

ISOLATION OF β -ASARON COMPOUND FROM SWEET FLAG RHIZOME (*Acorus calamus* L.) USING CHROMATOTRON METHODS

Suci Noviyannah Ansary¹, Virsa Handayani² and Ahmad Najib^{1*}

Abstract— Research on isolation of β -asaron compound from Sweet Flag rhizome *Acorus calamus* L.. The aims of this research are to isolate and to identify mayor compound β -asaron from Sweet Flag rhizome *Acorus calamus* L.. Sample from 150 gram dried rhizome, mascerated using n-hexane producing 4,22 grams thick extract. Isolation of n-hexane extract Sweet Flag rhizome *Acorus calamus* L. carried out by chromatotron methods using a comparison of eluen n-hexane : etil acetat (9:1). The isolate identified by UV-Vis spectroscopy, IR spectroscopy, and Gas Chromatography Mass Spectro. From the data result show that isolate is β -asaron.

Keywords— *Acorus calamus* L., β -asaron, chromatotron, isolation, Sweet Flag rhizome

I. INTRODUCTION

Sweet Flag rhizomes (*Acorus Calamus* L.) were included in Acoraceae tribe, known as a medicinal plant in India, USA, and Indonesia. Dringo (*Acorus Calamus* L.) can be used as a single agent or as a mixture of medicinal herbs [8].

The main chemical components (major compound) contained in this plant is cis-asaron, trans-asaron-patchoulen α , β -caryophyllen, humulen, methyl-eugenol, elemicine, cis- β -ocimene [6].

Previous research by Ari Wahyuni (2011) reported that the main active compounds in Dringo is β -asaron which are generally found in the rhizome, but are also found in the leaves.

Research on the chemical content and biological activity of the plant *Acorus Calamus* also been done that activity anthelmintic of the ethanol extract of *Acorus Calamus* who grew up in South Africa, antifungal, antioxidant, inhibition of FeCl₃ induced epileptogenesis in mice, antihepatotoksik and antioxidants, antihyperlipidemia and antibacterial [4].

II. MATERIAL AND METHODS

The sweet flag rhizomes (*Acorus calamus* L.) were obtained from the Bulukumba District, South Sulawesi Province - Indonesia.

A. Sample Extraction

Sample extraction by maceration using n-hexane solvent. Sweet flag rhizome powder (*Acorus Calamus* L.) macerated with n-hexane solvent. Maceration carried out for 3 days in a closed container and protected from light, stirring periodically. Extract the results of maceration (maserat) separated by the pulp, pulp macerated with n-hexane solvent. Maceration done 3 times. The filtrate obtained was collected further concentrated by using a rotary evaporator and the obtained extract n-hexane.

B. Thin Layer Chromatography Profile

N-hexane extract of *Acorus Calamus* L. reconstituted with the solvent n-hexane and then spotted on TLC plates berukuran 1 x 7 cm by using a capillary tube. Each plate has spotted insert into the chamber containing the eluent according to various comparison. Once eluted, the plates are removed from the chamber and aerated until the eluent evaporates. Then the resulting chromatograms profile observed in the visible UV 254 nm and 366 nm UV. Spotting obtained the observed and calculated nillai its R_f.

C. Isolation of β -asaron by Chromatotron Methods

Chromatotron plate with the stationary phase silica gel 60 PF₂₅₄ and 2 mm thick dried in an oven for 5 minutes at 50°C. The plates were then placed in the chamber and then the motor is turned on and the plate wetted with n-hexane stream. Eluent flow is stopped immediately after the first drops of n-hexane out. The sample chamber is inserted into the hole with

¹Phytochemistry Division,

²Pharmacognosy Division

Faculty of Pharmacy, Universitas Muslim Indonesia, Jl. Urip Sumiharjo KM 5, Makassar, Indonesia

the aid of a pipette and applied to the plate after the n-hexane had not come out.

D. Purification

a. Multi Eluen Methods

Isolates were then tested purity by using some variation of the eluent is based on different levels of polarity. Multi eluent method using TLC plates 60 F254 with a size of 1 x 7 cm. Single sighting spots indicating that the compound of isolates obtained a single chemical components.

b. Two Dimension Methods

Isolates obtained can also test the purity by using 2-dimensional manner isolates ditotal on the plates TLC 60 F254 with a size of 5 x 5 cm, then eluted using eluent is different to the first direction and the second direction, the elution process the latter is done by 90° rotating plate. Then the chromatogram profiles were observed in the UV 254 nm and 366 nm.

E. Identification of Pure Isolates

a. Identification by UV-Vis spectrophotometry

Pure isolates then identified using UV-Vis spectrophotometer. Compounds dissolved in methanol pa then snippets put into cuvette (sample compartments) that differ between monochromator and detector, resulting spectrum will be recorded on a recorder (Nurdin, 2014).

b. Identification by IR spectrophotometry

Pure isolates followed by identification using infrared spectrophotometry by placing the footage as a thin film between two layers of transparent sodium chloride, then placed in infrared light between monochromator with a detector, then recorded on a recording device (Nurdin, 2014).

c. Identification by GC-MS

Isolates obtained from the purification of the next sample is placed on a mobile phase will bring the sample passed through the stationary phase, so that most of the samples will be more likely to stick to the sample stationary and moving longer than the other components so that each component will be out on the stationary phase. The results are recorded on the recording device (Pavia , 2006).

III. RESULTS AND DISCUSSION

Sweet flag rhizome (*Acorus Calamus L.*) were taken in Bulukumba District, South Sulawesi Province. Sweet flag plant *Acorus Calamus L.* intact and still fresh directly determined in the Laboratory of Pharmacognosy Faculty of Pharmacy UMI-Phytochemistry. The result of determination shows the type of plant *Acorus Calamus L.* from Acoraceae tribe.

Simplicia rhizomes *Acorus Calamus L.* then extracted using maceration method. The extraction process is carried out using n-hexane solvent. Wherein the solvent is chosen because it has the ability to attract non-polar compounds.

TABLE 1. Results yield of n-hexane extract of the sweet flag rhizome (*Acorus calamus L.*)

Solvent	Sample Weight (g)	Quantity of Solvent (ml)	Extract Weight (g)	Yield Extract (%)
n-Heksan	150 g	2500 ml	4,22 g	3,55%

N-hexane extracts obtained, then in profile TLC using eluent n-hexane: ethyl acetate in a ratio of 8: 2 and 9: 1. Of the TLC profile, a good separation of the stains found on the plates using a ratio of 9:1.

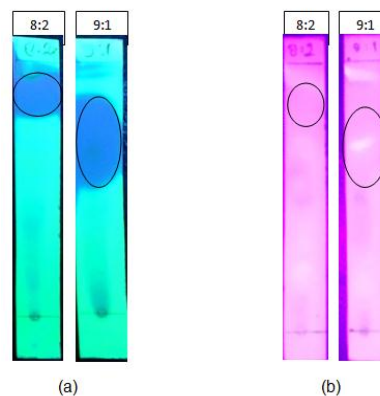


Figure 1. TLC profile of n-hexane extract of the sweet flag rhizome (*Acorus calamus L.*)

Description:

Stationary Phase: Silica Gel 60 F254

Mobile Phase : n-hexane: ethyl acetate

Plate Size : 1X7 cm

(a) Appear spotting UV254

(b) Appear spotting UV366

Isolation of β -asaron extracts of n-hexane was conducted using chromatotron, with silica gel 60 PF254 adsorbent as a stationary phase where chromatotron used plate thickness of 1 mm, using

the eluent n-hexane:ethyl acetate as the mobile phase with a ratio of 9: 1.

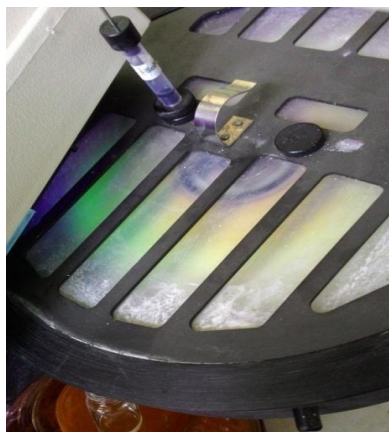


Figure 2. Instrument Chromatotron (Source: Personal Documentation)

In the radial thin layer chromatography is chromatotron, obtained 65 vials were combined into 3 fractions. TLC profiles performed on each fraction using the eluent n-hexane:ethyl acetate 9: 1.

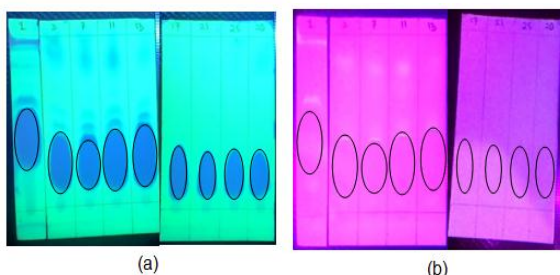


Figure 3. Profile fraction of the isolated chromatotron n-hexane extract of the Sweet flag rhizome (*Acorus calamus L.*)

Description:

Stationary Phase: Silica Gel 60 F254
 Mobile Phase : n-hexane: ethyl acetate (9:1)
 Plate Size : 1X7 cm

- (a) Appear spotting UV254
- (b) Appear spotting UV366

After the testing isolates with a single multi TLC eluent systems and two-dimensional.

TABLE 2. Results of Rf values and color patches on multi chromatography eluent isolates sweet flag rhizomes (*Acorus calamus L.*)

Mobile Phase (Eluen)	UV 254		UV 366	
	Value Rf	Spot Colour	Value Rf	Spot Colour
Aseton:EtOAc 6:4	0,87	Biru	0,87	Ungu

n-Hex:EtOAc 8:2	0,45	Biru	0,45	Ungu
Benzen:n-Hex 7:3	0,54	Biru	0,54	Ungu

TABLE 3. Results of Rf values and color patches on the two-dimensional chromatography isolates sweet flag rhizomes (*Acorus calamus L.*)

Direction Elution	UV 254		UV 366	
	Value Rf	Spot Colour	Value Rf	Spot Colour
Arah 1 n-Heksan : etil asetat (8:2)	0,42	Biru	0,42	Ungu
Arah 2 n-Heksan : etil asetat (9:1)	0,65	Biru	0,65	Ungu

Furthermore, the identification of the single isolates, using a spectrophotometer UV-Vis, IR Spectrophotometer, and GC-MS.

Spektro data interpretation UV-Visible show maximum absorption at a wavelength of 209.1 nm and 2,968 nm absorption is highest.

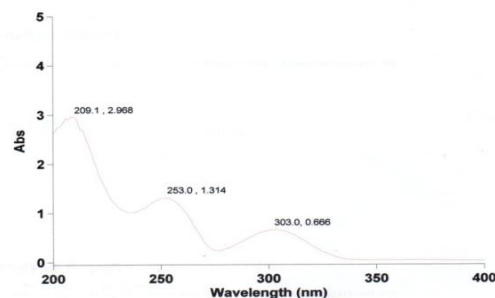


Figure 4. Diagram UV-Vis spectrophotometry data isolates the n-hexane extract of the sweet flag rhizome (*Acorus calamus L.*)

Subsequent analysis using IR spectrophotometer to determine the functional group of compounds that are in the isolates (Figure 5). IR spectrophotometry of observation, the absorption at 2959; 2927; 2873 cm⁻¹ indicate the presence of the CH stretching vibration of aromatic and CH₂; Aliphatic CH₃.

The presence of C-O bond is shown in a sharp peak absorption spectrum at wave numbers 1246 and 1292 cm⁻¹. Absorption band at 886 cm⁻¹ indicate a group C = C (cis). Absorption band at 1464 cm⁻¹ is the vibration of the C = C in the presence of the aromatic ring system.

TABLE 4. Results of single isolates identification of n-hexane extract of the sweet flag rhizome (*Acorus calamus* L.) in the spectrophotometer IR

Absorption Area (cm ⁻¹) Literatur	Absorption Area (cm ⁻¹) on Sample	Type Compounds	Gugus
3000-2850	2959; 2927; 2873	Alkana	C-H
1600-1475	1464	Alkena dan Aromatik	C=C
1300-1000	1292; 1246; 1136; 1061	Alkohol, Eter, Ester	C-O
1450-1375	1379		CH ₃

For the mass spectra of compounds isolated showed a molecular ion peak at $m/e = 208$ (M⁺) which indicates that the isolation of compounds having a molecular weight of 208 which is the same as the molecular weight of β -asaron. Having a fragmentation that is at m/e 15, 27, 39, 51, 77, 91, 105, 119, 137, 150, 165, 177, 193, and 208 [5].

TABLE 5. The results of the identification of a single isolate n-hexane extract of the sweet flag rhizome (*Acorus calamus* L.) in GC-MS.

Retention (minute)	m/z
8,76	15, 27, 39, 51, 77, 91, 105, 119, 137, 150, 165, 177, 193, 208

IV. CONCLUSION

In this research, it can be concluded that β -asaron compounds that have a molecular formula C₁₂H₁₆O₃ with chemical formula cis-1,2,4-trimetoksi-5- (1-propenyl) -benzen of Sweet Flag rhizomes (*Acorus calamus* L.) was isolated using chromatotron methods.

Acknowledgment

Authors are thankful to Rachmat Kosman, S.Si., M.,Kes., Apt who give a lot of support, and the Laboratory of Phytochemistry and Pharmacognosy-Chemistry, Faculty of Pharmacy, and was funded and provided by Muslim University of Indonesia..

References

[1] Ari, W, 2011, Isolasi dan Identifikasi Komponen Kimia Fraksi n-Heksana Daun Tumbuhan Jeringau (*Acorus calamus* L.) Asal Kabupaten Pinrang (Skripsi), Fakultas Farmasi Universitas Muslim Indonesia, Makassar.

[2] Fried, B. and Sherma, J., 1999, *Thin Layer Chromatography, 4th edition, Revised and Expanded*, New York, Marcel Dekker, Inc8.

[3] Fowles, I. A., 1998, *Gas Chromatography Analytical Chemistry by Open Learning*, John Wiley & Sons Ltd :Chichester.

[4] Hartati, S., Soemati, A., Irmawati E.A., 2012, *Isolasi β -asaron dari rimpang dringo (*Acorus calamus* Linn.) serta uji aktivitas antimikroba*, Jurnal Bahan Alam Indonesia, Fakultas Farmasi Universitas Indonesia, Depok.

[5] Jee Yeon, Lee., et al, 2004, Antifungal activity of β -asaron from rhizome of *Acorus gramineus*. *Journal of Agriculture and Food Chemistry*, 52, 776-780.

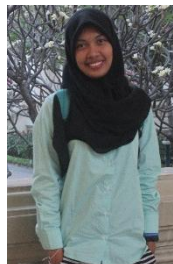
[6] Najib, A., 2010, *Isolasi Identifikasi Aktif Inhibitor α -Glukodase dari fraksi n-Butanol Rimpang *Acorus calamus* L. (Thesis)*, Depok: Fakultas Matematika & Ilmu Pengetahuan Alam Universitas Indonesia.

[7] Nurdin, M., 2014, *Isolasi dan Identifikasi Komponen Kimia Antioksidan Ekstrak Metanol Daun Jarak Kepyar (*Richinus communis* Linn.)*. (Essay), Fakultas Farmasi Universitas Muslim Indonesia, Makassar.

[8] Novianti, D.N., 2012, *Isolasi Uji Aktivitas Antioksidan dan Toksisitas Menggunakan *Artemia salina* Leach Dari Fraksi Aktif Ekstrak Metanol Daun Jambo-Jambo (Thesis)*, Depok: Fakultas Matematika & Ilmu Pengetahuan Alam Universitas Indonesia.

[9] Yande S.R., Harle U.N., Raygue D.T., Tuse D.T. and Vyawhare, 2008, Pharmacological profile of *Acorus calamus*, an Overview; *Pharmacognocny Review supplement*, vol 2 (4):22-26

About Author (s):



[If you never try, you will never know.
 Try until your limit]