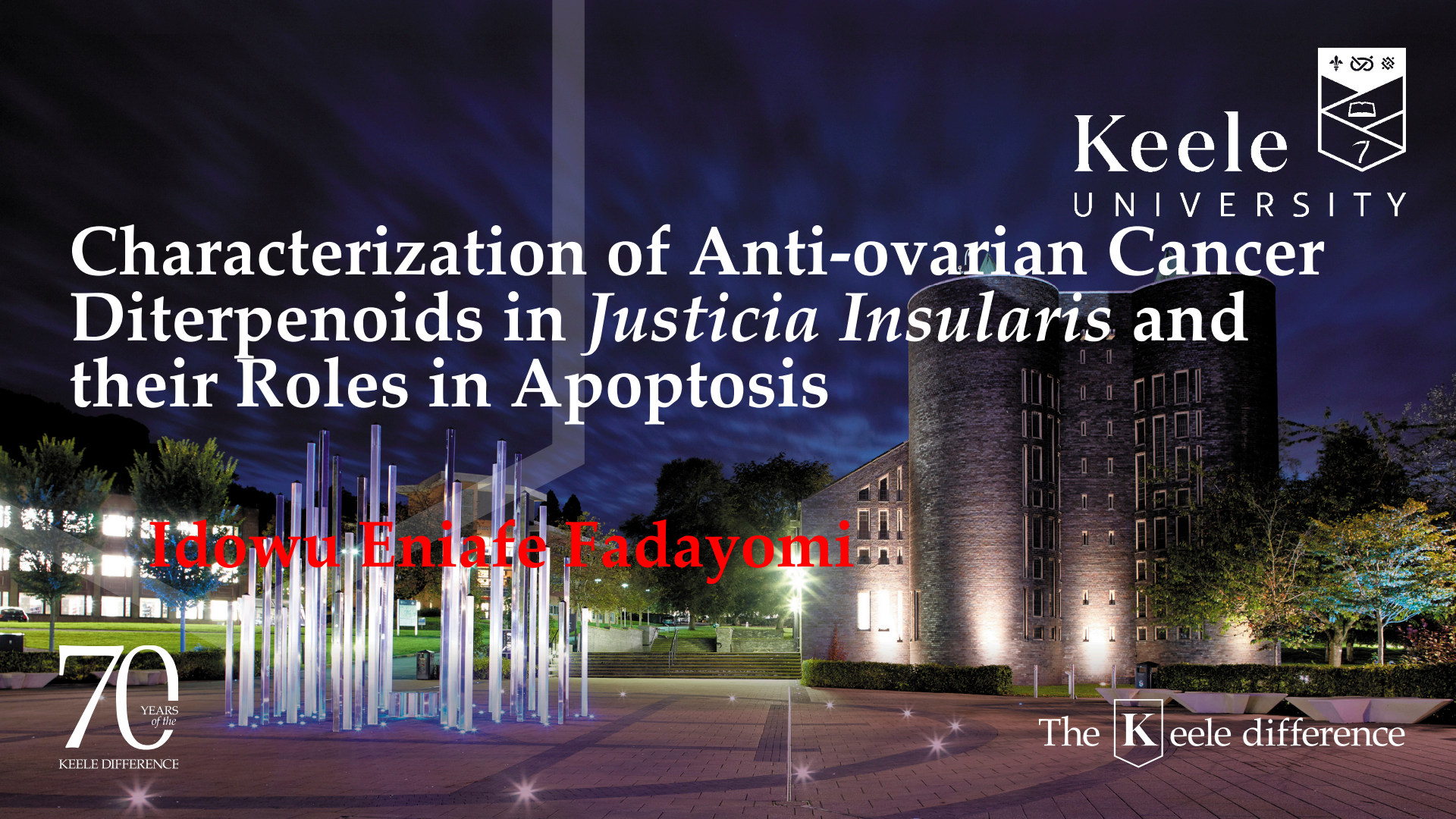
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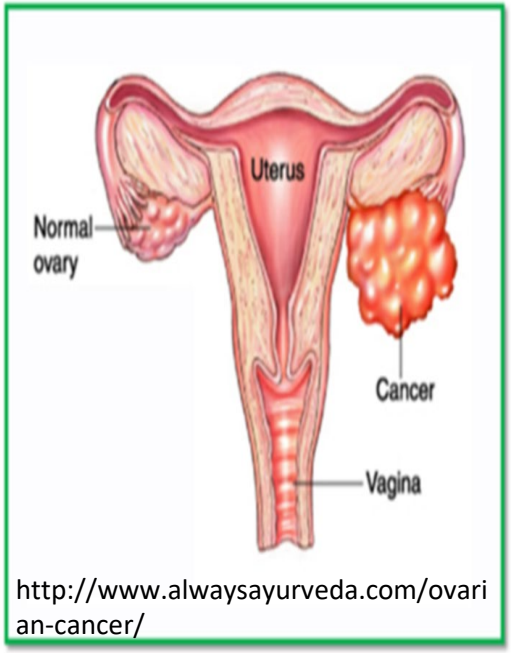
Characterization of Anti-ovarian Cancer Diterpenoids in *Justicia Insularis* and their Roles in Apoptosis
Idowu Eniafe Fadayomi
70 YEARS of the KEELE DIFFERENCE
The Keele difference

Background on Ovarian Cancer

- Ovarian cancer is the most severe gynaecological malignancy associated with the highest level of lethality worldwide.
- In the UK, over 6600 cases were recorded each year with above 4400 death.
- Seventh leading cause of cancer mortality among women with 140,000 mortality estimate yearly.
- Diagnosed in about quarter of a million women worldwide each year.
- In recent times, ovarian cancer have being known to be resistant to chemotherapeutic drugs.



http://www.alwaysayurveda.com/ovarian-cancer/

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Our research focus

- Our research is focused on the evaluation of the anti-ovarian cancer activities of some medicinal plants, identification of the bioactive natural compounds and mechanism of action.

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Justicia insularis (JI)

- *Justicia insularis* is an edible herbaceous plant in the family Acanthaceae, and mostly found in the tropical part of Africa.
- The leaves are used in making vegetable and groundnut soup and are even eaten as spinach when cooked.
- A medicinal plant widely used in the treatment of various diseases across Africa.
- The phytochemical analysis of *Justicia insularis* revealed the presence of alkaloids, flavonoids, saponin, anthocyanins, tanins, steroids and terpenes

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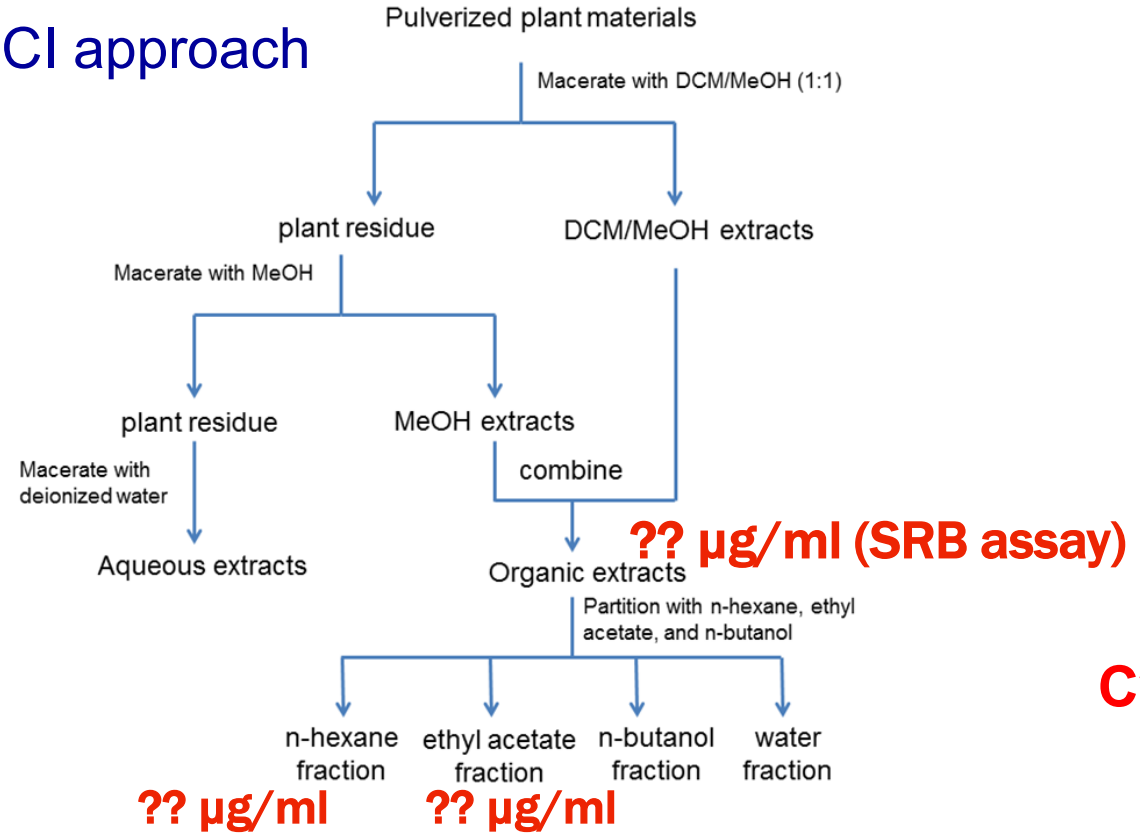
Research methods

Bioassay-guided fractionation and isolation

NCI approach



EtOAc fraction

CC

Sub-fractions

HPLC/MS/NMR

Active compounds

Cytotoxicity/selectivity /Apoptosis

Results

- Growth inhibitory activities of *J. insularis* extracts and sub fractions using SRB assay

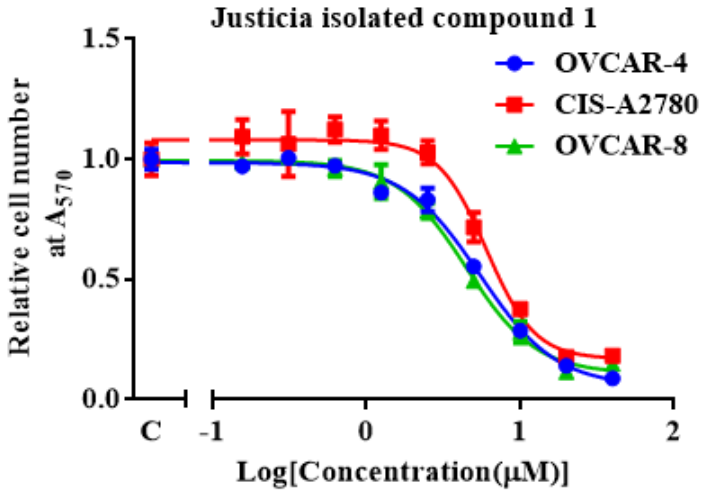
PLANT EXTRACTS	OVCAR-4 IC_{50} (µg/mL) ±SEM	OVCAR-8 IC_{50} (µg/mL) ±SEM
DCM/MeOH *J. insularis*	19.2±0.5	10.74±0.6
Aqueous *J. insularis*	>150	99.8±0.3

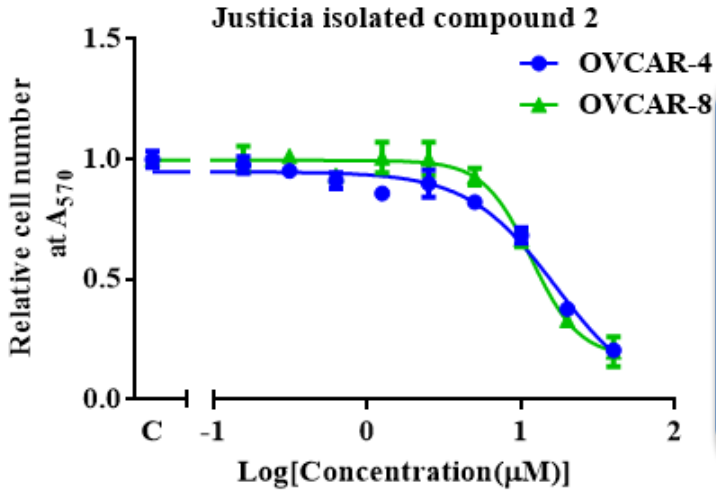
DCM/MeOH Partitioned fractions	OVCAR-4 IC_{50} (µg/mL) ±SEM	OVCAR-8 IC_{50} (µg/mL) ±SEM
N-Hex fraction	19.5±1.0	6.1±1.8
Ethylacetate (EA) fraction	25.7±1.5	7.5±1.7
n-Butanol fraction	188.4±4.6	101.7±2.2
Aqueous fraction	80.0±0.8	71.5±14.9

EA fractions	OVCAR-4 IC_{50} (µg/mL)
EA1	7.2±0.8
EA2	5.9±0.1
EA3	6.7±0.1
EA4	4.1±0.4
EA5	6.2±0.4
EA6	5.7±0.8
EA7	12.0±0.3
EA8	11.5±0.3
EA9	43.7±1.4
EA10	68.4±3.3

Growth inhibitory activities of *J. insularis* isolated compound 1 and 2

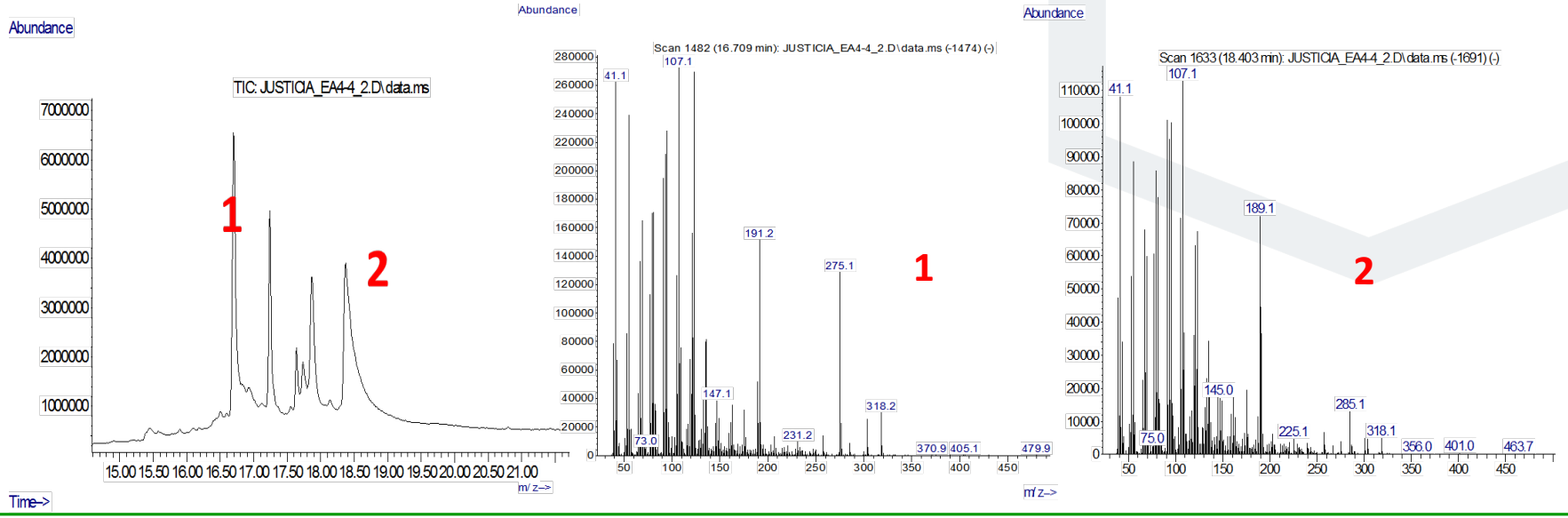
Compounds	OVCAR-4 (μM)	OVCAR-8 (μM)	CIS-A2780 (μM)	HOE (μM)	Selectivity index with OVCAR-8
Compound 1	5.7±0.3 (1.8 μg/ml)	4.4±0.2 (1.4 μg/ml)	8.1±0.8 (2.5μg/ml)	12.1±0.1 (3.9μg/ml)	3
Compound 2	16.6±2.8 (5.3 μg/ml)	11.8±0.5 (3.8 μg/ml)	Not determined	22.8±0.7 (7.3 μg/ml)	2
Carboplatin	17.6±4.6	8.2±2.2	> 40	13.0±3.7	1.6





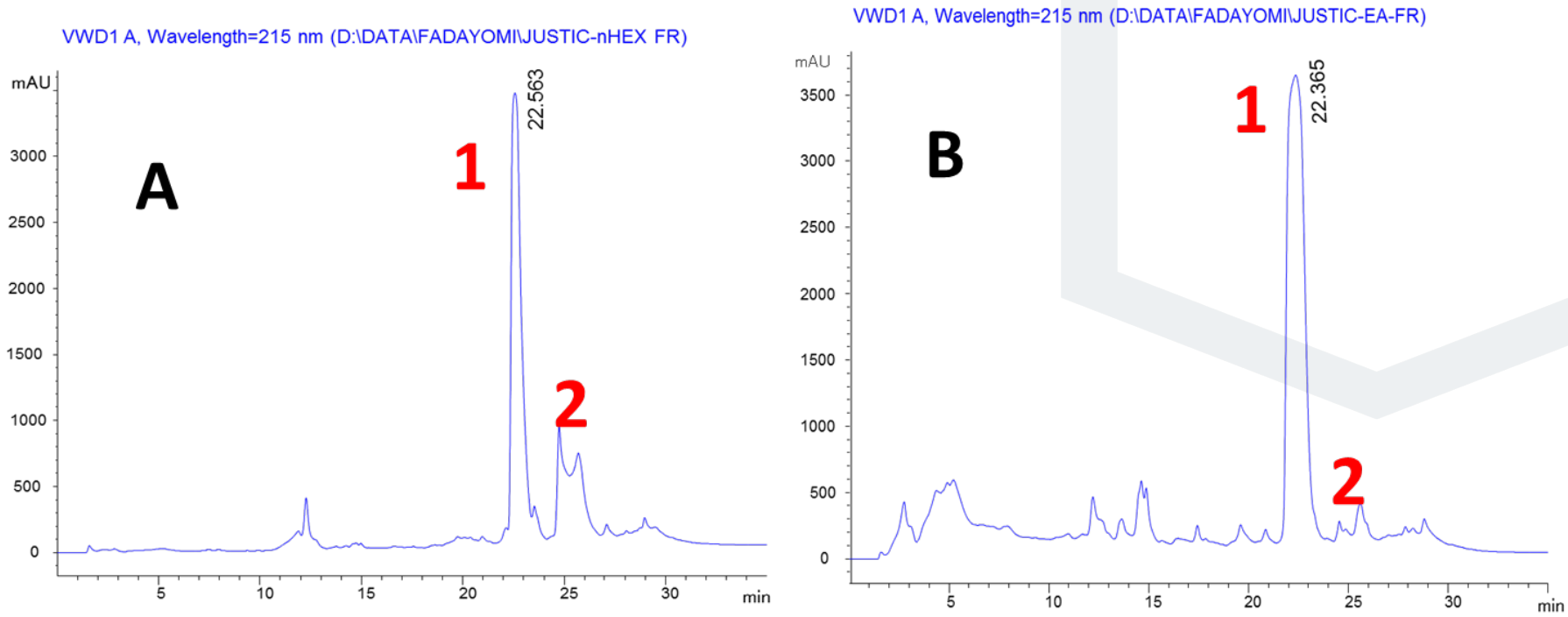
Mean concentration-response curve of growth inhibitory activities of *J. insularis* isolated compound 1 and 2 against ovarian cancer cell lines

GC MS Analysis of EA Sub fraction of *J.I*



GC MS chromatogram of EA sub-fraction 4-4 of *J insularis* showing the EI- MS spectra of the two compounds (1 and 2) isolated at different retention times.

Isolation and purification of the bioactive compounds of *J. insularis*



Analytical HPLC chromatogram of n-Hexane (A) and Ethylacetate (B) fractions respectively showing several peaks with the main compound indicated by retention time

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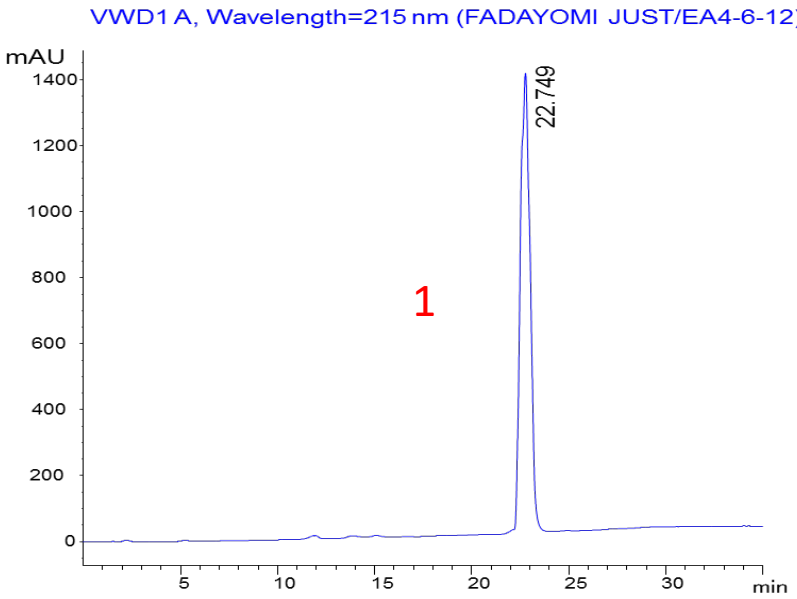
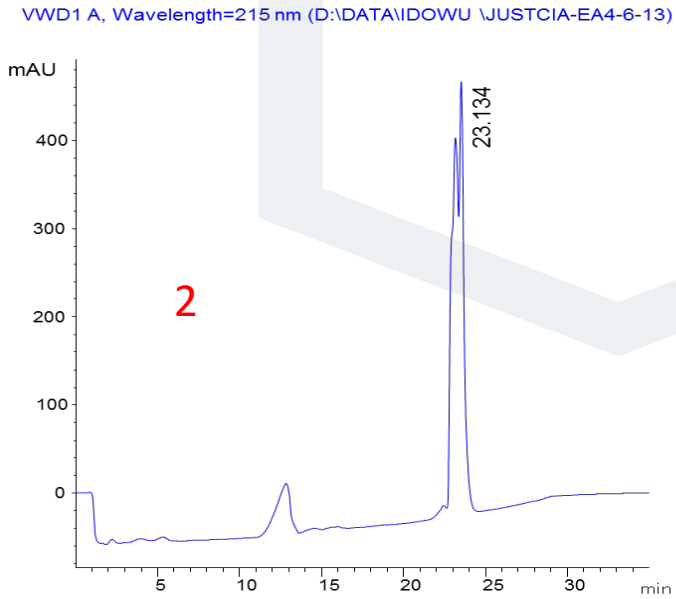
HPLC chromatogram of isolated compound 1 and 2 from *Il*





Analytical HPLC chromatogram of compound 1 and 2 isolated from *Il*

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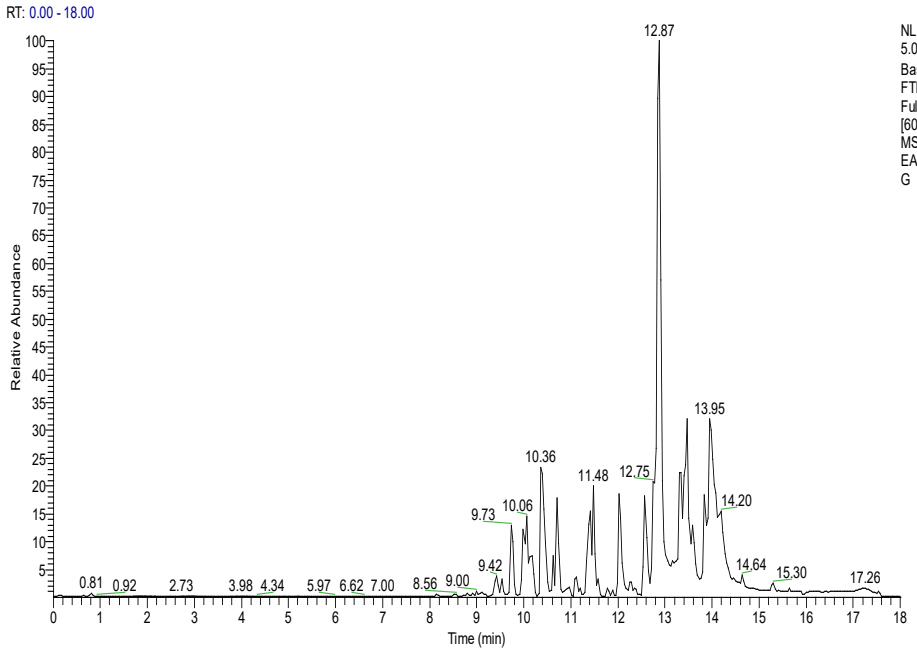
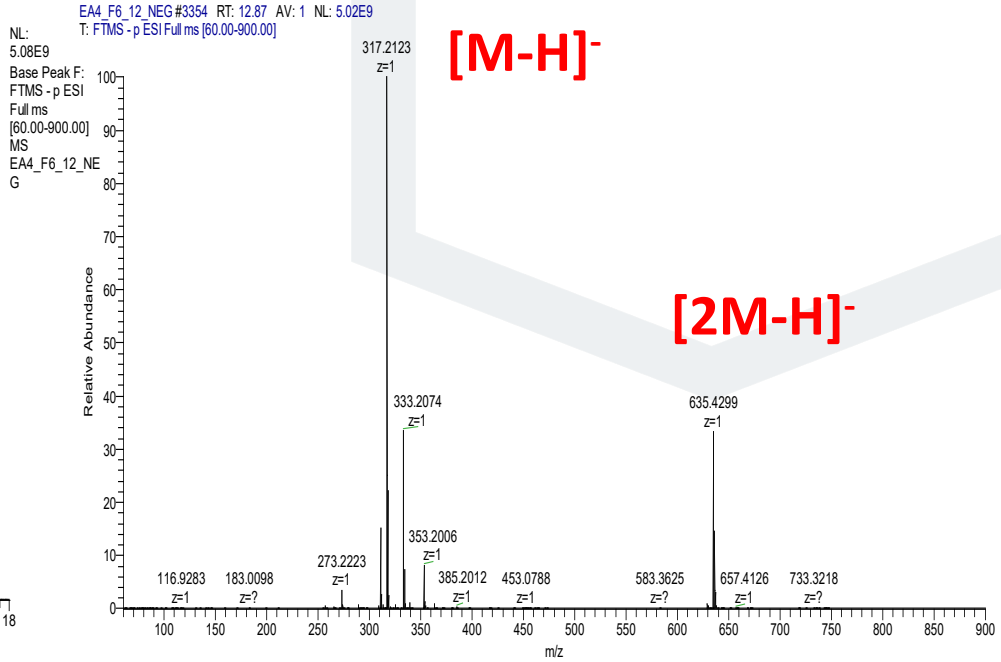
Identification of compound 1 and 2 with LC MS and NMR

1





[M-H]-

[2M-H]-

LC MS chromatogram of isolated compound 1 showing the (-)ESI-MS of the major peak (1) at retention time of 12.87.

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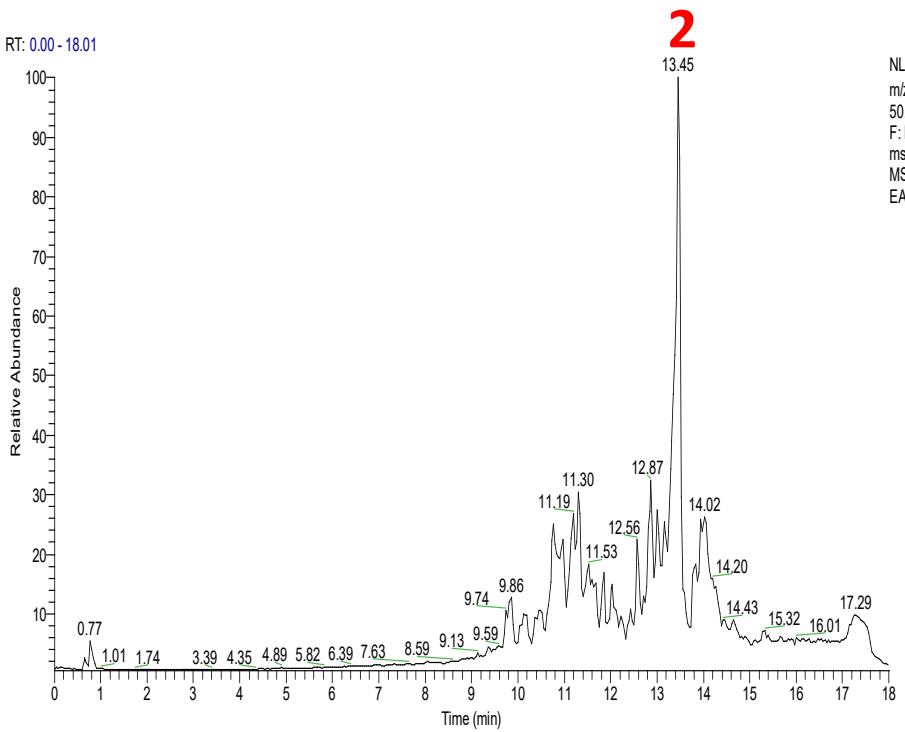
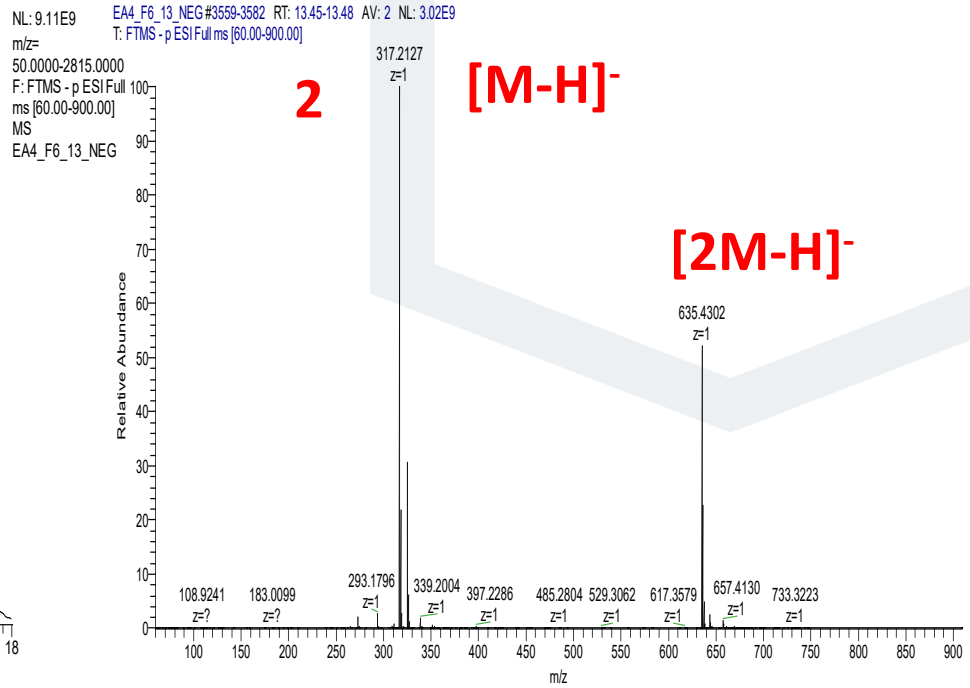
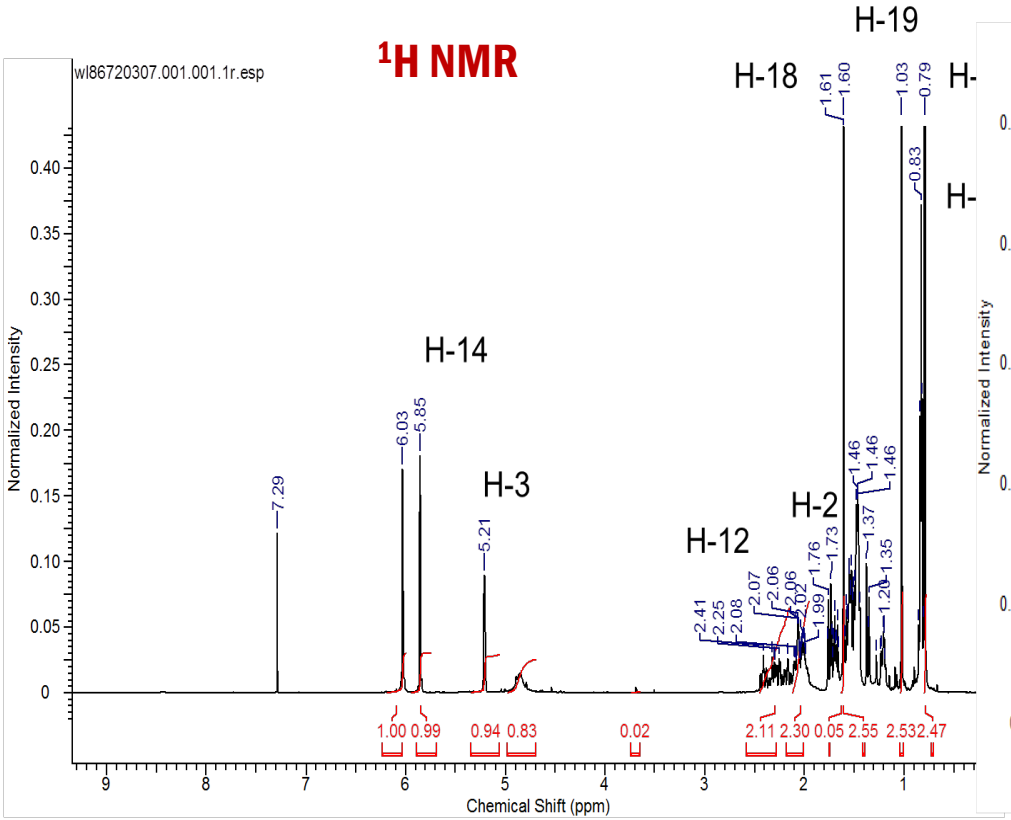
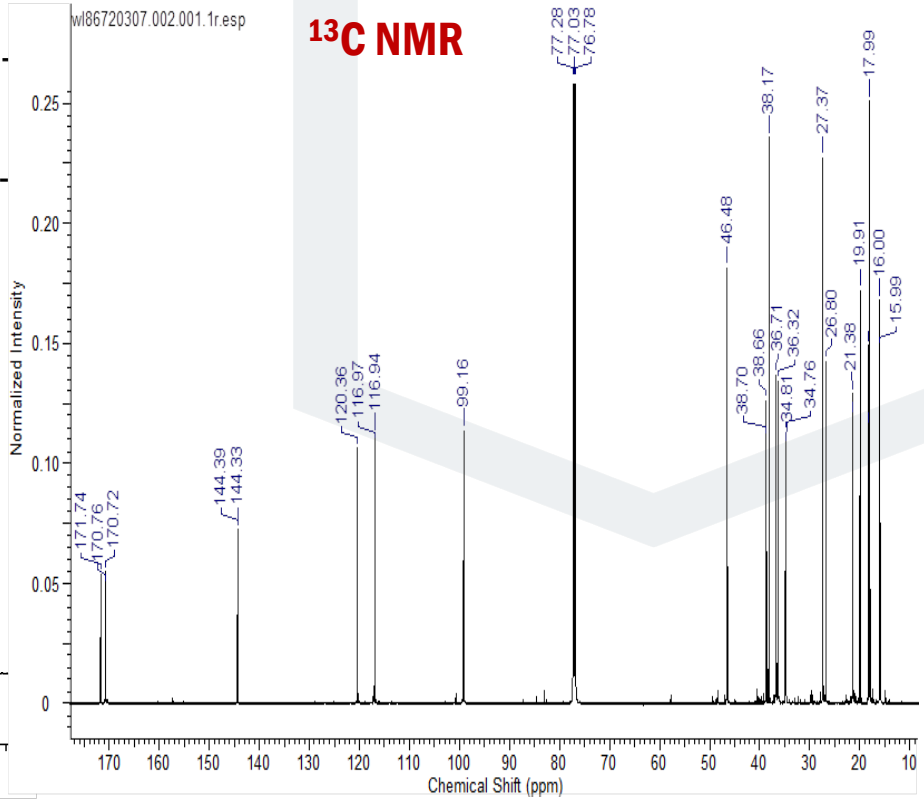
Identification of compound 1 and 2 with LC MS and NMR





LC MS chromatogram of isolated compound 2 showing the (-) ESI-MS of the major peak (2) at retention time of 13.45 .





NMR analysis of isolated bioactive compound 1

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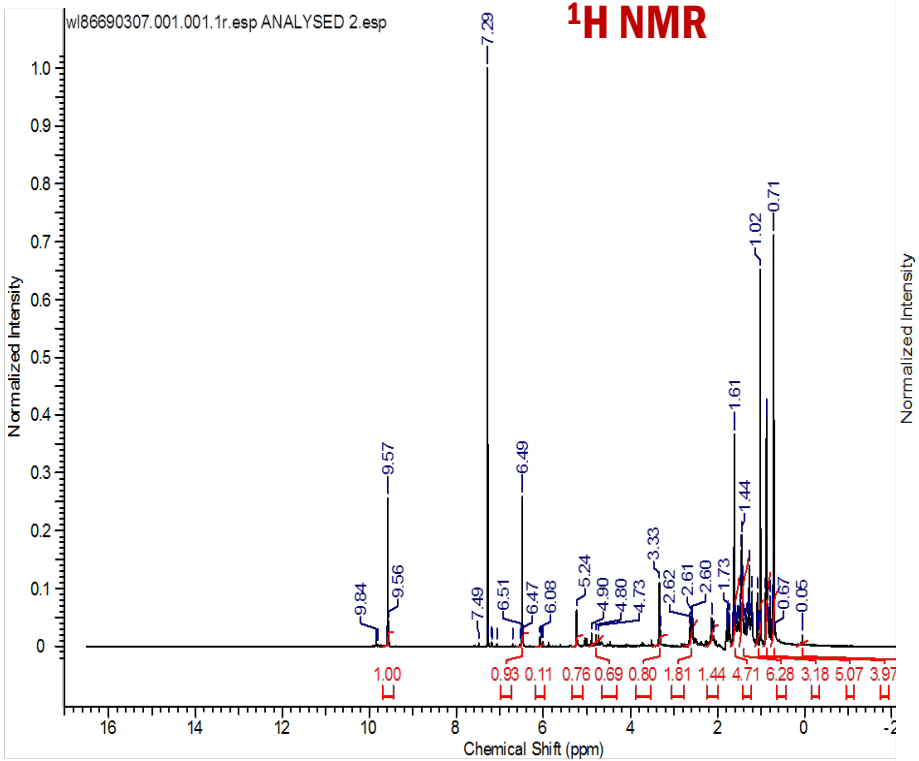
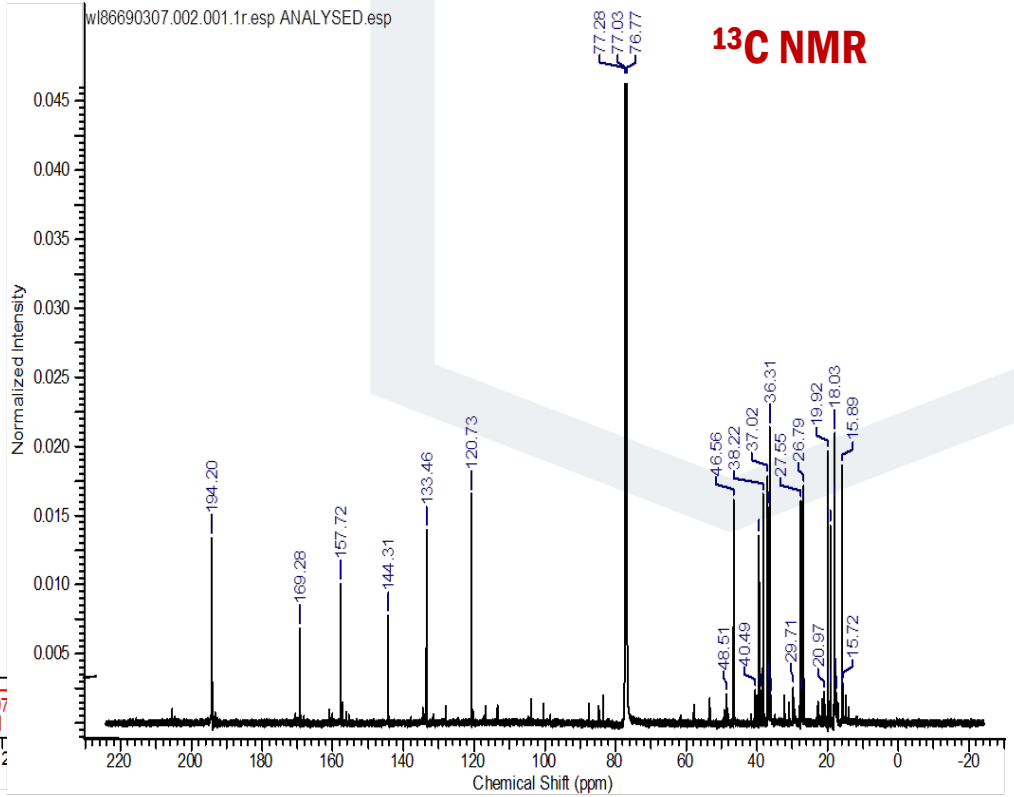
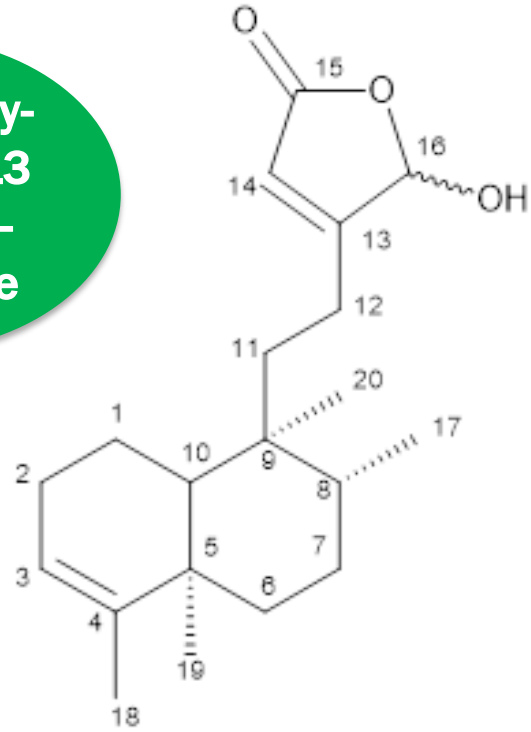




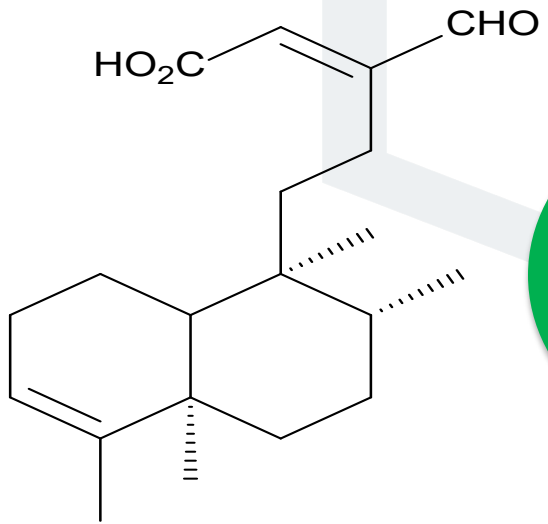
^{1}H NMR and ^{13}C NMR analysis of isolated bioactive compounds 2

Identification and chemical structure of compound 1 and 2

16α-hydroxy-cleroda-3,13 (14)Z-dien-15,16-olide



1



2

16-oxocleroda-3,13(14)E-dien-15-oic acid

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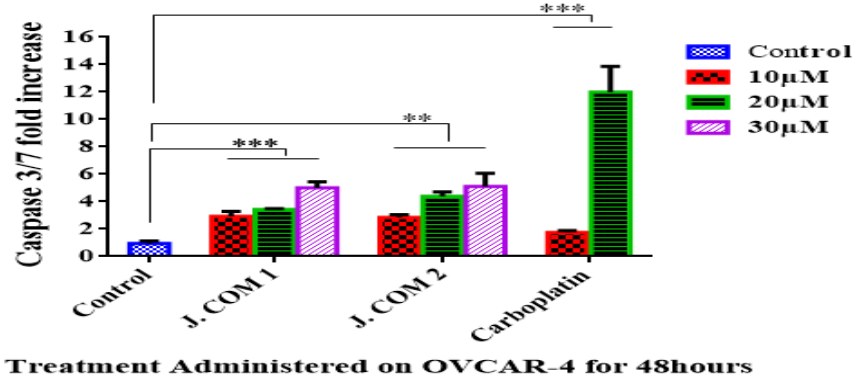
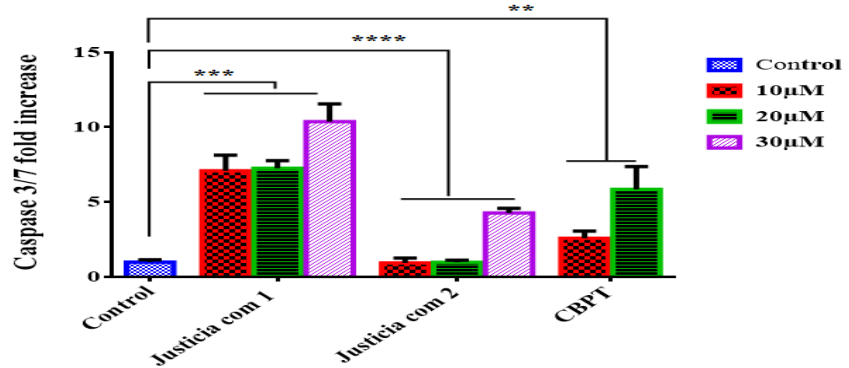
Analysis of Apoptotic activities of identified compounds 1 and 2

Caspase 3/7 activities of compound 1 and 2

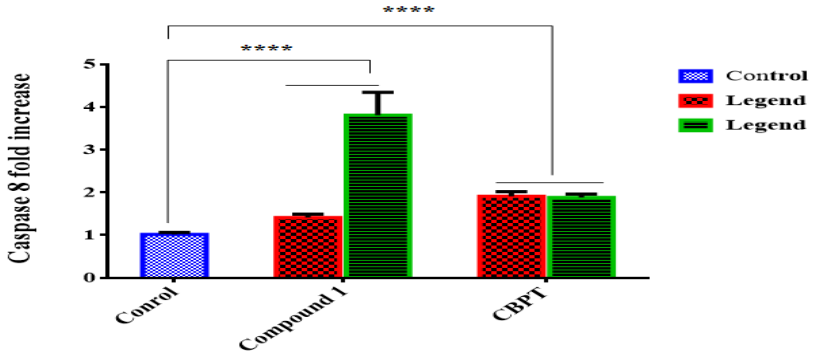


Treatment Administered on OVCAR-4 for 48hours

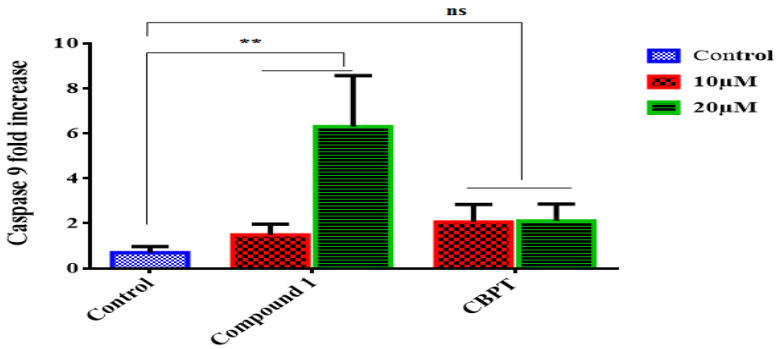


Treatment Administered on OVCAR-8 for 48hours

Caspase 8 and 9 activities of compound 1

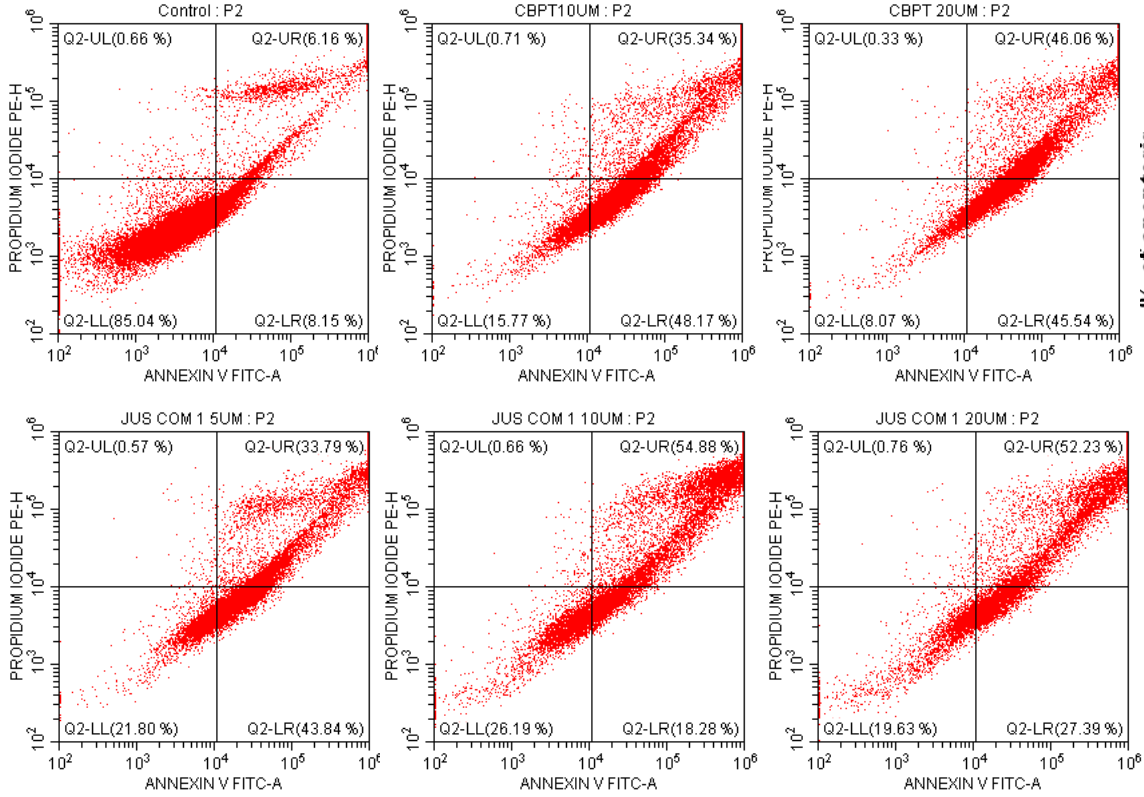


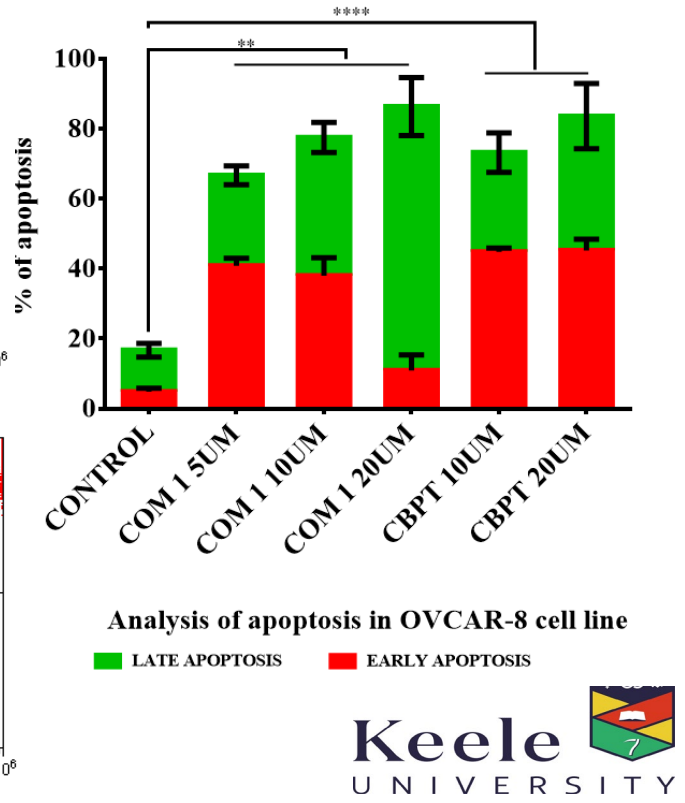
Treatment Administered on OVCAR-8 for 48hours



Treatment Administered on OVCAR-8 for 48hours

Analysis of Early and Late apoptosis in ovarian cancer cells





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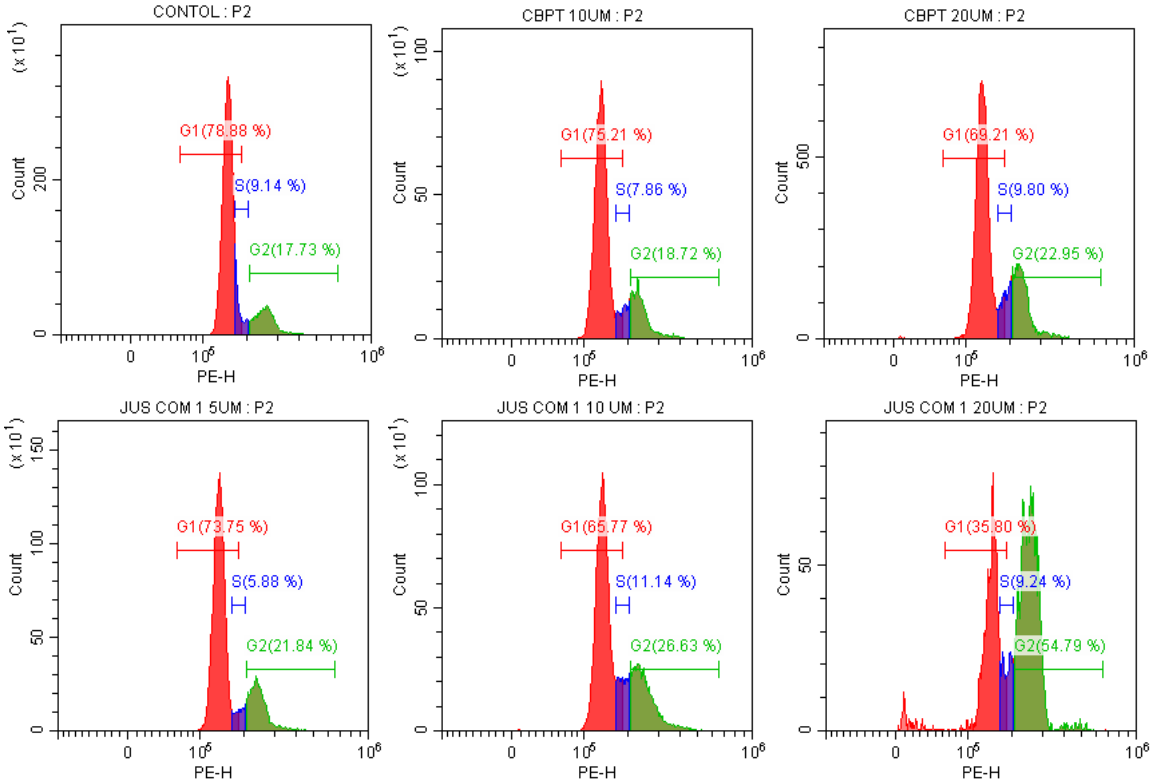
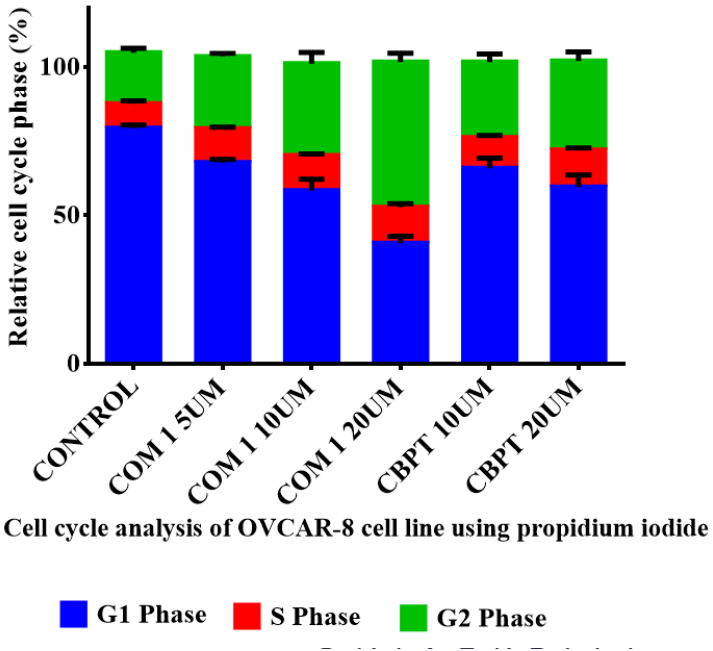
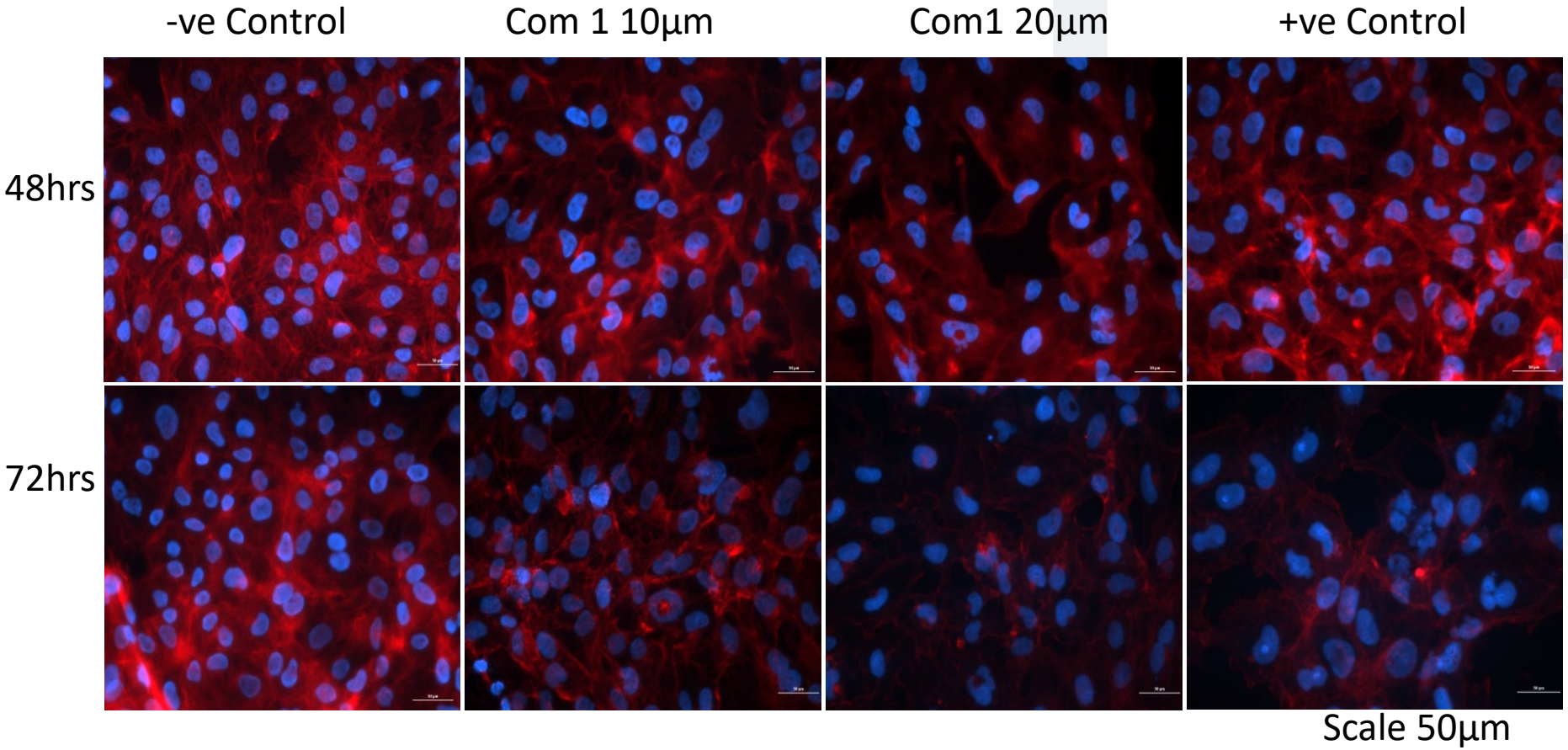
Cell cycle analysis of ovarian cancer cells in response to drug treatment





Morphological evaluation of the apoptotic activities of bioactive compound 1 using fluorescence microscopy



Conclusion

- This study shows that *J. insularis* has significant cytotoxic activities against ovarian cancer cell lines. It further identified 16α-hydroxy-cleroda-3,13(14)Z-dien-15,16-olide and 16-oxocleroda-3,13(14)E-dien-15-oic acid as the bio-active compounds in *J. insularis*.
- The cytotoxic activities of the bio-active compounds were further established to be induction of cell death/apoptosis by activation of caspase 3/7 activities through both intrinsic and extrinsic pathways.
- Further study will establish the roles of the identified diterpenoid compounds in gene regulating apoptosis.

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Thank you

It's the Keele difference.