches. The *Abroma augusta* L plant is found in tropical Asia, South and East Africa, and Australia.⁷ Based on the ethnobotany survey conducted by the author through an interview with a resident in Muaro Jambi District, Mendalo Darat Village, it is known that the broken steering plant (*Abroma augusta* L.) is used as traditional medicine. The community uses mucus from the bark to treat inflammation of the joints and for fractures. The previous study was done by Pastel and Dhanabal⁸ found that *Abroma augusta* Linn contained glycosides, alkaloids, carbohydrates, and steroids. Inflammation is usually treated using both steroid class anti-inflammatory drugs (AIS) and a nonsteroidal class of anti-inflammatory drugs (NSAIDs).^{5,9} These phytoconstituents had been reported to have many biological effects such as antibacterial, antidiabetic, and anti-inflammatory, based on our previous studies known that all parts of the plant *Abroma augusta* have anti-inflammatory activity. This study aims to analyze secondary metabolite compounds in *Abroma augusta* and characterize the potential of steroid compounds as anti-inflammatory using phytochemical screening, chromatography, GC-MS, IR spectrophotometer (FTIR), UV-Vis, and anti-inflammatory assay.

2. MATERIAL AND METHOD

Material and research tools

Fresh leaves of *Abroma augusta* L. were collected from the District of Muaro Jambi, Jambi 2019 (5 kg). The Chemical Solvents such as Methanol, n-hexane, ethyl acetate, acetone, aquadest, silica gel 40 (70-230) mesh were purchased from Merck (Germany). ASTM for column chromatography, glassware (Pyrex®), FeCl₃ 5%, NaOH 10 %, Mg-HCl, H₂SO₄ (p), 6% HCl, chloroform, TLC silica gel 60 F254.

Extraction, isolation, and fractionation

The leaves of *Abroma augusta* were dried for a week at room temperature in the laboratory, mashed and macerated in 8 L of n-hexane then for 24 hours, then filtered and the filtrate is collected. The filtrate obtained was combined and concentrated with a rotary evaporator until a thick extract was obtained. The n-hexane residue was then macerated successively with 8 liters of ethyl acetate and methanol for 24 hours, each filtrate was collected

and concentrated with a rotary evaporator. The methanol fraction was isolated and purified by column chromatography, then analyzed by thin-layer chromatography (TLC) to select the appropriate eluent, then separated by open column chromatography using silica gel 60 silent phases. Purification of the main components contained in the active fraction was carried out by column chromatography and recrystallization until a pure compound with a single stain was obtained at TLC.^{10,11}

Characterization of compound

We did a qualitative analysis of secondary metabolites of both extract and isolate by phytochemical screening.¹² Furthermore, the isolated compounds were characterized using a UV-Vis Spectrophotometer (Biochrom Libra S70), IR Spectrophotometer (FTIR) (Perkin Elmer), and GC-MS (Shimadzu GCMS-QP2010 Ultra, Australia). The analysis was carried out using a UV-Vis spectrophotometer, the crystalline fraction obtained was dissolved using methanol as a solvent. In the UV-Vis spectrophotometer and determined the wavelength to be 200-400 nm.¹⁰ For analysis using an IR spectrophotometer, crystals from the obtained fraction were used as much as 0.5 mg then mixed with 50 mg KBr and crushed homogeneously. The FTIR spectrophotometer is first performed with a blank baseline in the form of air. Samples were put into KBr cells and put into a device with a hole leading to the radiation source then analysis was carried out ranging from 2.5-micron wavelengths (ν 4000 cm^{-1}) to 25 microns (ν 400 cm^{-1}). In the GCMS tool, 1 μl of the pure isolate was injected into the GC-MS tool, then the column used was the capillary model number 19091S-433 HP-5MS 5% Methyl Siloxane with a length of 30 m, a diameter of 250 μm and a thickness of 0.25 μm . Oven temperatures were used between 100-220 $^{\circ}\text{C}$. The rate of temperature increase is 15 $^{\circ}\text{C}$ / minute and the flow velocity is 1.0 ml/minute. The carrier gas is pressurized helium 10.5 psi and a total rate of 140 ml/min and a split ratio of 1:50.^{10,13}

Anti-inflammatory assays

Anti-inflammatory effectiveness test of leaves of *Abroma augusta* using eight male white mice aged around two months with an average weight of 20-30 g. Mice were randomly divided into 4 groups, each group containing 3 mice (Table 1). Mice are acclimatized and fasted for 24 hours by being given a drink. The inaction of injection from the beginning of the treatment, half an hour after we treatment, the rats were given an injection both of extract and isolates on the sole of the left rear foot. The volume of Edema was measurement using calipers for 90 min at 30 min intervals.

Table 1. Grouping of Animal test for anti-inflammatory assays

No	Group Name	Total a mice host
1	Positive control	1
2	Negative control	1
3	The isolate of <i>Abroma augusta</i>	3
4	Extract of <i>Abroma augusta</i>	3

Calculation of determination of several test animals

Calculated according to the Federer formula: $(n-1)(t-1) \geq 15$

Where n: Number of experimental animals per group; and t: Number of groups

Diclofenac sodium dose calculation

$$\text{VAO} = \frac{\text{Body Weight (kg)} \times \text{Dose (mg/kgWeight)}}{\text{Concentration (mg/mL)}}$$

Determination of anti-inflammatory activity

Calculated percent edema and percent inhibition of edema by following the previous study.⁴

$$\% \text{ uдем} = \frac{V_t - V_o}{V_o} \times 100\%$$

Determination of phytochemical analysis

This research to find out the content of compounds from the roots of the *Abroma augusta* L, was carried out through phytochemical tests by looking at the color changes or reactions that occur in the sample caused by the reaction between the reagent and the sample.

3. RESULTS AND DISCUSSION

Percentage of yield

The percentage yield for the extract was obtained and tabulated in [table 2](#), by weighing the weight of each fraction and then calculated. The following results are obtained:

Tabel 2. The percent yield of *Abroma augusta* L extract with different solvent

Fraction	Fraction weight (gr)	% Yield
n-hexane	1.4016	0.14016
Ethyl- acetate	2.014	0.2014
Methanol	16.6159	1.66159

Among the three extracts, the methanol fraction gave the highest percentage yield of 1.66159%, then ethyl acetate 0.2014% and n-hexane 0.14016% gave the lowest percentage yield. That seems due to the ability of each solvent to extract compounds in the roots is different. Besides, also this is influenced by the root extracted of the plant, where the concentration of the chemical compound is not too high. Methanol extract shows the content of secondary metabolites alkaloids, tannins, steroids, and triterpenoids which are non-polar compounds ([Table 3](#)). Moreover, we found that the steroid group is the major compound of the secondary metabolites group in the isolate.

Table 3. Phytochemical screening of *A. augusta* L

Secondary Metabolites	Method	Result	
		Total Extract	Isolate
Alkaloids	Meyer reagent	+	-
	Dragendorff reagents	-	-
Flavonoids	HCl 2N + Mg powder	-	-
Tannins	FeCl ₃	+	-
Steroids	Lieberman Burchard	+	+
Triterpenoids	Lieberman Burchard	+	-
Saponins	Water (Boiled) shake until foam appears	-	-

(+) Present/ (-) Absent

Isolation of *Abroma augusta* L.

The isolation of bioactive compounds was carried out by column chromatography which begins with vacuum column chromatography to separate and simplify the total compounds in the extract, then continued for the isolation process using gravity column chromatography to obtain four sub-fractions, and the best fraction we continued for the second of the KKG process to obtain a pure of isolates. Finally, we found that fraction 2.1.1 ([Figure 1](#)) has a staining pattern. Somehow, we wonder what the compound in that isolate. We did the phytochemical screening of the fraction to determine the content of chemical compounds in the isolates. The results of the phytochemical test ([Table 3](#)) showed that the isolate contained a single compound is steroids. This data showed that the isolate is a natural polymer, which was supported by the results of three eluent tests that showed an elongated TLC pattern that indicated that the compound obtained was a polymer.

Characterization of isolate

To identify the groups of secondary metabolites found in the isolated methanol extract of *Abroma augusta*, the characterization was performed using a spectrophotometer.

Characterization using a UV-Vis spectrophotometer

The number of absorbance of the compound the isolates were obtained from the leaves of *Abroma augusta* L. against UV light which has absorption at a maximum wavelength of 265 nm (1.1487 A) (Figure 2). This data shows the characteristics of steroid compounds that indicate conjugated double bonds which may also be aromatic.¹⁴

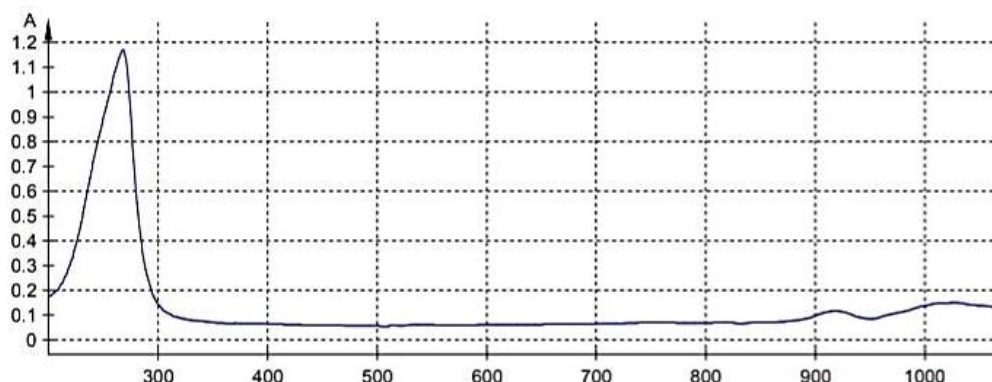


Figure 1. UV-Vis Spectrum of Isolate 2.1.1

Characterization using spectrophotometer of FT-IR

The FTIR analysis of compound F211 is used to find out functional groups of compounds, where the results of the FTIR spectrum of compound F.2.1.1 and comparison can be seen in Figures 3 and 4.

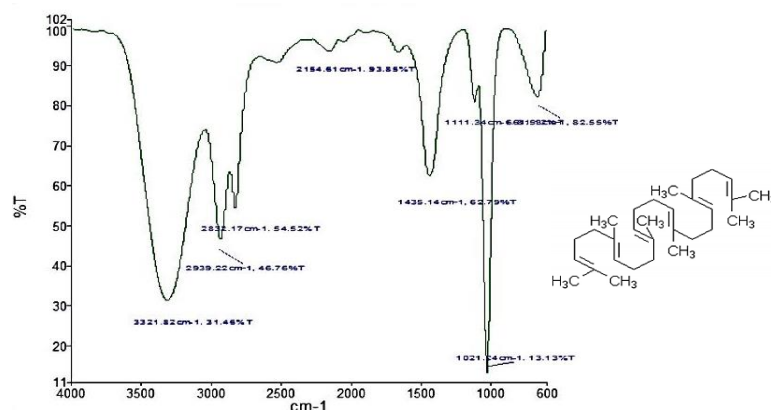


Figure 2. The FT-IR spectrum of Isolate 2.1.1 and Squalene structure interpretation

The results of the isolated spectrum (figure 3) showed that the existence of functional groups of a compound based on the electromagnetic spectrum in the IR region. Wavenumber 3321.82 cm^{-1} showed there is the existence of stretching absorption of O-H hydroxyl groups, and $3030,2$ is a C=C-H group. This assumption is strengthened by the existence of wave number at 2939 cm^{-1} which is belongs to an aliphatic CH group ($2850\text{--}2970\text{ cm}^{-1}$). The absorbance at wavenumber 668 cm^{-1} is caused by the C = C group which is an aromatic CH group. There is also a peak at 2924.09 cm^{-1} means the C-H strain of CH_3 . Moreover, the peaks in both 1643 cm^{-1} and 1512.19 cm^{-1} indicate the presence of aromatic C=C and wave numbers 1273.02 and 1226.73 cm^{-1} which indicate C-O uptake, this indicates the presence of terpenoid compounds or modified of squalene may occur even due to impure of the isolate.¹⁴ The FTIR spectrum of isolate F2.1.1 has high similarity with the previous results which is confirmed a similar compound.¹⁵

Tabel 4. A functional cluster of FTIR spectrophotometer

No	This Study (F211)	The previous Study*		Wavelength (cm ⁻¹)	Functional Group
		Squalene	Squalene (Modified)		
1	3321,82	3030,2	3031,2	3650-3500	C=C-H
2	-	3051,18		3100-3000	C- H
3	2939,22	2920,2	2920,2	3000-2850	C – H
	2832,17	2885,4	2885		
4	1435,14	1667,3	1737,7 1667,3	1650-1450	C = C -CO-O
5	1021,24	-	-	1300-1000	C – O alkohol

*Erizal, (2005)

According to previous research, the results of our study are suspected to have CO-O type bonds; C – H; C = C and C = C – H, it can be seen in [Table 4](#). Based on the results of the comparative spectrum in the table above with the isolated compound F2.1.1 have similarities with the results of Erizal¹⁵ study which stated positively the steroid group compounds. To find out specifically needed further characterization using GC-MS for the determination of compounds.

Characterization of molecules compound by GC-MS

To confirm the results of our FT-IR analysis, the characterization was carried out using the GC-MS spectrophotometer, to know the molecular weight of the compounds was obtained. The isolate that was characterized showed the pattern of fragmentation of MS data with molecular ions valued at m/z 410.0 which had similarities with MS data of squalene compounds obtained from *Saussurea obvallata* extract.¹⁶ The pattern of fragmentation between isolates and literature can be seen in [Figure 3](#).

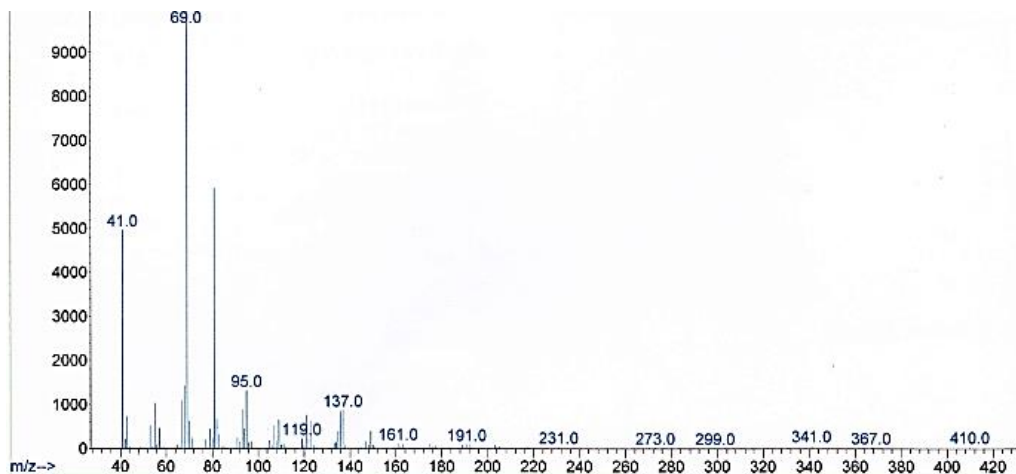


Figure 3. Spectrum GC-MS of isolate F211

Upon comparing with previous studies shows that indeed, the FT-IR spectrum of isolate F.2.1.1 corresponds to Squalene and GC-MS spectrum results also show that isolate F.2.1 has belonged to squalene which is a steroid group.

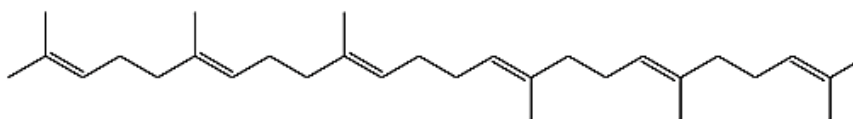


Figure 4. Structure of squalene: (*E*)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexane^{16,17}

Table 5. Chemical and physical properties of squalene

Properties	Value
Molecular weight	410.7 g mol ⁻¹
Melting point	-75°C
Refractive index	1.499
Viscosity at 25°C	12 cp
Density	0.858 g/ml
Boiling point at 25°C	285°C
Flash point	110°C
Iodine number	381 g/100 g
Infrared peaks	2728, 1668, 1446, 1380, 1150, 1180, 964, 835 cm ⁻¹
Surface tension	~32 mN/m

Anti-inflammatory assays

Anti-inflammatory testing was done by injecting test samples on experimental animals that have been injected with carrageenan. The results of measuring the diameter of the test animal's legs can be seen in [Table 6](#).

Table 6. Anti-inflammatory activity of both extract and isolate F2.1.1

Sample	The feet diameter after injection (mm)				% Decreased Edema
	0 min	30 min	60 min	90 min	
Positive control (0.14 mg)	0.46	0.42	0.39	0.38	39.67
Negative control	nd	nd	nd	nd	nd
Extract (5 mg)	0.36	0.33	0.31	0.31	31.66
Extract (10mg)	0.38	0.36	0.32	0.31	33
Extract (15mg)	0.33	0.30	0.29	0.27	28.66
Isolate F211 (5mg)	0.31	0.31	0.32	0.27	30
Isolate F211 (10mg)	0.28	0.29	0.28	0.27	28
Isolate F211 (15mg)	0.35	0.32	0.30	0.28	30

Based on the data in [Table 6](#) it can see a decrease in swelling of the legs of the test animals that have been given test samples, due to the activity of the isolate as an anti-inflammatory agent. The ability of anti-inflammatory activity can be seen from the % of edema of test animal feet. The formation of inflammation due to the induction of carrageenan consists of two phases. The first phase (early phase) is 1-2 hours after carrageenan injection which causes trauma. In the second phase (late phase) prostaglandin release occurs and is mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandin production by macrophages.⁶

Inflammatory and drug response

Inflammation is a response to inflammation of tissue damage due to various adverse stimuli, both chemical and mechanical stimuli, infections, and foreign objects such as bacteria and viruses.¹⁸ Anti-inflammatory drugs that are generally used are divided into two major groups, namely anti-inflammatory steroids and non-steroidal anti-inflammatory groups. However, both classes of drugs have side effects that are quite serious in their use. Anti-inflammatory steroids can cause peptic ulcers, decrease immunity to infections, osteoporosis, muscle atrophy, and fat tissue, increase intraocular pressure, and are diabetes.³ Some compounds that have anti-inflammatory activity from plants are classified as flavonoids. The development of natural-based compounds as an alternative to anti-inflammatory steroids and non-steroid which are known to have side effects on health.¹⁹ In our study we were able to obtain isolates from the *Abroma augusta* plant which were thought to be squalene compounds. The results of our isolate characterization using UV-Vis spectrophotometry and phytochemical tests confirm that isolate 2.11 is a steroid class. Further characterization using FT-IR and GC-

MS strengthens our hypothesis that the compound isolate 2.11 is squalene, by comparing with some previous studies.^{13,20} Squalene is a triterpene that intermediate in the biosynthesis of sterols in eukaryotes and a few bacteria,²¹ with the formula $C_{30}H_{50}$, an intermediate for the biosynthesis of phytosterol or cholesterol in animal, plants, and even humans, widespread in animal and vegetal kingdom.²² In previous research have reported that squalene (SQ) from virgin olive oil (VOO) possesses preventive effects against skin damage and anti-inflammatory properties.¹⁷ Hence, Squalene could be a useful natural product that might manage wound healing by its immunomodulation of macrophages, the main innate cells involve in wound healing.¹⁷ Squalene play role in reducing free radical oxidative stress and reduced intracellular levels of ROS, nitrites, pro-inflammatory enzymes (iNOS, COX-2, and MPO), and cytokines (TNF- α , IL-1 β , IL-6, and IFN- γ). Besides, squalene enhanced expression levels of anti-inflammatory enzymes (HO-1) and transcription factors (Nrf2 and PPAR γ).²³

CONCLUSION

Based on the results of this research it can be concluded that the results of UV-Vis and FTIR characterization show that the compound contained in isolate F211 is a steroid group and confirmed by GC-MS it is concluded that the steroid compounds obtained are squalene, it strengthened by the results of phytochemical test produce a brown positive steroid with a white needle-shaped crystal. Moreover, the anti-inflammatory activity of the extract and isolate F211 has the same potency as an anti-inflammatory with edema values approaching positive control results with a reduction in edema of 28-30%.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

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