

Supplementary Materials

Phytochemical Analysis of *Anastatica hierochuntica* and *Aerva javanica* Grown in Qatar: Their Biological Activities and Identification of Active Ingredients.

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Department of Chemistry and Earth Sciences, Chemistry Program, College of Arts and Sciences, Qatar University, P.O. Box 2713, Doha, Qatar

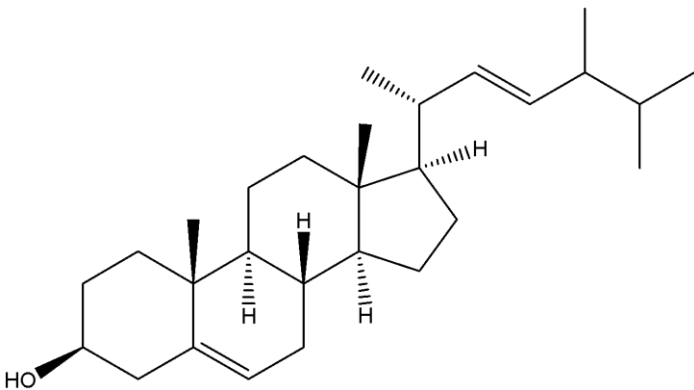
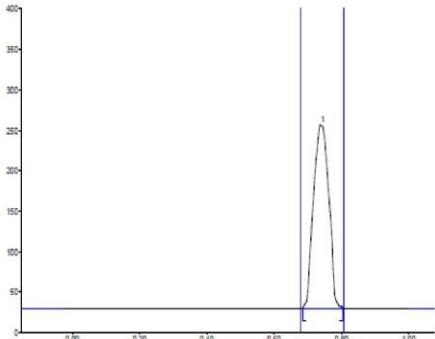
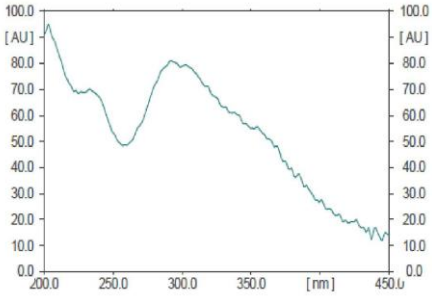
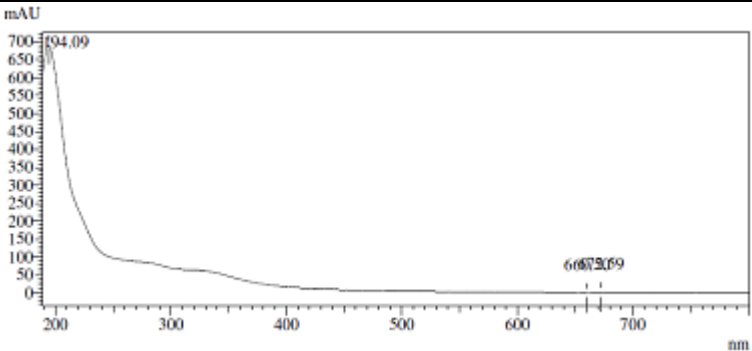
* Correspondence: author: lsreerama@qu.edu.qa

Anastatica Hierochuntica

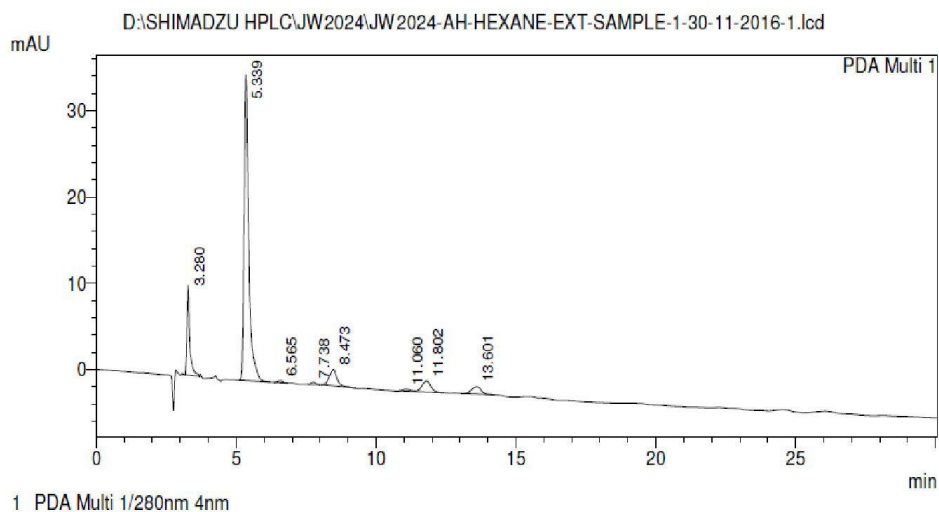
Phytochemical analysis of the Isolated Compounds

The hexane fraction was subjected to HPTLC and HPLC analysis. Three pure compounds were isolated and subjected to IR and NMR analysis for further identification.

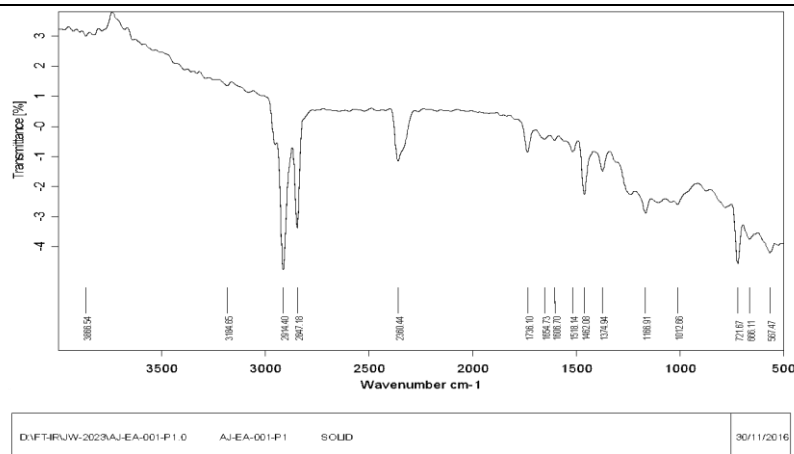
COMPOUND 1 (AH-HX-001-P1)

SPECIFICATON	SPECTRUM								
Probable structure	<p>β-Sitosterol</p> 								
CAMAG HPTLC Scanner III Mobile Phase: Hex: EtoAc (7:3) Rf: 0.74 λ max: 203nm	  <table data-bbox="985 1310 1414 1358"><thead><tr><th>T</th><th>Rf</th><th>Substance</th><th>Max. @</th></tr></thead><tbody><tr><td>2</td><td>0.74</td><td>Rf AutoGenerated6</td><td>203 nm</td></tr></tbody></table>	T	Rf	Substance	Max. @	2	0.74	Rf AutoGenerated6	203 nm
T	Rf	Substance	Max. @						
2	0.74	Rf AutoGenerated6	203 nm						
UV spectrum PDA detector: 200-800nm λ max: 194 nm									

SHIMADZU SPD-20A
HPLC
Column: Enable C₁₈
4.6x250mm (5micron)
Mobile Phase:
ACN:H₂O (15:85) with
0.1% H₃PO₄
 λ max: 280nm
Inj. Vol: 20 μ l
Flow rate: 1.0ml/min
Rt: 5.339 min

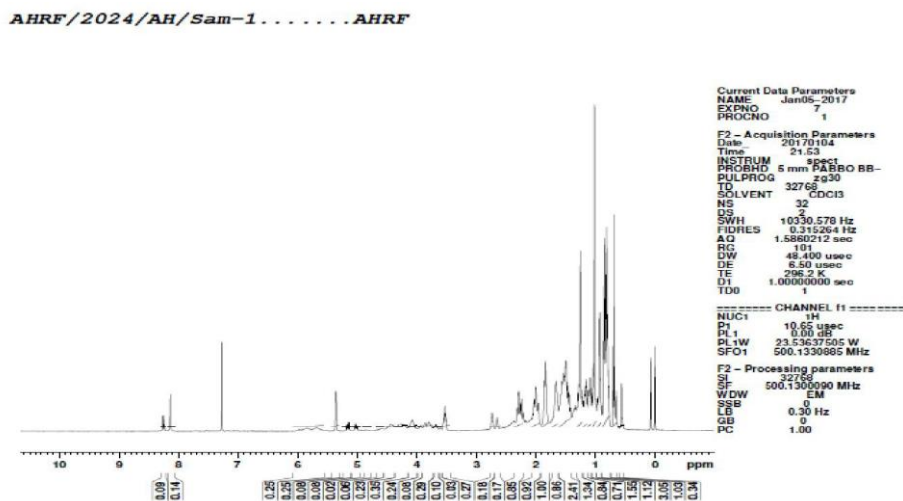


Bruker FT-IR
Wavelength scan:
500-4000cm⁻¹



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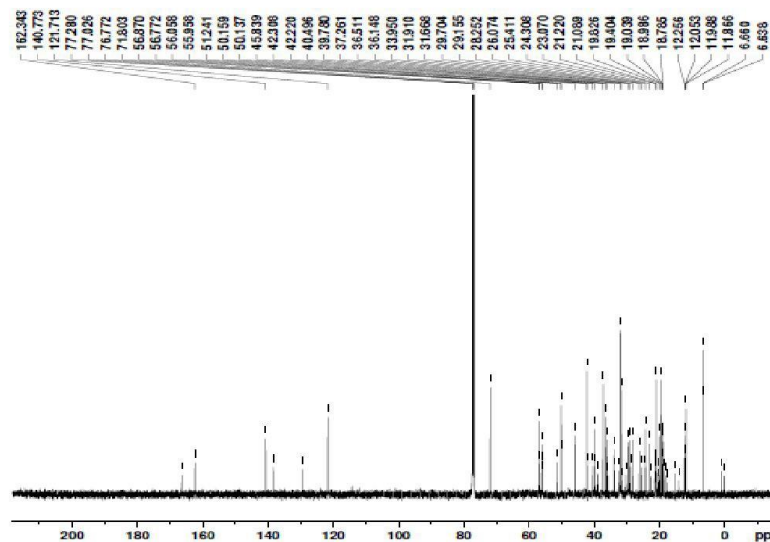
BRUKER NMR
400MHz
¹HNMR(CDCl₃):



BRUKER
400MHz
¹³CNMR(CDCl₃):

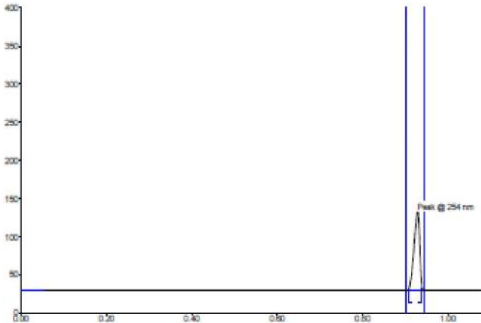
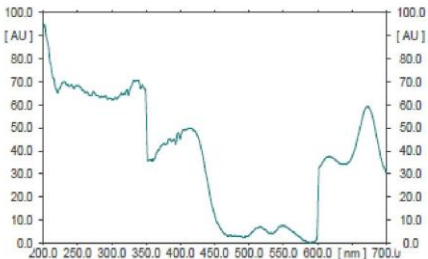
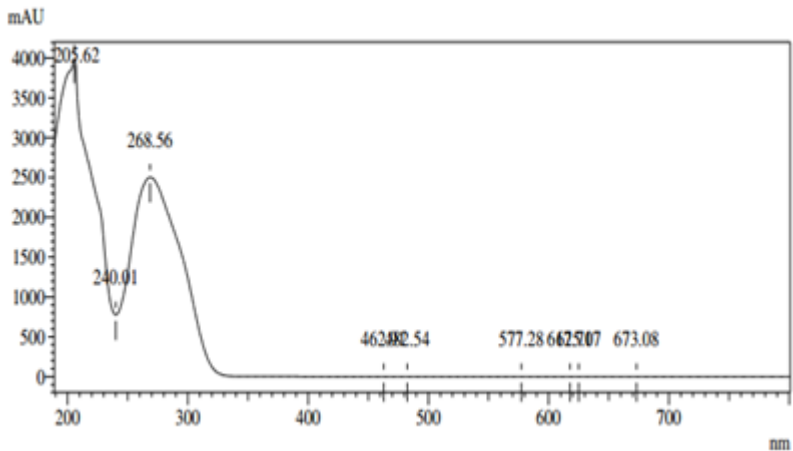
NMR

AHRE/2024/AH/Sam-1.....AHRE

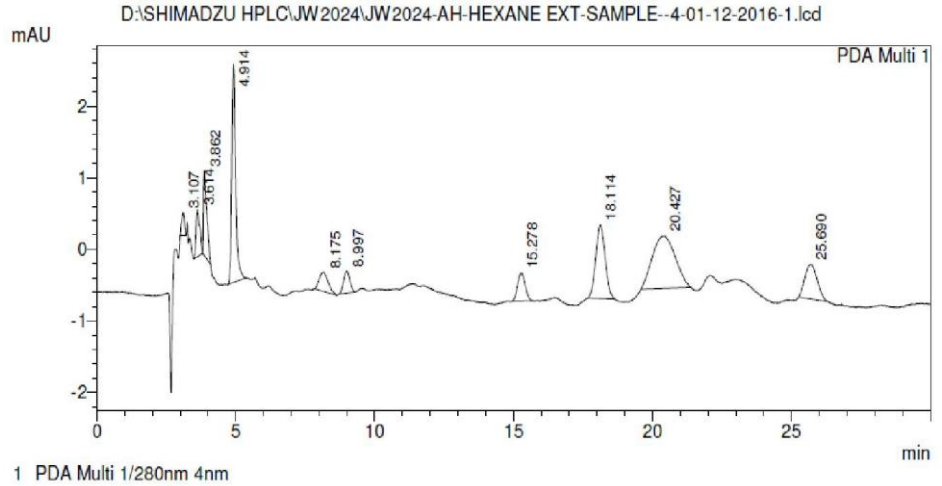


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PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 1600
DS 4
SWH 297.61.904 Hz
FIDRES 0.9052511 Hz
AQ 0.5505524 sec
RG 800
DW 16.800 usec
DE 0.50 usec
TE 297.3 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
===== CHANNEL f1 =====
NUC1 13C
P1 7.30 usec
PL1 0.00 dB
PL1W 70.93518745 W
SFO1 125.7703643 MHz
===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 00.00 usec
PL2 0.00 dB
PL2 17.51 dB
PL3 15.00 dB
PL12W 23.53637505 W
PL12W 0.41757909 W
PL13W 0.37325643 W
SFO2 500.1320005 MHz
F2 - Processing parameters
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GB 0
PC 1.40

COMPOUND 2 (AH-HX-001-P4)

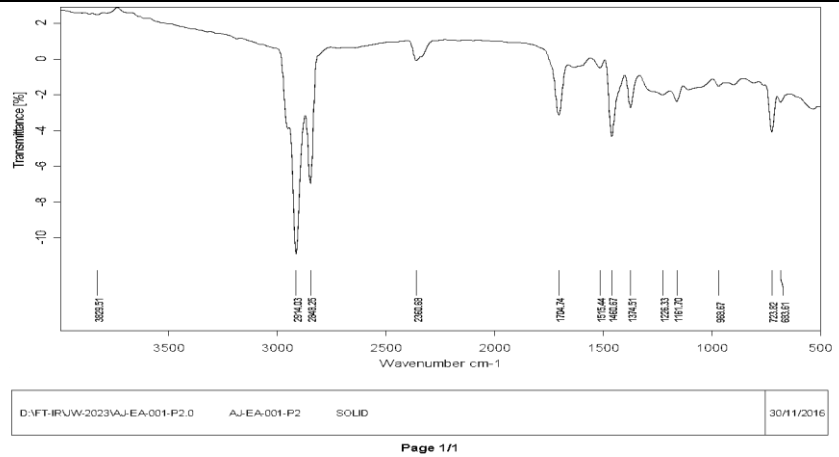
SPECIFICATON	SPECTRUM								
Probable structure	<div>Campesterol</div> <div><chem>CC(C)CC(C)C1CCC2C(C1)CCC3=C(C2)CCCC4C3CCCC(C4)O</chem></div>								
CAMAG HPTLC Scanner III Mobile Phase: Hex: EtoAc (7:3) Rf: 0.93 λ max: 202 nm	<div></div> <div></div> <table><tr><th>T</th><th>Rf</th><th>Substance</th><th>Max. @</th></tr><tr><td>1</td><td>0.93</td><td>Rf Peak</td><td>202 nm</td></tr></table>	T	Rf	Substance	Max. @	1	0.93	Rf Peak	202 nm
T	Rf	Substance	Max. @						
1	0.93	Rf Peak	202 nm						
UV spectrum PDA detector: 200-800nm λ max: 205, 268nm	<div></div>								

SHIMADZU SPD-20A HPLC
Column: Enable C₁₈,
4.6x250mm (5micron)
Mobile Phase: ACN:H₂O
(15:85) with 0.1% H₃PO₄
λ max: 280nm
Inj. Vol: 20μl
Flow rate: 1.0ml/min
Rt: 4.914 min



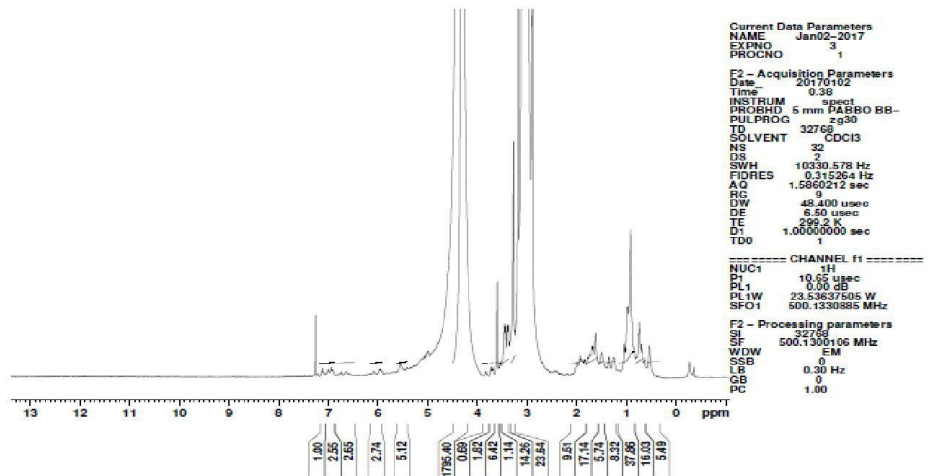
Bruker FT-IR
Wavelength
500-4000cm⁻¹

scan:



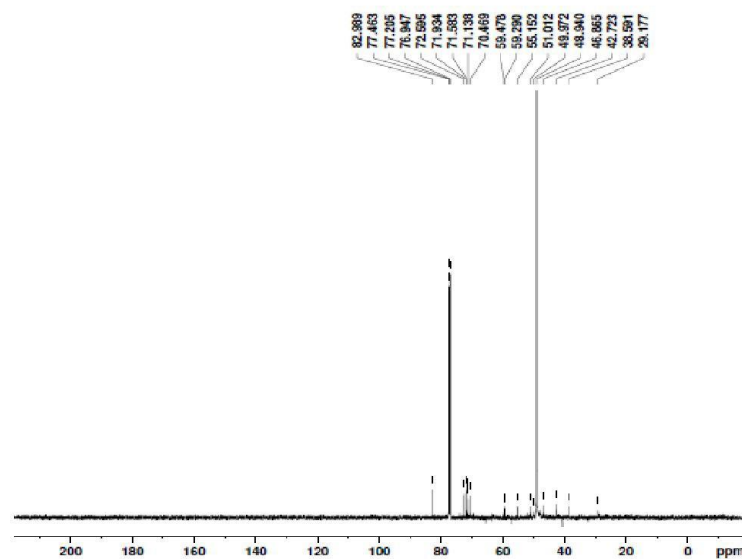
BRUKER NMR 400MHz
¹HNMR(CDCl₃):

AHRF/2024/AH/Sam-2...AHRF



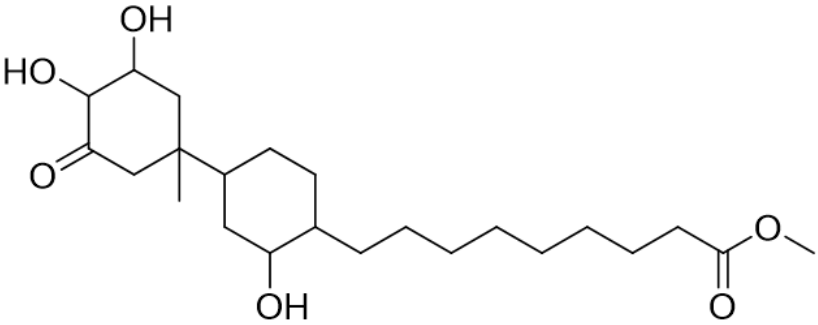
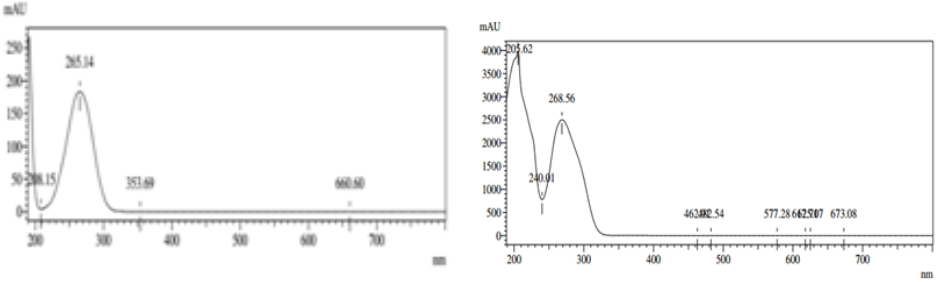
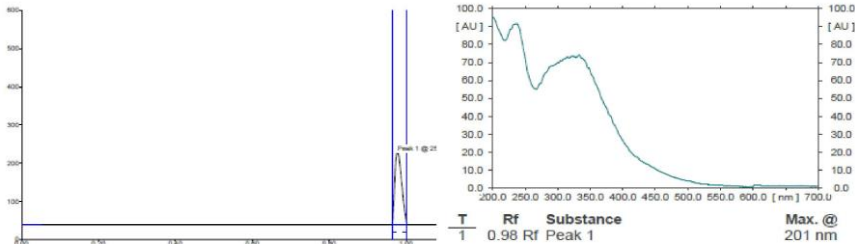
BRUKER NMR 400MHz
¹³CNMR(CDCl₃):

AHRF/2024/AH/Sam-2...AHRF



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PROCNO 1
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Time 1.23
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PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 1024
DS 4
SWH 29701.904 Hz
FIDRES 0.8062511 Hz
AQ 0.5505524 sec
RG 205
DW 16.600 usec
DE 6.50 usec
TE 300.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
===== CHANNEL f1 =====
NUC1 13C
P1 7.00 usec
PL1 0.00 dB
PL1W 70.93519745 W
SFO1 125.7703643 MHz
===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 00.00 usec
PL2 0.00 dB
PL12 17.51 dB
PL13 10.00 dB
PL2W 23.53637505 W
PL12W 0.41737588 W
PL13W 0.37302643 W
SFO2 500.132005 MHz
F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WOW 2M
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

COMPOUND 3 (AH-HX-001-P5)

SPECIFICATON	SPECTRUM
<p>Probable structure</p>	<p>Methyl- 9-(4-(3,4-dihydroxy-1'-methyl-5'-oxocyclohexyl)-2-hydroxycyclohexyl)nonanoate</p> 
<p>UV spectrum PDA detector: 200-800nm λ max: 205, 265nm</p>	
<p>CAMAG HPTLC Scanner III Mobile Phase: Hex: EtoAc (7:3) Rf: 0.09 λ max: 201 nm</p>	 <p> Peak 1 Rf 0.09 Substance Peak 1 Max. @ 201 nm </p>

SHIMADZU SPD-20A

HPLC

Column: Enable C₁₈

4.6x250mm (5micron)

Mobile Phase: ACN:H₂O
(15:85) with 0.1% H₃PO₄

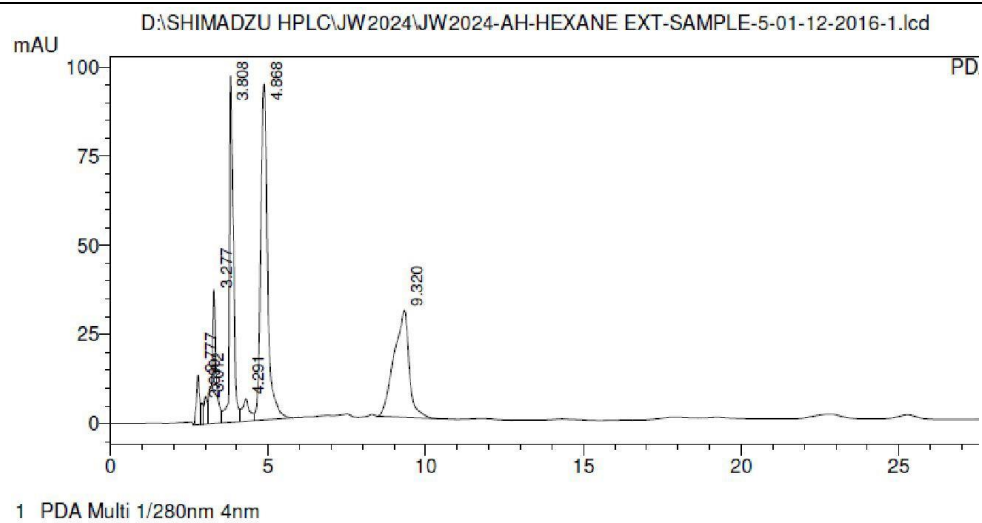
λ max: 280nm

Inj. Vol: 20μl

Flow rate: 1.0ml/min

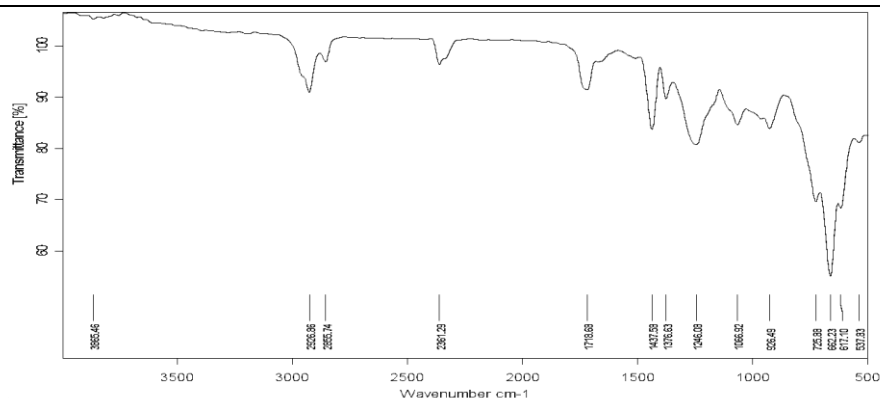
Rt: 3.808min and

4.868min



Bruker FT-IR
Wavelength
500-4000cm⁻¹

scan:



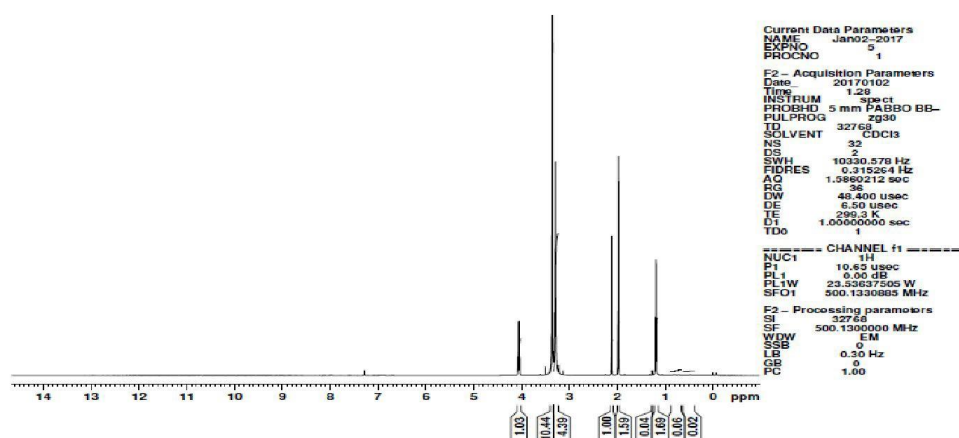
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30/11/2016

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BRUKER NMR 400MHz
¹HNMR(CDCI₃):

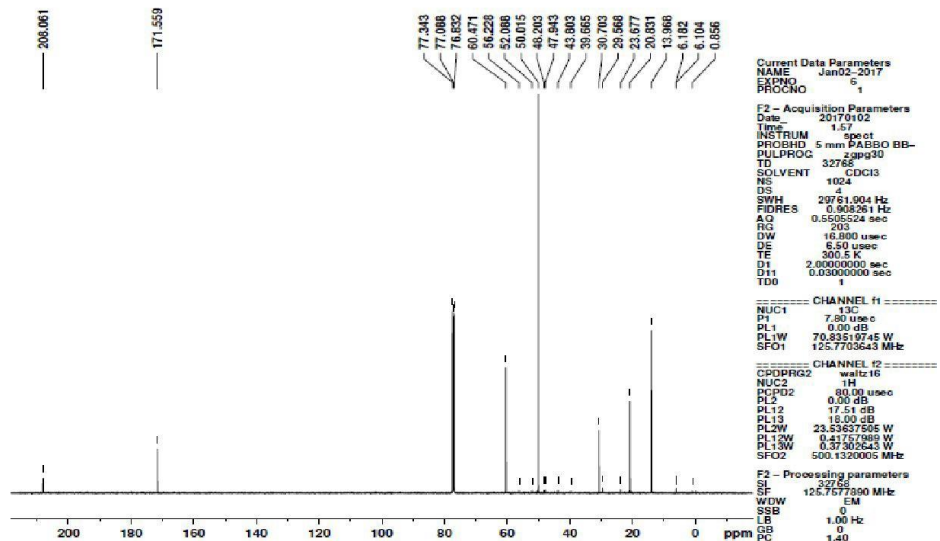
AHRF/2024/AH/Sam-3 . . . AHRF



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TD 32768
SOLVENT CDCl3
NS 32
DS 2
SWH 10030.579 Hz
FIDRES 0.315264 Hz
AQ 1.9850212 sec
RG 38
DW 48.400 usec
DE 6.50 usec
TE 296.3 K
D1 1.00000000 sec
TD0 1
===== CHANNEL f1 =====
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.130885 MHz
F2 - Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
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GB 0
PC 1.00

BRUKER NMR 400MHz
¹³CNMR(CDCI₃):

AHRF/2024/AH/Sam-3 . . . AHRF



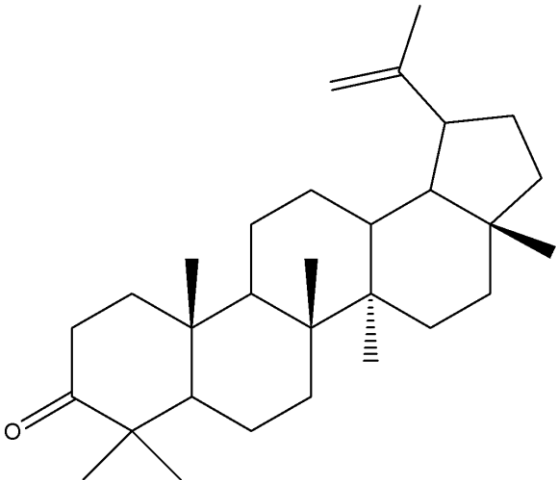
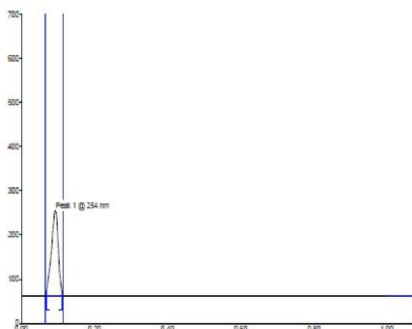
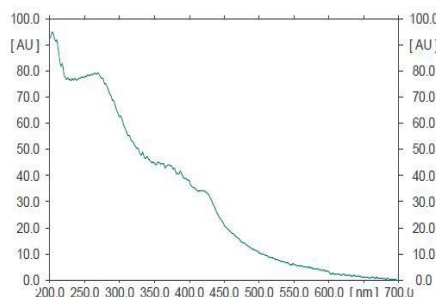
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PULPROG zgpg30
TD 32768
SOLVENT CDCl3
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SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 0.5505524 sec
RG 203
DW 18.890 usec
DE 6.50 usec
TE 300.5 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
===== CHANNEL f1 =====
NUC1 13C
P1 7.80 usec
PL1 0.00 dB
PL1W 70.93519745 W
SFO1 125.7703645 MHz
===== CHANNEL f2 =====
CROPRG2 waltz16
NUC2 1H
PCPRG2 80.00 usec
PL2 0.00 dB
PL12 17.51 dB
PL13 18.00 dB
PL2W 23.53637505 W
PL1W 0.41767989 W
PL13W 0.37302643 W
SFO2 500.1300005 MHz
F2 - Processing parameters
SI 32768
SF 125.7677050 MHz
WDW EM
SSB 1.00 Hz
LB 0
GB 0
PC 1.40

Aerva Javanica

Phytochemical analysis of the Isolated Compounds

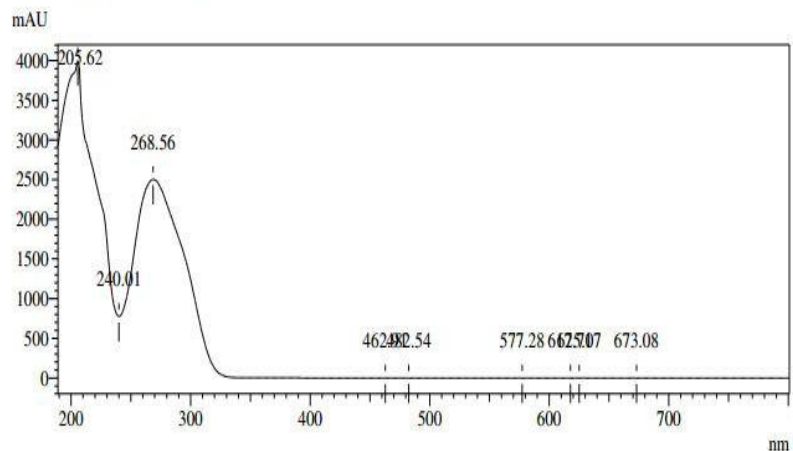
The ethyl acetate fraction was subjected to HPTLC and HPLC analysis. Three pure compounds were isolated and subjected to IR and NMR analysis for further identification.

COMPOUND 1 (AJ-EA-001-P1)

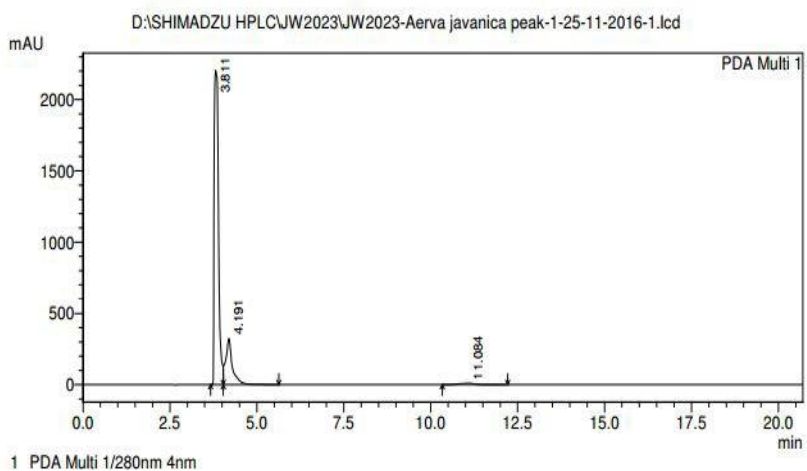
Probable structure	<p>Lupenone</p> 								
<p>CAMAG HPTLC Scanner III Mobile Phase: Hex: EtoAc (7:3) Rf: 0.09 λ max: 254nm</p>	  <table><tr><th>T</th><th>Rf</th><th>Substance</th><th>Max. @</th></tr><tr><td>1</td><td>0.09</td><td>Rf peak 2</td><td>254 nm</td></tr></table>	T	Rf	Substance	Max. @	1	0.09	Rf peak 2	254 nm
T	Rf	Substance	Max. @						
1	0.09	Rf peak 2	254 nm						

UV spectrum
PDA detector: 200-800nm
 λ max: 205, 268nm

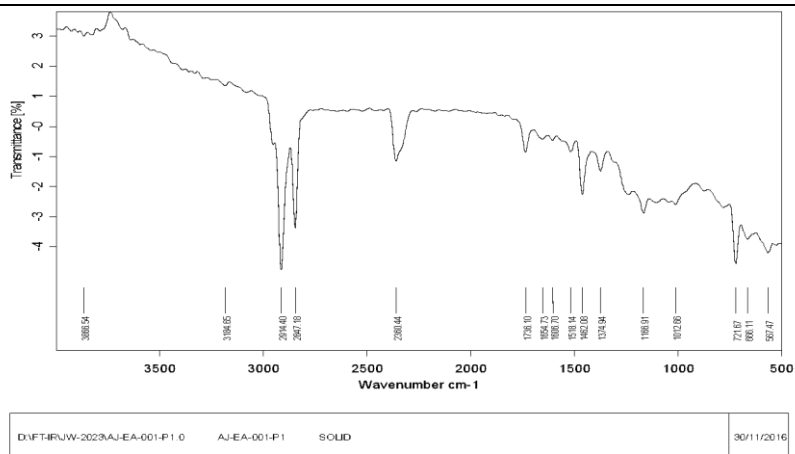
Retention Time : 3.811
Compound Name :
Spectrum Operation : None



SHIMADZU SPD-20A HPLC
Column: Enable C_{18}
4.6x250mm (5micron)
Mobile Phase: ACN:H₂O (15:85)
with 0.1% H₃PO₄
 λ max: 280nm
Inj. Vol: 20 μ l
Flow rate: 1.0ml/min
Rt: 3.811min

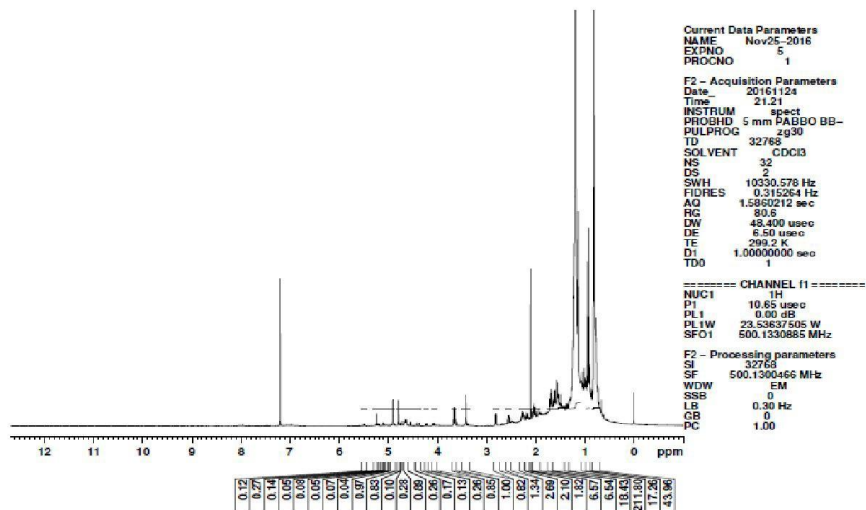


Bruker FT-IR
Wavelength scan:
500-4000cm⁻¹



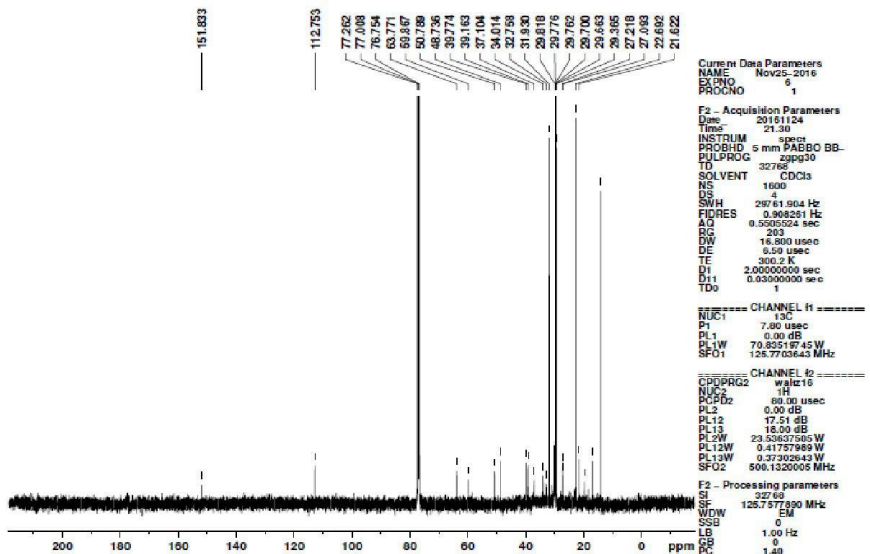
BRUKER NMR 400MHz
¹HNMR(CDCl₃):

AHRF/2023/AJ/Sam-1.....Prince

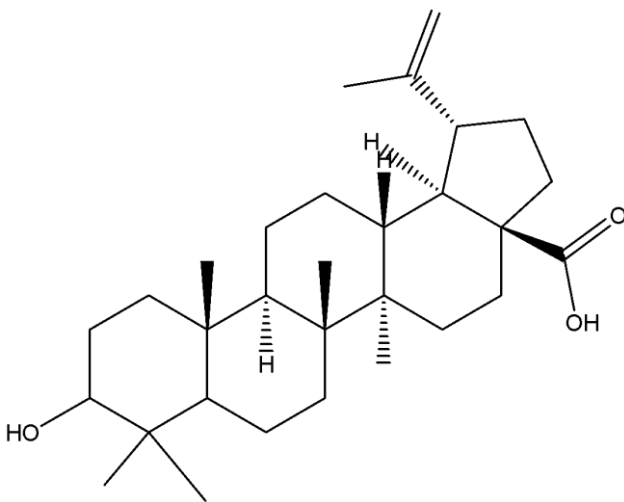
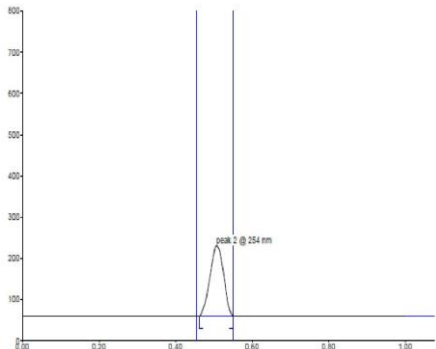
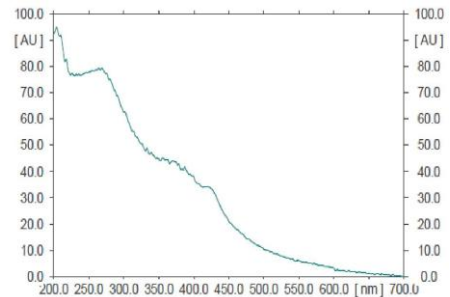
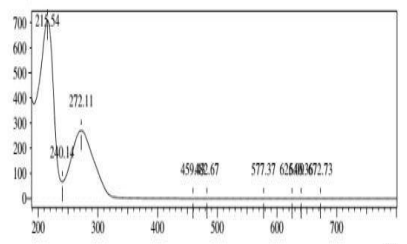
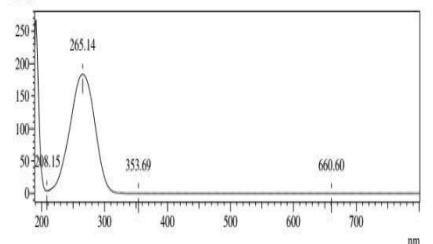


BRUKER NMR 400MHz
¹³CNMR(CDCl₃):

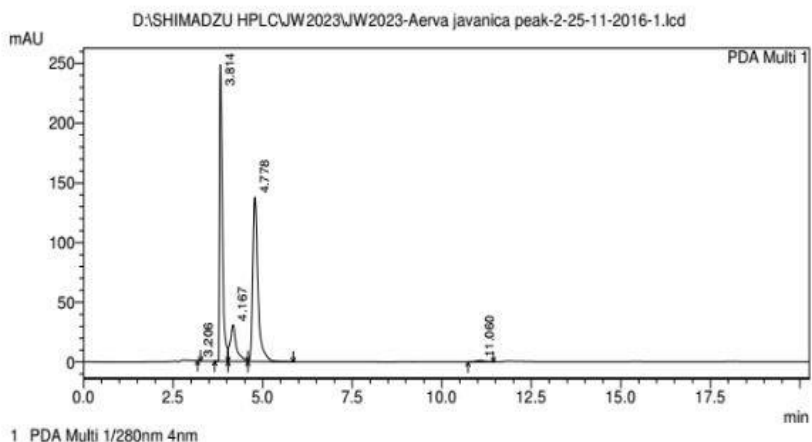
AHRF/2023/AJ/Sam-1.....Prince



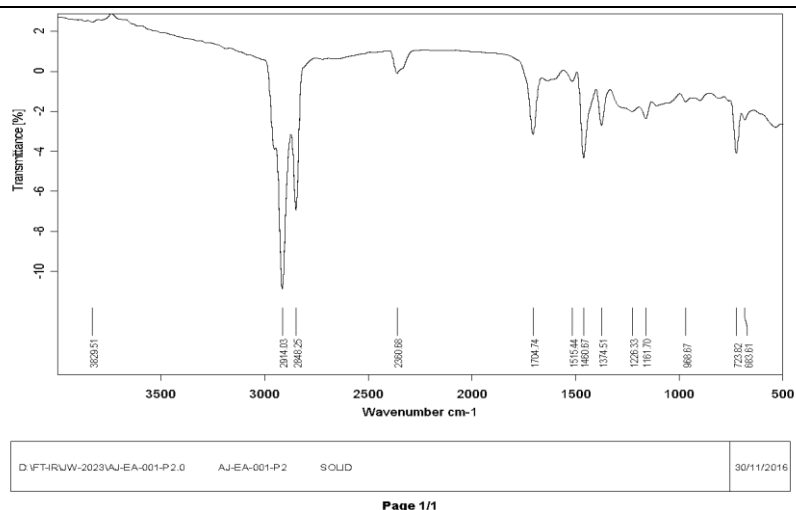
COMPOUND 2 (AJ-EA-001-P2)

SPECIFICATON	SPECTRUM								
Probable structure	<div>Betulinic acid</div> <div></div>								
<div>CAMAG HPTLC Scanner III</div> <div>Mobile Phase: Hex: EtoAc (7:3)</div> <div>Rf: 0.51</div> <div>λ max: 204nm</div>	<div></div> <div></div> <div><table><tr><th>T</th><th>Rf</th><th>Substance</th><th>Max. @</th></tr><tr><td>1</td><td>0.51</td><td>Rf peak 2</td><td>204 nm</td></tr></table></div>	T	Rf	Substance	Max. @	1	0.51	Rf peak 2	204 nm
T	Rf	Substance	Max. @						
1	0.51	Rf peak 2	204 nm						
<div>UV spectrum</div> <div>PDA detector</div> <div>λ max: Rt 3.814 – 215, 272nm</div> <div>Rt 4.778 – 265nm</div>	<div><div><div>Retention Time : 3.814</div><div>Compound Name :</div><div>Spectrum Operation : None</div></div><div></div></div> <div><div><div>Retention Time : 4.778</div><div>Compound Name :</div><div>Spectrum Operation : None</div></div><div></div></div>								

SHIMADZU SPD-20A HPLC
 Column: Enable C₁₈,
 4.6x250mm (5micron)
 Mobile Phase: ACN:H₂O
 (15:85) with 0.1% H₃PO₄
 λ max: 280nm
 Inj. Vol: 20μl
 Flow rate: 1.0ml/min
 Rt: 3.811min and 4.778min

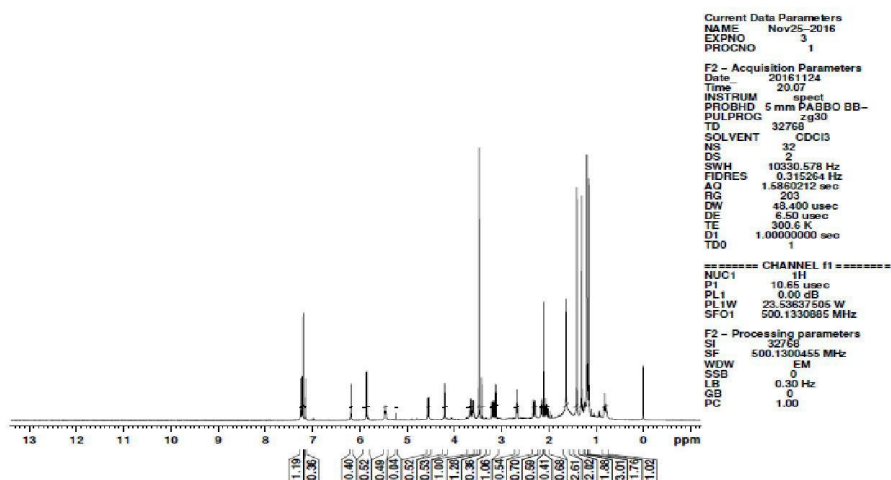


Bruker FT-IR
 Wavelength
 500-4000cm⁻¹ scan:



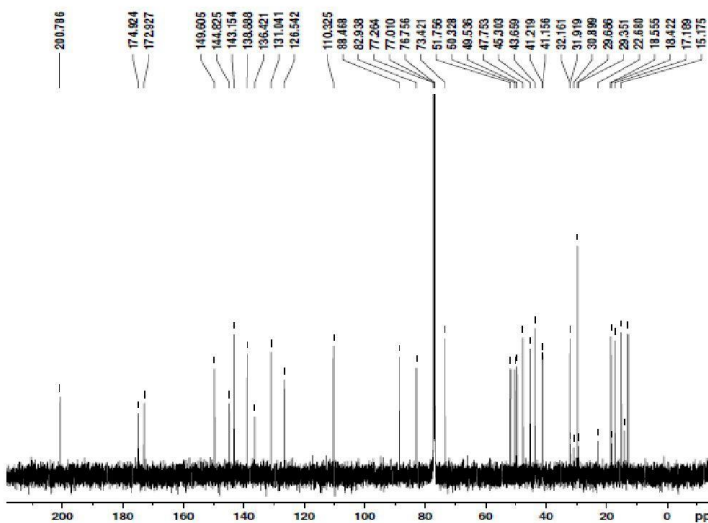
BRUKER NMR 400MHz
¹HNMR(CDCI₃):

AHRF/2023/AJ/Sam-2.....Prince



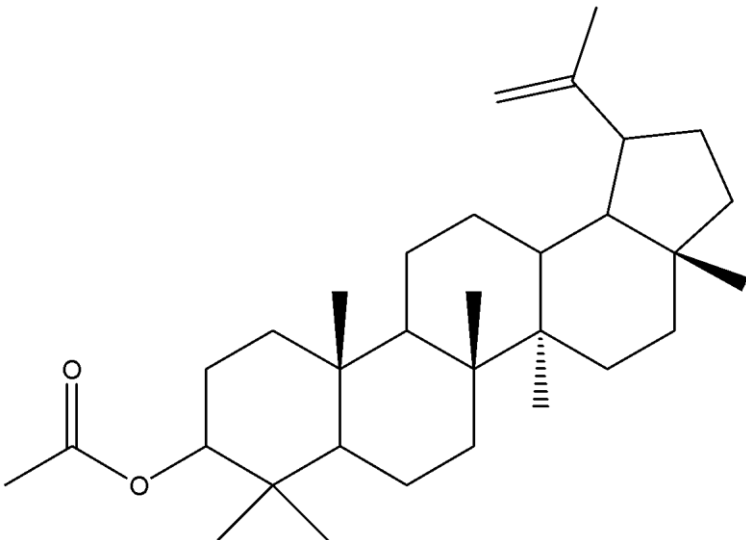
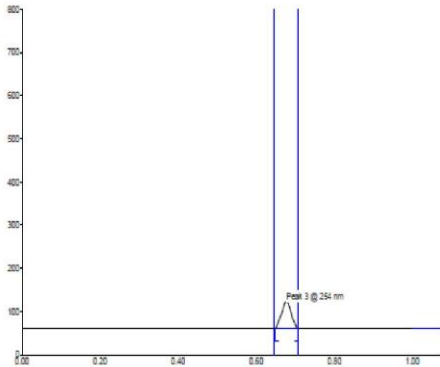
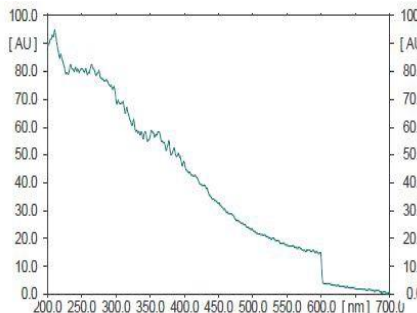
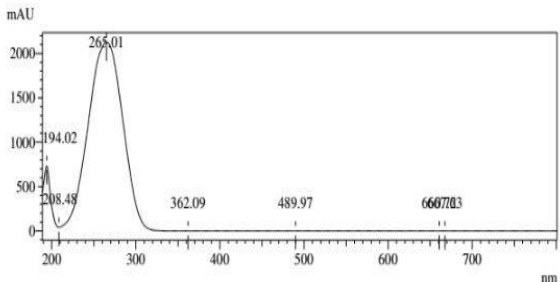
BRUKER NMR 400MHz
13CNMR(CDCl₃):

AHRF/2023/AJ/Sam-2.....Prince

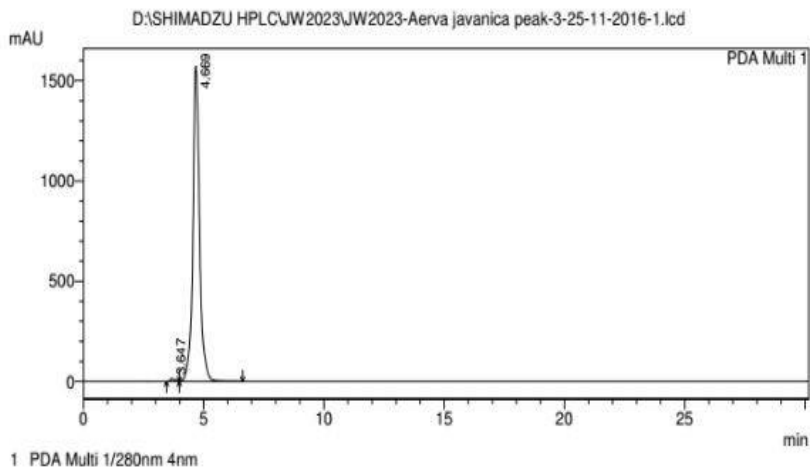


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PROCNO 1
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PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 1600
DS 4
SWH 29761.904 Hz
FIDRES 0.500261 Hz
AQ 0.5902524 sec
RG 253
RW 16.000 usec
TE 301.2 K
DE 6.50 usec
DT 2.0000000 sec
D11 0.0300000 sec
TD0 1
----- CHANNEL f1 -----
NUC1 13C
P1 7.60 usec
PL1 0.00 dB
PL1W 70.83510745 W
SFO1 125.7703643 MHz
----- CHANNEL f2 -----
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.81 dB
PL13 16.00 dB
PL1W 23.33037505 W
PL2W 0.41757995 W
PL3W 0.37302643 W
SFO2 500.1320095 MHz
F2 - Processing parameters
SI 32768
SF 125.7677990 MHz
WDW EM
SSS 0
LB 1.00 Hz
GB 0
PC 1.40

COMPOUND 3 (AJ-EA-001-P6)

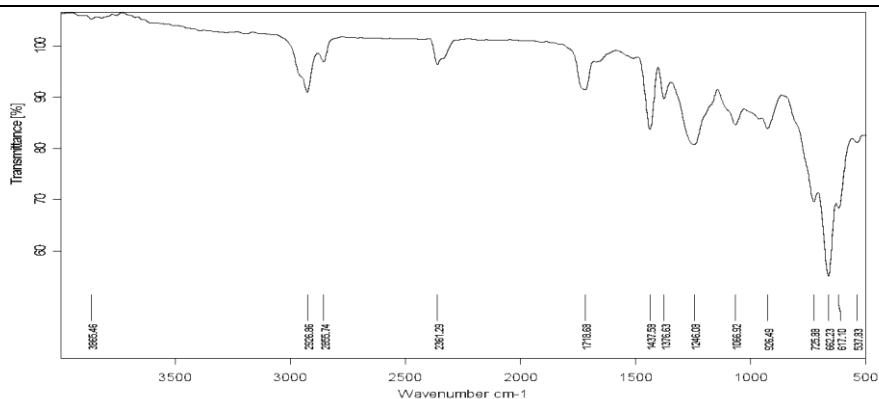
SPECIFICATON	SPECTRUM								
Probable structure	<div>Lupeol acetate</div> <div></div>								
CAMAG HPTLC Scanner III Mobile Phase: Hex: EtoAc (7:3) Rf: 0.68 λ max: 254nm	<div></div> <div><table><thead><tr><th>T</th><th>Rf</th><th>Substance</th><th>Max. @</th></tr></thead><tbody><tr><td>1</td><td>0.68</td><td>Rf Peak 3</td><td>254 nm</td></tr></tbody></table></div>	T	Rf	Substance	Max. @	1	0.68	Rf Peak 3	254 nm
T	Rf	Substance	Max. @						
1	0.68	Rf Peak 3	254 nm						
UV spectrum PDA detector λ max: 265nm	<div>Retention Time : 4.669 Compound Name : Spectrum Operation : None</div> <div></div>								

SHIMADZU SPD-20A HPLC
 Column: Enable C₁₈,
 4.6x250mm (5micron)
 Mobile Phase: ACN:H₂O
 (15:85) with 0.1% H₃PO₄
 λ max: 280nm
 Inj. Vol: 20μl
 Flow rate: 1.0ml/min
 Rt: 4.669min



Bruker FT-IR
 Wavelength
 500-4000cm⁻¹

scan:



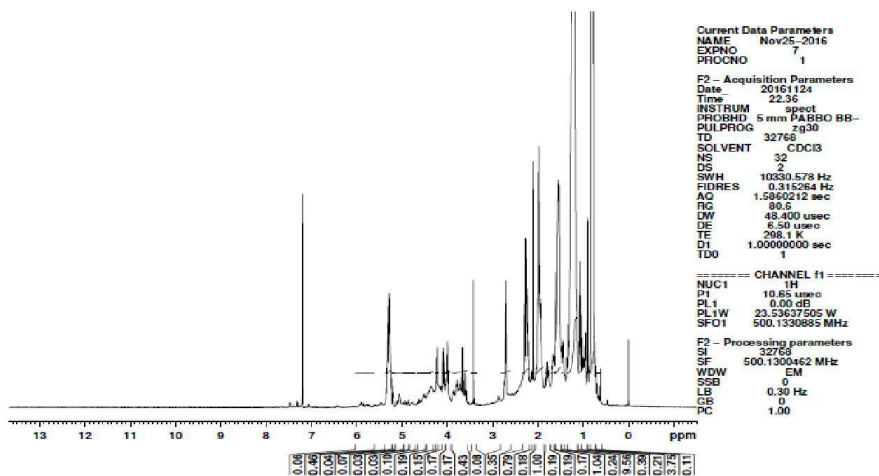
D:\FT-IR\JW-2023\AJ-EA-001-P3.1 AJ-EA-001-P3 SOLID

30/11/2016

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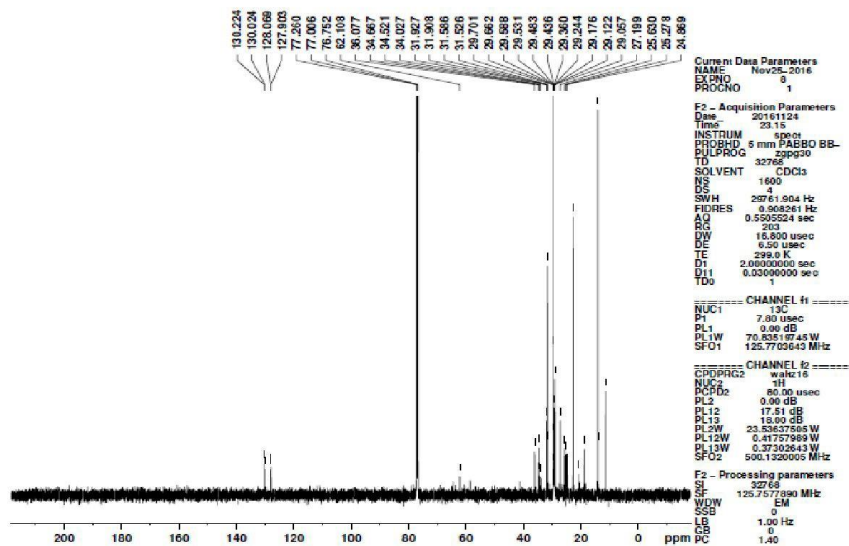
BRUKER NMR 400MHZ
¹HNMR(CDCl₃):

AHRE/2023/AJ/Sam-3.....Prince



BRUKER NMR 400MHz
13CNMR(CDCI₃):

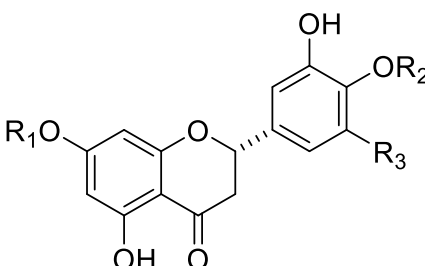
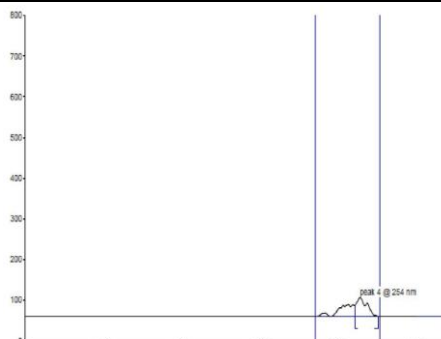
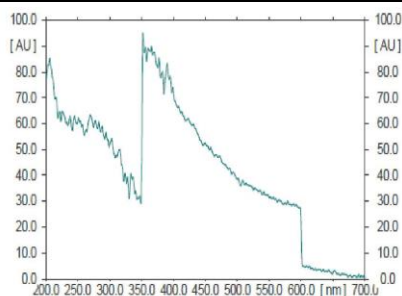
AHRE/2023/AJ/Sam-3.....Prince

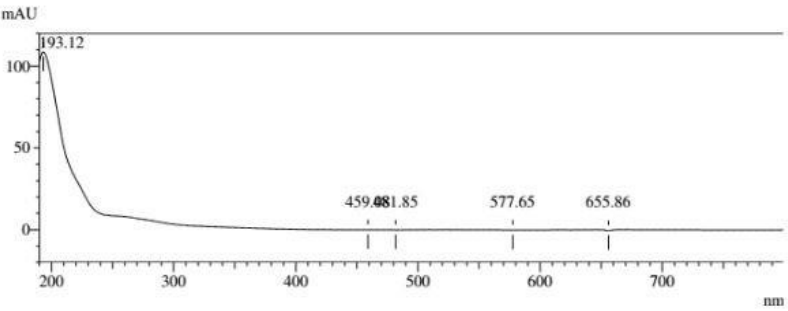
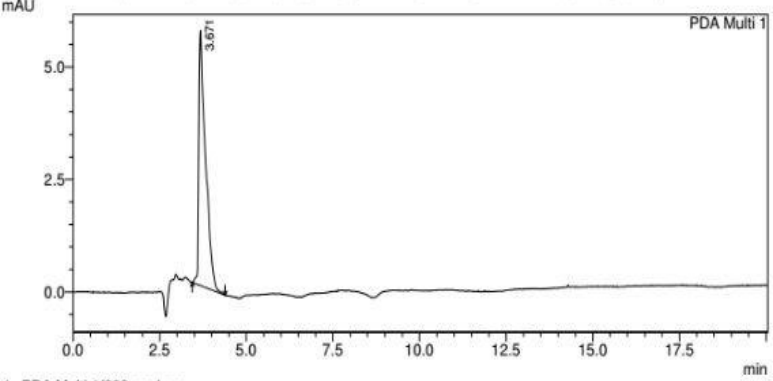
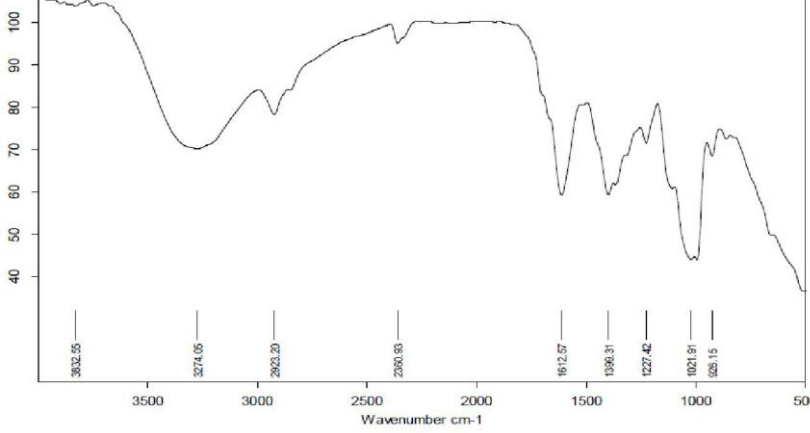


METHANOL EXTRACT

The methanol fraction were subjected to HPTLC and HPLC analysis. One pure compound was isolated and subjected to IR and NMR analysis for further identification.

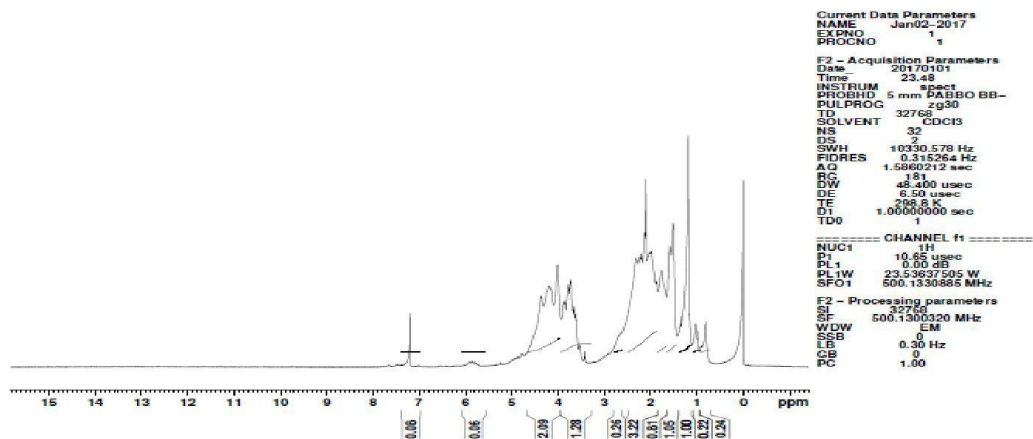
COMPOUND 4 (AJ-ME-001-P3)

SPECIFICATON	SPECTRUM								
Probable structure	<p>Persinoside A and B</p> <div></div> <p>Persinoside A R₁=Me, R₂=Glc, R₃ = H Persinoside B R₁=Glc, R₂=H, R₃ = OH</p>								
CAMAG HPTLC Scanner III Mobile Phase: Hex: EtoAc (7:3) Rf: 0.81 λ max: 352nm	<div></div> <div></div> <table><tr><th>T</th><th>Rf</th><th>Substance</th><th>Max. @</th></tr><tr><td>1</td><td>0.86</td><td>Rf peak 4</td><td>352 nm</td></tr></table>	T	Rf	Substance	Max. @	1	0.86	Rf peak 4	352 nm
T	Rf	Substance	Max. @						
1	0.86	Rf peak 4	352 nm						

<p>UV spectrum PDA detector: 200-800nm λ max: 195nm</p>	<p>Retention Time : 3.671 Compound Name : Spectrum Operation : None</p> 
<p>SHIMADZU SPD-20A HPLC Column: Enable C₁₈ 4.6x250mm (5micron) Mobile Phase: ACN:H₂O (15:85) with 0.1% H₃PO₄ λ max: 280nm Inj. Vol: 20μl Flow rate: 1.0ml/min Rt: 3.671min</p>	<p>D:\SHIMADZU HPLC\JW2023\JW2023-AERVA JAVANICA-PEAK-4-29-11-2016-2.lcd</p>  <p>1 PDA Multi 1/280nm 4nm</p>
<p>Bruker FT-IR Wavelength scan: 500-4000cm⁻¹</p>	 <p>©FTIR\JW-2023\AJ-EA-001-P4.0 AJ-EA-001-P4 SOLID 30/11/2016</p>

BRUKER NMR 400MHz
¹HNMR(CDCl₃):

AHRF/2023/AJ/Sam-4...AHRF



BRUKER NMR 400MHz
¹³CNMR(CDCl₃):

AHRF/2023/AJ/Sam-4...AHRF

