รายงานวิจัยฉบับสมบูรณ์

โครงการ “การศึกษาองค์ประกอบทางเคมี และสารออกฤทธิ์ทางชีวภาพในเปลือกลูกเนียง”

ผู้วิจัย
ดร.หทัยชนก วงษ์เทพ

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

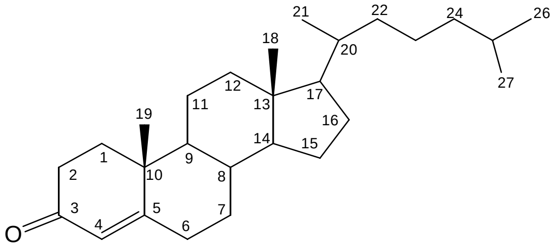
กิตติกรรมประกาศ

ผู้วิจัยขอขอบคุณฝ่ายวิชาการ สำนักงานกองทุนสนับสนุนการวิจัย ที่ได้อนุมัติ งบประมาณสนับสนุนการศึกษาวิจัยนี้ และขอขอบคุณ สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยวลัยลักษณ์ ศูนย์เครื่องมือวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยวลัยลักษณ์ ศูนย์เครื่องมือวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยสงขลานครินทร์ ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ ที่ให้ความอนุเคราะห์ในการศึกษาวิจัย

บทคัดย่อ

การแยกองค์ประกอบทางเคมีจากเปลือกลูกเนียง (*Archidendron jiringa*) สามารถแยกสารประกอบบริสุทธิ์ออกมาได้ 14 สาร ได้แก่ 4-cholesten-3-one **(AJ1)** stigmasterol **(AJ2)** β-sitosterol **(AJ3)** betulinic acid **(AJ4)** oleanolic acid **(AJ5)** deguelin **(AJ6)** lonchocarpusone **(AJ7)** rotenone **(AJ8)** elliptone **(AJ9)** rotenonone **(AJ10)** $6a\alpha$, $12a\alpha$-$12a$-hydroxyelliptone **(AJ11)** $12a\alpha$-hydroxyrotenone **(AJ12)** β-sitosterol glycoside **(AJ13)** และ β-stigmasterol glycoside **(AJ14)** โครงสร้างของสารเหล่านี้วิเคราะห์โดยข้อมูลทางสเปกโทรสโกปี UV IR NMR และเปรียบเทียบกับสารที่เคยมีรายงานแล้ว

สารประกอบบริสุทธิ์ที่แยกได้นำไปทำการทดสอบฤทธิ์ทางชีวภาพพบว่า สาร AJ4 AJ5 AJ6 AJ8 AJ9 และ AJ12 มีฤทธิ์ต้านเชื้อวัณโรค (anti TB) ด้วยค่า MIC 25-100 μg/ml ส่วนสาร AJ6 AJ8 AJ9 AJ10 AJ11 และ AJ12 มีฤทธิ์ต้านเซลล์มะเร็งช่องปาก (anti KB cell) ด้วยค่า ED$_{50}$ 0.1-2.3 μg/ml



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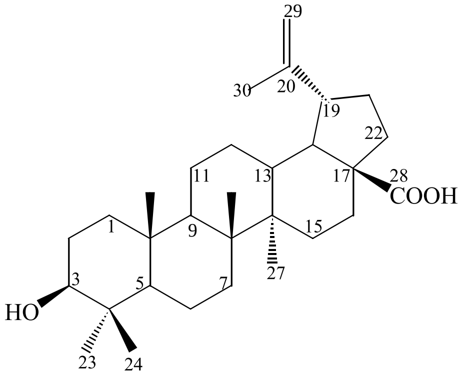

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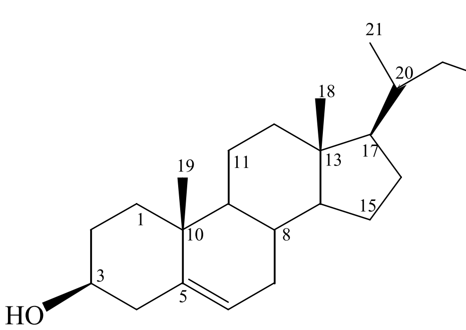

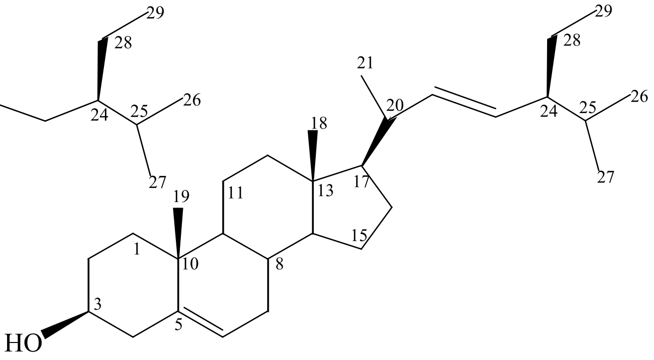

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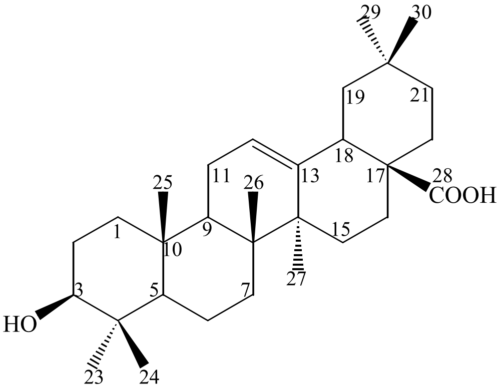

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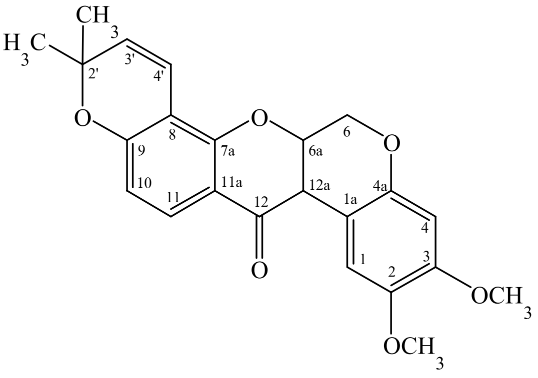

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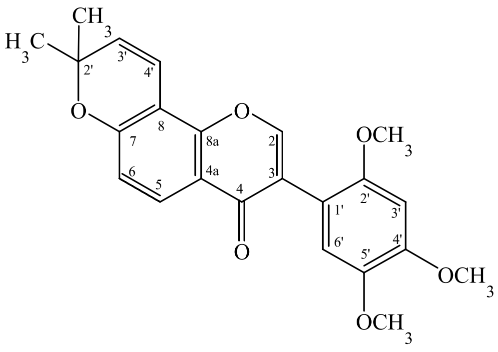

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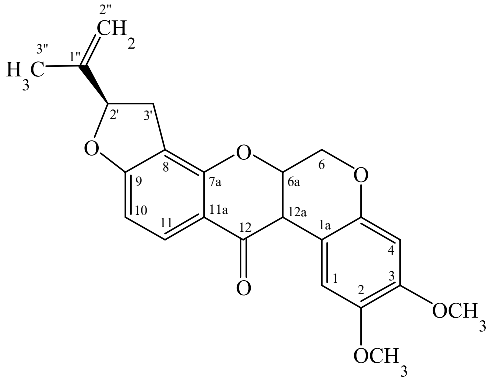

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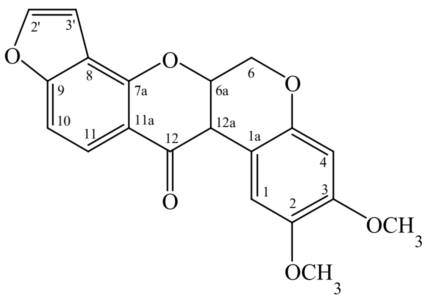

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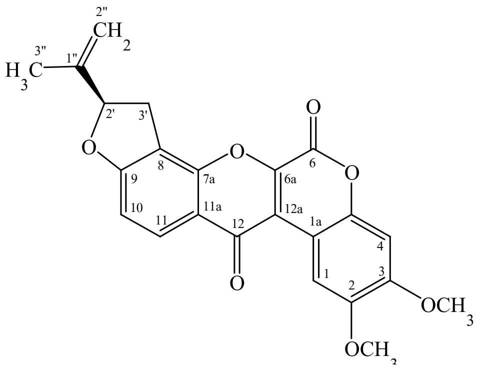

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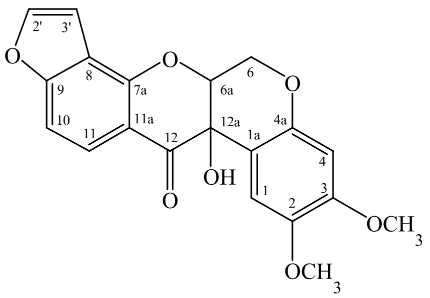

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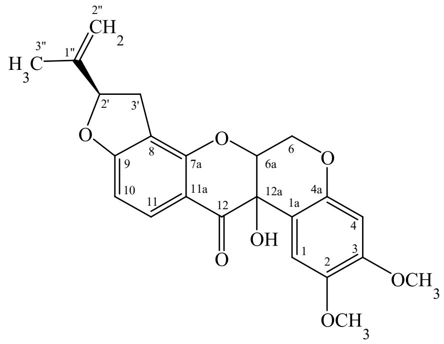

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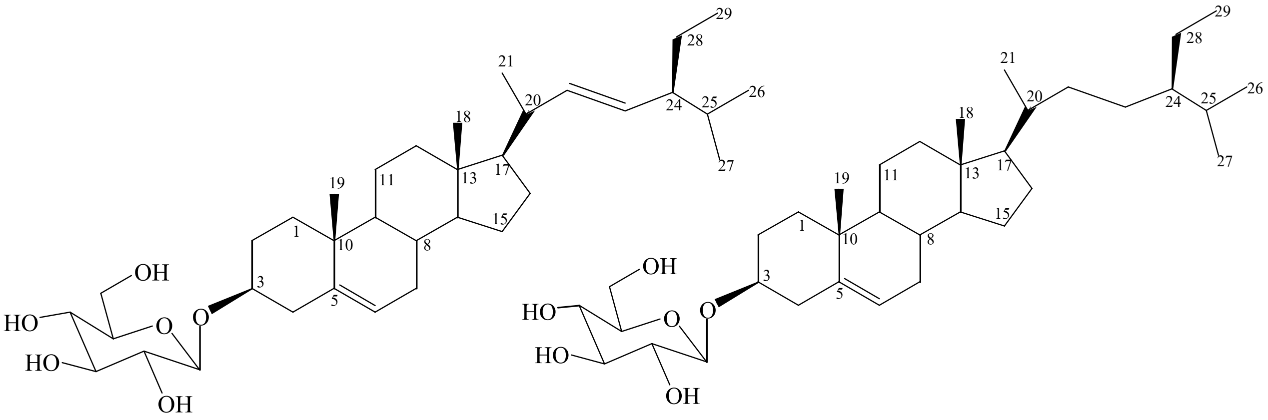


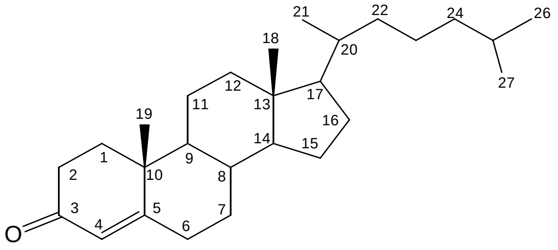
AJ13

AJ14

ABSTRACT

Isolation of the chemical constituents from the bean shells of *Archidendron jiringa* yielded fourteen known compounds: 4-cholesten-3-one **(AJ1),** stigmasterol **(AJ2),** β-sitosterol **(AJ3)**, betulinic acid **(AJ4),** oleanolic acid **(AJ5),** deguelin **(AJ6),** lonchocarpusone **(AJ7),** rotenone **(AJ8),** elliptone **(AJ9),** rotenonone **(AJ10),** 6*a*α, 12*a*α-12*a*-hydroxyelliptone **(AJ11),** 12*a*α-hydroxyrotenone **(AJ12),** β-sitosterol glycoside **(AJ13)** and β-stigmasterol glycoside **(AJ14)**. Their structures were elucidated on the basis of UV, IR and NMR spectroscopic data and comparison with reported value.

Some of the isolated compounds were screened for their biological activities. AJ4, AJ5, AJ6, AJ8, AJ9 and AJ12 showed antituberculous activity with the MIC of 25-100 μg/ml. AJ6, AJ8, AJ9, AJ10, AJ11 and AJ12 showed anti KB activity with the ED_{50} of 0.1-2.3 μg/ml.



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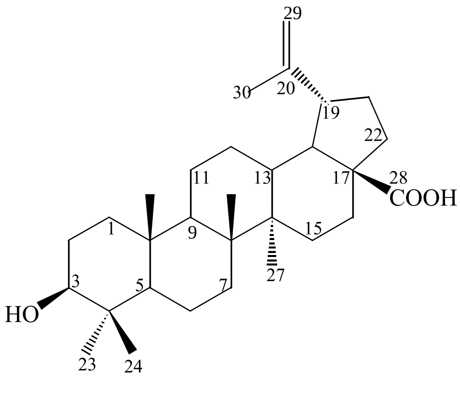

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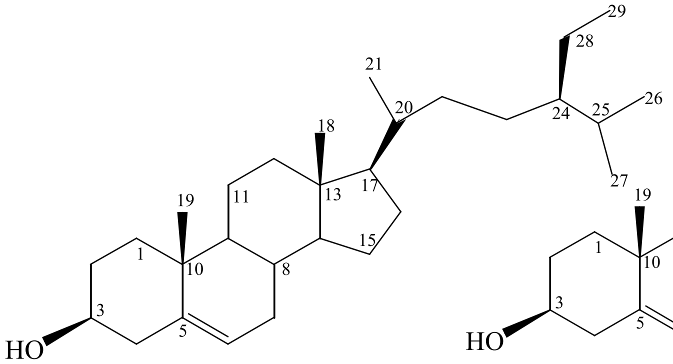

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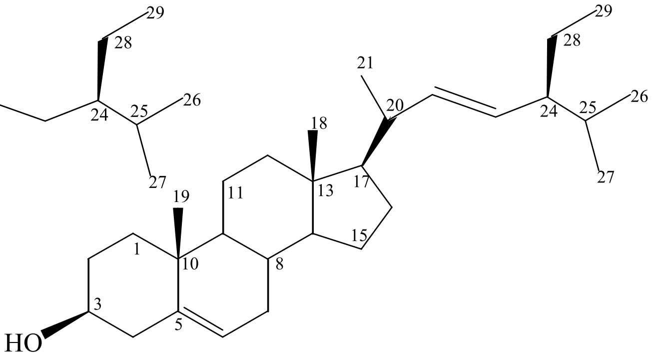

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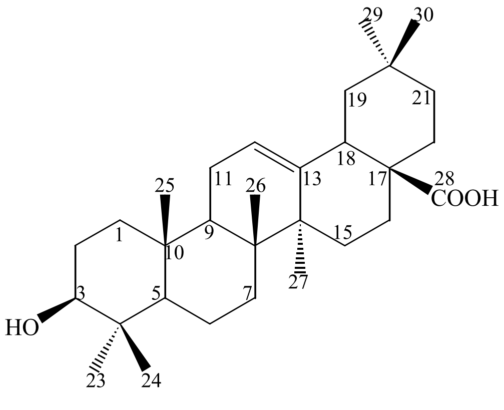

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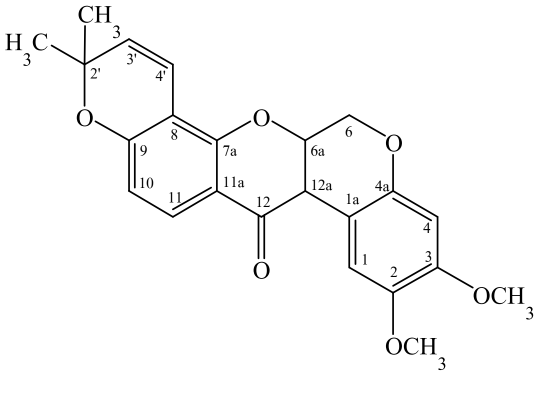

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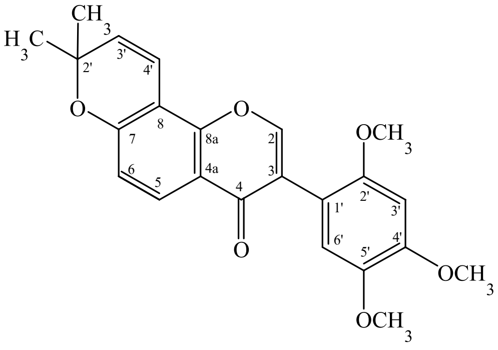

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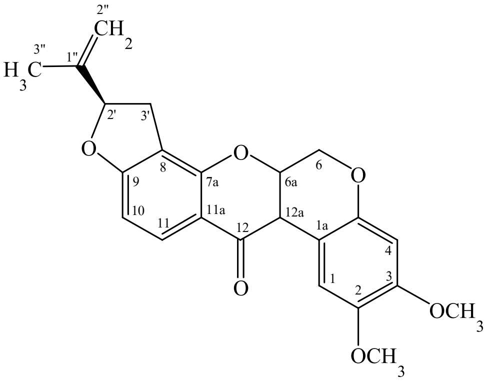

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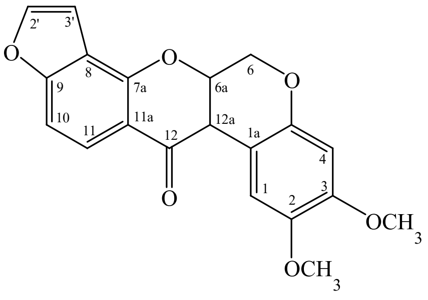

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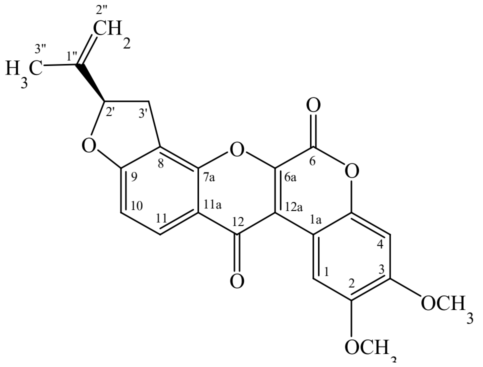

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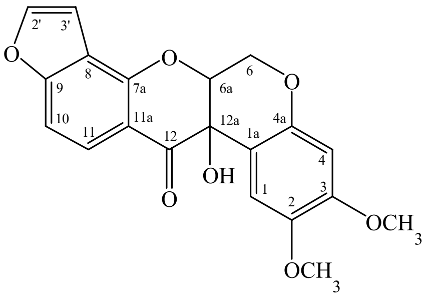

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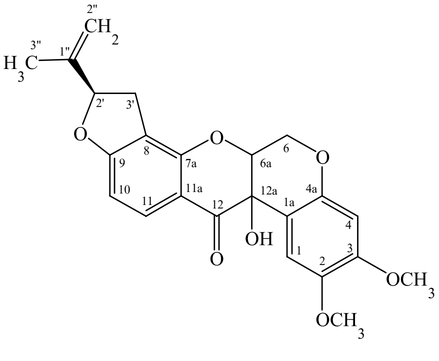

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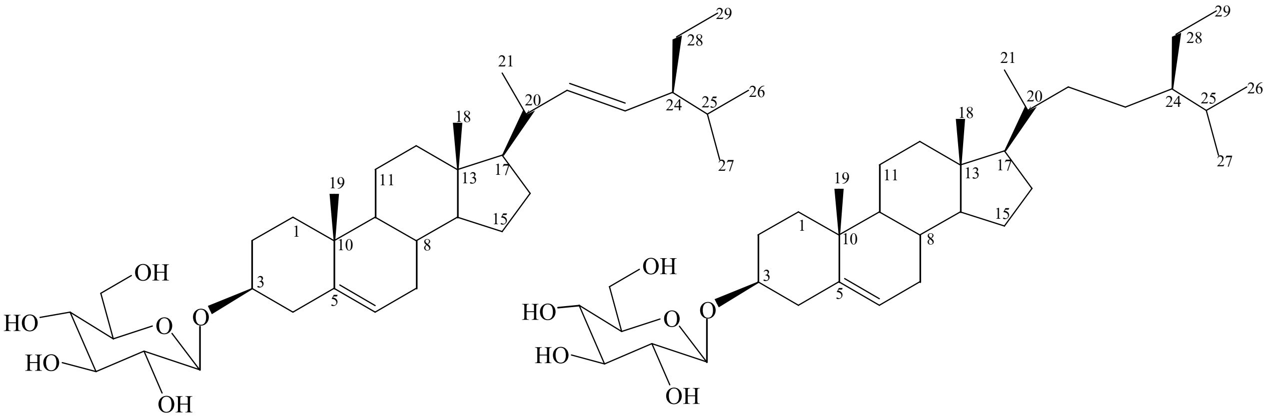

AJ13 **AJ14**

EXECUTIVE SUMMARY

ในปัจจุบันเป็นที่ทราบกันดีแล้วว่าการศึกษาหาองค์ประกอบทางเคมีในพืชเพื่อค้นหาสารตัวใหม่ที่มีฤทธิ์ทางชีวภาพ กำลังได้รับความสนใจอย่างมากในประเทศไทย อันเนื่องมาจากความต้องการผลิตยาขึ้นมาใช้ได้เองในประเทศ เพื่อลดการนำเข้ายาบางชนิดทีมีราคาแพง ประกอบกับที่ตั้งของประเทศไทยซึ่งอยู่ในเขตร้อนชื้น ทำให้พืชมีความหลากหลายทางชีวภาพมาก ในภาคใต้ของประเทศไทยก็เป็นพื้นที่หนึ่งที่มีความหลายหลายทางชีวภาพมาก เนื่องจากลักษณะภูมิประเทศมีความหลากหลายเช่น พื้นที่ชายฝั่งทะเล พื้นที่ภูเขา พื้นที่ป่า เป็นต้น และนอกจากนั้นยังมีพืชเฉพาะอีกถิ่นมากมาย

ดังนั้นในโครงการนี้จึงมีวัตถุประสงค์เพื่อศึกษาหาองค์ประกอบทางเคมีและสารที่ออกฤทธิ์ทางชีวภาพของเปลือกลูกเนียง ซึ่งเป็นพืชท้องถิ่นที่พบได้ทั่วไปในทางภาคใต้ของประเทศไทย

เนียง เป็นไม้ยืนต้นเปลือกสีเทา ใบประกอบรูปขนนก 2 ชั้น ใบย่อยรูปไข่มนจนถึงแกมขอบขนาน ดอกเป็นช่อแยกแขนง ผลเป็นฝักขดไปมา เมล็ดมีเปลือกสีเขียว ผลของเนียงเรียกว่า “ลูกเนียง” เป็นที่นิยมรับประทานกันมากในท้องถิ่น จนมีการค้าลูกเนียงเพาะอย่างกว้างขว้าง ในขั้นตอนการทำลูกเนียงเพาะนั้นจะต้องมีการกะเทาะเอาเปลือกหนาที่หุ้มเมล็ดทิ้งไป ทำให้เกิดขยะขึ้นเป็นจำนวนมาก ดังนั้นในการศึกษาครั้งนี้นอกจากจะศึกษาหาองค์ประกอบทางเคมีและสารที่ออกฤทธิ์ทางชีวภาพของเปลือกลูกเนียงเพื่อประโยชน์ทางการแพทย์แล้วนั้นยังเป็นการกำจัดขยะอีกทางหนึ่งด้วย

ในการศึกษาทดลองครั้งนี้จะเริ่มจากการเก็บเปลือกลูกเนียงที่กะเทาะออกใหม่ นำมาผึ่งให้แห้งในที่ร่ม จากนั้นนำไปสกัดเย็นด้วยตัวทำละลายที่ต่างขั้วกัน เมื่อได้สารสกัดหยาบแล้วนำไปทดสอบฤทธิ์ทางชีวภาพ และนำส่วนสกัดหยาบที่มีฤทธิ์ทางชีวภาพนั้นมาแยกหลาย

ขั้นตอน ด้วยวิธีทางโครมาโทกราฟฟี เพื่อให้ได้สารประกอบบริสุทธิ์ จากนั้นนำสารประกอบบริสุทธิ์นั้นไปทดสอบฤทธิ์ทางชีวภาพ ซึ่งได้ผลดังนี้คือ

การแยกองค์ประกอบทางเคมีจากเปลือกลูกเนียง (*Archidendron jiringa*) สามารถแยกสารประกอบบริสุทธิ์ออกมาได้ 14 สาร ได้แก่ AJ1-AJ14 โครงสร้างของสารเหล่านี้ศึกษาวิเคราะห์โดยข้อมูลทางสเปกโทรสโกปี UV IR NMR และเปรียบเทียบกับสารที่เคยมีรายงานแล้ว สารประกอบบริสุทธิ์ที่แยกได้นำไปทำการทดสอบฤทธิ์ทางชีวภาพพบว่า สาร AJ4 AJ5 AJ6 AJ8 AJ9 และ AJ12 มีฤทธิ์ต้านเชื้อวัณโรค (anti TB) ด้วยค่า MIC 25-100 μg/ml ส่วนสาร AJ6 AJ8 AJ9 AJ10 AJ11 และ AJ12 พบว่ามีฤทธิ์ต้านเซลล์มะเร็งช่องปาก (anti KB) ด้วยค่า ED_{50} 0.1-2.3 μg/ml

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CHAPTER 3: RESULTS AND DISCUSSION

ABBREVIATIONS AND SYMBOL

s = *singlet*

d = *doublet*

t = *triplet*

m = *multiplet*

dd = *doublet of doublet*

dt = *doublet of triplet*

br s = *broad singlet*

br t = *broad triplet*

g = gram

kg = kilogram

mg = milligram

mL = milliliter

nm = nanometer

cm^3 = cubic centimeter

ft. = feet

in. = inch

% = percent

cm^{-1} = reciprocal centimeter (wave number)

δ = chemical shift relative to TMS

J = coupling constant

$[\alpha]_D$ = specific rotation

λ_{max} = maximum wavelength

ν = absorption frequencies

ε = molar extinction coefficient

ABBREVIATIONS AND SYMBOL (continued)

calc. = calculated

m/z = a value of mass divided by charge

°C = degree celcius

MHz = Megahertz

ppm = part per million

c = concentration

EIMS = Electron Impact Mass Spectra

IR = Infrared

UV = Ultraviolet-Visible

MS = Mass Spectroscopy

NMR = Nuclear Magnetic Resonance

2D NMR = Two Dimentional Nuclear Magnetic Resonance

COSY = Correlated Spectroscopy

DEPT = Distortionless Enhancement by Polarization Transfer

HMBC = Heteronuclear Multiple Bond Correlation

HMQC = Heteronuclear Multiple Quantum Coherence

NOE = Nuclear Overhauser Effect Spectroscopy

PLC = Preparative Thin Layer Chromatography

TMS = tetramethylsilane

$CDCl_3$ = deuterochloroform

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CHAPTER 1

INTRODUCTION

Introduction and Literature Reviewed

Botanical Description

Common Name: Djenkol tree (Indonesia), krakos (Cambodia), jering (Malaysia), niang-yai (Thailand), jenkol, genkol, yiniking, yi-ring, ma-niang, cha-niang, niang, kra-niang
Scientific Name: *Archidendron jiringa* (Jack) I. C. Nielsen [*Abarema jiringa* Kosterm, *Albizzia jiringa* (Jack) Kurz, *Inga jiringa* (Jack) Jack, *Mimosa jiringa* Jack, *Pithecellobium jiringa* (Jack) Prain, *Pithecellobium lobatum* Benth., *Zygia jiringa* (Jack) Kosterm]
Botanical Family: Mimosaceae (Mimosa)

Physical Description: The djenkol bean has a broad, round shape and reddish color. The beans grow in large, dark purple pods (3-9 beans/pod) on the djenkol tree, which grows to 25 m (82 ft) in height. These beans resemble a large, flattened horse chestnut, and the crushed bean emits a faint sulfurous odor.

Exposure

Djenkol beans are a local delicacy in Indonesia, Malaysia, southern Thailand, and Myanmar. The beans are commercially available in markets during most of the year and are consumed raw, roasted, or fried.

Principal Toxins

Chemical Composition

The djenkol bean contains the sulfur-containing amino acid, djenkol acid (CAS RN: 498-59-9, $C_{11}H_{23}N_3S_3O_6$) in the range of about 0.3-1.3 g/100 g wet weight.[1] This compound is a cysteine thioacetal of formaldehyde as displayed in Fig 1.

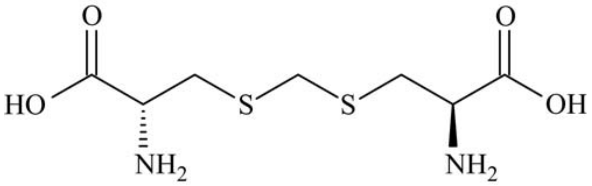


Fig 1. Chemical structure of djenkol acid.

Moreover, chemical constituents isolated from *Archidendron jiringa* (Jack) I. C. Nielsen were summarized by faculty of pharmacy Mahidol university.[2] It was reported that several compounds presented in the plant as follows: (+)-catechin; (-)-catechin,epi: 3-O-gallate; (+)-catechin-3'-O-gallate; (+)-catechin-4'-O-gallate; folate; gallic acid; (+)-gallocatechin; gallocatechin,epi: 3-O-gallate; gallocatechin-3'-7-di-O-gallate; gallocatechin-3'-O-gallate; gallocatechin-4'-7-di-O-gallate; Gallocatechin-4'-O-gallate; procyanidin B-3; procyanidin B-4; prodelphinidin B-1; vitamin B1.

Physiochemical Properties

Boiling, frying, or roasting the djenkol bean does not necessarily prevent toxicity as a case report temporally associated the development of acute renal failure with the ingestion of fried djenkol beans.[3] Typically, the ingestion of djenkol beans produces a pungent smell in the breath and urine of affected individuals.[4]

Mechanism of Toxicity

The mechanism of renal failure has not been clearly established. Animal studies demonstrate variable amounts of acute tubular necrosis with occasional glomerular involvement after the oral administration of djenkolic acid extracted from djenkol beans with 70% ethanol and water.[1] The peak incidence of djenkolism occurs in the rainy season (September- January) corresponding to the bloom time of the djenkol tree.[5]

Dose Response

Most people eat djenkol beans without ill effects, and there is no clear dose-response effect for the development of djenkolism. Although the occurrence of renal failure is frequently associated with the consumption of raw beans with low fluid intake,[6] the development of djenkolism does not depend on the method of preparation or the age of the fruit. The dose of djenkol beans associated with acute renal failure is highly variable (1-20 beans).[7] The development of djenkol bean poisoning can occur after numerous symptom-free meals of djenkol beans, and the attack rate can be low in large families consuming similar amounts of djenkol beans. Prior poisoning with djenkol beans does not confer immunity or hypersensitivity to subsequent meals of djenkol beans.

Toxicokinetics

There are inadequate data to determine the toxicokinetics of djenkol acid in humans.

Clinical Response

Acute Effects

The clinical features of djenkolism range from asymptomatic hematuria, dysuria, and abdominal pain to gross hematuria, severe flank pain, and acute renal failure. Mild djenkolism develops within 2-12 hours after ingesting djenkol beans, manifest by hypogastric cramps, flank pain, vomiting, diarrhea, dysuria, hematuria, and opaque (“milky”) urine. Case reports associate acute djenkolism with the development of acute renal failure, manifest by nausea, vomiting, bilateral flank pain, gross hematuria, hypertension, and transient oliguria.[3,8]

Phase-contrast microscopy of urinary sediment and renal biopsy reported in this case report was consistent with acute tubular necrosis rather than glomerular disease. There was no evidence of ureteral obstruction. Symptoms usually resolve within 3 days with supportive care.[7] Rare case reports associate the consumption of djenkol beans with the development of urethral stones, obstruction of the ureters, and urinary retention.[9]

Chronic Effects

In a study of 609 school children aged 7-11 years in an urban areaof Thailand, 78% of the children had a history of djenkol bean consumption with 38% ingesting these beans within 24 hours of the interview.[10] The presence of hematuria was about 4-fold (crude odds ratio _ 3.7) higher in children with a history of djenkol bean consumption compared with children not consuming djenkol beans. There was no significant difference in the presence of crystalluria or pyuria between these two groups.

Diagnostic Testing

Urinary abnormalities in cases of renal failure associated with the ingestion of djenkol beans include hematuria, proteinuria, and needlelike crystals of djenkolic acid. Crystals of djenkol acid are usually, but not always, present in the urine of patients with djenkolism. Persistent or recurrent microscopic hematuria in children consuming djenkol beans is not usually associated with clinical effects, but there are few data on the long-term effects of hematuria secondary to chronic djenkol bean consumption.[10] Renal biopsies of patients with djenkolism typically demonstrate evidence of acute tubular necrosis with little interstitial nephritis, ureteral obstruction, or glomerular lesions.[3]

Treatment

Treatment is supportive with careful monitoring of renal function. Generous fluid intake and alkalinization potentially increase the dissolution of djenkol crystals (pKa _ 5.1), although there are few clinical data to confirm the efficacy of this treatment. Decontamination measures are unnecessary. Most patients recover without sequelae.

CHAPTER 2

EXPERIMENTAL

2.1 Instruments and chemicals

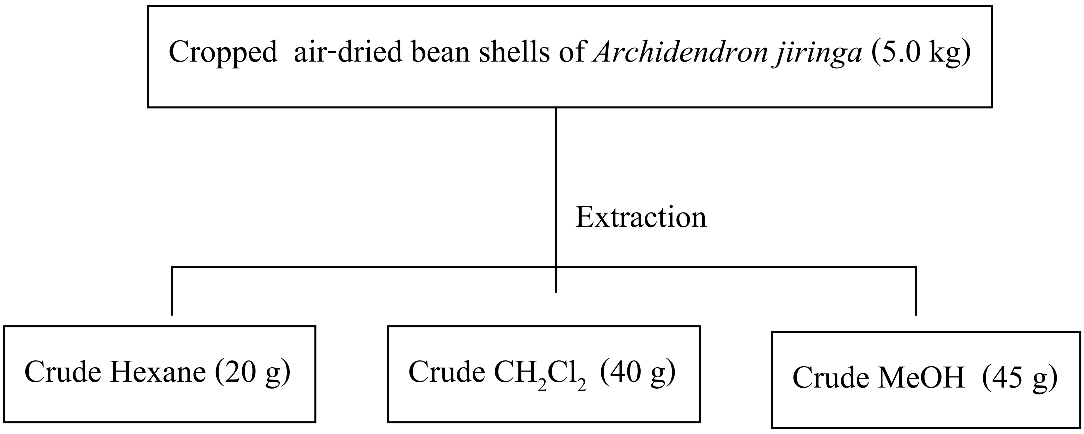
Melting points were determined on an Electrothermal melting point apparatus and recorded in °C. Optical rotation was measured in methanol solution with sodium D line (590 nm) on an Automatic Polarimeter (AD-100, ATAGO). Ultraviolet (UV) absorption spectra were recorded using a UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded in wavelengths (nm) and absorbance calculated in log ε in chloroform or methanol solution. Infrared spectra were recorded on a Perkin-Elmer Spectrum One spectrophotometer and major bands (ν) were recorded in wave number (cm^{-1}). Mass spectra (EI or FAB) were recorded on a MAT 95 XL Mass spectrometer, Thermofinnigan. The NMR spectra were determined on a 300 MHz, Avance DPX-300, Bruker spectrometer and a 500 MHz, UNITY INOVA, Varian spectrometer with TMS as an internal standard. Spectra was measured in deuterochloroform solution and recorded as δ value (ppm) downfield from TMS. Column chromatography was performed on silica gel type 100 (0.060 – 0.200, Merck) and Sephadex LH-20, TLC aluminum sheets (silicagel 60GF 254, Merck) for TLC analysis and detection was performed by UV lamp and spraying with reagent (1% vanillin in H_2SO_4). Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use.

2.2 Plant material

The bean shells of *Archidendron jiringa* were collected at Khiriwong village, Nakhon Si Thummarat province in the southern part of Thailand.

2.3 Extraction

The air-dried bean shells of *Archidendron jiringa* (5.0 kg) were cropped into small pieces and extracted twice with hexane (20 L), dichloromethane (30 L) and methanol (20 L) at room temperature, seven days each time. Each extract was filtered and solvent evaporated under pressure to yield the crude extract (**Scheme 1**).



Scheme 1 Extraction of the bean shells of *Archidendron jiringa.*

2.4 Biological activities of the crude extract from the bean shells of Archidendron jiringa.

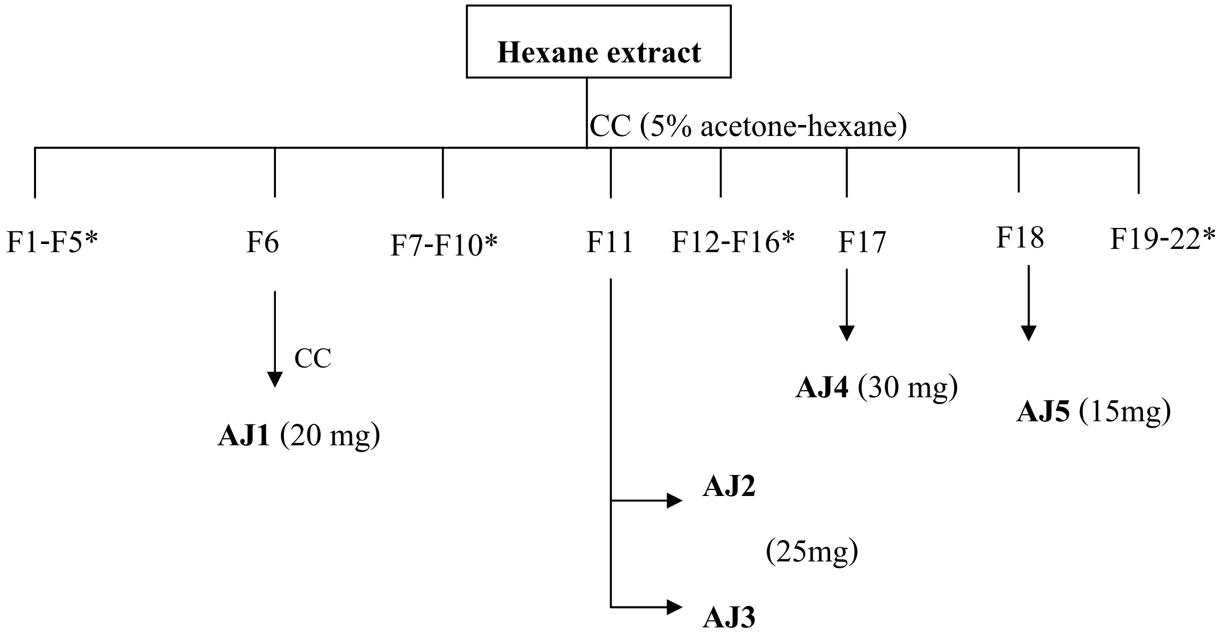
Table 1 Biological activities of the crude extract.

Crude extract	Anti TB MIC(μg/ml)	Antimalarial IC_{50}(μg/ml)	Cytotoxicity ED_{50}(μg/ml)		
			KB	BC	Vero-cell
Hexane	100	Inactive	Inactive	Inactive	Inactive
CH_2Cl_2	100	Inactive	2.71	Inactive	2.30
MeOH	Inactive	Inactive	Inactive	Inactive	Inactive

The crude hexane and dichloromethane extracts exhibited antimicobacterial activity with MIC value 100 μg/ml. It was therefore of interest to investigate the chemical constituents of the crude hexane and dichloromethane extracts.

2.5 Investigation of the crude hexane extract from the bean shells of Archidendron jiringa.

The hexane extract (20g) was separated into fractions by using column chromatography on a silica gel (900 g) column and eluted with 5% acetone-hexane. The solvent was removed by evaporation under a reduced pressure. Crude materials were checked on TLC using UV detector and vanilline-H_2SO_4 standard spray reagent. Fractions with similar TLC chromatogram were combined to afford 20 fractions, as shown in **Scheme 2**. The selected fractions were further purified to yield five pure compounds.



*Not further investigated

Scheme 2 Isolation of compounds **AJ1 - AJ5** from the bean shells of *Archidendron jiringa.*

Fraction 6 (95.0 mg) was purified by column chromatography eluting with 10% dichloromethane-hexane to afford ten fractions. The seventh fraction (34.9 mg) was further purified by preparative TLC on silica gel developing with 40% dichloromethane-hexane to give compounds **AJ1** (20 mg) as a white solid.

Fraction 11 (1.20 g) was subjected to column chromatography and eluted with the mixed solvent of 20% dichloromethane-hexane to afford ten fractions. The third fraction (188.5 mg) was obtained as a white solid mixture (25 mg) of **AJ2** and **AJ3**.

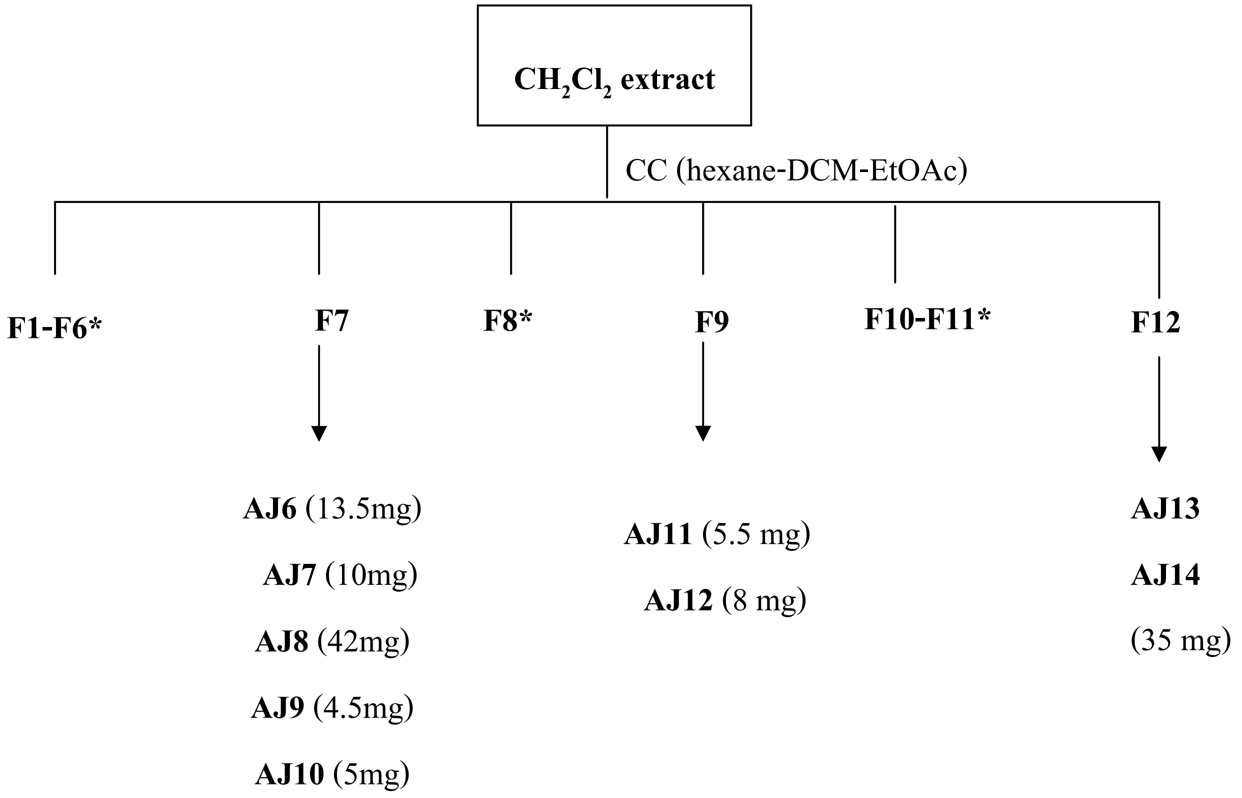
Fraction 17 (1.3 g) was rechromatographed on silica gel column chromatography using hexane enriched with dichloromethane, ethyl acetate and methanol to afford 4 subfractions. **Subfraction 17.1** was washed with dichloromethane to afford colorless crystals of compound **AJ4** (30 mg).

Fraction 18 (1.20 g) was further separated on column chromatography and eluted with the mixed solvent of 10% acetone-hexane to yield five fractions.

Subfraction 18.3 (80 mg) was subjected to column chromatography and eluted with 20% acetone-hexane to give five subfractions. The third fraction (34 mg) was purified on preparative TLC, eluting with 40% ether-hexane. The white solid of **AJ5** was obtained (15 mg).

2.6 Investigation of the crude dichloromethane extract from the bean shells of Archidendron jiringa.

The crude dichloromethane extract (40 g) was subjected to column chromatography by silica gel as the stationary phase and eluted with gradient elution of hexane, dichloromethane and ethylacetate. On the basis of their TLC characteristic, the collected fractions which contained the same major components were combined; fractions 1 – 12 were obtained. The selected fractions were further purified to yield seven pure compounds (**Scheme** 3).



*Not further investigated

Scheme3 Isolation of compounds **AJ6-AJ14** from the bean shells of *Archidendron jiringa.*

Fraction 7 (14 g) was subjected to sephadex column chromatography and eluted with 50%dichloromethane in methanol to yield subfractions 7.1 and 7.2.

Subfractions 7.1 was isolated by sephadex column chromatography, eluted with 20% dichloromethane in methanol to yield five subfractions.

Subfraction 7.1.3 (0.86 g) was further purified by column chromatography using silica gel and eluted with 20% chloroform in dichloromethane and gradient up to 100% dichloromethane to give eight subfractions.

Subfraction 7.1.3.2 (48.9 mg) was separated by preparative thin layer chromatography with 3% acetone in chloroform to give **AJ6** (13.5 mg) as a white solid.

Subfraction 7.1.3.4 (27.3 mg) was further purified by column chromatography using 10% acetone in hexane to give **AJ7** (10 mg) as a white solid.

Subfraction 7.2 was isolated by silica gel column chromatography, eluted with 10% acetone in hexane and gradient up to 50% acetone in hexane to yield subfractions 7.2.1 and 7.2.2

Subfraction 7.2.1 (3.37 g) was rechromatographed on a silica gel column, and eluted with 10% acetone in hexane to afford **AJ8** (42 mg) and **AJ10** (5 mg) as yellow crystals.

Subfraction 7.2.2 (1.63 g) was rechromatographed on a silica gel column three times, and eluted with 100% chloroform to afford **AJ9** (4.5 mg) as a white solid.

Fraction 9 (1.90 g) was isolated by silica gel column chromatography, eluted with 50% hexane in dichloromethane, increasing polarity with ethyl acetate in dichloromethane and finally with 100% ethyl acetate, to give six subfractions.

Subfraction 9.4 (22.2 mg) was separated by preparative thin layer chromatography with 10% acetone in hexane and followed by sephadex column chromatography, eluted with 50% dichloromethane in methanol to give **AJ11** (5.5 mg) as a pale yellow viscous oil.

Subfraction 9.5 (32.7 mg) was separated by preparative thin layer chromatography with 20% acetone in hexane to give **AJ12** (8.0 mg) as a colourless viscous oil.

Fraction 12 (3.4 g) was seperated to give subfractions 12.1 (45 mg) as a white powder and subfractions 12.2 (3.3 g) as a brown viscous liquid. Subraction 12.1 was further purified by recrystallization from a mixture of methanol and dichloromethane to give white powder as the mixture of **AJ13** and **AJ14** (35 mg).

Compound AJ1: a white solid; mp = 118-119 °C; $[\alpha]_D^{27}$ +50.2° (c = 0.02, $CHCl_3$); IR (neat) ν_{max} cm^{-1}: 1646 (C=O stretching), 2987 (C-H stretching); ^1H NMR ($CDCl_3$) (500 MHz) and ^{13}C NMR ($CDCl_3$) (125 MHz): see **Table 2**.

Compounds AJ2 and **AJ3:** Colorless crystals; mp: 135-136°C; IR (KBr) ν_{max}(cm^{-1}) 3435, 2973, 2937, 2842 and 1637; ^1H NMR ($CDCl_3$) (δ ppm) (500 MHz): δ 5.40-5.39 (*m*), 5.19 (*dd*, *J* = 15.0, 8.5 Hz), 5.06 (*dd*, *J* = 15.0, 8.5 Hz), 3.59-3.53 (*m*), 2.33 (*ddd*, *J* = 13.0, 5.0, 2.0 Hz), 2.30-2.24 (*m*), 2.28 (*qd*, *J* = 11.5, 2.5 Hz), 2.30-2.24 (*m*), 2.08-1.98 (*m*), 1.90-1.84 (*m*), 1.76-1.67 (*m*), 1.65-1.45 (*m*), 1.34-1.21 (*m*), 1.06 (*d*, *J* = 7.5 Hz), 1.04 (*s*), 0.96 (*d*, *J* = 6.5 Hz), 0.89 (*d*, *J* = 4.0 Hz), 0.88 (*d*, *J* = 1.5 Hz), 0.86 (*d*, *J* = 4.0 Hz), 0.85 (*d*, *J* = 1.5 Hz), 0.84 (*d*, *J* = 6.5 Hz), 0.73 (*s*), 0.71 (*s*).

Compound AJ4: White solid; mp: 254-256°C; IR (KBr) ν_{max} (cm^{-1}): 3443, 2944, 2871, 1688, 1452 and 883; ^1H NMR ($CDCl_3$) (δ ppm) (500 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 3.**

Compound AJ5: White solid; mp: 205-208°C; IR (KBr) ν_{max} (cm^{-1}): 3420, 2927, 2867, 1492, 1049 and 824; ^1H NMR ($CDCl_3$) (δ ppm) (500 MHz) and ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 5**.

Compound AJ6 : White solid; $[\alpha]_D^{25}$: +20.4° (c = 1.3 x 10^{-4}, MeOH); UV-Vis (MeOH) λ_{max}(nm) (log ε) : 205 (5.52), 239 (1.92), 271 (4.23) and 297 (4.08); IR (neat) ν_{max}(cm^{-1}) : 2933 (C-H stretching), 1675 (C=O stretching), 1598, 1513 (in plane ring stretching), 732 (out of plane H bending); ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (δ ppm) : see **Table 7** .

Compound AJ7 : White solid; $[\alpha]_D^{25}$: -68.3° (c = 1.2 x 10^{-4}, MeOH); UV-Vis (MeOH) λ_{max}(nm) (log ε) : 206 (4.37), 230 (4.30), 258 (4.33) and 298 (3.96); EIMS *m/z* (% relative intensity) : 395 (23), 394 $[M]^+$ (100), 379 (68), 363 (31), 349 (19); IR (neat) ν_{max} (cm^{-1}) : 2953 (C-H stretching), 1669 (C=O stretching), 1602, 1512 (in plane ring stretching); ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (δ ppm) : see **Table 9** .

Compound AJ8: White solid; m.p. 165-166 °C; $[\alpha]_D^{25}$: +54.40° (c = 5.1 x 10^{-4}, MeOH); EIMS *m/z* (% relative intensity) : 394 $[M]^+$ (14), 192 (78), 191 (16), 86 (53), 84 (100), 83 (30); UV-Vis (MeOH) λ_{max}(nm) (log ε) : 211 (4.53), 237 (4.17) and 294 (4.24); IR (neat) ν_{max}(cm^{-1}) : 2937 (C-H stretching), 1672 (C=O stretching), 1609, 1513 (in plane ring stretching); ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (δ ppm) : see **Table10** .

Compound AJ9: White solid; m.p. 178-179 °C; $[\alpha]_D^{25}$: +41.0° (c = 1.4 x 10^{-4}, MeOH); EIMS *m/z* (% relative intensity) : 352 $[M]^+$ (47), 192 (100), 191 (35), 86 (52), 84 (85); UV-Vis (MeOH) λ_{max} (nm) (log ε) : 211 (4.58), 237 (4.33), 274 (4.25) and 294 (4.21); IR (neat) ν_{max}(cm^{-1}) : 3124 (unsaturated C-H stretching), 2938 (C-H stretching), 1676 (C=O stretching), 1614, 1512 (in plane ring stretching), 733 (out of plane H bending); ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (δ ppm) : see **Table 12** .

Compound AJ10 : Yellow crystals; $[\alpha]_D^{25}$: +68.4° (c = 9.4 x 10^{-5}, $CHCl_3$); EIMS *m/z* (% relative intensity) : 407 (27), 406 $[M]^+$ (100), 391 (58); UV-Vis (MeOH) λ_{max} (nm) (log ε) : 255 (4.33), 290 (3.79) and 330 (3.92); IR (neat) ν_{max}(cm^{-1}) : 2927 (C-H stretching), 1748 (C=O stretching) and 1626, 1511 (in plane ring stretching) ; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (δ ppm) : see **Table 14** .

Compound AJ11 : Colourless viscous oil; $[\alpha]_D^{25}$: +69.1° (c = 8.7 x 10^{-5}, MeOH); UV-Vis (MeOH) λ_{max} (nm) (log ε) : 203 (3.11), 239 (3.04), 277 (2.46); IR (neat) $\nu_{\max}$ (cm^{-1}): 3416 (O-H stretching), 2938 (C-H stretching), 1678 (C=O) stretching), 1615, 1510 (in plane ring stretching) ; ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (δ ppm) : see **Table 16** .

Compound AJ12 : Colourless viscous oil; $[\alpha]_D^{25}$: +34.9° (c = 1.1 x 10^{-4}, MeOH); EIMS *m/z* (% relative intensity) : 410 $[M]^+$ (22), 208 (100), 207 (34), 86 (43), 84 (65), 83 (24); UV-Vis (MeOH) $\lambda_{\max}$ (nm) (log ε) : 210 (4.53), 238 (4.25) and 294 (4.74); IR (neat) $\nu_{\max}$ (cm^{-1}) : 3467 (O-H stretching), 2937 (C-H stretching), 1674 (C=O stretching), 1610, 1511 (in plane ring stretching); ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (δ ppm) : see **Table18**.

Compounds AJ13 and AJ14 : White solid; mp: 269-270°C; IR (KBr) $\nu_{\max}$ 3402 (O-H stretching), 2934 (C-H stretching), 1073 (C-O stretching) cm^{-1}; ^{1}H NMR ($CDCl_3$) (500 MHz): 5.37 *m*, 4.42 *d, J* = 8.0 Hz, 3.84 *dd, J* = 12.0, 3.0 Hz, 3.78 *dd, J* = 12.0, 4.0 Hz, 3.61 - 3.55 *m*, 3.51-3.45 *m*, 3.41-3.40 *m*, 3.32 - 3.26 *m*, 2.39 *ddd J* = 13.5, 5.0 and 2.5 Hz, 2.29 - 2.24 *m*, 2.02 - 1.96 *m*, 1.70 - 1.40 *m*, 1.25 *s*, 1.00 *s*, 0.96 *d, J* = 6.5 Hz, 0.86 s, 0.84 *d, J* = 3.0 Hz, 0.83 *d, J* = 4.0 Hz, 0.80 *s*, 0.68 *s*.

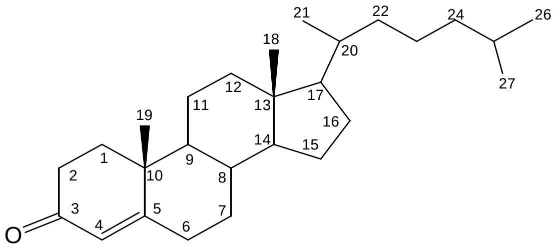
CHAPTER 3

RESULTS AND DISCUSSION

3.1 . Structure elucidation of compounds from the bean shells of Archidendron jiringa.

The hexane and dichloromethane extracts from the bean shells of *Archindendron jiringa* were separated by column chromatography. Repeated chromatographic separation of the crude hexane extract resulted in the isolation of five compounds, **AJ1-AJ5**. The crude dichloromethane extract resulted in the isolation of seven compounds, **AJ6-AJ14**. The structures of the isolated compounds were elucidated using 1D, 2D NMR spectroscopic data, their physical and spectral data were compared with known data, compound **AJ1-AJ12** were successfully identified.

3.1.1 Compound AJ1



Compound **AJ1** was obtained as a white solid, mp = 118-119 $^{\circ}$C. Its IR spectrum exhibited absorption band at 1646 cm^{-1} for carbonyl group. This compound showed green color with vanillin sulfuric acid reagent, indicating the steroid characteristic.

The ^{1}H NMR spectrum revealed five methyl groups, two of them showed *singlets* at δ 0.71 and 1.18 which could be assigned to H-18 and H-19, respectively. The other ones resonated as *doublet* at δ 0.92 (J = 6.5 Hz), 0.85 (J = 4.5 Hz), and 0.83 (J = 4.5 Hz), which were assigned to H-21, H-26 and H-27, respectively. One olefinic proton appeared at δ 5.72 (*br s*), could be assigned to H-4. Thus the compound **AJ1** was determined to be 4-cholesten-3-one.[11]

Table 2 The NMR spectral data of **AJ1**.

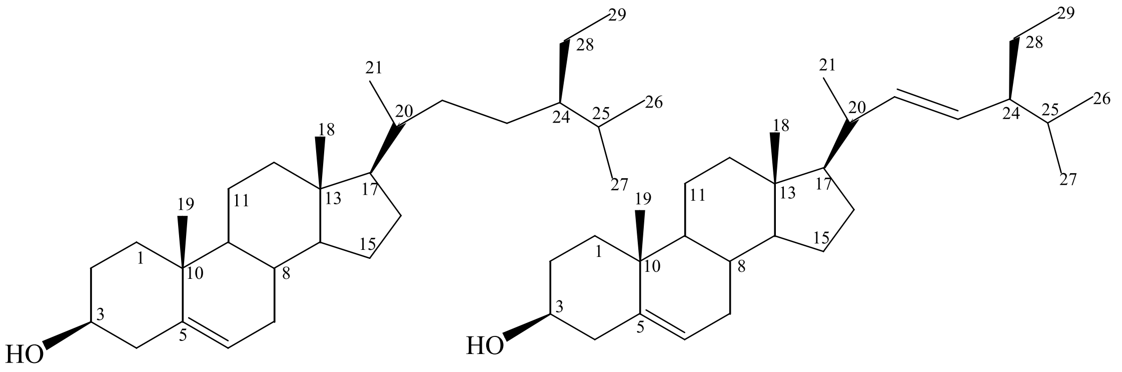
Position	δ_C**# (ppm)**	δ_H **(ppm)**
1	39.3 (CH_2)	1.24 (1H, *m*), 1.68 (1H, *m*)
2	30.1 (CH_2)	1.50 (1H, *m*), 1.84 (1H, *m*)
3	66.4 (CH)	3.97 (1H, *m*)
4	51.0 (CH_2)	2.13 (1H, *m*), 1.71 (1H, *m*)
5	79.4 (C)	-
6	130.7 (CH)	6.52 (1H, *d*, 8.5)
7	135.4 (CH)	6.26 (1H, *d*, 8.5)
8	82.2 (C)	-
9	34.7 (CH)	1.32 (1H, *m*)
10	36.9 (C)	-
11	20.8 (CH_2)	1.97 (1H, *m*), 1.50 (1H, *m*)
12	36.9 (CH_2)	2.02 (1H, *m*), 1.73 (1H, *m*)
13	44.5 (C)	-
14	51.7 (CH)	1.54 (1H, *m*)
15	28.6 (CH_2)	1.42 (1H, *m*), 1.84 (1H, *m*)
16	23.4 (CH_2)	1.37 (1H, *m*), 1.77 (1H, *m*)
17	56.2 (CH)	1.74 (1H, *m*)
18	21.8 (CH_3)	0.84 (3H, *s*)
19	18.2 (CH_3)	0.88 (3H, *s*)
20	39.7 (CH)	1.22 (1H, *m*)
21	19.6 (CH_3)	1.00 (3H, *d*, 6.5)
22	132.3 (CH)	5.23 (1H, *dd*, 7.5, 15)
23	135.2 (CH)	5.15 (1H, *dd*, 8.5, 15)
24	42.8 (CH)	1.98 (1H, *m*)
25	33.0 (CH)	1.86 (1H, *m*)

Table 2 (Continued)

Position	δ_C**# (ppm)**	δ_H **(ppm)**
26	19.9 (CH_3)	0.92 (3H, *d*, 7.0)
27	20.6 (CH_3)	0.81 (3H, *d*, 7.0)
28	17.6 (CH_3)	0.83 (3H, *d*, 5.0)

Carbon type was deduced from DEPT experiments.

3.1.2 Compounds AJ2 and AJ3

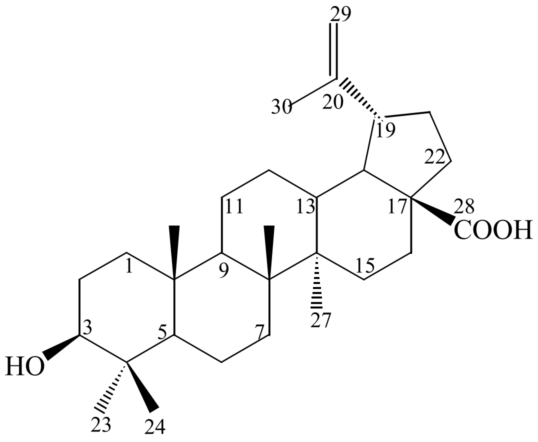


AJ2 **AJ3**

Stigmasterol **(AJ2)** and *β*-sitosterol **(AJ3)** were isolated as colorless crystals with melting point 135-136°C. The IR spectrum showed absorption bands at 3260 and 1664 cm^{-1} indicating the presence of hydroxyl and olefinic groups in the molecule.

The ^{1}H and ^{13}C NMR spectra were consistent with those reported for stigmasterol and *β*-sitosterol. Compounds **AJ2** and **AJ3** were therefore assigned as a mixture of stigmasterol and *β*-sitosterol in a ratio of 1:3 by the relative integral of their olefinic protons.

3.1.3 Compound AJ4



Compound **AJ4** was isolated as white solids, with molecular formula $C_{30}H_{48}O_3$ base on the $[M-H]^-$ ion at *m/z* 455 in the EIMS experiment. Its IR spectrum indicated the absorptions for hydroxyl (3443 cm^{-1}) and carboxyl group (1688 cm^{-1}).

The ^{13}C NMR spectrum (**Table 3**) revealed 30 carbon signals and which were shown by DEPT experiment to be six methyl carbon signals (δ 27.99, 19.14, 16.13, 16.03, 15.35 and 14.69), eleven methylene carbon signals (δ 109.71, 38.87, 37.04, 34.30, 32.15, 30.55, 29.70, 27.39, 25.48, 20.84 and 18.28), six methine carbon signals (δ 79.02, 55.32, 50.49, 49.25, 46.90 and 38.69) and six quaternary carbon signals (δ 150.42, 56.34, 42.43, 40.68, 38.40 and 37.20), one carboxylic acid (δ 180.02), revealing that **AJ4** is a triterpenic acid having five rings.

The ^1H NMR spectrum of **AJ4** (**Table 3**) showed the signals for five tertiary methyl groups at δ 0.75, 0.82, 0.93, 0.97, 0.98, one isopropenyl moiety at δ 1.69, 4.62 and 4.75 indicating a lupane-type skeleton. All Protons directly bond to carbon atoms of compound **AJ4** assigned by the HMQC spectrum and ^1H -^{13}C long-range were assigned by HMBC.

Comparison of ^1H and ^{13}C spectral data between compound **AJ4** and betulinic acid (**Table 4**) showed similarity. Thus, compound **AJ4** was identified as betulinic acid.[12]

Table 3 ^{1}H and ^{13}C NMR spectral data and HMBC correlations of compound **AJ4.**

Position	**$\delta_C$$^\#$(ppm)**	**δ_H (ppm)**	**HMBC correlations**
1	38.87(CH$_2$)	*	
2	27.39(CH$_2$)	*	
3	79.02(CH)	3.20 (1H, *dd*, *J* = 5.0, 11.5 Hz)	C-2, C-4
4	38.40(C)	-	
5	55.32(CH)	0.67 (1H, *m*)	
6	18.28(CH$_2$)	*	
7	34.30(CH$_2$)	1.42 (2H, *m*)	
8	40.68(C)	-	
9	50.49(CH)	1.27 (1H, *m*)	
10	37.20(C)	-	
11	20.84(CH$_2$)	1.37 (2H, *m*)	
12	25.48(CH$_2$)	*	
13	38.69(CH)	2.19 (1H, *dt*, *J* = 3.0, 12.5 Hz)	
14	42.43(C)	-	
15	30.55(CH$_2$)	*	
16	32.15(CH$_2$)	*	
17	56.34(C)	-	
18	46.90(CH)	1.62 (1H, *t*, *J* = 11.5 Hz)	

Table 3 (Continued)

Position	$\delta_C^{\#}$(ppm)	δ_H (ppm)	HMBC correlations
19	49.25(CH)	3.01 (1H, *dt*, *J* = 4.5, 10.5 Hz)	C-20, C-18, C-13
20	150.42(C)	-	
21	29.72(CH_2)	*	
22	37.04(CH_2)	1.96 (2H, *t*, *J* = 3.5 Hz)	
23	27.99(CH_3)	0.93 (3H, *s*)	C-3, C-5, C-24, C10
24	15.35(CH_3)	0.75 (3H, *s*)	C-5, C-6, C-23
25	16.03(CH_3)	0.82 (3H, *s*)	C-5, C-9, C-1
26	16.13(CH_3)	0.97 (3H, *s*)	C-9, C-7, C-14
27	14.69(CH_3)	0.98 (3H, *s*)	C-8, C-13, C-15
28	180.02(C)	-	
29	109.71(CH_2)	4.75 (1H, *d*, *J* = 2.0 Hz) and 4.62 (1H, *s*)	C-20, C19
30	19.14(CH_3)	1.69 (3H, *s*)	

Carbon type was deduced from DEPT experiments.

*The chemical shifts of proton resonated at δ 1.20-2.00 ppm.

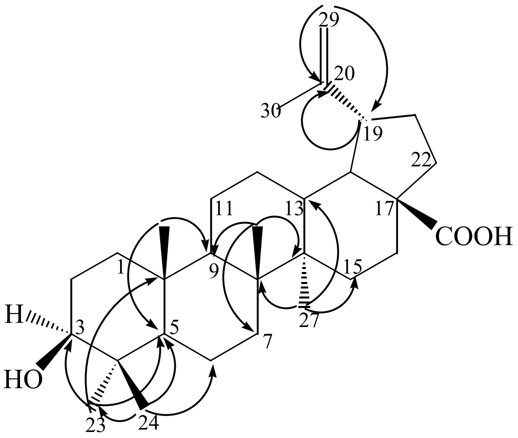


Fig.2 Selected HMBC correlations of compound **AJ4.**

Table 4 Comparison of ^{1}H and ^{13}C NMR spectral data between compound **AJ4** and Betulinic acid.

Position	**Compound AJ4**		**Betulinic acid**	
	δ_C# (ppm)	**δ_H (ppm)**	**δ_C# (ppm)**	**δ_H (ppm)**
1	38.87	*	38.7	
2	27.39	*	27.4	
3	79.02	3.20 (1H, *dd*, *J* = 5.0, 11.5 Hz)	78.9	3.19 (1H, *dd J* = 4.7, 10.0 Hz)
4	38.40	-	38.8	
5	55.32	0.67 (1H, *m*)	55.3	
6	18.28	*	18.3	
7	34.30	1.42 (2H, *m*)	34.3	
8	40.68		40.7	
9	50.49	1.27 (1H, *m*)	50.5	
10	37.20		37.2	
11	20.84	1.37 (2H, *m*)	20.8	
12	25.48	*	25.5	
13	38.69	2.19 (1H, *dt*, *J* = 3.0, 12.5 Hz)	38.4	
14	42.43	-	42.4	
15	30.55	*	30.5	
16	32.15	*	32.1	
17	56.34	-	56.3	
18	46.90	1.62 (1H, *t*, *J* = 11.5 Hz)	46.8	
19	49.25	3.01 (1H, *dt*, *J* = 4.5, 10.5 Hz)	49.2	2.99 (1H, *dd J* = 5.0, 11.5 Hz)

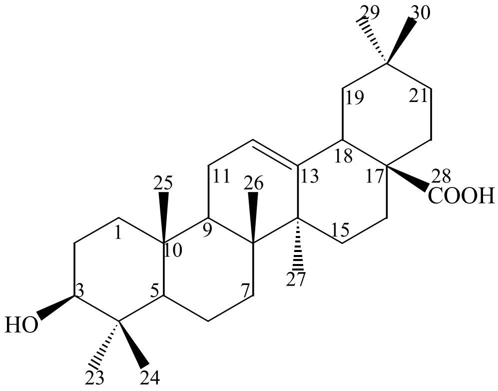
Table 4 (Continued)

Position	Compound KC5		Betulinic acid	
	$\delta_{C}^{\#}$ (ppm)	δ_{H} (ppm)	δ_{C} #(ppm)	δ_{H} (ppm)
20	150.42	-	150.3	
21	29.72	*	29.7	
22	37.04	1.96 (2H, *t*, *J* = 3.5 Hz)	37.0	
23	27.99	0.93 (3H, *s*)	27.9	0.93 (3H, *s*)
24	15.35	0.75 (3H, *s*)	15.3	0.75 (3H, *s*)
25	16.03	0.82 (3H, *s*)	16.0	0.82 (3H, *s*)
26	16.13	0.97 (3H, *s*)	16.1	0.96 (3H, *s*)
27	14.69	0.98 (3H, *s*)	14.7	0.97 (3H, *s*)
28	180.02	-	180.5	
29	109.71	4.75 (1H, *d*, *J* = 2.0 Hz) 4.62 (1H, *s*)	109.6	4.73 (1H, *d*, *J* = 1.5 Hz) 4.60 (1H, *d*, *J* = 1.5 Hz)
30	19.14	1.69 (3H, *s*)	19.4	1.68 (3H, *s*)

Carbon type was deduced from DEPT experiments.

*The chemical shifts of proton resonated at δ 1.20-2.00 ppm.

3.1.4 Compound AJ5



Compound **AJ5** was isolated as a white solid. Its molecular formula, $C_{30}H_{46}O_3$, was established by its EIMS mass spectrum (*m/z* 454 $[M]^+$). The IR spectrum showed the presence of a hydroxyl group at 3420 cm^{-1} and carbonyl group at 1693 cm^{-1}.

The ^1H NMR spectrum (**Table 5**) revealed the presence of seven tertiary methyl groups [δ 0.75, 0.78, 0.90, 0.91, 0.93, 0.99 and 1.14 (each 3H, *s*)]. The ^{13}C NMR spectrum (**Table 5**) revealed 30 carbon signals which were shown by DEPT experiments to be seven methyls (δ 15.31, 15.53, 17.07, 23.05, 27.16, 28.08 and 33.05), ten methylenes (δ 18.28, 22.94, 23.38, 25.90, 27.66, 32.40, 32.60, 33.77, 38.38 and 45.85), four methines (δ 41.02, 47.60, 55.18 and 79.01), six quaternary carbons (δ 30.66, 37.05, 38.74, 39.24, 41.60 and 46.47), one carboxylic acid (δ 182.21) and two olefinic carbons (δ 122.64 and 143.55) revealing that **AJ5** is a triterpenic acid having five rings.

The singlet signals for seven methyls in the ^1H NMR spectrum and olefinic carbon signals at 122.64 and 143.55 coresponding to C-12 and C-13, respectively in the ^{13}C NMR spectra data indicated the presence of Δ^{12}-oleanane skeleton in compound **AJ5**. This spectral feature indicated that **AJ5** belongs to an oleanane-type triterpene having a carboxylic function. Its physical and spectroscopic data were the same as those reported for oleanolic acid in the literature.[13] An identity was also confirmed by comparison with an authentic sample (**Table 6**). Compound **AJ5** was therefore identified as oleanolic acid.

Table 5 ^{1}H and ^{13}C NMR spectral data and HMBC correlations of compound **AJ5.**

Position	**$\delta_C$$^\#$(ppm)**	**δ_H (ppm)**	**HMBC correlations**
1	38.38 (CH_2)	1.68 (2H, *m*)	
2	23.38 (CH_2)	1.60 (2H, *m*)	
3	79.01 (CH)	3.22 (1H, *dd*, *J* = 4.5, 11.5 Hz)	C-2, C-4
4	38.74 (C)	-	
5	55.18 (CH)	0.75 (1H, *m*)	
6	18.28 (CH_2)	1.54 (1H, *m*), 1.39 (1H, *m*)	
7	32.40 (CH_2)	1.44 (2H, *m*)	
8	39.24 (C)	-	
9	47.60 (CH)	*	
10	37.05 (C)	-	
11	22.94 (CH_2)	*	
12	122.64 (CH_2)	5.29 (2H, *t*, *J* = 3.5 Hz)	
13	143.55 (C)	-	
14	41.60 (C)	-	
15	27.66 (CH_2)	*	
16	25.90 (CH_2)	*	
17	46.67 (C)	-	
18	41.02 (CH)	2.82 (1H, *dd*, *J* = 4.5, 14.5 Hz)	
19	45.85 (CH_2)	*	
20	30.66 (C)	-	

Table 5 (Continued)

Position	**$\delta_C^{\#}$(ppm)**	**δ_H (ppm)**	**HMBC correlations**
21	33.77 (CH_2)	*	
22	32.60 (CH_2)	*	
23	28.08 (CH_3)	0.99 (3H, *s*)	C-3, C-5, C-10
24	15.53 (CH_3)	0.78 (3H, *s*)	C-5, C-6, C26
25	15.31 (CH_3)	0.91 (3H, *s*)	C-9, C-5
26	17.07 (CH_3)	0.75 (3H, *s*)	C-9, C7, C-14
27	27.16 (CH_3)	1.14 (3H, *s*)	C-8, C15, C13
28	182.21 (C)	-	
29	33.05 (CH_3)	0.90 (3H, *s*)	C-21, C-19, C-30
30	23.05 (CH_3)	0.93 (3H, *s*)	C21, C22

Carbon type was deduced from DEPT experiments.

*The chemical shifts of proton resonated at δ 1.20-2.00 ppm.

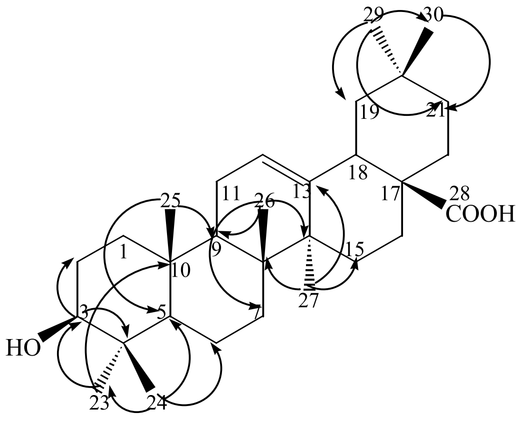


Fig.3 Selected HMBC correlations of compound **AJ5.**

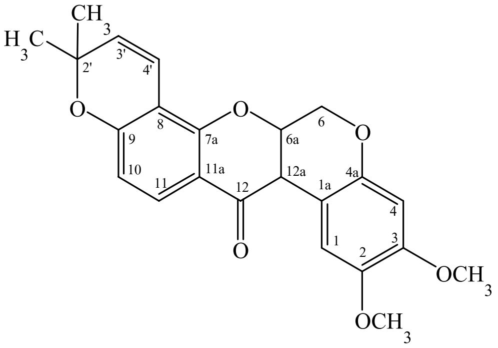
Table 6 Comparison of ^{13}C NMR spectral data between compound **AJ5** and Oleanolic acid.

Position	**Compound AJ5** δ_C **(ppm)**	**Oleanolic acid** δ_C **(ppm)**
1	38.38	38.5
2	23.38	23.4
3	79.01	78.7
4	38.74	38.7
5	55.18	55.2
6	18.28	18.3
7	32.40	32.6
8	39.24	39.3
9	47.60	47.6
10	37.05	37.0
11	22.94	23.1
12	122.64	122.1
13	143.55	143.4
14	41.60	41.6
15	27.66	27.6
16	25.90	27.4
17	46.67	46.6
18	41.02	41.3
19	45.85	45.8
20	30.66	30.6

Table 6 (continued)

Position	**Compound KC6** δ_C **(ppm)**	**Oleanolic acid** δ_C **(ppm)**
21	33.77	33.8
22	32.60	32.3
23	28.08	28.1
24	15.53	15.6
25	15.31	15.3
26	17.07	16.8
27	27.16	26.0
28	182.21	181.0
29	33.05	33.1
30	23.05	23.6

3.1.5 Compound AJ6



Compound **AJ6** was isolated as a white solid, m.p. 171-172 °C, $[\alpha]_D^{25}$: +20.4° (c = 1.3 x 10^{-4}, MeOH). In the UV-Vis spectrum, strong absorptions at 205, 239, 271 and 297 nm were detected. The IR spectrum exhibited absorption bands of a conjugated carbonyl group at 1675 cm^{-1}.

The ^{1}H and ^{13}C NMR spectral data of compound **AJ6** were assigned with information provided from DEPT, HMQC and HMBC spectral data (**Table 7**).

The ^{13}C NMR spectrum showed 23 signals for 23 carbon atoms. Analysis of the DEPT spectral data for this compound suggested the presence of four methyl carbons (δ 27.66, 27.99, 55.35 and 55.84), a methylene carbon (δ 65.79), eight methine carbons (δ 43.91, 71.96, 100.48, 110.04, 110.97, 115.25, 128.06 and 128.16), nine quaternary carbons (δ 77.17, 104.29, 108.63, 112.26, 143.41, 146.95, 149.03, 156.44 and 159.59) and a carbonyl carbon (δ 188.71).

Comparison of the ^{1}H NMR spectral data of **AJ6** and deguelin[14] revealed close structural similarities (**Table** 8). The ^{1}H NMR spectrum of compound **AJ6** showed two olefinic proton signals (δ6.63 and 5.54 : each 1H, *d*, J = 10.2 Hz) and two methyl protons (δ1.39 and 1.46 : each 3H, *s*) characteristic of a dimethylchromene moiety. It also showed the characteristic signals of rotenoid (δ 4.91 :1H, *t*, J = 3.3 Hz; 4.17 :1H, *d*, J = 12.0 Hz; 4.62 :1H, *dd*, J =

12.0, 3.3 Hz and 3.84 :1H, *d*, *J* = 3.9 Hz), signals of aromatic protons (δ 6.46, 6.79 : each 1H, *s*; 6.44 :1H, *d*, *J* = 8.7 Hz and 7.73 :1H, *d*, *J* = 8.7 Hz) and signals of two methoxy protons (δ 3.84 and 3.77 : each 3H, *s*).

The COSY data for compound **AJ6** indicated correlations between H-6a/H-12a; H-3′/H-2′ and H-10/H-11 which supported the structure.

The structure was supported by the following HMBC correlations : H-1/C-1a, C-4a, C-3; H-2/C-1a, C-4a, C-3; H-6/C-4a, C-1a; H-11/C-7a, C-9, C-12; H-4′/C-7a, C-9, C-2′ and H-3′/C-8 (**Fig. 4**). Compound **AJ6** was therefore identified as deguelin.[15]

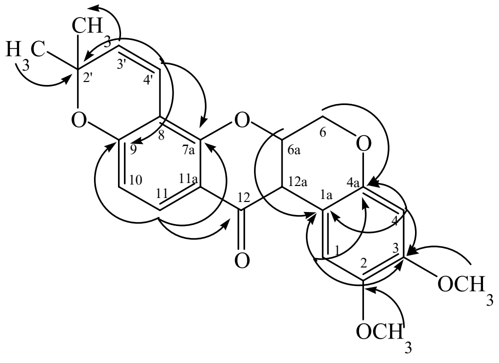


Fig. 4 Selected HMBC correlations of compound **AJ6**.

Table 7 ^{1}H and ^{13}C NMR spectral data and HMBC correlations of compound **AJ6.**

Position	$\delta^{\#}_{C}$ (ppm)	δ_{H} (ppm)	**HMBC** correlations
1	110.04 (CH)	6.79 (1H, *s*)	C-1a, C-4a and C-2
1a	104.30 (C)	-	-
2	149.03^{a} (C)	-	-
3	143.41^{a} (C)	-	-
4	100.48 (CH)	6.46 (1H, *s*)	C-1a, C-4a and C-2
4a	146.95 (C)	-	-
6	65.79 (CH_2)	α 4.62 (1H, *dd*, *J* = 12.0, 3.3 Hz)	C-12a, C-6a and C-4a C-6a and C-1a
		β 4.17 (1H, *d*, *J* = 12.0 Hz)	
6a	71.96 (CH)	4.91 (1H, *t*, *J* = 3.3 Hz)	C-1a
7a	156.44 (C)	-	-
8	108.63 (C)	-	-
9	159.60 (C)	-	-
10	110.97 (CH)	6.44 (1H, *d*, *J* = 8.7 Hz)	-
11	128.16 (CH)	7.73 (1H, *d*, *J* = 8.7 Hz)	C-7a, C-9 and C-12
11a	112.26 (C)	-	-
12	188.71 (C=O)	-	-
12a	43.91 (CH)	3.84 (1H, *d*, *J* = 3.9 Hz)	-
$2'$	77.18 (C)	-	-
$3'$	128.06 (CH)	5.54 (1H, *d*, *J* = 10.2 Hz)	C-1$''$/2$''$ and C-2$'$

Table 7 (continued)

Position	$\delta^{\#}_{C}$ (ppm)	δ_{H} (ppm)	**HMBC** correlations
4□	115.25 (CH)	6.63 (1H, d, J = 10.2 Hz)	C-2,C-7a and C-9
1□□	27.99 b (CH3)	1.46 (3H, s)	C-2□ and C-3□
2□□	27.66 b (CH3)	1.39 (3H, s)	C-2□ and C-3□
2-OMe	55.35c (OCH3)	3.81 (3H, s)	C-2
3-OMe	55.84c (OCH3)	3.77 (3H, s)	C-3

[a, b, c] Assignments with the same superscripts may be interchanged.

[#] Carbon type deduced from DEPT experiment.

Table 8 Comparison of ^{1}H and ^{13}C NMR spectral data between **AJ6** and deguelin.[15]

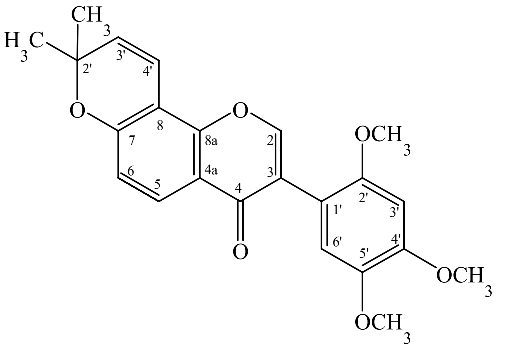
Position	**AJ6**		**deguelin**	
	δ_{C} (ppm)	δ_{H} (ppm)	δ_{C} (ppm)	δ_{H} (ppm)
1	110.04	6.79 (1H, *s*)	110.7	6.72 (*s*)
1a	104.30	-	105.0	-
2	143.41[a]	-	144.1	-
3	149.03[a]	-	149.8	-
4	100.48	6.46 (1H, *s*)	101.2	6.38 (*s*)
4a	146.95	-	147.7	-

Table 8 (continued)

Position	**AJ6**		**deguelin**	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
6	65.79	α 4.62 (1H, *dd*, *J* = 12.0, 3.3 Hz) β 4.17 (1H, *d*, *J* = 12.0 Hz)	66.5	α 4.56 (*dd*,*J* = 12.4, 3.2 Hz) β 4.11 (*d*, *J* = 12.4 Hz)
6a	71.96	4.91 (1H, *t*, *J* = 3.3 Hz)	72.7	4.84 (*m*)
7a	156.44	-	158.0	-
8	108.63	-	109.4	-
9	159.60	-	160.3	-
10	110.97	6.44 (1H, *d*, *J* = 8.7 Hz)	111.7	6.38 (*d*, *J* = 8.8 Hz)
11	128.16	7.73 (1H, *d*, *J* = 8.7 Hz)	128.8	7.67 (*d*, *J* = 8.8 Hz)
11a	112.26	-	113.0	-
12	188.71	-	189.4	-
12a	43.91	3.84 (1H, *d*, *J* = 3.9 Hz)	44.6	3.77 (1H, *d*, *J* = 3.6 Hz)
2′	77.18	-	77.9	-
3′	128.06	5.54 (1H, *d*, *J* = 10.2 Hz)	128.9	5.48 (*d*, *J* = 10.0 Hz)
4′	115.25	6.63 (1H, *d*, *J* = 10.2 Hz)	116.0	6.57 (*d*, *J* = 10.0 Hz)
1″	27.66[b]	1.39 (3H, *s*)	28.4	1.38 (*s*)
2″	27.99[b]	1.46 (3H, *s*)	28.7	1.32 (*s*)
2-OMe	55.35[c]	3.81 (3H, *s*)	56.1	3.70 (*s*)
3-OMe	55.84[c]	3.77 (3H, *s*)	56.7	3.73 (*s*)

[a, b, c] Assignments with the same superscripts may be interchanged.

3.1.6 Compound AJ7



Compound **AJ7** was isolated as a white solid, $[\alpha]_D^{25}$: -68.3° (c = 1.2 x 10^{-4}, MeOH). The molecular formula was determined as $C_{23}H_{22}O_6$ by LREI-MS, *m/z* 394 $[M]^+$. This molecular formula indicated thirteen units of unsaturation in the molecule. The UV-Vis spectrum with absorption bands at 206, 230, 258 and 298 nm was typical for an isoflavone.[16] The IR spectrum displayed an absorption band for a carbonyl group at 1669 cm^{-1}.

The ^1H and ^{13}C NMR spectral data of this compound were assigned with information provided from DEPT, HMQC and HMBC spectral studies (**Table 9**).

The ^1H NMR spectrum of compound **AJ7** suggested the presence of a dimethylchromene isoflavone moiety, with a sharp *singlet* at δ 7.97 (1H), two *doublets* with *ortho*-coupled protons at δ 6.84 (1H, J = 9.0 Hz) and 8.03 (1H, J = 8.7 Hz), two *doublets* with olefinic protons at δ 6.84 (1H, J = 10.5 Hz) and 5.70 (1H, J = 9.9 Hz) and a *singlet* with two methyl protons at δ 1.50 (6H). It also suggested three *singlets* with three methoxy protons at δ 3.78, 3.85 and 3.90 (each 3H) and two *singlets* with two aromatic protons at δ 6.63 and 6.95 (each 1H).

The ^{13}C NMR spectrum of compound **AJ7** was showed 23 signals for 23 carbon atoms. Analysis of the DEPT spectral data of this compound suggested the presence of five methyl carbons (δ27.61, 55.70, 56.10 and 56.44), seven methine carbons (δ 97.97, 114.57, 114.85, 114.97, 126.23, 129.71 and 153.46), ten quaternary carbons (δ 77.13, 108.75, 111.84, 114.85, 117.86, 121.05, 142.63, 149.32, 151.43 and 156.68) and a carbonyl carbon (δ 175.35).

The COSY data for compound **AJ7** indicated correlations between H-5/H-6 and H-4′/H-5′ which supported the structure.

The structure was supported by the following HMBC correlations : H-2/C-4, C-3, C-1, C-2′; H-5/C-4, C-2; H-6/C-7, C-8a; H-3′/C-1′; H-6′/C-3, C-2′, C-4′, C-5′ and H-3″/C-2″, C-8 (**Fig. 5**). Compound **AJ7** was therefore identified as the previously reported lonchocarpusone.[17]

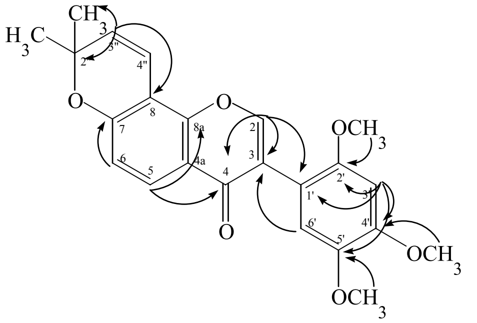


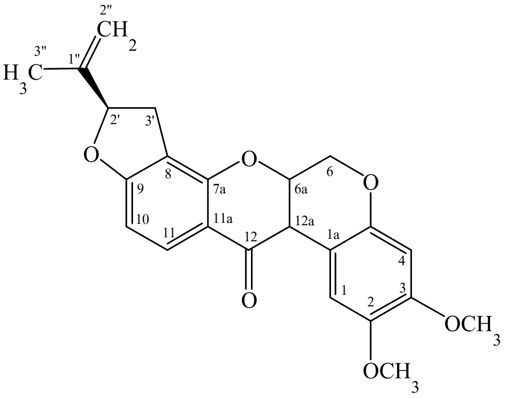
Fig. 5 Selected HMBC correlations of compound **AJ7**.

Table 9 ^{1}H and ^{13}C NMR spectral data and HMBC correlations of compound **AJ7.**

Position	$\delta^{\#}_{C}$ (ppm)	δ_{H} (ppm)	HMBC correlations
2	153.46 (CH)	7.97 (1H, *s*)	C-4, C-2′, C-3′ and C-1′
3	121.05 (C)	-	-
4	175.35 (C=O)	-	-
4a	117.94 (C)	-	-
5	126.23 (CH)	8.04 (1H, *d*, *J* = 8.7 Hz)	C-4 and C-8a
6	114.57 (CH)	6.84 (1H, *d*, *J* = 9.0 Hz)	C-7 and C-8a
7	156.68 (C)	-	-
8	108.75 (C)	-	-
8a	153.46 (C)	-	-
1′	111.84 (C)	-	-
2′	151.43 (C)	-	-
3′	97.97 (CH)	6.63 (1H, *s*)	C-2′, C-4′, C-5′ and C-1′
4′	142.63 (C)	-	-
5′	149.32 (C)	-	-
6′	114.97 (CH)	6.95 (1H, *s*)	C-2′, C-4′, C-5′, C-3 and C-1′
2″	77.13 (C)	-	-
3″	129.71 (CH)	5.70 (1H, *d*, *J* = 9.9 Hz)	2″-Me, C-2″ and C-8
4″	114.57 (CH)	6.81 (1H, *d*, *J* = 10.5 Hz)	C-7 and C-8a
2″-Me	28.61 (CH_3)	1.50 (6H, *s*)	C-3″ and C-2″
2′-OMe	56.44[a] (OCH_3)	3.78 (3H, *s*)	C-2′
4′-OMe	55.70[a] (OCH_3)	3.93 (3H, *s*)	C-4′
5′-OMe	56.10[a] (OCH_3)	3.86 (3H, *s*)	C-5′

[a] Assignments may be interchanged.

3.1.7 Compound AJ8



Compound **AJ8** was isolated as a white solid, m.p. 165-166 °C, $[\alpha]_D^{25}$: +54.40^0 (*c* = 5.1 x 10^{-4}, MeOH) and its molecular formula was determined as $C_{23}H_{22}O_6$ by LREI-MS, *m/z* 394 $[M]^+$. This molecular formula indicated thirteen units of unsaturation in the molecule. In the UV spectrum, strong absorption bands at 211, 237 and 294 nm were detected. The IR spectrum exhibited an absorption band of a conjugated carbonyl group at 1672 cm^{-1}.

The ^{13}C and ^1H NMR spectra of this compound were assigned with the information provided from DEPT, HMQC and HMBC spectral data **(Table 10)**.

The ^1H NMR spectrum of compound **AJ8** showed the presence of four characteristic rotenoid signals (δ 4.55 : 1H, *dd*, *J* = 12.0, 3.0 Hz; 4.12 :1H, *d*, *J* =12.0 Hz; 4.90 : 1H, *s*, and 3.79 : 1H, *d*, *J* = 3.9 Hz) and four signals of aromatic protons (δ 6.44 and 7.79 : each 1H, *d*, *J* = 8.7 Hz ; 6.41 and 6.76 :each 1H, *s*) The ^1H NMR spectrum also showed two *doublets of doublet* signals (δ 3.24 : 1H, *J* = 15.6, 8.6 Hz and 2.88 : 1H, *J* = 15.9, 8.4 Hz), a *triplet* signal (δ 5.15 :1H, *J* = 8.7 Hz), a *singlet* signal of a methyl proton (δ 1.74 : 3H) and two *singlet* signals of olefinic protons (δ 4.90 and 5.04 : each 1H, *s*) suggesting the presence of an isopropenylfuran unit. In addition, the ^1H NMR spectrum showed two *singlet* signals of methoxy protons (δ 3.75 and 3.76 : each 3H, *s*).

The ^{13}C NMR and the DEPT spectral data indicated the existence of three methyl carbons (δ 16.63, 55.36 and 55.86), three methylene carbons (δ30.79, 65.78 and 112.01), seven methine carbons (δ 44.13, 71.76, 87.34, 100.48, 104.38, 110.05 and 129.48), nine quaternary carbons (δ 109.92, 112.48, 112.85, 142.56, 143.44, 146.92, 149.07, 157.44 and 166.87) and a carbonyl carbon (δ 188.46).

The COSY data for compound **AJ8** indicated correlations between H-3′/H-2′ ; H-12a/H-6a and H-10/H-11.

The structure of compound **AJ8** was supported by the following HMBC correlations : H-1/C-3, C-12a; H-4/C-4a, C-2, C-3; H-6/C-12a, C-4a, C-12; H-6a/ C-11a; H-10/C-8, C-9; H-11/C-11a, C-7a, C-12; H-3′/C-2″, C-7a, C-9; H-2′/C-3″, C-2″, C-8, C-1″; H-2″/C-3″, C-1″ and H-3″/C-1″ **(Fig. 6)**.

The ^{1}H and ^{13}C NMR spectral data of compound **AJ8** were compared with the spectral data of rotenone **(Table 11)**.[14] Compound **AJ8** was identified as rotenone.

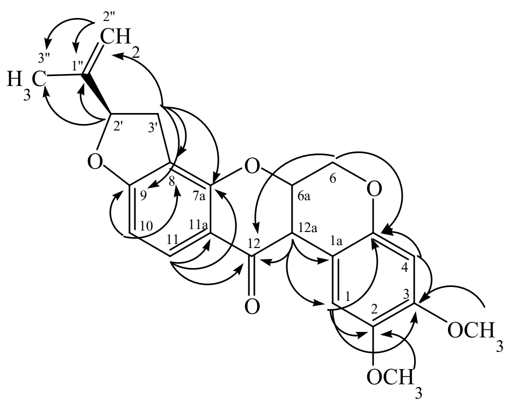


Fig. 6 Selected HMBC correlations of compound **AJ8**.

Table 10 ^{1}H and ^{13}C NMR spectral data and HMBC correlations of compound **AJ8**

Position	$\delta^{\#}_{C}$ **(ppm)**	δ_{H} **(ppm)**	**HMBC correlations**
1	110.05 (CH)	6.76 (1H, *s*,)	C-12a, C-4, C-2, C-4a and C-3
1a	109.93 (C)	-	-
2	143.44 (C)	-	-
3	149.07 (C)	-	-
4	100.48 (CH)	6.41 (1H, *s*,)	C-2, C-4a and C-3
4a	146.92 (C)	-	-
6	65.78 (CH_2)	α 4.55 (1H, *dd*, *J* = 12.0, 3.0 Hz) β 4.12 (1H, *d*, *J* = 12.0 Hz)	C-12a, C-6a, C-4a and C-12 C-12a, C-6a, C-7a and C-12
6a	71.76 (CH)	4.90 (1H, *s*,)	C-11a and C-8
7a	157.44 (C)	-	-
8	112.48 (C)	-	-
9	166.87 (C)	-	-
10	104.38 (CH)	6.44 (1H, *d*, *J* = 8.7 Hz)	C-8, C-7a and C-9
11	129.48 (CH)	7.79 (1H, *d*, *J* = 8.7 Hz)	C-8, C-11a, C-7a, C-9 and C-12
11a	112.85 (C)	-	-

Table 10 (continued)

Position	$\delta^{\#}_{C}$ (ppm)	δ_{H} (ppm)	HMBC correlations
12	188.41 (C=O)	-	-
12a	44.13 (CH)	3.79 (1H, *d*, J = 3.9 Hz)	C-1, C-11a and C-12
2′	87.34 (CH)	5.15 (1H, *t*, J = 8.7 Hz)	C-3″, C-3′, C-2″, C-8, C-1″ and C-9
3′	30.79 (CH_2)	α 3.24 (1H, *dd*, J = 15.6, 8.6 Hz) β 2.88 (1H, *dd*, J = 15.9, 8.1 Hz)	C-5, C-10, C-2″, C-11, C-2, C-7a and C-9
1″	142.56 (C)	-	-
2″	112.01 (CH_2)	4.90 (1H, *s*) 5.04 (1H, *s*)	C-3″, C-2′, C-1″ and C-8 C-3″, C-3′, C-2′ and C-1″
3″	16.63 (CH_3)	1.74 (3H, *s*)	C-2′ and C-1″
2-OMe	55.86[a] (OCH_3)	3.75 (3H, *s*)	C-1 and C-2
3-OMe	55.36[a] (OCH_3)	3.76 (3H, *s*)	C-4 and C-4a

[a] Assignments with the same superscripts may be interchanged.

[#] Carbon type deduced from DEPT experiment.

Table 11 Comparison of ^{1}H and ^{13}C NMR data between **AJ8** and rotenone.

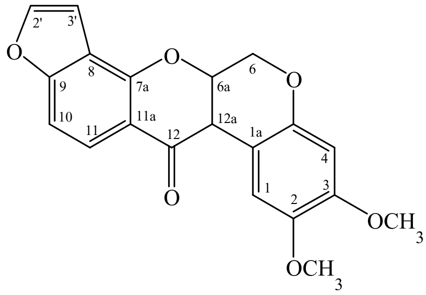
Position	**AJ8**		**rotenone**	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	110.05	6.76 (1H, *s*)	110.4	6.77, (*s*)
1a	109.93	-	104.7	-
2	143.44	-	143.9	-
3	149.07	-	149.5	-
4	100.48	6.41 (1H, *s*)	100.9	6.46 (*s*)
4a	146.92	-	147.4	-
6	65.78	α 4.55 (1H, *dd*, *J* = 12.0, 3.0 Hz) β 4.12 (1H, *d*, *J* = 12.0 Hz)	66.3	α 4.62 (*dd*, *J* = 12.0, 3.0 Hz) β 4.19 (*d*, *J* = 12.0 Hz)
6a	71.76	4.90 (1H, *s*)	72.2	4.94 (*m*)
7a	157.44	-	156.1	-
8	112.48	-	113.0	-
9	166.87	-	167.4	-
10	104.38	6.44 (1H, *d*, *J* = 8.7 Hz)	104.7	6.52 (*d*, *J* = 8.6 Hz)
11	129.48	7.79 (1H, *d*, *J* = 8.7 Hz)	130.0	7.84 (*d*, *J* = 8.6 Hz)
11a	112.85	-	114.7	-

Table 11 (continued)

Position	AJ8		rotenone	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
12	188.41	-	188.9	-
12a	44.13	3.79 (1H, *d*, *J* = 3.9 Hz)	44.6	3.8 (partly absorbed)
2′	87.34	5.15 (1H, *t*, *J* = 8.7 Hz)	87.8	5.25 (*t*, *J* = 8.8 Hz)
3′	30.79	α 3.24 (1H, *dd*, *J* = 15.6, 8.6 Hz) β 2.88 (1H, *dd*, *J* = 15.9, 8.1 Hz)	31.3	α 3.33 (*dd*, *J* = 15.8, 9.8 Hz) β 2.95 (*dd*, *J* = 15.8, 8.0 Hz)
1″	142.56	-	143.0	-
2″	112.01	4.90 (1H, *s*) 5.04 (1H, *s*)	112.6	4.94 (*s*) 5.08 (*s*)
3″	16.63	1.74 (3H, *s*)	17.2	1.77 (*s*)
2-OMe	55.86[a]	3.76 (3H, *s*)	55.8	3.77 (*s*)
3-OMe	55.36[a]	3.75 (3H, *s*)	56.3	3.81 (*s*)

[a] Assignments with the same superscripts may be interchanged.

3.1.8 Compound AJ9



Compound **AJ9** was isolated as a white solid, m.p. 178-179 °C, $[\alpha]_D^{25}$: +41.0° (*c* = 1.4 x 10^{-4}, MeOH). The molecular formula was determined as $C_{20}H_{16}O_6$ by LREI-MS, *m/z* 352 $[M]^+$. This molecular formula indicated thirteen units of unsaturation in the molecule. The UV-Vis spectrum showed maxima at 211, 237, 274 and 294 nm. The IR spectrum exhibited a band for a conjugated carbonyl group at 1676 cm^{-1}.

The ^1H and ^{13}C NMR spectral data for this compound were assigned with information provided by DEPT, HMQC and HMBC spectral data (**Table 12**).

The ^1H NMR spectrum of compound **AJ9** exhibited two *singlet* signals for methoxy groups (δ 3.76 and 3.80 : each 3H) and four aromatic proton signals (δ 6.47, 6.77 : each 1H, *s* ; 7.14 : 1H, *dd*, *J* = 8.7, 0.9 Hz and 7.89 : 1H, *d*, *J* = 9.0 Hz). It also exhibited the following two *doublet* signals (δ 3.95 : 1H, *J* = 4.2 Hz and 4.23 : 1H, *J* = 12.3 Hz); a *doublet* of *doublet* signal (δ 4.71 : 1H, *J* = 12.0, 3.0 Hz) and a *triplet* signal (δ 5.08 : 1H, *J* = 3.0 Hz) indicating the presence of a rotenoid unit. In addition, the ^1H NMR spectrum showed a *double of double* signal (δ 6.93 : 1H, *J* = 2.1, 0.9 Hz) and a *double* signal (δ 7.57 : 1H, *J* = 2.1 Hz) characteristic of benzofuranoic protons.

The ^{13}C NMR and DEPT spectral data of compound **AJ9** indicated the existence of two methyl carbons (δ 55.36 and 55.86), a single methylene carbon (δ 65.72), eight methine carbons (δ 44.24, 72.44, 100.55, 104.36, 106.19, 109.95, 123.52 and 144.44), eight quaternary carbons (δ 104.05, 113.10, 116.72, 144.44, 146.99, 149.16 and 159.81) and a carbonyl carbon (δ 189.36).

The COSY data for compound **AJ9** indicated correlations between H-12a/ H-6/H-6a; H-10/H-11 and H-2′/H-3′.

The structure was supported by the following HMBC correlations : H-1,H-4/ C-2, C-3, C-4a, C-1a; H-6/C-12a, C-4a; H-12a/C-1a, C-11a, C-4a, C-12; H-10/ C-11a, C-8, C-9; H-11/C-12. Finally, the presence of two benzofuranoic proton (H-2′ and H-3′) correlated with C-9 (**Fig. 7**).

The ^{1}H and ^{13}C NMR spectral data of compound **AJ9** was compared with the spectral data of 6*a*α, 12*a*α-12*a*-hydroxyelliptone (**Table 13**).[18] Thus, compound **AJ9** was identified as elliptone.

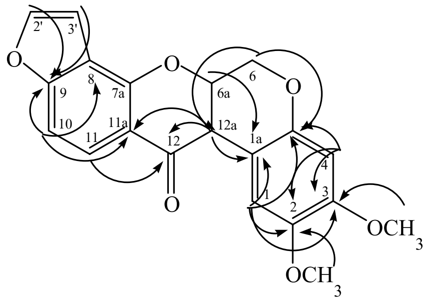


Fig. 7 Selected HMBC correlations of compound **AJ9**.

Table 12 ^{1}H and ^{13}C NMR spectral data and HMBC correlations of compound **AJ9**.

Position	$\delta^{\#}_{\mathrm{C}}$ **(ppm)**	δ_{H} **(ppm)**	**HMBC correlations**
1	109.95 (CH)	6.77 (1H, *s*)	C-12a, C-1a, C-3, C-4a and C-2
1a	104.04 (C)	-	-
2	144.44 (C)	-	-
3	149.16 (C)	-	-
4	100.55 (CH)	6.47 (1H, *s*)	C-1a, C-3, C-4a, C-2 and C-12
4a	146.99 (C)	-	-
6	65.72 (CH_2)	α 4.71 (1H, *dd*, *J* = 12.0, 3.0 Hz) β 4.23 (1H, *d*, *J* = 12.3 Hz)	C-12a, C-6a, C-4a and C-12 C-6a and C-11a
6a	72.44 (CH)	5.08 (1H, *t*, *J* = 3.0 Hz)	C-1a
7a	149.16 (C)	-	-
8	116.72 (C)	-	-
9	159.81 (C)	-	-
10	106.19 (CH)	7.14 (1H, *dd*, *J* = 8.7, 0.9 Hz)	C-11a, C-8 and C-9
11	123.52 (CH)	7.89 (1H, *d*, *J* = 9.0 Hz)	C-12
11a	113.10 (C)	-	-

Table 12 (continued)

Position	$\delta^{\#}_{C}$ **(ppm)**	δ_H **(ppm)**	**HMBC correlations**
12	189.36 (C=O)	-	-
12a	44.24 (CH)	3.95 (1H, *d*, *J* = 4.2 Hz)	C-1a, C-1, C-4a, C-12 and C-11a
$2'$	144.44 (CH)	7.57 (1H, *d*, *J* = 2.1 Hz)	C-8 and C-9
$3'$	104.36 (CH)	6.93 (1H, *dd*, *J* = 2.1, 0.9 Hz)	C-9
2-OMe	55.85[a] (OCH_3)	3.76 (3H, *s*)	C-3
3-OMe	55.36[a] (OCH_3)	3.80 (3H, *s*)	C-2

[a] Assignments with the same superscripts may be interchanged.

[#] Carbon type deduced from DEPT experiment.

Table 13 Comparison of ^{1}H and ^{13}C NMR spectral data between **AJ9** and 6*a*α, 12*a*α-12*a*-hydroxyelliptone.

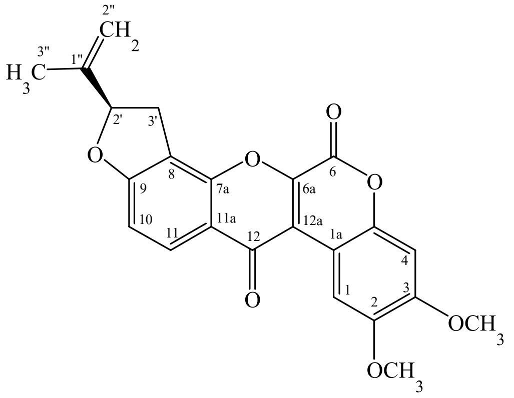
Position	**AJ9**		**6*a*α, 12*a*α-12*a*-hydroxyelliptone**	
	δ_C (ppm)	**δ_H (ppm)**	**δ_C (ppm)**	**δ_H (ppm)**
1	109.95	6.77 (1H, *s*)	109.3	6.55 (1H, *s*)
1a	104.04	-	108.4	-
2	144.44	-	144.0	-
3	149.16	-	151.2	-
4	100.55	6.47 (1H, *s*)	101.1	6.49 (1H, *s*)
4a	146.99	-	148.4	-
6	65.72	α 4.71 (1H, *dd*, *J* = 12.0, 3.0 Hz) β 4.23 (1H, *d*, *J* = 12.3 Hz)	63.8	α 4.72 (1H, *dd*, *J* = 14.3, 2.6 Hz) β 4.56 (1H, *d*, *J* = 14.3 Hz)
6a	72.44	5.08 (1H, *t*, *J* = 3.0 Hz)	77.2	4.74 (1H, *br s*)
7a	149.16	-	155.8	-
8	116.72	-	117.2	-
9	159.81	-	160.6	-
10	106.19	7.14 (1H, *dd*, *J* = 8.7, 0.9 Hz)	107.1	7.17 (1H, *dd*, *J* = 8.8, 1.1 Hz)
11	123.52	7.89 (1H, *d*, *J* = 9.0 Hz)	123.9	7.87 (1H, *d*, *J* = 8.8 Hz)
11a	113.10	-	112.0	-

Table 13 (continued)

Position	**AJ9**		**6*a*α, 12*a*α-12*a*-hydroxyelliptone**	
	δ_C **(ppm)**	δ_H **(ppm)**	δ_C **(ppm)**	δ_H **(ppm)**
12	189.36	-	192.2	-
12a	44.24	3.95 (1H, *d*, *J* = 4.2 Hz)	67.7	4.49 (1H, *br s*, 12*a*-OH)
2′	144.44	7.57 (1H, *d*, *J* = 2.1 Hz)	145.1	7.56 (1H, *d*, *J* = 2.2 Hz)
3′	104.36	6.93 (1H, *dd*, *J* = 2.1, 0.9 Hz)	104.8	6.91 (1H, *dd*, *J* = 2.2, 1.1 Hz)
2-OMe	55.85[a]	3.76 (3H, *s*)	56.4	3.71 (3H, *s*)
3-OMe	55.36[a]	3.80 (3H, *s*)	55.8	3.80 (3H, *s*)

[a] Assignments with the same superscripts may be interchanged.

3.1.9 Compound AJ10



Compound **AJ10** was isolated as a yellow solid, $[\alpha]_D^{25}$: +68.4° ($c = 9.4 \times 10^{-5}$, $CHCl_3$), and its molecular formula was determined as $C_{23}H_{18}O_7$ by LREI-MS, *m/z* 406 $[M]^+$. This molecular formula indicated fifteen units of unsaturation in the molecule. In the UV-Vis spectrum, strong absorptions at 255, 290 and 330 nm were detected. The IR spectrum showed the presence of a conjugated carbonyl group at 1672 cm^{-1}.

The ^1H and ^{13}C NMR spectral data of this compound were assigned with information provided from DEPT and HMQC spectral data **(Table 14)**.

The ^1H NMR spectrum of **AJ10** showed two methoxy protons (δ 3.74 and 3.81 : each 3H, *s*) and four signals for aromatic protons (δ 6.76 and 7.95 : each 1H, *d*, $J = 8.7$ Hz; 8.78 and 6.67 : each 1H, *s*). The ^1H NMR spectrum also showed two *doublets of doublet* signals (δ 3.10 : 1H, $J = 15.9$, 7.8 Hz and 3.43 : 1H, $J = 16.2$, 10.2 Hz), a *triplet* signal (δ 5.23 : 1H, $J = 8.7$ Hz), a *singlet* signal for a methyl group (δ 1.60 : 3H) and a signal for olefinic protons (δ 4.78 : 1H, *s* and 4.93 : 1H, *s*) all indicative of isopropenylfuran.

The ^{13}C NMR spectrum showed a total of 23 carbons with two carbonyl carbon (δ 165.53 and 197.78) and DEPT experiment showed three methyl carbons (δ 16.15, 55.22 and 55.32), two methylene carbons (δ 30.40 and 112.05), five methine carbons (δ 87.50, 98.59, 107.17, 108.73 and 127.31), eleven quaternary carbons (δ 108.52, 112.72, 117.41, 137.11, 141.59, 144.28, 145.91, 150.24, 150.45, 150.85 and 165.53).

The COSY data for compound **AJ10** indicated correlations between H-2$'$/H-3$'$ and H-8/H-9.

Comparison of the ^{1}H NMR spectral data of compounds **AJ10** and **AJ8** revealed close structural similarities (**Table 15**). The spectrum of compound **AJ10** does not show signals characteristic of rotenoid observed in compound **AJ8**. Thus, compound **AJ10** was identified as rotenonone.[19]

Table 14 ^{1}H and ^{13}C NMR spectral data of compound **AJ10.**

position	$\delta^{\#}_{C}$ **(ppm)**	δ_{H} **(ppm)**
1	107.17 (CH)	8.78 (1H, *s*)
1a	108.52 (C)	-
2	144.28 ^{a} (C)	-
3	150.24 $^{a, b}$ (C)	-
4	98.59 (CH)	6.67 (1H, *s*)
4a	150.45 ^{b} (C)	-
6	165.53 (C=O)	-
6a	145.91 ^{b} (C)	-
7a	150.85 ^{b} (C)	-
8	112.72 (C)	-
9	165.53 (C)	-
10	108.73 (CH)	6.76 (1H, *d*, J = 8.7 Hz)
11	127.31 (CH)	7.94 (1H, *d*, J = 8.7 Hz)
11a	117.41 (C)	-
12	197.78 (C)	-
12a	137.11 (C)	-
2′	87.50 (CH)	5.23 (1H, *t*, J = 8.7 Hz)
3′	30.40 (CH_2)	3.10 (1H, *dd*, J = 15.9, 7.8 Hz)
		3.43 (1H, *dd*, J = 16.2, 10.2 Hz)
1″	141.59 (C)	-

Table 14 (continued)

position	**$\delta^{\#}_{C}$ (ppm)**	**δ_{H} (ppm)**
2″	112.05 (CH_2)	4.78 (1H, *s*) 4.93 (1H, *s*)
3″	16.15 (CH_3)	1.60 (3H, *s*)
2-OMe	55.32^{c} (CH_3)	3.74 (3H, *s*)
3-OMe	55.22^{c} (CH_3)	3.81 (3H, *s*)

$^{a, b, c}$ Assignments with the same superscripts may be interchanged.

$^{\#}$ Carbon type deduced from DEPT experiment.

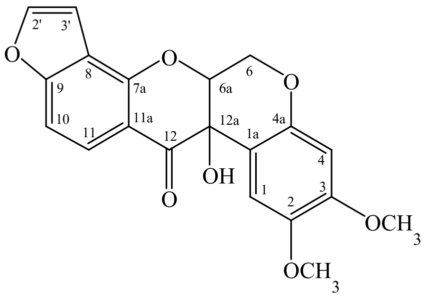
Table 15 Comparison of ^{1}H and ^{13}C NMR spectral data between **AJ10** and **AJ8.**

Position	**AJ10**		**AJ8**	
	δ_C **(ppm)**	δ_H **(ppm)**	δ_C **(ppm)**	δ_H **(ppm)**
1	107.17	8.78 (1H, *s*)	110.05	6.76 (1H, *s*)
1a	108.52	-	109.93	-
2	144.28[a]	-	143.44	-
3	150.24[a,b]	-	149.07	-
4	98.59	6.67 (1H, *s*)	100.48	6.41 (1H, *s*)
4a	150.45[b]	-	146.92	-
6	165.53	-	65.78	4.55 (1H, *dd*, J = 12.0, 3.0 Hz) 4.12 (1H, *d*, J = 12.0 Hz)
6a	145.91[b]	-	71.76	4.90 (1H, *s*)
7a	150.85[b]	-	157.44	-
8	112.72	-	112.48	-
9	165.53	-	166.87	-
10	108.73	6.76 (1H, *d*, J = 8.7 Hz)	104.38	6.44 (1H, *d*, J = 8.7 Hz)
11	127.31	7.94 (1H, *d*, J = 8.7 Hz)	129.48	7.79 (1H, *d*, J = 8.4 Hz)
11a	117.41	-	112.85	-
12	197.78	-	188.41	-
12a	137.11	-	44.13	3.79 (1H, *d*, J = 3.9 Hz)

Table 15 (continued)

Position	AJ10		AJ8	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
2′	87.50	5.23 (1H, *t*, J = 8.7 Hz)	87.34	5.15 (1H, *t*, J = 8.7 Hz)
3′	30.40	3.43 (1H, *dd*, J = 16.2, 10.2 Hz) 3.10 (1H, *dd*, J = 15.9, 7.8 Hz)	30.79	3.24 (1H, *dd*, J = 15.6, 8.6 Hz) 2.88 (1H, *dd*, J = 15.9, 8.1 Hz)
1″	141.59	-	142.56	-
2″	112.05	4.78 (1H, *s*) 4.93 (1H, *s*)	112.01	4.90 (2H, *s*) 5.04 (1H, *s*)
3″	16.15	1.60 (3H, *s*)	16.63	1.74 (3H, *s*)
2-OMe	55.32[c]	3.74 (3H, *s*)	55.36	3.76 (3H, *s*)
3-OMe	55.22[c]	3.81 (3H, *s*)	55.86	3.75 (3H, *s*)

3.1.10 Compound AJ11



Compound **AJ11** was isolated as a colourless viscous oil, $[\alpha]_D^{25}$: +69.1° (c = 8.7 x 10^{-5}, MeOH). The UV-Vis spectrum showed maxima at 203, 239 and 277 nm. The IR spectrum exhibited bands for a conjugated carbonyl group at 1725 cm^{-1} and a hydroxy group at 3416 cm^{-1} .

The ^{1}H and ^{13}C NMR spectral data of compound **AJ11** were assigned based on DEPT and HMQC spectral data (**Table 16**).

The ^{1}H NMR spectrum of compound **AJ11** exhibited four aromatic proton signals (δ 6.49 and 6.54 : each 1H, *s*; δ 7.17 : 1H, *d*, J = 8.7 Hz; δ 7.86 : 1H, *d*, J = 8.7 Hz). It also exhibited the following signals due to protons on carbon atoms bearing oxygen atoms (δ 4.47, 1H, *s*; 7.70 : 1H, *dd*, J = 12.3, 2.4 Hz and 4.53 : 1H, *dd*, J = 12.4, 1.8 Hz) and two *singlet* signals for methoxy groups (δ 3.80 and 3.71 : each 3H). In addition, the characteristic signals of benzofuranoic protons (δ 7.57 and 6.91 : each 1H, *d*, J = 2.4 and 2.1 Hz , respectively) were observed.

The ^{13}C NMR and DEPT spectra suggested that compound **AJ11** contained a methylene carbon (δ 63.85); seven methine carbons (δ 77.22, 101.23, 104.83, 107.13, 109.43, 123.91 and 145.16); nine quaternary carbons (δ 67.77, 108.84, 112.11, 117.39, 144.13, 148.53, 151.22, 155.85 and 160.69) ; two methoxy carbons (δ 55.88 and 56.45) and a carbonyl group (δ 192.25).

The ^{1}H and ^{13}C NMR spectral data compared with the literature were found to be in close agreement with the data for the known compound 6*a*α, 12*a*α-12*a*-hydroxyelliptone **(Table 17).**[18] The identity of compound **AJ11** was further supported by the UV-Vis, and IR data which were in agreement with the literature values for 6*a*α, 12*a*α-12*a*-hydroxyelliptone.[18]

Table 16 The ^{1}H and ^{13}C spectral data of compound **AJ11.**

Position	$\delta^{\#}_{\mathrm{C}}$ (ppm)	δ_{H} (ppm)
1	109.43 (CH)	6.54 (1H, *s*)
1a	108.84 (C)	-
2	144.13 (C)	-
3	151.22 (C)	-
4	101.23 (CH)	6.49 (1H, s)
4a	148.53 (C)	-
6	63.85 (CH_2)	4.53 (1H, *dd*, *J* = 12.4, 1.8 Hz) 4.70 (1H, *dd*, *J* = 12.3, 2.4 Hz)
6a	77.22 (CH)	4.74 (1H, *s*)
7a	155.85 (C)	-
8	117.39 (C)	-
9	160.69 (C)	-
10	107.13 (CH)	7.17 (1H, *d*, *J* = 8.7 Hz)
11	123.91 (CH)	7.86 (1H, *d*, *J* = 8.7 Hz)
11a	112.11 (C)	-
12	192.25 (C)	-
12a	67.77 (C)	-
2′	145.16 (CH)	7.57 (1H, *d*, *J* = 2.4 Hz)
3′	104.83 (CH)	6.91 (1H, *d*, *J* = 2.1 Hz)
2-OMe	56.45 (OCH_3)	3.76 (3H, *s*)
3-OMe	55.88 (OCH_3)	3.80 (3H, *s*)

Carbon type deduced from DEPT experiment.

Table 17 Comparison of ^{1}H and ^{13}C NMR spectral data between **AJ11** and 6*a*α, 12*a*α-12*a*-hydroxyelliptone.

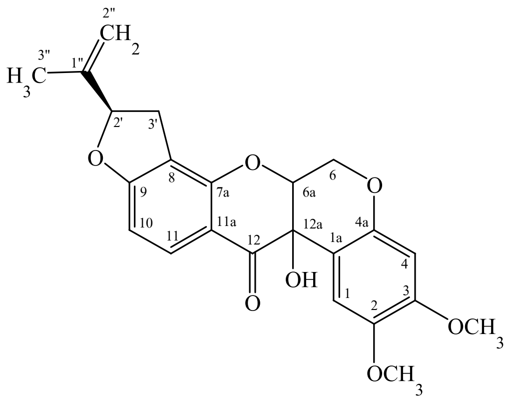
Position	AJ11		6*a*α, 12*a*α-12*a*-hydroxyelliptone	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	109.43	6.54 (1H, *s*)	109.3	6.55 (1H, s)
1a	108.84	-	108.4	-
2	144.13	-	144.0	-
3	155.25	-	151.2	-
4	101.23	6.49 (1H, s)	101.1	6.49 (1H, s)
4a	148.53	-	148.4	-
6	63.85	4.53 (1H, *dd*, *J* = 12.4, 1.8 Hz) 4.70 (1H, *dd*, *J* = 12.3, 2.4 Hz)	63.8	4.56 (1H, *d*, *J* =14.3 Hz) 4.72 (1H, *dd*, *J* =14.3, 2.6 Hz)
6a	77.22	4.74 (1H, *s*)	77.2	4.74 (1H, *br s*)
7a	161.01	-	155.8	-
8	115.03	-	117.2	-
9	166.02	-	160.6	-
10	107.13	7.17 (1H, *d*, *J* = 8.7 Hz)	107.1	7.17 (1H, *dd*, *J* = 8.8, 1.1 Hz)
11	123.91	7.86 (1H, *d*, *J* = 8.7 Hz)	123.9	7.87 (1H, *d*, *J* = 8.8 Hz)
11a	112.11	-	112.0	-
12	192.25	-	192.2	-
12a	67.77	-	67.7	4.49 (1H, *br s*, 12*a*-OH)

Table 17 (continued)

Position	**AJ11**		**6*a*α, 12*a*α-12*a*-hydroxyelliptone**	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
$2'$	145.16	7.57 (1H, *d*, *J* = 2.4 Hz)	145.1	7.56 (1H, *d*, *J* = 2.2 Hz)
$3'$	104.83	6.91 (1H, *d*, *J* = 2.1 Hz)	104.8	6.91 (1H, *dd*, *J* = 2.2, 1.1 Hz)
2-OMe	56.45[a]	3.76 (3H, *s*)	56.4	3.71 (3H, *s*)
3-OMe	55.88[a]	3.80 (3H, *s*)	55.8	3.80 (3H, *s*)

[a] Assignments with the same superscripts may be interchanged.

3.1.11 Compound AJ12



Compound **AJ12** was isolated as a colourless viscous oil, $[\alpha]_D^{25}$: +34.9° (c = 1.1 x 10^{-4}, MeOH) and its molecular formula was analysed as $C_{23}H_{22}O_7$ by LREI-MS, *m/z* 410 $[M]^+$. This molecular formula indicated thirteen units of unsaturation in the molecule. In the UV-Vis spectrum, strong absorption bands at 210, 238 and 294 nm were detected. The IR spectrum exhibited an absorption band of a conjugated carbonyl group at 1674 cm^{-1} and a hydroxy group at 3467 cm^{-1} .

The ^1H and ^{13}C NMR spectra for this compound were assigned based on DEPT, HMQC and HMBC spectral data **(Table 18)**.

The ^1H NMR spectrum of compound **AJ12** exhibited two *singlet* methoxy protons (δ 3.72 and 3.82 : each 3H) and four aromatic protons (δ 6.52 : 1H, *d*, J = 9.6 Hz; 7.81 : 1H, *d*, J = 8.7 Hz; 6.55 and 6.48 : each 1H, *s*). It also exhibited the following signals due to protons on carbon atoms bearing oxygen atoms (δ 4.47 (1H) and 4.59 (2H) each : *dd*, J = 13.2, 2.4 Hz). In addition, the characteristic signals of an isopropenylfuran moiety (δ 4.94 and 5.07 : each 1H, *s*; 2.90 : 1H, *dd*, J = 15.9 , 8.4 Hz; 3.25 : 1H, *dd*, J = 15.6, 9.9 Hz; 5.21 : 1H, *t*, J = 9.3 Hz and 1.74, 3H, *s*) were observed.

The ^{13}C NMR and DEPT spectral data indicated the existence of three methyl carbons (δ 17.10, 55.88 and 56.43), three methylene carbons (δ 31.16, 63.87 and 112.70), six methine carbons (δ 76.09, 87.99, 101.13, 105.33, 109.51 and 130.11), ten quaternary carbons (δ 67.59, 108.82, 111.78, 113.22, 142.90, 144.06, 148.43, 151.20, 157.71 and 168.05) and a carbonyl group (δ 191.11).

Comparison of the ^{1}H and ^{13}C NMR spectral data of compounds **AJ12** and **AJ8** revealed their close structural similarity **(Table 19)**. However, the spectrum of compound **AJ12** exhibits signals due to protons on carbon atoms bearing oxygen atoms (δ 4.47 and 4.59), which were not observed in compound **AJ8**.

To ensure that the structure of compound **AJ12** was correct, the COSY and HMBC data for compound **AJ12** were confirmed. The COSY data for compound **AJ12** indicated correlations between H-3′/H-2′ and H-10/H-11. The HMBC spectrum presence the correlations : H-1/C-2, C-1a; H-4/ C-3, C-4a; H-6/ C-4a, C-7a, C-12a; H-6a/ C-12; H-10/ C-8, C-9, C-11a; H-11/ C-7a, C-12; H-3′/ C-8, C-9, C-1″ and H-2″/ C-1″, C-3″ (**Fig. 8**).

The data from the ^{1}H and ^{13}C NMR spectra when compared to the literature were found to be in close agreement with the data for the known compound 12aα-hydroxyrotenone **(Table 20)**.[20] Thus, compound **AJ12** was considered to be 12*a*α-hydroxyrotenone.

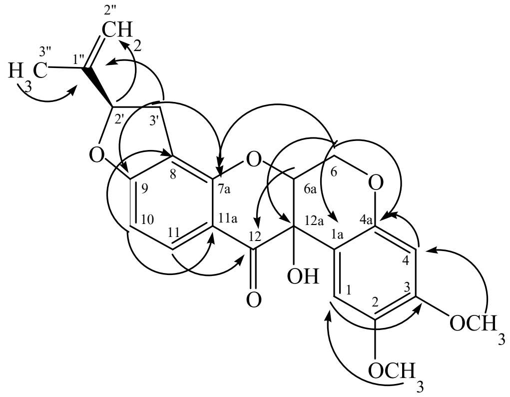


Fig. 8 Selected HMBC correlations of compound **AJ12**.

Table 18 ^{1}H and ^{13}C NMR spectral data and HMBC correlations of compound **AJ12**.

Position	$\delta^{\#}_{C}$ (ppm)	δ_{H} (ppm)	**HMBC correlations**
1	109.51 (CH)	6.55 (1H, *s*)	C-3, C-4a, C-2, C-1a, C-4 and C-12a
1a	108.82 (C)	-	-
2	144.06^{a} (C)	-	-
3	151.20^{a} (C)	-	-
4	101.13 (CH)	6.48 (1H, *s*)	C-3, C-4a, C-2, C-1a and C-12a
4a	148.43 (C)	-	-
6	63.87 (CH_2)	4.59 (1H, *dd*, *J* = 13.2, 2.4 Hz) 4.47 (1H, *dd*, *J* = 13.2, 2.4 Hz)	C-7a, C-4a, C-1a, C-6a and C-12a
6a	76.09 (CH)	4.59 (1H, *br s*)	C-12, C-4a, C-1a, C-12a and C-6

Table 18 (continued)

Position	$\delta^{\#}_{\mathrm{C}}$ (ppm)	δ_{H} (ppm)	**HMBC correlations**
7a	157.71 (C)	-	-
8	111.78 (C)	-	-
9	168.05 (C)	-	-
10	105.33 (CH)	6.52 (1H, *d*, *J* = 9.6 Hz)	C-9, C-7a, C-11a and C-8
11	130.11 (CH)	7.81 (1H, *d*, *J* = 8.7 Hz)	C-12, C-9 and C-7a
11a	113.22 (C)	-	-
12	191.11 (C)	-	-
12a	67.59 (C)	-	-
2′	87.99 (CH)	5.21 (1H, *t*, *J* = 9.3 Hz)	C-9, C-1″ and C-2″
3′	31.16 (CH$_2$)	2.90 (1H, *dd*, *J* = 15.9, 8.1 Hz) 3.25 (1H, *dd*, *J* = 15.6, 9.6 Hz)	C-9, C-7a, C-1″, C-11a, C-8, C-10 and C-2′
1″	142.90 (C)	-	-
2″	112.70 (CH$_2$)	4.94 (1H, *s*)	C-1″, and C-2′
		5.07 (1H, *s*)	C-1″, C-2′ and C-3″
3″	17.10 (CH$_3$)	1.76 (3H, *s*)	C-1″, C-2″ and C-2′
2-OMe	56.43^{b} (OCH$_3$)	3.82 (3H, *s*)	C-3
3-OMe	55.88^{b} (OCH$_3$)	3.72 (3H, *s*)	C-2

[a, b] Assignments with the same superscripts may be interchanged.

[#] Carbon type deduced from DEPT experiment.

Table 19 Comparison of ^{1}H and ^{13}C NMR spectral data between **AJ12** and **AJ8.**

Position	AJ12		AJ8	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	109.51	6.55 (1H, *s*)	110.05	6.76 (1H, *s*)
1a	108.82	-	109.93	-
2	144.06[a]	-	143.44	-
3	151.20[a]	-	149.07	-
4	101.13	6.48 (1H, *s*)	100.48	6.41 (1H, *s*)
4a	148.43	-	146.92	-
6	63.87	4.59 (1H, *dd*, *J* = 13.2, 2.4 Hz) 4.47 (1H, *dd*, *J* = 13.2, 2.4 Hz)	65.78	α 4.55 (1H, *dd*, *J* = 12.0, 3.0 Hz) β 4.12 (1H, *d*, *J* = 12.0 Hz)
6a	76.09	4.59 (1H, *br s*)	71.76	4.90 (1H, *s*)
7a	157.71	-	157.44	-
8	111.78	-	112.48	-
9	168.05	-	166.87	-
10	105.33	6.52 (1H, *d*, *J* = 9.6 Hz)	104.38	6.44 (1H, *d*, *J* = 8.7 Hz)
11	130.11	7.81 (1H, *d*, *J* = 8.7 Hz)	129.48	7.79 (1H, *d*, *J* = 8.7 Hz)
11a	113.22	-	112.85	-
12	191.11	-	188.41	-
12a	67.59	-	44.13	3.79 (1H, *d*, *J* = 3.9 Hz)
2′	87.99	5.21 (1H, *t*, *J* = 9.3 Hz)	87.34	5.15 (1H, *t*, *J* = 8.7 Hz)

Table 19 (continued)

Position	**AJ12**		**AJ8**	
	δ_C (ppm)	**δ_H (ppm)**	**δ_C (ppm)**	**δ_H (ppm)**
$3'$	31.16	3.25 (1H, *dd*, *J* = 15.6, 9.6 Hz) 2.90 (1H, *dd*, *J* = 15.9, 8.1 Hz)	30.79	α 3.24 (1H, *dd*, *J* = 15.6, 8.6 Hz) β 2.88 (1H, *dd*, *J* = 15.9, 8.1 Hz)
$1''$	142.90	-	142.56	-
$2''$	112.70	4.94 (1H, *s*) 5.07 (1H, *s*)	112.01	4.90 (1H, *s*) 5.04 (1H, *s*)
$3''$	17.10	1.76 (3H, *s*)	16.63	1.74 (3H, *s*)
2-OMe	56.43 [b]	3.82 (3H, *s*)	55.86	3.76 (3H, *s*)
3-OMe	55.88 [b]	3.72 (3H, *s*)	55.36	3.75 (3H, *s*)

[a, b] Assignments with the same superscripts may be interchanged.

Table 20 Comparison of ^{1}H and ^{13}C NMR spectral data between **AJ12** and 12aα-hydroxyrotenone.

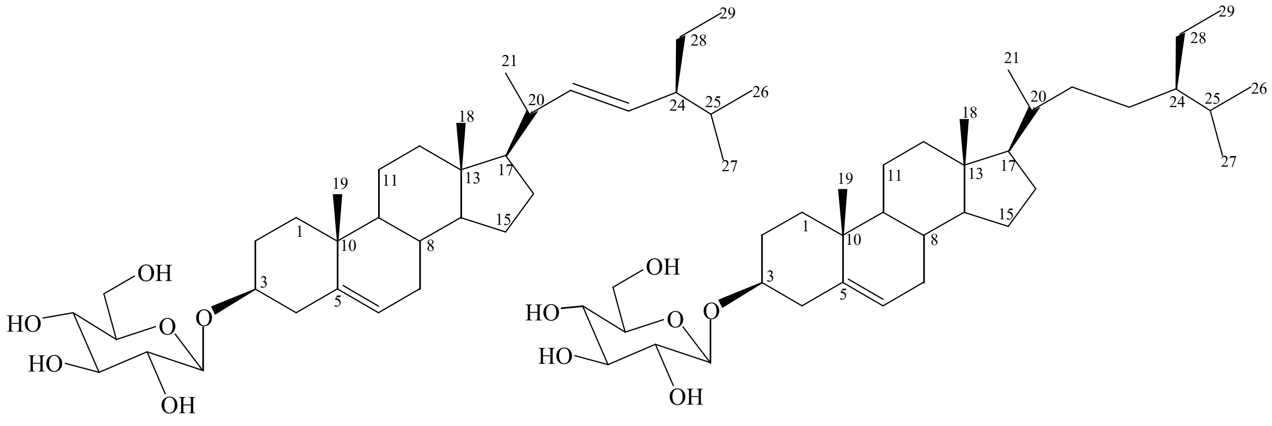
Position	**AJ12**		**12aα-hydroxyrotenone**	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	109.51	6.55 (1H, *s*)	112.9	7.82 (1H, *s*)
1a	108.82	-	109.93	-
2	144.06[a]	-	143.5	-
3	151.20[a]	-	150.9	-
4	101.13	6.48 (1H, *s*)	100.3	6.42 (1H, *s*)
4a	148.43	-	149.3	-
6	63.87	4.59 (1H, *dd*, *J* = 13.2, 2.4 Hz) 4.47 (1H, *dd*, *J* = 13.2, 2.4 Hz)	61.6	4.67 (1H, *dd*, *J* = 11.0, 4.6 Hz) 4.38 (1H, *dd*, *J* = 9.7, 4.6 Hz)
6a	76.09	4.59 (1H, *br s*)	76.6	4.48 (1H, *dd*, *J* = 11.3, 9.7 Hz)
7a	157.71	-	156.7	-
8	111.78	-	112.6	-
9	168.05	-	166.8	-
10	105.33	6.52 (1H, *d*, *J* = 9.6 Hz)	105.3	6.62 (1H, *d*, *J* = 8.2 Hz)
11	130.11	7.81 (1H, *d*, *J* = 8.7 Hz)	130.9	7.88 (1H, *d*, *J* = 8.7 Hz)
11a	113.22	-	114.2	-
12	191.11	-	187.2	-

Table 20 (continued)

Position	**AJ12**		**12aα-hydroxyrotenone**	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
12a	67.59	-	66.1	2.85 (1H, *s*, 12a-OH)
$2'$	87.99	5.21 (1H, *t*, $J = 9.3$ Hz)	87.8	5.33 (1H, *dd*, $J = 9.7, 8.2$ Hz)
$3'$	31.16	3.25 (1H, *dd*, $J = 15.6, 9.6$ Hz) 2.90 (1H, *dd*, $J = 15.9, 8.1$ Hz)	31.2	3.38 (1H, *dd*, $J = 15.9, 9.7$ Hz) 3.02 (1H, *dd*, $J = 15.9, 8.2$ Hz)
$1''$	142.90	-	142.9	-
$2''$	112.70	4.94 (1H, *s*) 5.07 (1H, *s*)	112.6	4.96 (1H, *s*) 5.11 (1H, *s*)
$3''$	17.10	1.76 (3H, *s*)	17.1	1.79 (3H, *s*)
2-OMe	56.43^{b}	3.82 (3H, *s*)	56.3	3.92 (3H, *s*)
3-O Me	55.88^{b}	3.72 (3H, *s*)	55.7	3.84 (3H, *s*)

[a, b] Assignments with the same superscripts may be interchanged.

3.1.12 Compounds AJ13 and AJ14



AJ13 **AJ14**

Compounds **AJ13** and **AJ14** were obtained as white powder, melting at 269-270°C. The IR spectrum showed the presence of the hydroxy group at 3435 cm^{-1} and C-O signal at 1023 cm.$^{-}$ ^{1} .It has similar spectral data to mixture of β-sitosterol and β-stigmasterol except for the additional signals at δ 4.42-3.23 of a sugar moiety. Comparison of its spectrum with those of mixture of β-sitosterol and β-stigmasterol glycoside confirmed that compounds **AJ13** and **AJ14** contained both β-sitosterol and β-stigmasterol glycosides in a ratio 8:1 by the relative integral of their olefinic protons.

3.2 . Biological activities of the pure compounds from the bean shells of Archidendron jiringa.

Table 21 Biological activities of the pure compounds**.**

Code	Anti TB MIC(μg/ml)	Cytotoxicity ED$_{50}$(μg/ml)	
		KB	Vero-cell
AJ1	inactive	*	*
AJ2 and AJ3	inactive	*	*
AJ4	25	*	*
AJ5	50	*	*
AJ6	25	0.37	*
AJ7	*	*	*
AJ8	100	0.62	*
AJ9	100	0.50	*
AJ10	inactive	2.29	39.7
AJ11	*	0.90	*
AJ12	25	0.12	*
AJ13 and AJ14	inactive	*	*

*not tested

3.3 . Conclusions

Chemical investigation of constituents from the bean shells of A. jiringa led to the isolation of fourteen known compounds AJ1-AJ14. Six pure compounds: AJ4, AJ5, AJ6, AJ8, AJ9 and AJ12 showed anti TB with the MIC of 25-100 μg/ml. AJ6, AJ8, AJ9, AJ10, AJ11 and AJ12 showed anti KB with the ED_{50} of 0.1-2.3 μg/ml.

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APPENDICES

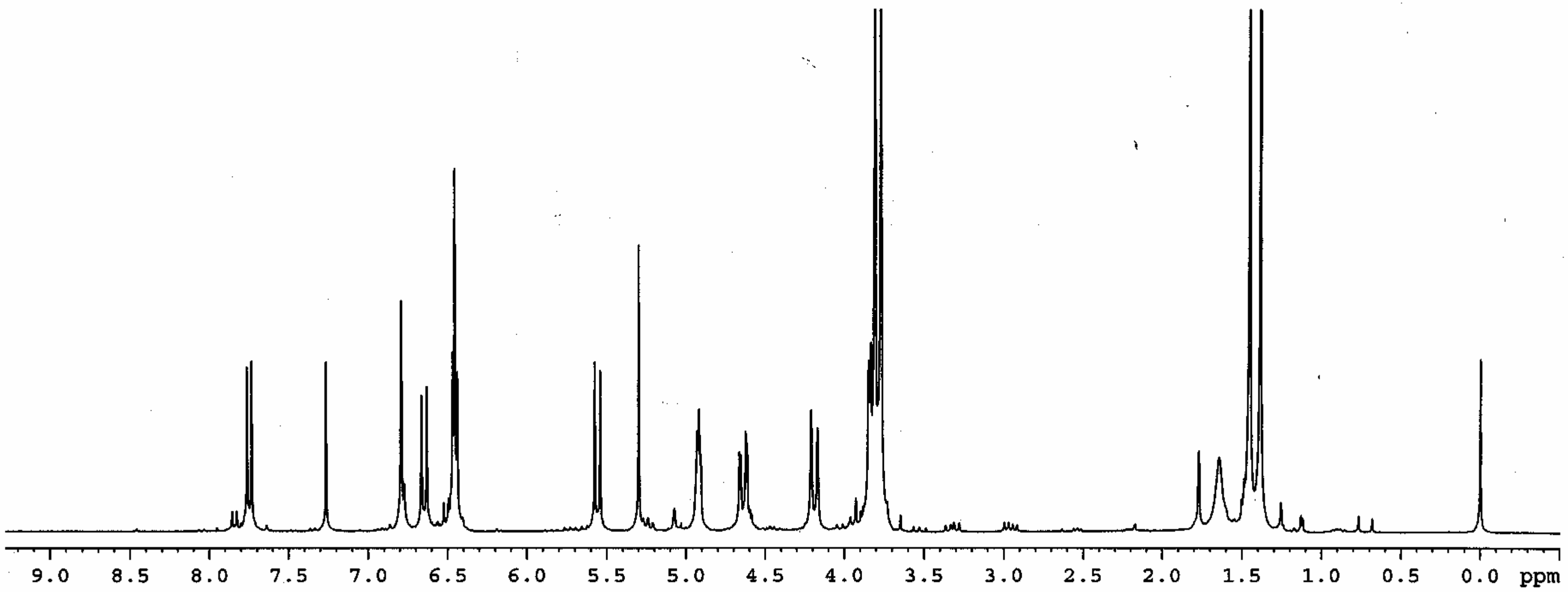

Fig. 9 The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of compound **AJ6**

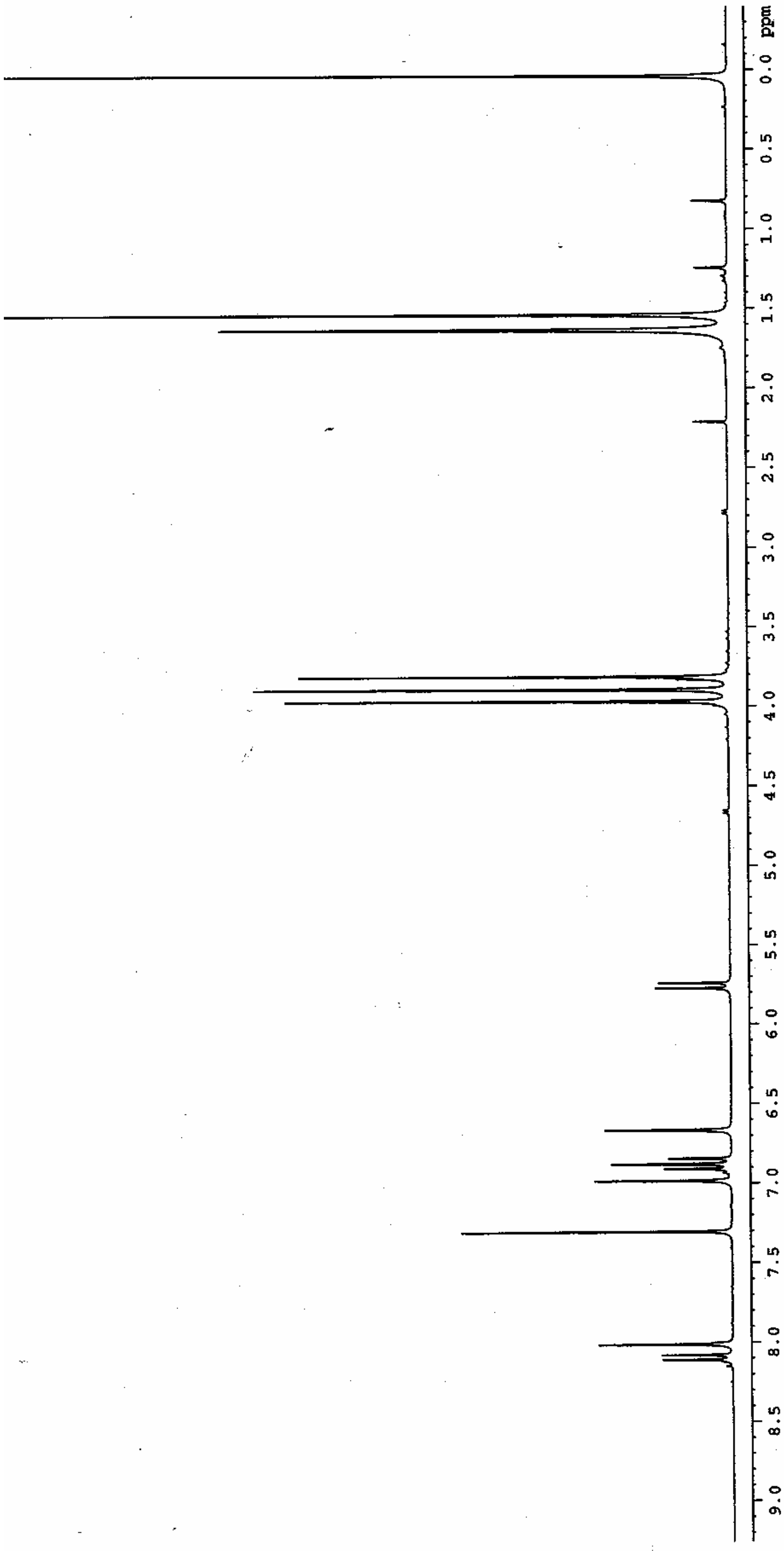

Fig.10 The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of compound **AJ7**

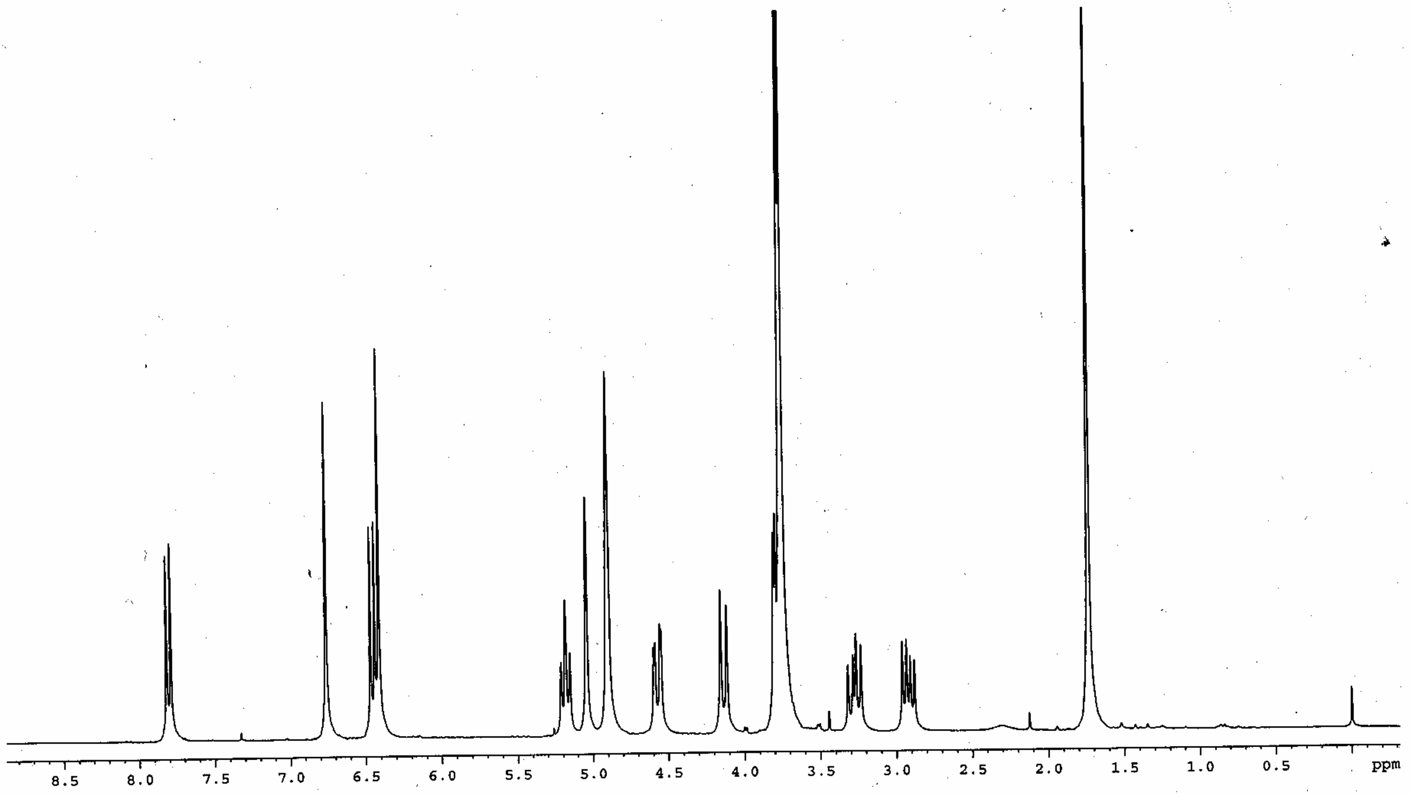

Fig.11 The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of compound **AJ8**

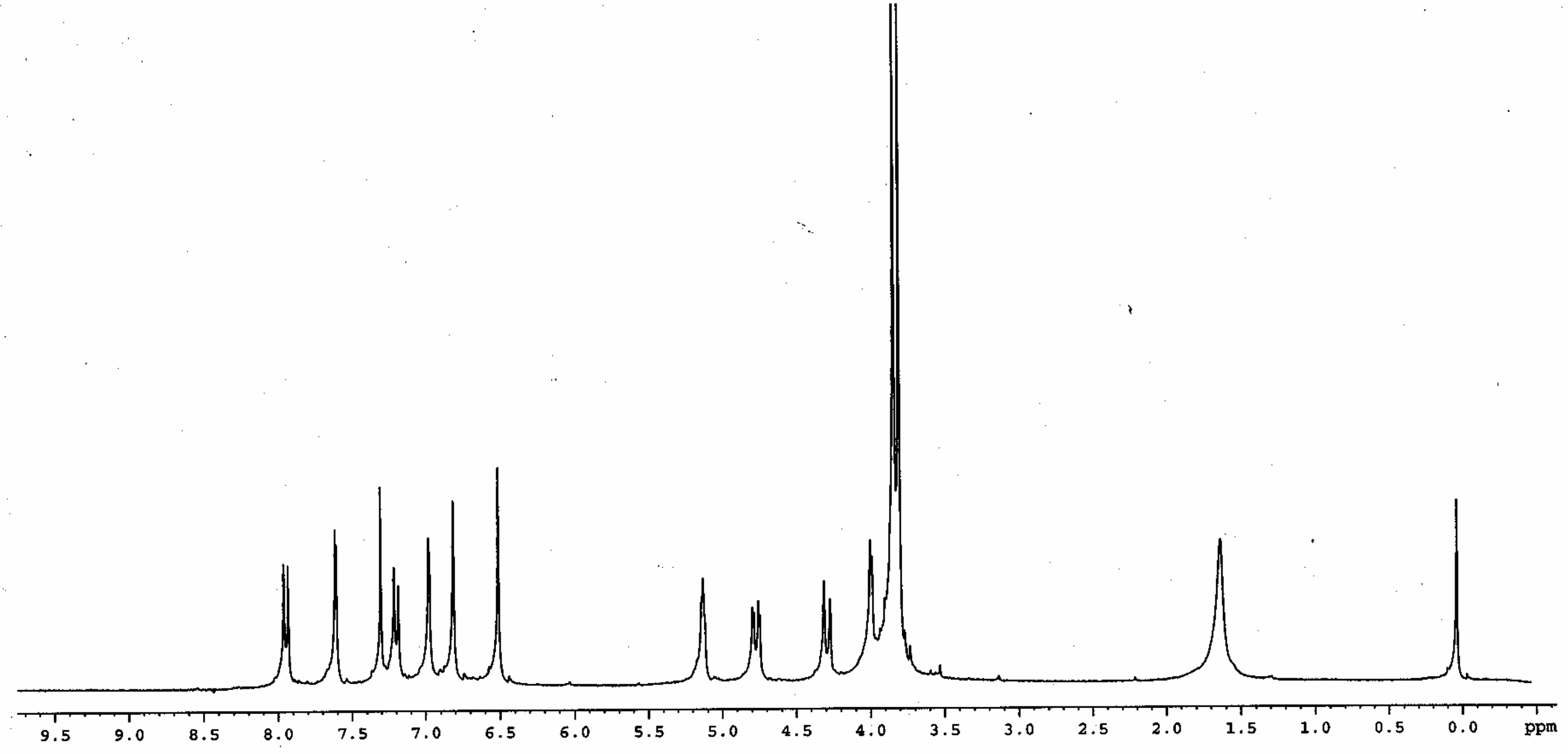

Fig.12 The ^{1}H NMR (300 MHz, CDCl$_3$) spectrum of compound **AJ9**

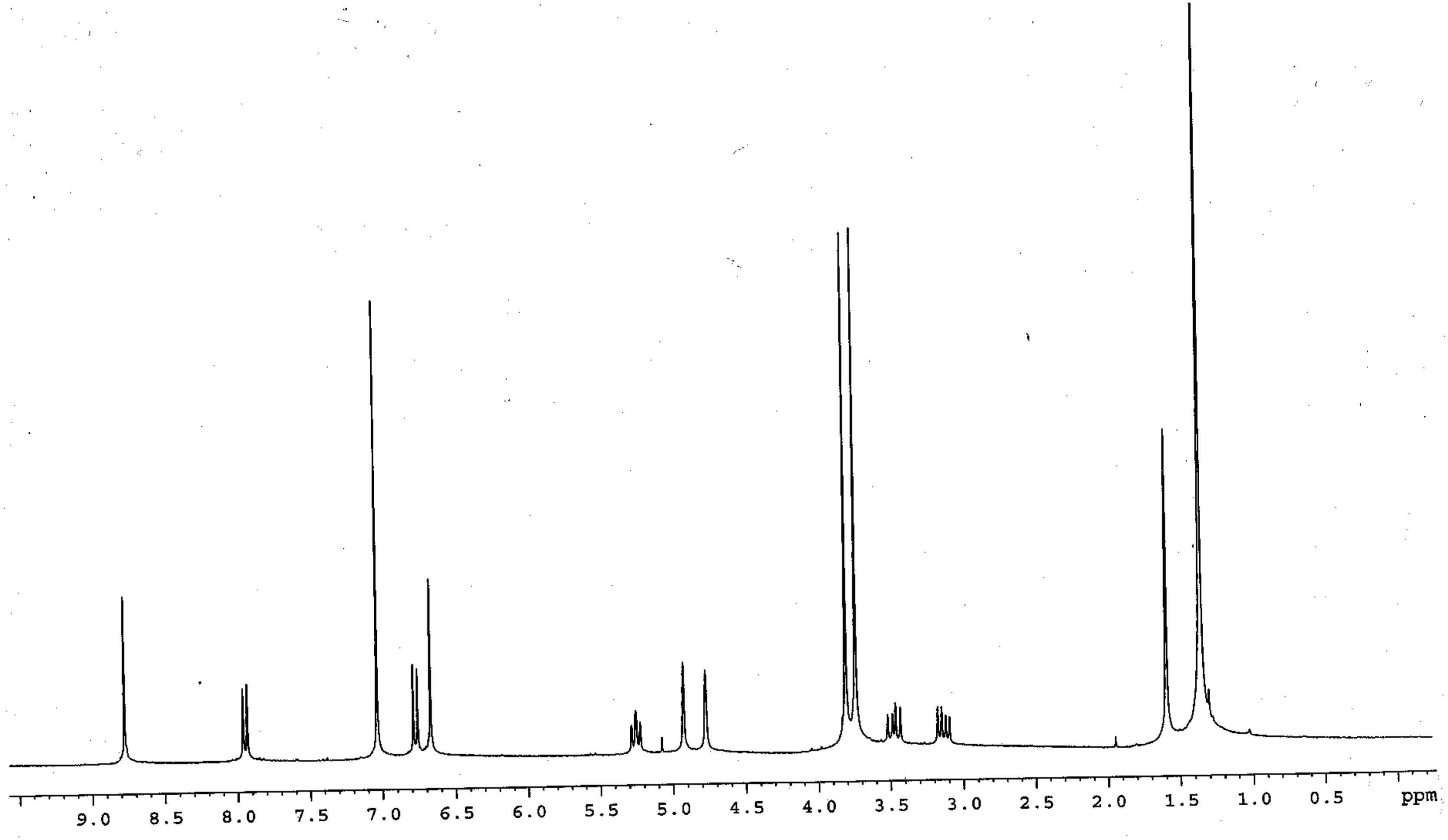

Fig.13 The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of compound **AJ10**

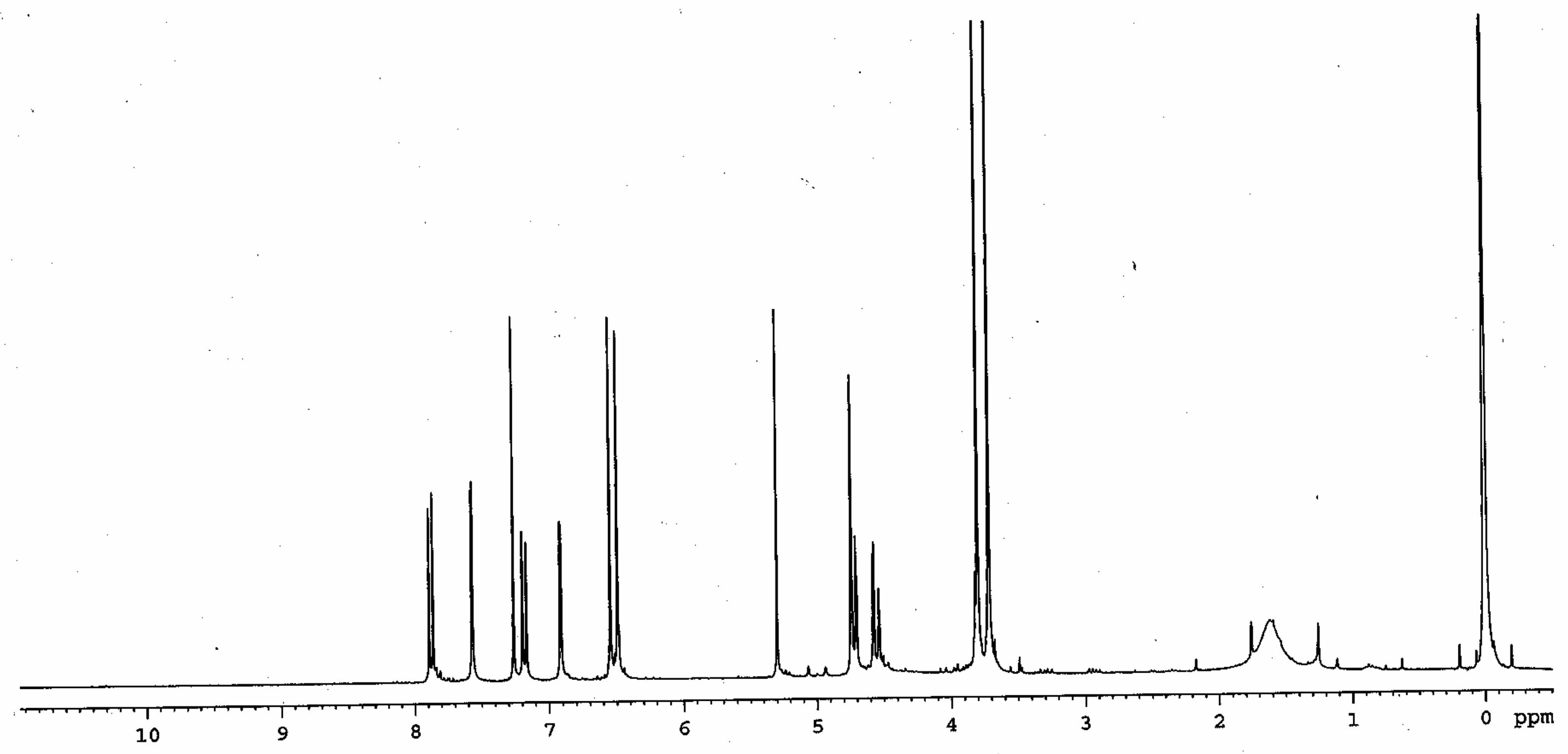

Fig.14 The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of compound **AJ11**

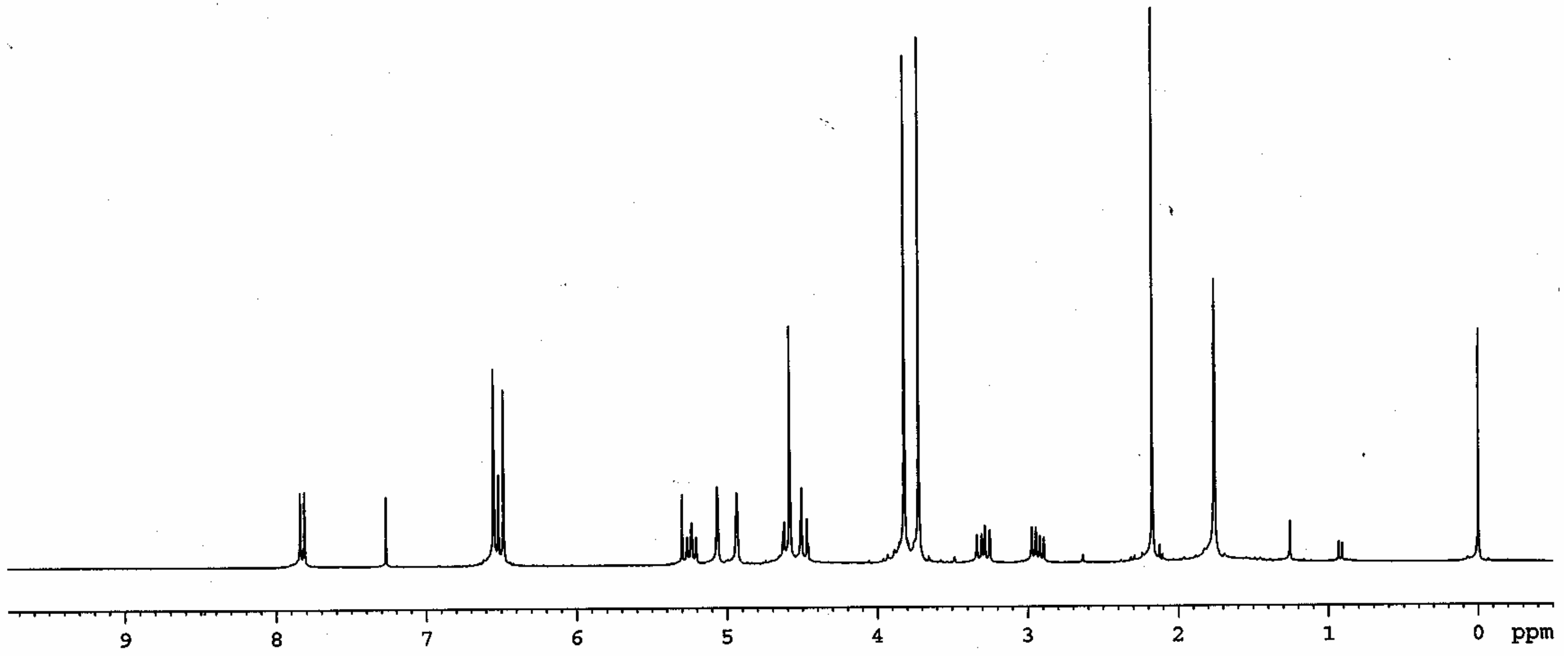

Fig.15 The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of compound **AJ12**