ANALYSIS

Vol.13, 2019

ISSN 2278–540X EISSN 2278–5396

DRUG DISCOVERY

Isolation and antiproliferative characterisation of bergenin from root extract of *Uvariodendron anisatum* Verdec (Annonaceae)

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Article History
Received: 24 February 2019
Accepted: 29 April 2019
Published: May 2019

Citation
Onyancha JM, Gikonyo NK, Wachira SW, Gicheru MM. Isolation and antiproliferative characterisation of bergenin from root extract of *Uvariodendron anisatum* Verdec (Annonaceae). *Drug Discovery*, 2019, 13, 55-78

ABSTRACT

Uvariodendron anisatum (annonaceae) is a rare, endemic and indigenous medicinal plant in Kenya. The plant root decoction is used to remove placenta in women if it is retained during child deliveries. It is also known to management of impotency in men. Despite this folkloric claim, information about phytochemical composition and biological activities are needed. The objective of this study

was to carryout isolation and antiproliferative characterisation of methanol extract of the whole root, fractions thereof and isolated compound. Extract was prepared by maceration using methanol. Fractions of petroleum ether, dichloromethane, ethylacetate, acetone and methanol were prepared by column chromatography. The compound was achieved by column and thin layer chromatography and structural elucidation was done using spectral data and comparison with published literature. Methyl thiazole tetrazolium (MTT) assay was used to study the effects of the extract, fractions and isolated compound against cancers of the prostate and breast (DU-145 and HCC 1395 respectively). Vero cells were used as a control celline. The isolated compound was identified as anhydrous bergenin. The extract revealed high antiproliferative effects against normal cell line (CC_{50} = 3.3±0.2µg/ml) whereas it was moderately antiproliferative against prostate and breast cancer cell lines (IC_{50} =.81.7±3.9 and 50.6±2.9µg/ml) respectively. Polar fractions revealed weak antiproliferative effects whereas the non polar fractions and the isolated compounds did not reveal antiproliferative effects against cancer and normal cell lines. It was concluded that the extract of *U. Anisatum* was highly antiproliferative against normal cell line but moderately cytotoxic against prostate and breats cancer cell lines. The antiproliferaive effects were not attributed to bergenin and it was recommended that continued isolation and bioassay studies are necessary to determine the compounds that are responsible for antiproliferative effects.

Keywords: Annonaceae, Cell lines, Chromatography, Fractionation, Kenya, Medicinal plants, Spectroscopy

1. INTRODUCTION

Uvariodendron anisatum Verdec. (annonaceae) is a shrub or small tree that grows up to 15 m tall and 40 cm diameter. It is rare, vulnerable and endemic to Kenya [1]. The plant occurs in natural forest areas of Emali (Makueni county), Karura (Nairobi county), Kianjiru and Kiangome hills (Embu county) [2]. Locally it is referred to as mutonga (Kikuyu) and mutongu (Meru and the Mbeere people). *Uvariodendron anisatum* root decoction is drunk to enhance labour and removal of retained after birth in women. On the other hand, the root infusion is drunk to manage impotence in men [3]. Inspite of the folkloric claims of the root extracts, information related to phytochemical composition and biological activities of *Uvariodendron anisatum* extracts are limited [4]. The current study saught to evaluate antiproliferative activities of methanol extract, fractions and compound obtained from *Uvariodendron anisatum* root. Structural elucidation of the isolated compound was described in this paper based on spectroscopic analysis and comparisons of spectra with available literature.

2. MATERIALS & METHODS

Extraction and activity guided fractionation

Plant material

Roots of *Uvariodendron anisatum* were collected from Kiangombe forest, Embu county at GPS coordinates S 00˚ 33′ 41.0″; E 037˚ 12′ 12.", Elevation 1662 m on February 2015. The plant was authenticated and a voucher specimen (JMO-1-2015) was deposited at East Africa herbarium (Nairobi). The duplicate voucher was deposited at the school of Pharmacy Herbarium (Mount Kenya University). The collected roots were washed in clean water and immediately they were cut into small pieces. The dried root chips were evenly spread on the drying benchunder the shade for one week after which they were ground using a miller. Powder (250 g) was extracted by soaking in methanol (500 ml) three times using a 1 litre conical flask. The mixture was filtered and reduced *in vacuo*. It yielded a total extract of red brown sticky solid (65.5 g) which was kept at 4 ^{0}C in the refrigerator.

Activity guided fractionation

The dried methanol extract of *U. anisatum* root (10 g) were loaded loaded onto a column which was packed with 110 g silica gel, column elution was done isocratically using petroleum ether (200 ml), dichloromethane (200 ml), ethyl acetate (200 ml), acetone (300 ml) and methanol (300 ml) as mobile phases. Thin layer chromatography of the individual fractions was done, after which the fractions that had similar spots were combined and allowed to air dry in the hood, there after they were weighed and packed into labelled sample bottles. Five fractions were obtained and were identified accordingly as petroleum ether, dichloromethane, ethyl acetate, acetone and methanol fractions. Each fraction was assayed for cytotoxic activity against two human cancer cell lines (prostate cancer- DU-145 and breast cancer- HCC 1395) and one normal cell line (vero).

Isolation of anhydrous

About seven and half grams (7.5 g) of combined fractions D and E from *Uvariodendron anisatum* root methanol extract were loaded onto column packed with 100 g of silica gel. The column was subjected to gradient elusion using a mixture of ethyl acetate and acetone in the percentage ratio of 100:0 (250 ml), 25:75 (250 ml), 50:50 (250 ml), 75:25 (250 ml), 0:100 (250 ml) and acetone: methanol 25:75 (250 ml), 50:50 (250 ml), 75:25 (250 ml), and 100:0 (300 ml). Fractions (180) of about 5 ml were collected into clean test tubes, thin layer chromatography was perfomed and fractions that had similar profile were pooled and combined. Fractions 32 to 77 yielded a compound which formed on the wall of the test tube as small white salt like crystals, upon solvent evaporation, the compound was washed dow the test tube using methanol. The purity of the compound was ascertained as it formed a single spot on a TLC. The compound was harvested using spatula, it was weighed, labelled and packed in clean amber coloured sample bottle.

Spectral data

White star like crystals (MeOH), melting point; 235-238 ^{o}C; Rf values; 0.29 and 0.53 for mobile phase; 9:1 and 8:2 (dichloromethane:methanol respectively), UV: λ_{max}(MeoH):273 nm, IR: ν^{KBr}_{max}(cm^{-1}):3388,3198,1702, 1602, 1461, 1401, 1343, 1234, 1098, 1070, 991, 697 and 617, ES-MS m/z: 327.06 $(M-H)^-$. ^{1}H NMR (400 MHz, MeOD) δ 8.44 (s, 1H), 8.34 (s, 1H), 7.08 (s, 1H), 5.04 (d, *J* = 10.4 Hz, 1H), 4.91 (dd, *J* = 4.6, 0.8 Hz, 1H), 4.64 (dd, *J* = 4.9, 0.8 Hz, 1H), 4.20 – 4.01 (m, 3H), 3.89 (s, 3H), 3.93 – 3.66 (m, 3H), 3.51 (ddd, *J* = 9.7, 8.5, 4.9 Hz, 1H), 2.88 – 2.80 (m, 3H), 2.05 (p, *J* = 2.2 Hz, 2H).^{13}C NMR (101 MHz, (C_3D_6CO) δ 162.8, 150.8, 148.2, 140.4, 118.7, 116.1, 109.3, 82.0, 80.0, 74.6, 73.0, 71.1, 61.8, 59.8.

Antiproliferative assay

Cell culture

Monkey kidney epithelial cell (vero) were used as normal mammalian cells. HCC 1395 cultured in Roswell Park Memorial Institute (RPMI-1640) while DU-145 and vero in Eagle's Minimum Essential Medium (EMEM) containing 10 % fetal bovine serum (FBS).

MTT assay

Standard 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was used. Two human cancer cell lines were used in this study, breast cancer (HCC 1395) and Prostate cancer (DU-145). The assay was done by using microtitre 96 well plates, 100 µlof respective growth medium were poured into each well and seeded with 20,000 cells per well. Cells were allowed to attach overnight and concentration ranging from 0-1000 µg/ml of crude drug, fractions, pure compounds and standard drug (cyclophosphamide) were made using dimethyl sulfoxide (DMSO) as a diluent. The test drugs were added in triplicate to respective wells. The plates containing media, cells and drugs were incubated for 48 h at 37^{o}C, 5% CO_2 and relative humidity 95%. After the 48th hour, MTT reagent (10 µl) was added to each well followed with further incubation for 4 hours. Supernatant was aspirated and 100 µl of DMSO solution were added to each well to solubilize MTT crystals and the plates were read for colour absorbance on an ELISA scanning multiwell spectrophotometer (Multiskan Ex labsystems) at 562 nm and 620 nm as a reference. Fluorouracil was used as positive controls.

Measurement of antiproliferative effects

The method is based on the principle that mitochondrial dehydrogenase enzyme, which occur in viable cells, cause cleavage to tetrazolium ring of pale yellow MTT and form formazan which is blue in colour and is impermeable and accumulated in health cells. The amount of formazan is proportional to the number of life cells and this is indicated by highest optical density recorded at the control wells (wells with media and cell but without drug). Percentage cell cytotoxicity was calculated using following formula:

$$\text{Percentage cytotoxicity} = \frac{\text{Ac} - \text{At}}{\text{Ac}} \times 100$$

Where Acis absorbance of cells without treatment (control cells), At is absorbance of treated cells

Statistical analysis

Raw data for MTT assay was transfered for processing using Microsoft Excel 2016 to compute optical densities. The concentration of extracts, fractions and the isolated compound that inhibited growth fifty percent of cells (IC_{50}) was determined using Graphpad Prism Version 7.04 software. IC_{50} valuesof three independent experiments were expressed as mean ± standard error of the mean

(SEM). Antiproliferative effects were analysed in respect to the American National Cancer Institute criteria, crude extracts that revealed IC_{50}values less than50 µg/ml were classified as active while those with IC_{50}< 30 µg/ml were considered to be remarkably active [5]. The Nuclear magnetic resonance raw data of the isolated compound was analysed using Mest ReNova software (Mestre lab Research Chemistry Software Solutions). The spectrometric data that was obtained was printed out and compared with authentic data for structural prediction. The resolved structures were drawn using Chem Draw Ultra 8.0 (Cambridgesoft Corp.).

3. RESULTS AND DISCUSSION

Structural elucidation of isolated compounds

Anhydrous bergenin (Figure 1) was isolated as white star like crystals (115 mg) from methanol fraction of *U. anisatum* whole root extract. It had a melting point of 235-358ºC. The compound was observed as a dark and yellow spot at short Ultra violet wavelength (λ 254 nm) and resublimed iodine respectively. It was neither visible in long Ultra violet wavelength (λ 365 nm) nor 1% vanillin spray. The electron spray mass spectrometry (ES) indicated a molecular ion $[M-H]^-$ with mass-to-charge ratio (*m/z*) of 327.06. Proton NMR spectrum revealed a signal for one aromatic proton at δ_H7.08 (H-7) and a signal for methoxy proton at δ_H3.93 – 3.66 (H-12). Carbon 13 spectrum exhibited signal at δ_c162.8 ppm and at δ_c δ 59.8 ppm which indicated the presence carbonyl (C=O) and methoxy (O-CH_3) groups respectively.

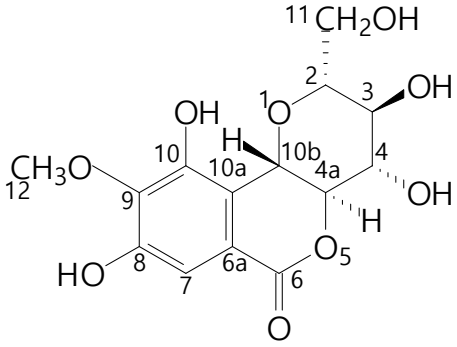


Figure 1: Anhydrous Bergenin.

Fourier Transform Infra-red (FTIR) spectrum at wavenumber 1702 cm^{-1} indicated the presence of a strong carbonyl bond (C=O) while absorption bands at 3201-3643 cm^{-1} showed OH groups with intermolecular hydrogen bonds. Comparison of ^1H and ^{13}C NMR, FTIR and UV-Vis λ_{max} (MeOH): 173 spectral data with existing literature provided a strong prediction of the isolated compound as anhydrous bergenin with molecular formula $C_{14}H_{16}O_9$ [6]. The calculated molecular weight of bergenin is 328.273 [7].

Anhydrous bergenin was isolated for the first time from methanol extract of *Uvariodendron anisatum* root in this study. However, this compounds is commonly isolated from higher plants including *Connarus monocarpus* [8], *Flueggea microcarpa* [9], *Peltoboykinia watanabei* and *Boykinialy coctonifolia* [10], *Mallotus repandus* [11], *Mallotus japonicas* [12], *Peltophoruma fricanum* [13], *Ardisia colorata* [14], *Vateria indica* [15], *Ficus glomerate* [16], *Hopea sangal* [6], *Endopleura uchi* [17], *Endopleura uchi* [18], *Vatica odorata* [19], *Astilberivularis* [20], *Dryobalanops aromatica* [21], *Bergenia ligulate* [22], *Corylopsis coreana* [23], *Caesalpinia decapetala* [24] *Syzygium cumin* [25] and *Peltophorum pterocarpum* [6].

Table 1 Assignment of ^{13}C NMR (101 MHz, (C_3D_6CO) and ^1H NMR (400 MHz, MeOD) chemical shifts for isolated compound [6]

C	^{1} H NMR δ for isolated compound		^{13}C NMR δ for isolated compound	^{13}C NMR δ for bergenin literature
	δ_H, ppm	H		
2	3.51, ddd,	1H, H-2	81.9	81.8
3	3.51, ddd,	1H, H-3	71.1	70.8
4	4.64, dd	1H, 4-H	74.6	73.8
4a	4.91, dd	1H, 4a-H	80.0	79.9

6	-	-	162.8	163.5
6a	-	-	118.7	118.2
7	7.08, s	1H, 7-H	109.3	109.6
8	8.44, s	1H, 8-OH	150.8	151.1
9	-	-	140.4	140.7
10	8.34, s	1H, 10-OH	148.2	148.2
10a			116.1	116.1
10b	5.04, d	1H, 10b-H	73.0	72.2
11	-	-	61.8	61.2
12	3.89, s	3H, 12-H	59.8	60.0

Antiproliferative effects of extract, fractions and isolated bergenin

Methanol extract of *Uvariodendron anisatum* whole root exhibited potent antiproliferative effects against normal (vero) cell lines with 3.3±0.2 µg/ml (Table 2). Loss of antiproliferative activity against normal (vero) cells during bioassay guided fractionation and purification was noted. The IC_{50} values observed were in the range of 167.0 ± 8.6 to greater than 1000 µg/ml compared to that of the extract (3.3±0.2µg/ml). According to Ngeny *et al.,* [29] the fractions and the isolated compound were classified not having antiproliferative activity against normal cell lines since their IC_{50} values was more than 100µg/ml.

Concentrations that inhibited proliferarion of prostate cancer (DU-145) and breast cancer (HCC 1395) cells by half (IC_{50}) were 81.7±3.9µg/ml and 50.6±2.9µg/ml respectively. A decrease in antiproliferative effects during bioassay guided activity studies was noted with ethyl acetate, dichloromethane, petroleum ether fractions and the isolated compound against cancer cells. High IC_{50} values increasing from 468.3 ± 24.2µg/ml to more than 1000 µg/ml for prostate cancer cells (DU-145) were recorded. Similary such an increase in IC_{50} values was observed for breast cancer cells (HCC 1395) from 296.3 ± 44.8µg/ml to more than 1000 µg/ml. The range of IC_{50} values of acetone and methanol fractions (53.3±11.6µg/ml to 87.7±7.8µg/ml) against DU-145 and HCC 1395 was similar to the value of the extract 50.6±2.9µg/ml to 81.7±3.9µg/ml noting that there were no improved activities due to fractionation.

Bioassay guided fractionation of *U. anisatum* root methanol extract resulted did not improve the antiproliferative activities of the extract. Otherwise it led to loss of activities against normal cells. Situations where fractionation or purification leads to loss instead of potentiating activity have been reported [26]. The loss of activities has been attributed to inherent chemical instability of the active compound during fractionation or purification or due to los of synergistic interactions of the active components in the crude extract [27, 28] demonstrated that anticancer activities was by the compounds that were confined in the polar fractions (acetone and methanol). In contrast, the fractions were not cytotoxic against normal (vero) cells (CC_{50}> 100 µg/ml. It was observed that there was complete loss of anticancer activities with petroleum ether extract where IC_{50} values where above 1000 µg/ml against cancer cells. Nevertheless, the isolated compound (anhydrous bergenin) did not exhibit antiproliferative activities against the cancer cells

Table 2 IC_{50} values (µg/ml) of extract, fractions and isolated bergenin

Drug samples	**IC_{50} of selected cell lines in µg/ml**		
	DU-145	**HCC 1395**	**Vero**
Methanol crude extract	81.7±3.9	50.6±2.9	3.3±0.2
Petroleum ether fraction	>1000	>1000	653.3 ± 53.3
Dichloromethane fraction	533.0 ± 15.3	473.7 ± 3.0	784.3 ± 23.5
Ethyl acetate fraction	468.3 ± 24.2	296.3 ± 44.8	369.3 ± 23
Acetone fraction	66.7±18.3	87.7±7.8	399.3 ± 72.6

Methanol fraction	53.3±11.6	74.2±0.8	167.0 ± 8.6
Begenin anhydrous	>1000	>1000	>1000
Fluorouracil (standard)	18.3±6.1	38.8±7.6	185 ± 8

Data are represented as Mean± SEM of IC_{50} (µg/ml) from three independent experiments.

The isolated compound (anhydrous bergenin) was inactive against all the selected cancer cell lines. It demonstated IC_{50} value of more than1000 µg/ml for HCC 1395 (breast cancer cells) and 748±80 against DU-145 (prostate cancer cells) (Table 2). These findings are consistent with studies done by other researchers that reported inactivity of bergenin against prostate cancer cell lines (DU-145 and LNCaP), murine leukemia p-388 cells, human leukemia cell line(HL-60), human breast cancer cell line (MCF-7), human liver cancer cell line (HepG2), human lung cancer cell line (A-549) and human hepatic cell line (WRL-68) [19, 21,23].

Nevertheless, bergenin has been reported to be responsible of many other activities in plant extracts such as antioxidant [14, 23], anti-inflammatory and antitussive [26], immunomodulatory [27], antidiabetic, antifungal [18], hepatoprotective [28], neuroprotective [29], antiviral [20] and anti-HIV [30].

4. CONCLUSION

Benzenoid compounds (anhydrous and hydrousbergenin) were isolated for the first time from methanol extract of *Uvariodendron anisatum* root using a simple isolation procedure. The isolation of these compounds increases plant diversity which contain anhydrous and hydrate bergenin. Although the polar fractions (ethyl acetate and methanol) yielded anhydrous and hydrate bergenin were active against two cancer cell lines (DU-145, and HCC 1395) and one normal cell line (vero), the isolated compounds were inactive. Therefore, the compounds that confer the crude extract cytotoxic activities reside in the polar fractions, they act either singly or in combination with anhydrous or hydrate bergenin. The researchers recommend further bioactivity guided isolation in search of compounds with cytotoxic activities from the polar fractions of *U. anisatum root* methanol extract. The none polar fractions (petroleum ether, dichloromethane and ethyl acetate)

Acknowledgement

The Authors acknowledge the Dr. Mary Akinyi of Kenya Methodist University for organizing for the acquisition of mass spectrometry and Nuclear magnetic resonance spectral data. The directorate of Research and development at Mount Kenya University (Kenya) is also highly appreciated for providing enabling research laboratories and funds for manuscript handling and publication. The authors equally give credit to the Centre of Traditional Medicine and Drug Research at Kenya Medical Research institute for cytotoxicity work and pre-review of the manuscript for publication.

Author contributions

Onyancha designed and carried out the study while Prof.Gikonyo, Prof. Gicheru and Dr. Sabina gave professional guidance and supervision of the work. The manuscript was prepared by Onyancha while all the other authors read and corrected the manuscript in readiness for publication.

Funding:

This study has not received any external funding.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Peer-review:

External peer-review was done through double-blind method.

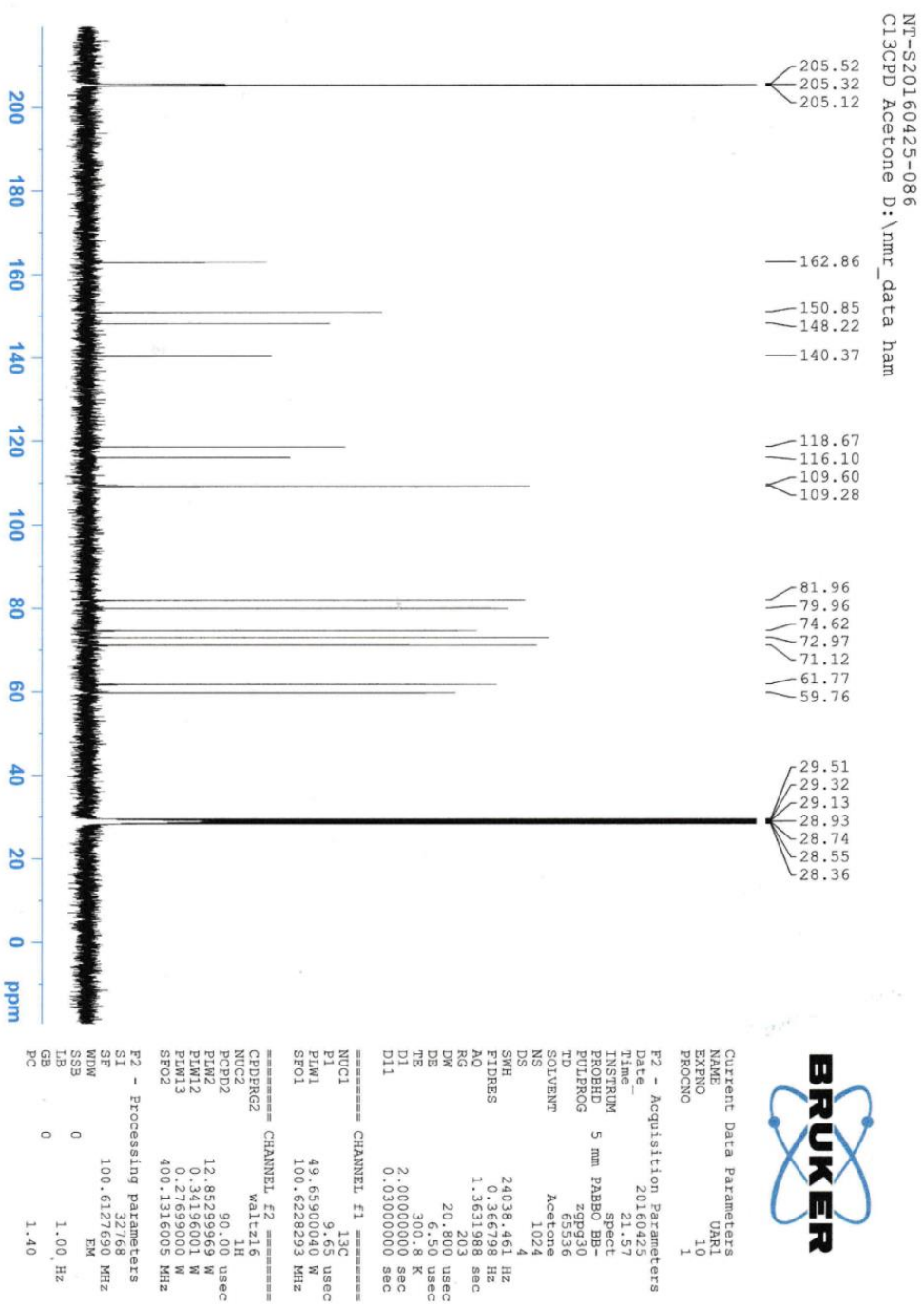
Data and materials availability:

All data associated with this study are present in the paper.

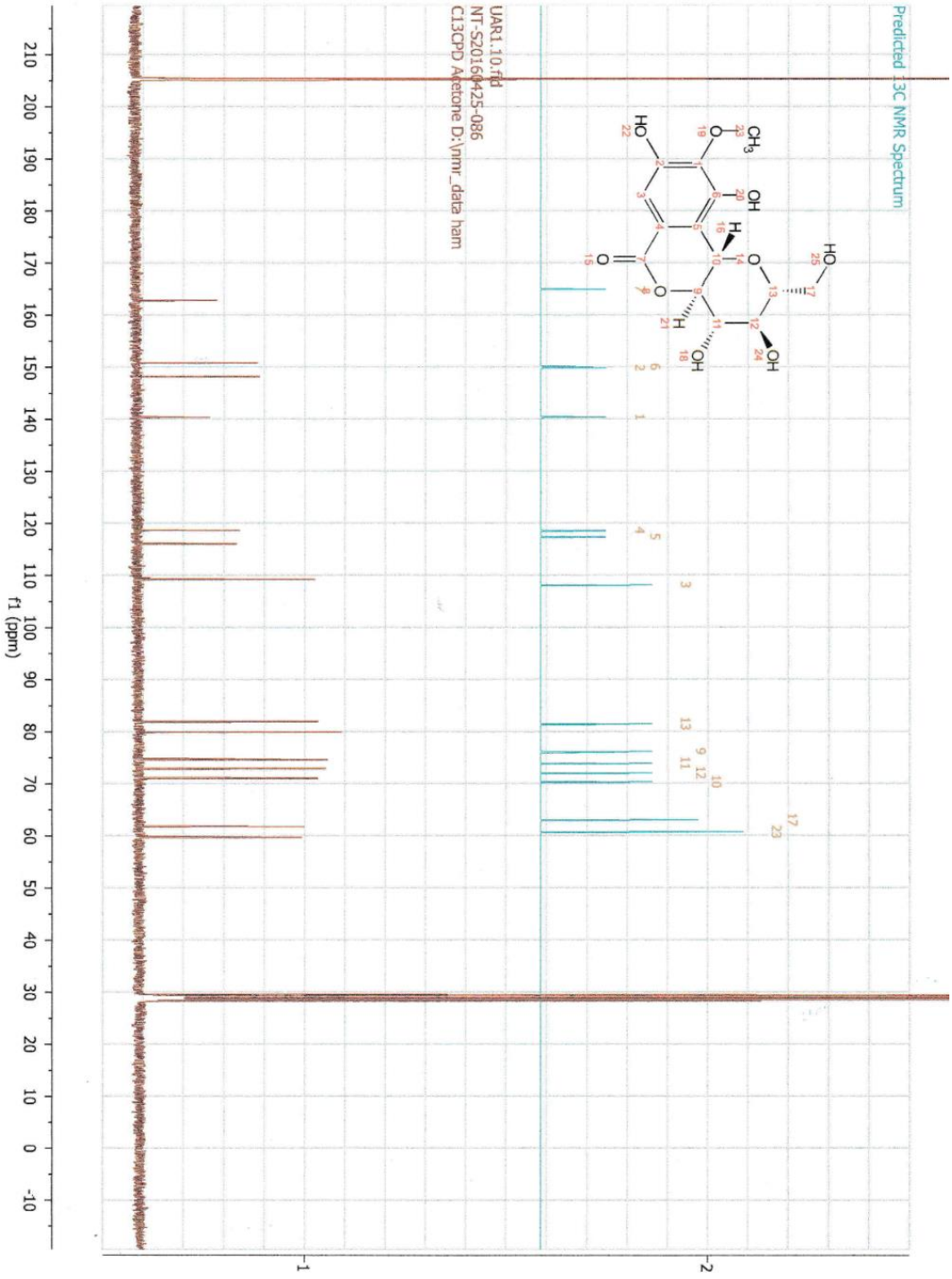
SUPPLEMENTARY MATERIALS

i. Chemical characterisation

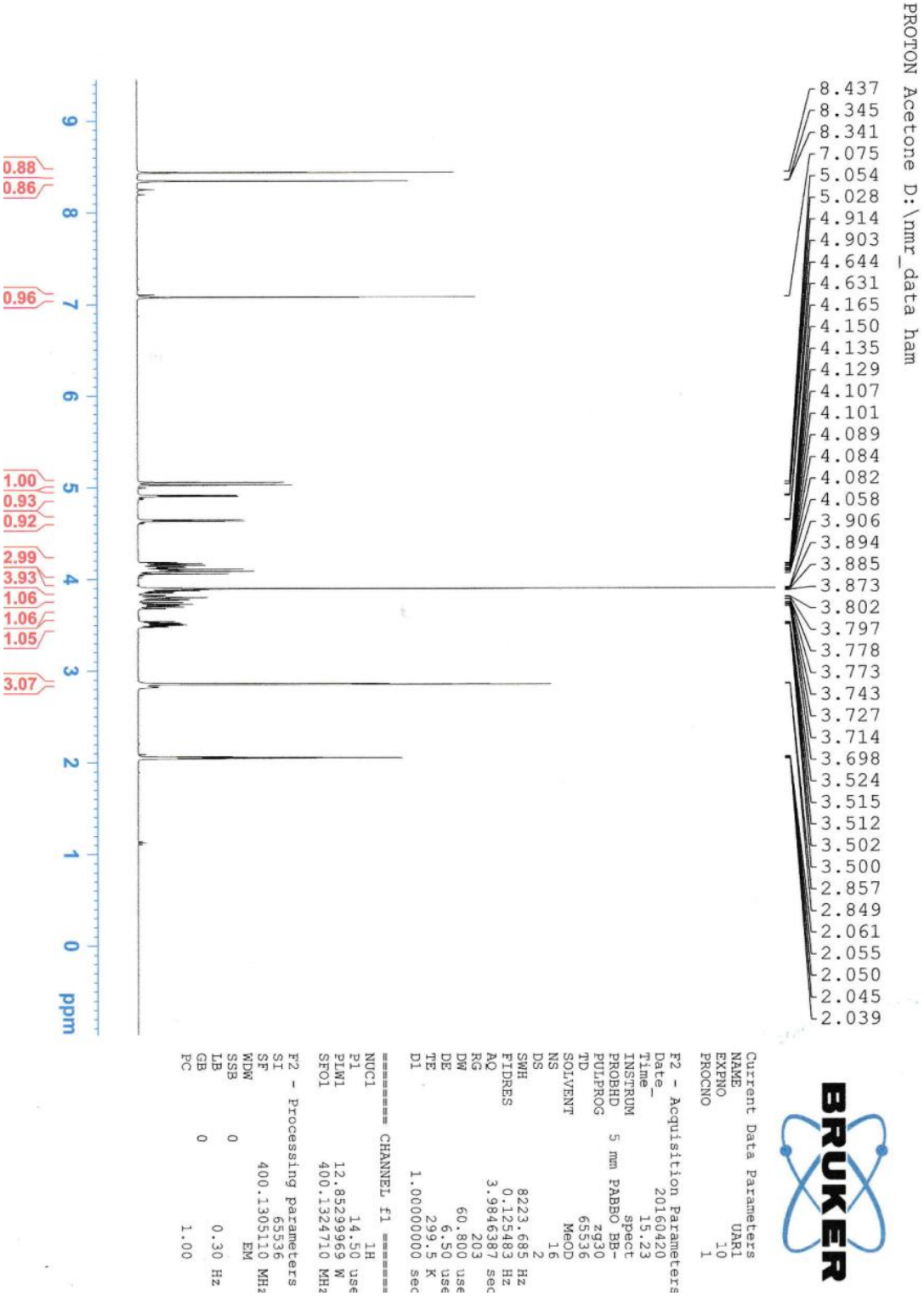
1. 13CNMR spectrum of bergenin



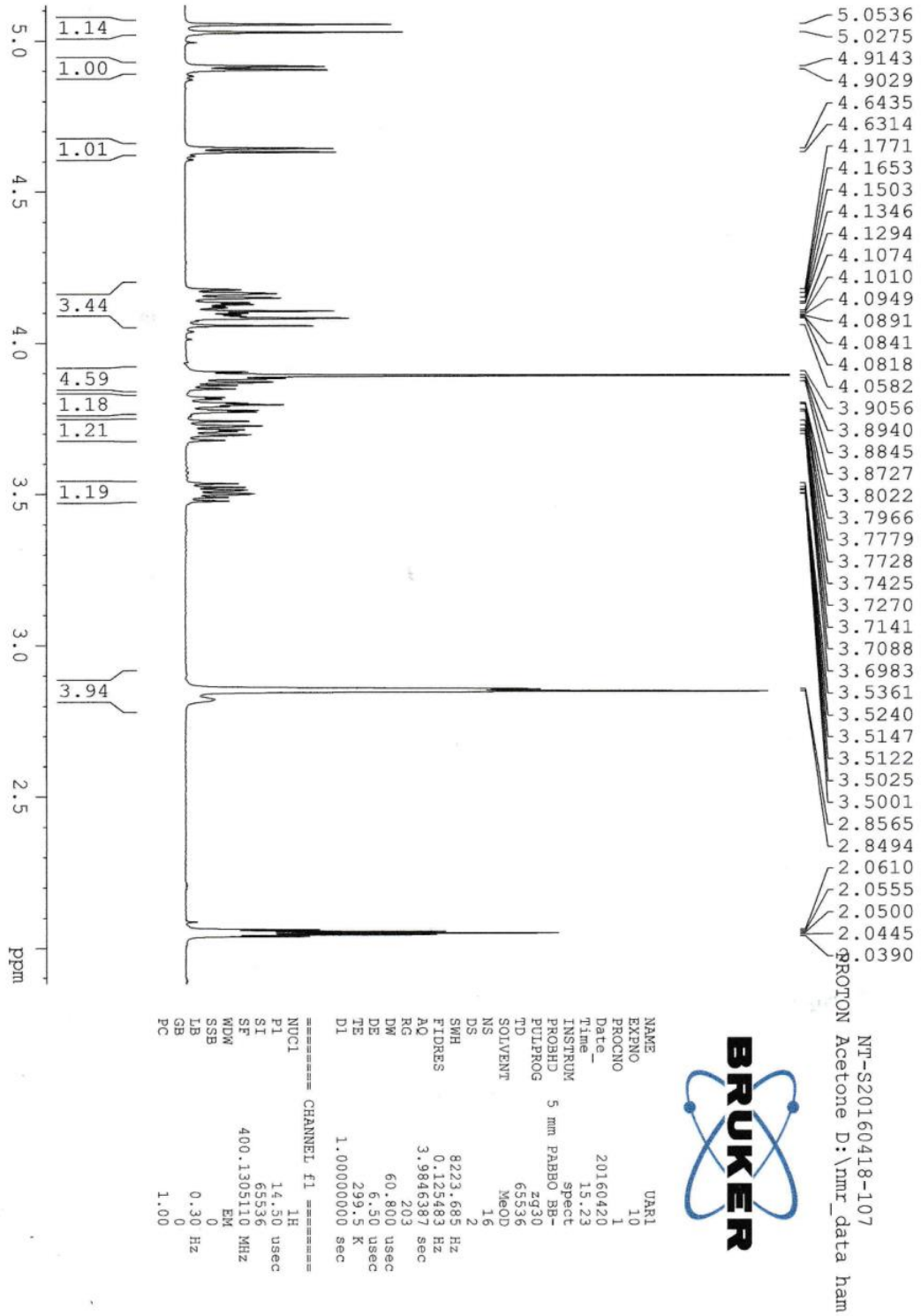
2. Graphical superimposition of ^{13}C NMR spectrum of bergenin (1) and (2)



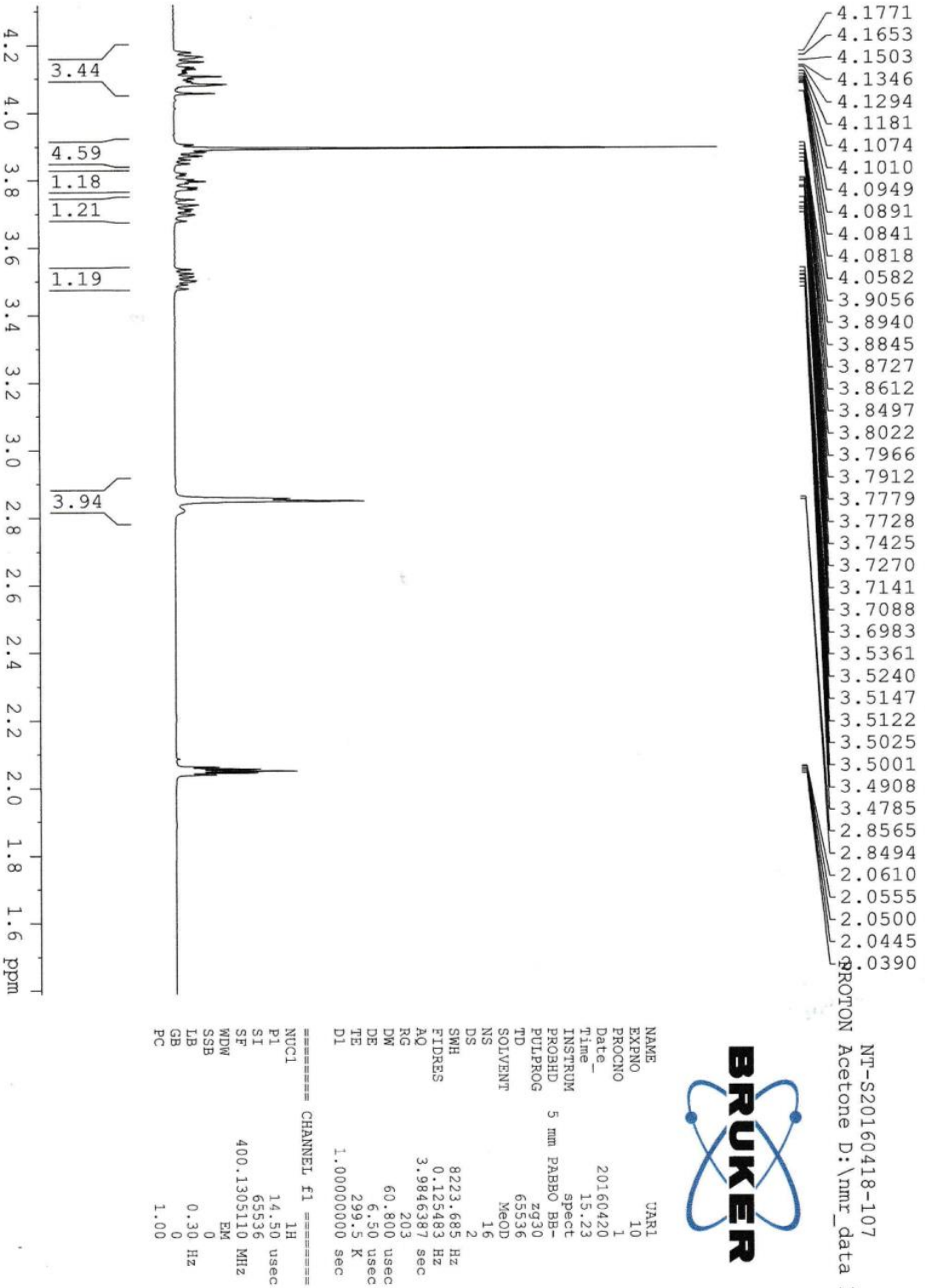
3. ^{1}H NMR spectrum of bergenin (δ H 2.04 to 8.44)



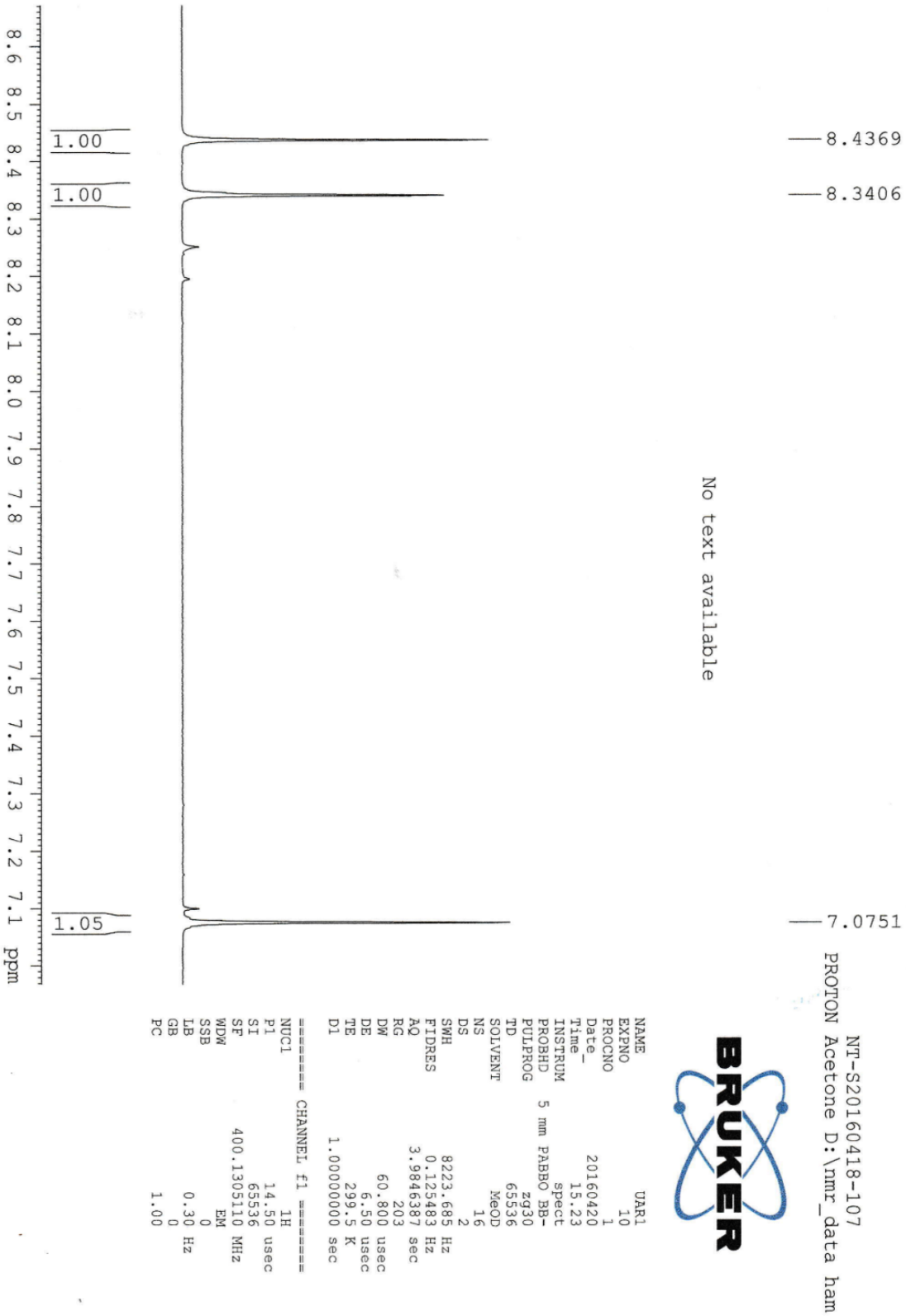
4. ^{1}H NMR spectrum of bergenin (Expanded δ H 2.04 to 5.05)



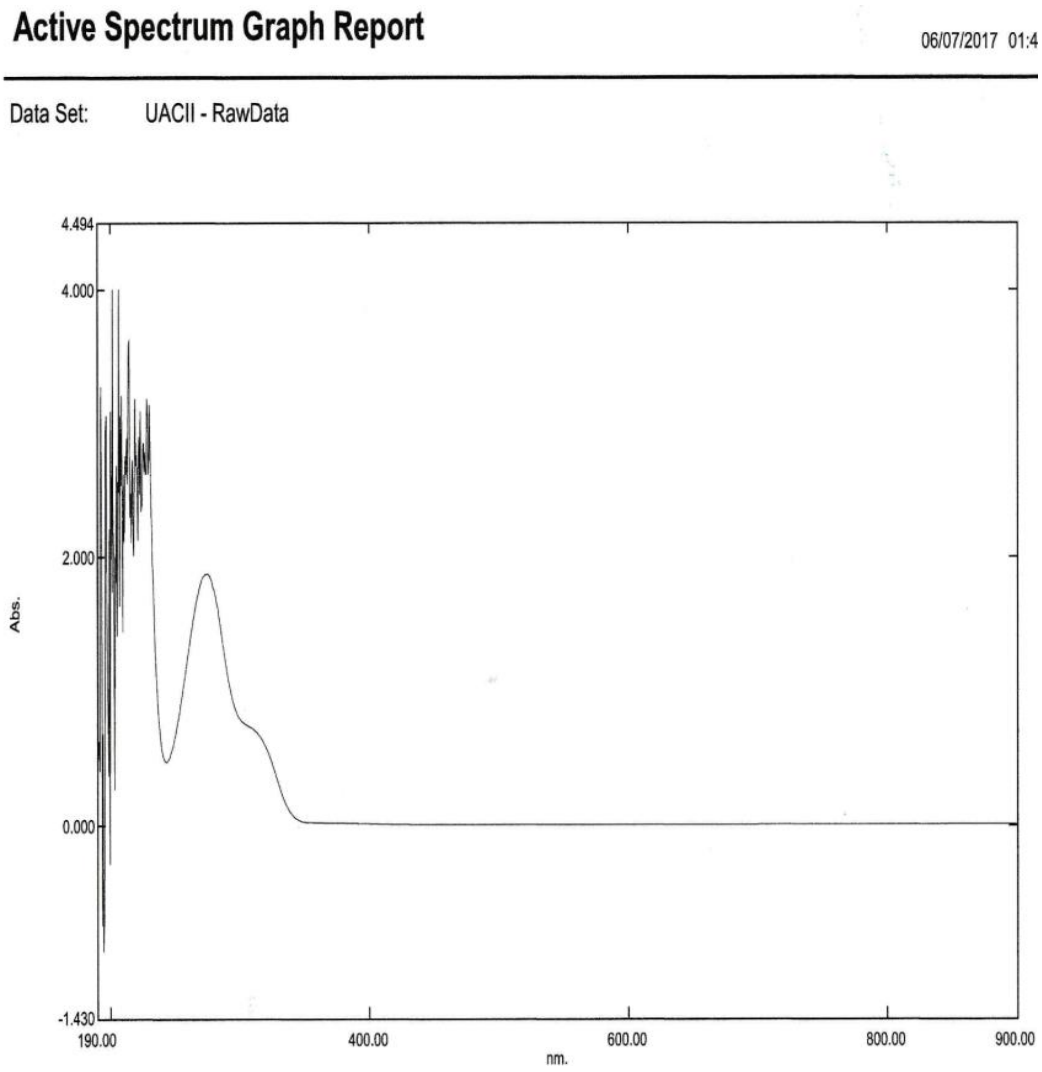
5. ^{1}H NMR spectrum ofbergenin (Expanded δ H 2.04 to 4.18)



6. ^{1}H NMR spectrum of bergenin (Expanded δ H 7.08 to 8.44)



7. Mass spectrum of bergenin



分析数据

Analysis Data

NO.项目编号

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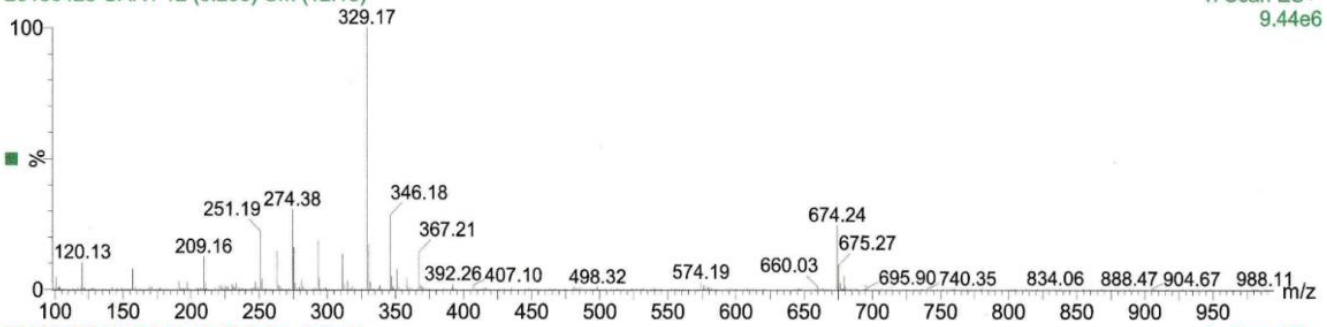

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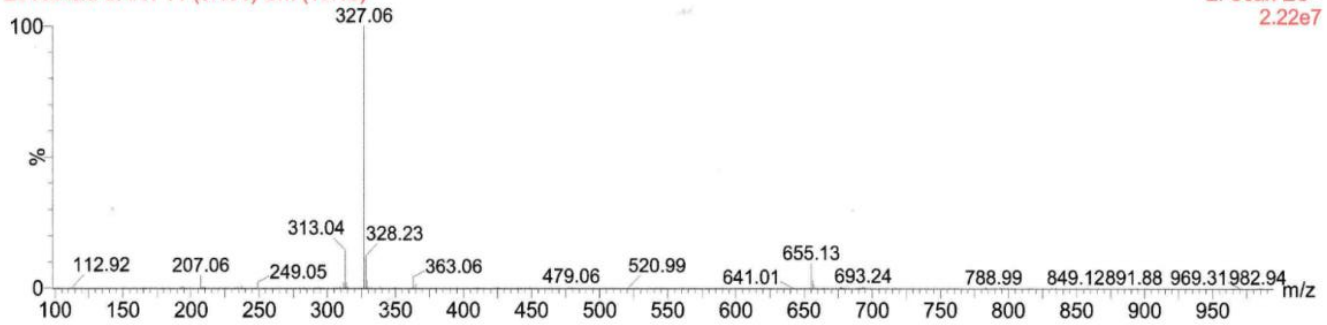

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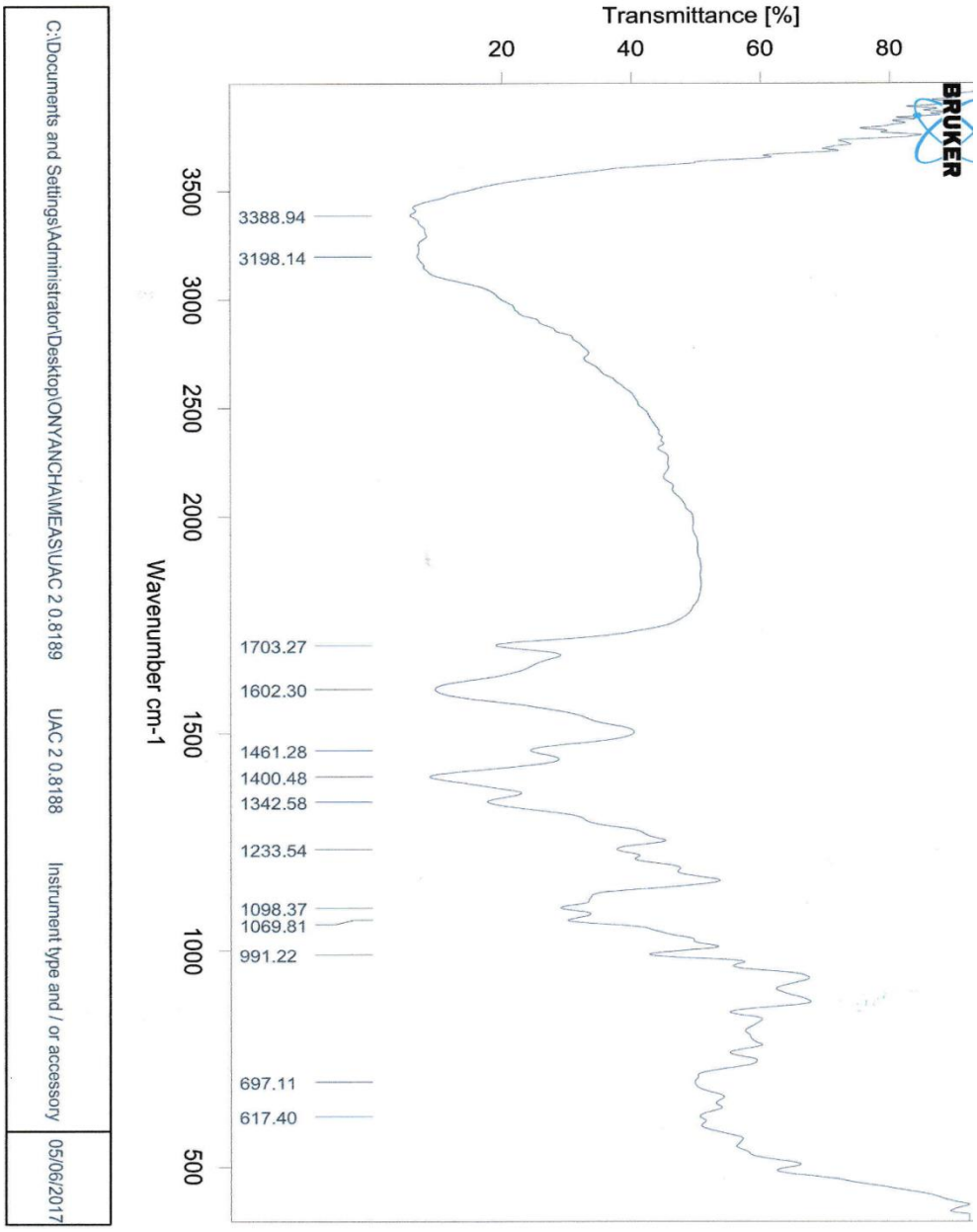
8. UV spectrum of bergenin

9. FTIR spectrum of bergenin



ii. Antiproliferative characterization MTT (based on optical density) and Cytotoxicity results

(1) Mean IC_{50} values for *U. anisatum* methanol extract against DU-145 cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against DU-145						Mean IC_{50} ± SEM n=3
		i IC_{50} = 81.7		ii IC_{50} = 88.3		iii IC_{50} =75		
A	0.00	0.502	0.00	0.503	0.00	0.586	0.00	
B	1.37	0.452	10.16	0.466	7.36	0.512	12.63	81.7 ± 3.9
C	4.12	0.427	14.94	0.437	13.12	0.467	20.31	

D	12.35	0.394	21.51	0.414	17.69	0.455	22.35
E	37.04	0.301	40.04	0.349	30.62	0.353	39.76
F	111.11	0.209	58.36	0.219	56.46	0.249	57.50
G	333.33	0.154	69.32	0.161	67.99	0.151	74.23
H	1000	0.089	82.27	0.127	74.75	0.091	84.47

(2) Mean IC_{50} values for *U. anisatum* methanol extract against HCC 1395 cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against HCC 1395						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 45.00		ii IC_{50} = 51.7		iii IC_{50} = 55		
A	0.00	0.911	0.00	0.955	0.00	0.917	0.00	50.6 ± 2.9
B	1.37	0.856	6.04	0.698	26.91	0.777	15.27	
C	4.12	0.631	30.74	0.696	27.12	0.714	22.14	
D	12.35	0.539	40.83	0.603	36.86	0.592	35.44	
E	37.04	0.456	49.95	0.489	48.80	0.46	49.84	
F	111.11	0.438	51.92	0.455	52.36	0.45	50.93	
G	333.33	0.225	75.30	0.409	57.17	0.379	58.67	
H	1000			0.045	95.29	0.039	95.75	

(3) Mean IC_{50} values for *U. anisatum* methanol extract against Vero cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against vero						Mean IC_{50} ± SEM n=3
		i IC_{50}=3.68		ii IC_{50}=3.38		iii IC_{50}=2.85		
A	0.00	0.456	0.00	0.53	0.00	0.68	0.00	3.3±0.2
B	1.37	0.264	42.11	0.452	14.72	0.442	35.00	
C	4.12	0.223	51.10	0.2	62.26	0.247	63.68	
D	12.35	0.163	64.25	0.146	72.45	0.041	93.97	
E	37.04	0.143	68.64	0.135	74.53	0.028	95.88	
F	111.11	0.079	82.68	0.133	74.91	0.026	96.18	
G	333.33	0.065	85.75	0.051	90.38	0.015	97.79	
H	1000	0.02	95.61	0.004	99.25	0.003	99.56	

(4) Mean IC_{50} values for *U. anisatum* Petroleum ether fraction against DU-145 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against DU-145						Mean IC_{50} ± SEM n = 3
		i IC_{50} > 1000		ii IC_{50} > 1000		iii IC_{50} >1000		
A	0.00	0.624	0.00	0.609	0.00	0.534	0.00	> 1000
B	1.37	0.584	6.41	0.593	2.63	0.51	4.49	
C	4.12	0.568	8.97	0.587	3.61	0.509	4.68	
D	12.35	0.552	11.54	0.578	5.09	0.496	7.12	
E	37.04	0.495	20.67	0.561	7.88	0.479	10.30	
F	111.11	0.486	22.12	0.552	9.36	0.468	12.36	
G	333.33	0.472	24.36	0.467	23.32	0.461	13.67	
H	1000	0.362	41.99	0.418	31.36	0.385	27.90	

(5) Mean IC_{50} values for *U. anisatum* Petroleum ether fraction against HCC 1395 cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against HCC 1395						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 813		ii IC_{50} = 815		iii IC_{50} = 908		
A	0.00	1.028	0.00	1.004	0.00	0.917	0.00	845.3 ± 31.3
B	1.37	0.92	10.51	0.923	8.08	0.881	3.93	
C	4.12	0.86	16.34	0.92	8.37	0.857	6.54	
D	12.35	0.845	17.80	0.878	12.55	0.768	16.25	
E	37.04	0.791	23.05	0.869	13.45	0.767	16.36	
F	111.11	0.762	25.88	0.803	20.02	0.705	23.12	
G	333.33	0.681	33.75	0.67	33.27	0.696	24.10	
H	1000	0.452	56.03	0.437	56.47	0.422	53.98	

(6) Mean IC_{50} values for *U. anisatum* Petroleum ether fraction against Vero cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against vero						Mean IC_{50} ± SEM n=3
		i IC_{50} =760		ii IC_{50} = 600		iii IC_{50} = 600		
A	0.00	0.718	0.00	0.701	0.00	1.011	0.00	653.3 ± 53.3
B	1.37	0.714	0.56	0.691	1.43	0.908	10.19	
C	4.12	0.686	4.46	0.675	3.71	0.74	26.81	
D	12.35	0.673	6.27	0.662	5.56	0.727	28.10	
E	37.04	0.633	11.84	0.615	12.27	0.725	28.29	
F	111.11	0.583	18.80	0.6	14.41	0.72	28.78	
G	333.33	0.502	30.08	0.499	28.82	0.633	37.39	
H	1000	0.279	61.14	0.13	81.46	0.32	68.35	

(7) Mean IC_{50} values for *U. anisatum* dichloromethane fraction against DU-145 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against DU-145						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 523		ii IC_{50}= 563		iii IC_{50} = 513		
A	0.00	0.626	0.00	0.599	0.00	0.643	0.00	533.0 ± 15.3
B	1.37	0.624	0.32	0.593	1.00	0.628	2.33	
C	4.12	0.615	1.76	0.569	5.01	0.619	3.73	
D	12.35	0.6	4.15	0.566	5.51	0.588	8.55	
E	37.04	0.578	7.67	0.509	15.03	0.54	16.02	
F	111.11	0.495	20.93	0.508	15.19	0.496	22.86	
G	333.33	0.433	30.83	0.43	28.21	0.428	33.44	
H	1000	0.009	98.56	0.051	91.49	0.021	96.73	

(8) Mean IC_{50} values for *U. anisatum* dichloromethane fraction against HCC 1395 cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against HCC 1395						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 813		ii IC_{50} = 815		iii IC_{50} = 908		
A	0.00	1.028	0.00	1.004	0.00	0.917	0.00	845.3 ± 31.3

B	1.37	0.92	10.51	0.923	8.08	0.881	3.93
C	4.12	0.86	16.34	0.92	8.37	0.857	6.54
D	12.35	0.845	17.80	0.878	12.55	0.768	16.25
E	37.04	0.791	23.05	0.869	13.45	0.767	16.36
F	111.11	0.762	25.88	0.803	20.02	0.705	23.12
G	333.33	0.681	33.75	0.67	33.27	0.696	24.10
H	1000	0.452	56.03	0.437	56.47	0.422	53.98

(9) Mean IC_{50} values for *U. anisatum* dichloromethane fraction against Vero cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against vero						Mean IC_{50} ± SEM n=3
		i IC_{50} =793		ii IC_{50} = 820		iii IC_{50} =740		
A	0.00	0.71	0.00	0.74	0.00	0.651	0.00	784.3 ± 23.5
B	1.37	0.684	3.66	0.718	2.97	0.65	0.15	
C	4.12	0.675	4.93	0.704	4.86	0.594	5.70	
D	12.35	0.653	8.03	0.703	5.00	0.588	9.68	
E	37.04	0.624	12.11	0.665	10.14	0.54	16.97	
F	111.11	0.62	12.68	0.657	11.22	0.529	18.74	
G	333.33	0.583	17.89	0.587	20.68	0.483	25.81	
H	1000	0.258	63.66	0.293	60.41	0.229	64.82	

(10) Mean IC_{50} values for *U. anisatu m*ethyl acetate fraction against DU-145 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against DU-145						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 495		ii IC_{50} = 420		iii IC_{50} = 490		
A	0.00	0.252	0.00	0.221	0.00	0.232	0.00	468.3 ± 24.2
B	1.37	0.247	1.98	0.221	0.00	0.228	1.72	
C	4.12	0.245	2.78	0.207	6.33	0.228	1.72	
D	12.35	0.237	5.95	0.206	6.79	0.227	2.16	
E	37.04	0.231	8.33	0.205	7.24	0.225	3.02	
F	111.11	0.191	24.21	0.147	33.48	0.223	3.88	
G	333.33	0.164	34.92	0.118	46.61	0.142	38.79	
H	1000	0.007	97.22	0.06	72.85	0.03	87.07	

(11) Mean IC_{50} values for *U. anisatu m*ethyl acetate fraction against HCC 1395 cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against HCC 1395						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 328		ii IC_{50} = 353		iii IC_{50} = 208		
A	0.00	0.472	0.00	0.452	0.00	0.496	0.00	296.3 ± 44.8
B	1.37	0.417	11.65	0.437	3.32	0.492	0.81	
C	4.12	0.41	13.14	0.428	5.31	0.477	3.83	
D	12.35	0.391	17.16	0.394	12.83	0.468	5.65	
E	37.04	0.385	18.43	0.38	15.93	0.404	18.55	
F	111.11	0.359	23.94	0.364	19.47	0.339	31.65	
G	333.33	0.234	50.42	0.234	48.23	0.117	76.41	

H	1000	0.011	97.67	0.007	98.45	0.042	91.53	

(12) Mean IC_{50} values for *U. anisatu m*ethyl acetate fraction against Vero cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against vero						Mean IC_{50}± SEM n=3
		i IC_{50}=413		ii IC_{50}=335		iii IC_{50}=360		
A	0.00	1.222	0.00	1.266	0.00	1.264	0.00	369.3±23.0
B	1.37	1.216	0.49	1.251	1.18	1.255	0.71	
C	4.12	1.205	1.39	1.193	5.77	1.081	14.48	
D	12.35	1.195	2.21	1.167	7.82	1.07	15.35	
E	37.04	1.105	9.57	1.166	7.90	1.024	18.99	
F	111.11	1.057	13.50	1.133	10.51	0.95	24.84	
G	333.33	0.691	43.45	0.635	49.84	0.661	47.71	
H	1000	0.022	98.20	0.109	91.39	0.09	92.88	

(13) Mean IC_{50} values for *U. anisatum* acetone fraction against DU-145 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against DU-145						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 32.5		ii IC_{50} = 72.5		iii IC_{50} = 95		
A	0.00	0.222	0.00	0.219	0.00	0.256	0.00	66.7 ± 18.3
B	1.37	0.219	1.35	0.204	6.85	0.243	5.08	
C	4.12	0.209	5.86	0.202	7.76	0.237	7.42	
D	12.35	0.189	14.86	0.199	9.13	0.209	18.36	
E	37.04	0.101	54.50	0.148	32.42	0.199	22.27	
F	111.11	0.088	60.36	0.067	69.41	0.108	57.81	
G	333.33	0.056	74.77	0.061	72.15	0.004	98.44	
H	1000	0.032	85.59	0.014	93.61	0	100	

(14) Mean IC_{50} values for *U. anisatum* acetone fraction against HCC 1395 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against HCC 1395						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 103		ii IC_{50} = 82.5		iii IC_{50} = 77.5		
A	0.00	0.984	0.00	0.951	0.00	0.899	0.00	87.7 ± 7.8
B	1.37	0.922	6.30	0.869	8.62	0.898	0.11	
C	4.12	0.911	7.42	0.864	9.15	0.896	0.33	
D	12.35	0.901	8.43	0.781	17.88	0.805	10.46	
E	37.04	0.865	12.09	0.774	18.61	0.796	11.46	
F	111.11	0.444	54.88	0.289	69.61	0.156	82.65	
G	333.33	0.094	90.45	0.094	90.12	0.129	85.65	
H	1000	0.051	94.82	0.085	91.06	0.114	87.32	

(15) Mean IC_{50} values for *U. anisatum* acetone fraction against Vero cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against vero			Mean IC_{50} ± SEM n=3
		i IC_{50} = 485	ii IC_{50} = 255	iii IC_{50} = 458	

A	0.00	0.884	0.00	0.809	0.00	0.836	0.00	
B	1.37	0.745	15.72	0.766	5.32	0.697	16.63	
C	4.12	0.738	16.52	0.689	14.83	0.627	25.00	
D	12.35	0.729	17.53	0.688	14.96	0.596	28.71	399.3 ± 72.6
E	37.04	0.725	17.98	0.602	25.59	0.594	28.95	
F	111.11	0.658	25.57	0.526	34.98	0.576	31.10	
G	333.33	0.502	43.21	0.337	58.34	0.443	47.00	
H	1000	0.216	75.57	0.039	95.18	0.298	64.35	

(16) Mean IC_{50} values for *U. anisatum* methanol fraction against DU-145 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against DU-145						Mean IC_{50}± SEM n=3
		i IC_{50} = 77.5		ii IC_{50} = 37.04		iii IC_{50} = 20		
A	0.00	0.244	0.00	0.25	0.00	0.197	0.00	
B	1.37	0.241	1.23	0.249	0.40	0.193	2.03	
C	4.12	0.211	13.52	0.236	5.60	0.147	25.38	
D	12.35	0.17	30.33	0.227	9.20	0.13	34.01	44.9±17.1
E	37.04	0.17	30.33	0.125	50.00	0.082	58.38	
F	111.11	0.088	63.93	0.124	50.40	0.055	72.08	
G	333.33	0.059	75.82	0.024	90.40	0.051	74.11	
H	1000	0.049	79.92	0.016	93.60	0.029	85.28	

(17) Mean IC_{50} values for *U. anisatum* methanol fraction against HCC 1395 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against HCC 1395						Mean IC_{50}± SEM n=3
		i IC_{50}=72.5		ii IC_{50}=75		iii IC_{50}=75		
A	0.00	1.002	0.00	0.939	0.00	0.945	0.00	
B	1.37	0.899	10.28	0.877	6.60	0.921	2.54	
C	4.12	0.865	13.67	0.863	8.09	0.851	9.95	
D	12.35	0.828	17.37	0.86	8.41	0.802	15.13	74.2±0.8
E	37.04	0.706	29.54	0.716	23.75	0.731	22.65	
F	111.11	0.252	74.85	0.26	72.31	0.258	72.70	
G	333.33	0.151	84.93	0.153	83.71	0.152	83.92	
H	1000	0.105	89.52	0.099	89.46	0.033	96.51	

(18) Mean IC_{50} values for *U. anisatum* methanol fraction against Vero cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against vero						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 178		ii IC_{50} = 173		iii IC_{50} = 150		
A	0.00	0.786	0.00	0.815	0.00	0.841	0.00	
B	1.37	0.75	4.58	0.807	0.98	0.805	4.28	
C	4.12	0.742	5.60	0.751	7.85	0.797	5.23	
D	12.35	0.679	13.61	0.718	11.90	0.676	19.62	167 ± 8.6
E	37.04	0.565	28.12	0.642	21.23	0.643	23.54	
F	111.11	0.476	39.44	0.501	38.53	0.475	43.52	

G	333.33	0.189	75.95	0.161	80.25	0.147	82.52
H	1000	0.122	84.48	0.083	89.82	0.113	86.56

(19) Mean IC_{50} values for bergenin anhydrous against DU-145 cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against DU-145						Mean IC_{50} ± SEM n = 3
		i IC_{50c}>c1000		ii IC_{50} > 1000		iii IC_{50} > 1000		
A	0.00	0.564	0.00	0.572	0.00	0.575	0.00	> 1000
B	1.37	0.528	6.38	0.563	1.57	0.551	4.17	
C	4.12	0.517	8.33	0.553	3.32	0.545	5.22	
D	12.35	0.497	11.88	0.528	7.92	0.54	6.09	
E	37.04	0.496	12.06	0.497	13.11	0.537	6.61	
F	111.11	0.421	25.35	0.482	15.73	0.457	20.52	
G	333.33	0.416	26.24	0.479	16.26	0.453	21.22	
H	1000	0.364	35.46	0.374	34.62	0.326	43.30	

(20) Mean IC_{50} values for bergenin anhydrous against HCC 1395 cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against HCC 1395						Mean IC_{50} ± SEM n = 3
		i IC_{50} > 1000		ii IC_{50} > 1000		iii IC_{50} > 1000		
A	0.00	1.261	0.00	1.219	0.00	1.359	0.00	>1000
B	1.37	1.231	2.38	1.197	1.8	1.13	16.89	
C	4.12	1.212	3.89	1.173	3.77	1.13	16.89	
D	12.35	1.162	7.85	1.14	6.48	1.122	17.44	
E	37.04	1.122	11.02	1.129	7.38	1.102	18.91	
F	111.11	1.107	12.21	1.103	9.52	1.09	19.79	
G	333.33	1.036	17.84	1.067	12.47	1.048	22.88	
H	1000	0.873	30.77	0.77	36.83	0.882	35.10	

(21) Mean IC_{50} values for bergenin anhydrous against Vero cell lines

Well	Conc. (µg/ml)	Absorbance/ % Cytotoxicity against vero						Mean IC_{50} ± SEM n = 3
		i IC_{50} >1000		ii IC_{50} > 1000		iii IC_{50} > 1000		
A	0.00	1.083	0.00	0.818	0.00	0.878	0.00	> 1000
B	1.37	0.812	25.02	0.802	1.96	0.874	0.46	
C	4.12	0.798	26.32	0.79	3.42	0.861	1.94	
D	12.35	0.753	30.47	0.774	5.38	0.836	4.78	
E	37.04	0.7	35.36	0.764	6.60	0.816	7.06	
F	111.11	0.668	38.32	0.701	14.30	0.765	12.87	
G	333.33	0.582	46.26	0.608	25.67	0.618	29.61	
H	1000	0.578	46.63	0.517	36.80	0.49	44.19	

(22) Mean IC_{50} values for Fluorouracil against DU-145 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against DU-145						Mean IC_{50} ± SEM n = 3
		i IC_{50} = 11.9		ii IC_{50} = 12.5		iii IC_{50} = 30.5		
A	0.00	0.231	0.00	0.22	0.00	0.219	0.00	18.3 ± 6.1
B	1.37	0.176	23.81	0.132	40.00	0.152	30.59	
C	4.12	0.152	34.20	0.129	41.36	0.131	40.18	
D	12.35	0.113	51.08	0.11	50.00	0.119	45.66	
E	37.04	0.088	61.9	0.094	57.27	0.106	51.60	
F	111.11	0.075	67.53	0.089	59.55	0.083	62.00	
G	333.33	0.069	70.13	0.078	64.55	0.083	62.00	
H	1000	0.042	81.82	0.046	79.09	0.044	79.91	

(23) Mean IC_{50} values for Fluorouracil against HCC 1395 cell lines

Well	Conc. (µg/ml)	Absorbance and % Cytotoxicity against HCC 1395						Mean IC_{50} ± SEM n=3
		i IC_{50} = 53.5		ii IC_{50} = 34.5		Iii IC_{50} = 28.4		
A	0.00	0.474	0.00	0.483	0.00	0.497	0.00	38.8 ± 7.6
B	1.37	0.442	6.75	0.47	2.69	0.44	11.47	
C	4.12	0.433	8.65	0.449	7.04	0.421	15.29	
D	12.35	0.421	11.18	0.448	7.25	0.418	15.90	
E	37.04	0.302	36.29	0.215	55.49	0.158	68.21	
F	111.11	0.016	96.62	0.007	98.55	0.005	98.99	
G	333.33	0.003	99.37	0.003	99.38	0.003	99.40	
H	1000	0.001	99.79	0.001	99.79	0.003	99.40	

(24) Mean IC_{50} values for Fluorouracil against Vero cell lines

(a) Well \ Conc. (µg/ml)		Absorbance and % Cytotoxicity against vero				Mean IC_{50} ± SEM n = 2
		i IC_{50} = 110		ii IC 50 = 260		
A	0.00	0.395	0.00	0.41	0.00	185 ± 75
B	1.37	0.247	37.47	0.262	36.09	
C	4.12	0.235	40.51	0.245	40.24	
D	12.35	0.233	41.01	0.225	45.12	
E	37.04	0.214	45.82	0.217	47.07	
F	111.11	0.195	50.06	0.215	47.57	
G	333.33	0.191	51.65	0.201	50.98	
H	1000	0.188	52.41	0.2	51.22	

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