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Cytotoxic Activity of Coleon Diterpenoids from *Plectranthus mutabilis* Codd.

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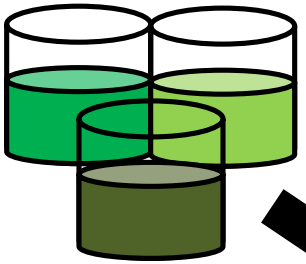
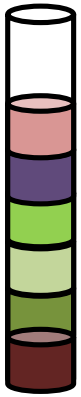
Graphical Abstract

16 Plectranthus spp.



Ultrasound-assisted Extraction method



Bio-guided isolation of the most active extract: *P. mutabilis* Codd.

Structure elucidated 1D- and 2D- NMR

Cytotoxicity Study

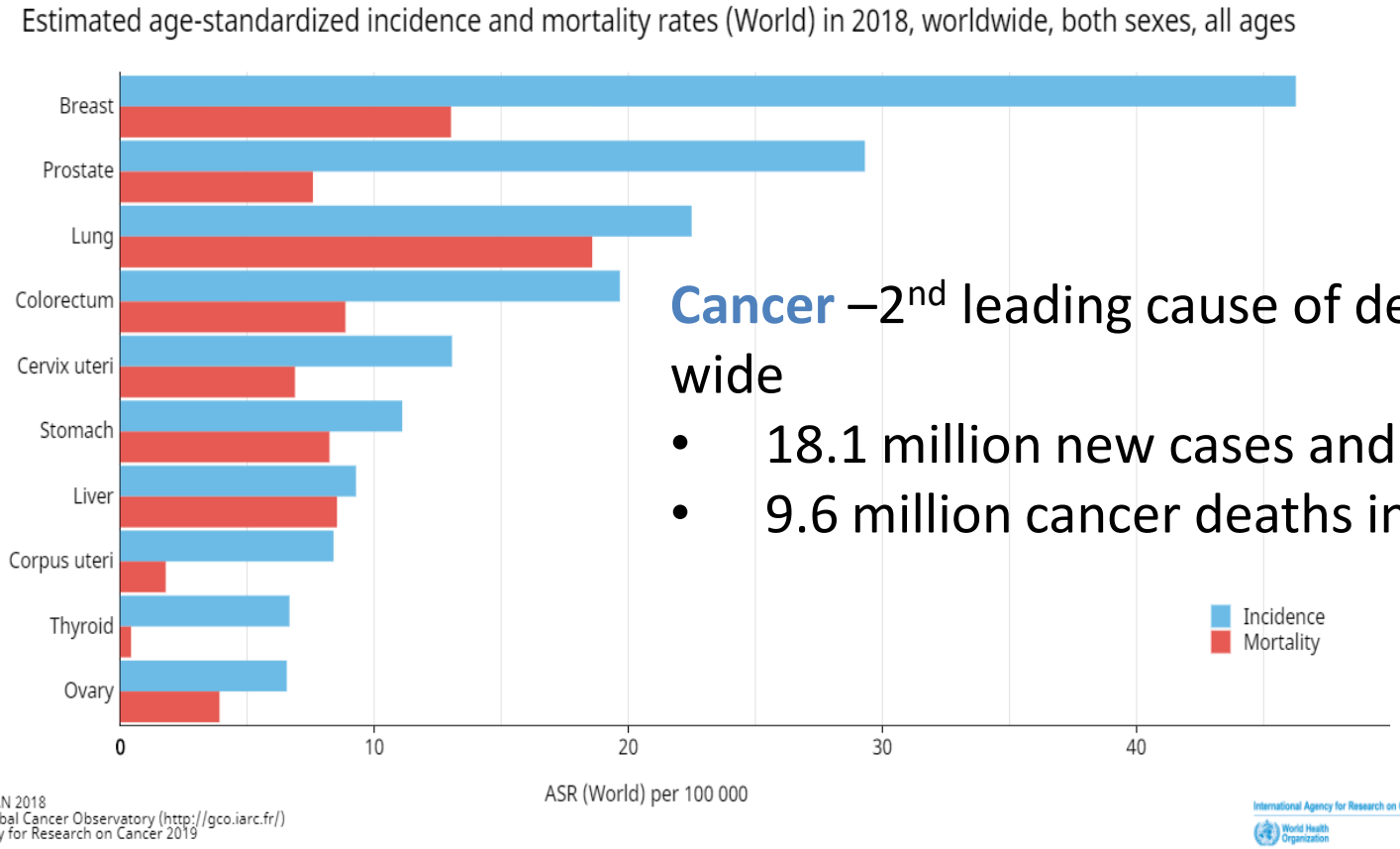
Toxicity study:
- General toxicity (*Artemia salina*)
- Cytotoxicty (Cancer and normal Human cell lines)

Abstract

Plectranthus plants are known source of abietane diterpenoids with antitumor activity. The objective of this study was to evaluate the biological activity of sixteen *Plectranthus* spp. acetonic extracts and identify the bioactive compounds in the most effective extracts. *P. mutabilis* had the highest extraction yield (30.03%, dry weight % w/w). All extracts were screened for their general toxicity using the *Artemia salina* model [4]. Thus, the antitumor activity of the five most toxic extracts was explored in three different cancer cell lines: HCT116, MCF-7 and NCI-H460. *P. mutabilis* with high cytotoxic activity was subjected to a bio-guided fractionation using the *A. salina* general toxicity assay. Column chromatography on silica or polyamide, with gradient systems of increasing polarity allowed to achieve the diterpenoid coleon U (1) and 8α,9α-epoxycoleon U quinone (2). The complete structure characterization was done mainly by 1D- and 2D-NMR, and comparison with literature data. Compound 1 showed moderate cytotoxicity on sensitive and resistant (ABCB1 overexpressing) human colon adenocarcinoma and normal cell lines showing slight selectivity towards resistant cells. Moreover, this compound is not an inhibitor of ABCB1 transporter based on the intracellular accumulation of the ABCB1 substrate rhodamine 123. Further phytochemical studies are ongoing.

Keywords: *Plectranthus*; *P. mutabilis*; Isolation; characterization; Cytotoxicity

Introduction

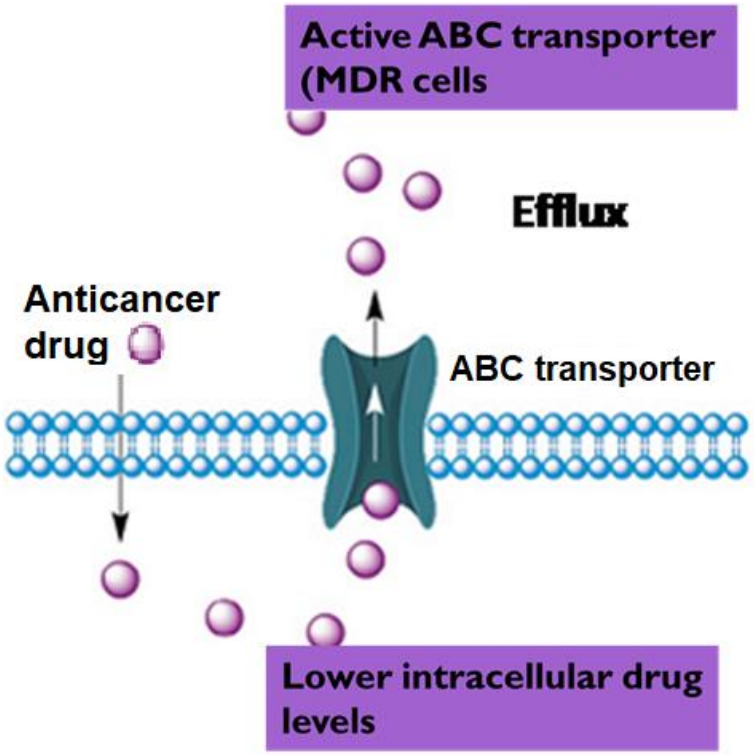


Cancer –2nd leading cause of death world wide

- 18.1 million new cases and
- 9.6 million cancer deaths in 2018

WHO, 2018
https://www.who.int/health-topics/cancer#tab=overview

Introduction



Multi-drug-resistant (MDR)

- *Major challenge to cancer therapy*
- Need to develop new reversal MDR agents

Introduction

Plectranthus spp.

- Natural products: source of bioactive compounds
- *Plectranthus genus* (Lamiaceae) uses: different types of cancer
- Source of bioactive compounds:
  - abietane-type diterpenoids
    - ➢ antibacterial, antifungal and **antitumoral**

Objective

Screen the biological activity of sixteen *Plectranthus* spp extracts:

- General toxicity
  - (*Artemia salina* model)
- Cytotoxicity
  - (Human cell lines)

Identify the bioactive compound/s in the most active extract

In this study: sixteen *Plectranthus* species

sixteen *Plectranthus* spp.

Previous Studies	Never studied
P. swynnertonii S. Moore	*P. ramosior* Benth. Van Jaarsv.
P. welwischii (Briq. Codd)	*P. woodii* Gürke
P. ciliatus E. Mey ex BenthP.	*P. inflexus* (Thunb.) Vahl ex Benth
P. cylindraceus Hochst, ex Benth	*P. mutabilis* Codd
P. spicatus E. Mey ex Benth	*P. xerophylus* Codd
P. crassus N.E.Br.	*P. mzimvubuensis* Van Jaarsv.
P. welshii	*P. lippio*. Druce
	P. lucidus (Benth.) Van Jaarsv. and T.J. Edwards
	P. petiolaris E. Mey ex Benth

Ultrasound-assisted Extraction

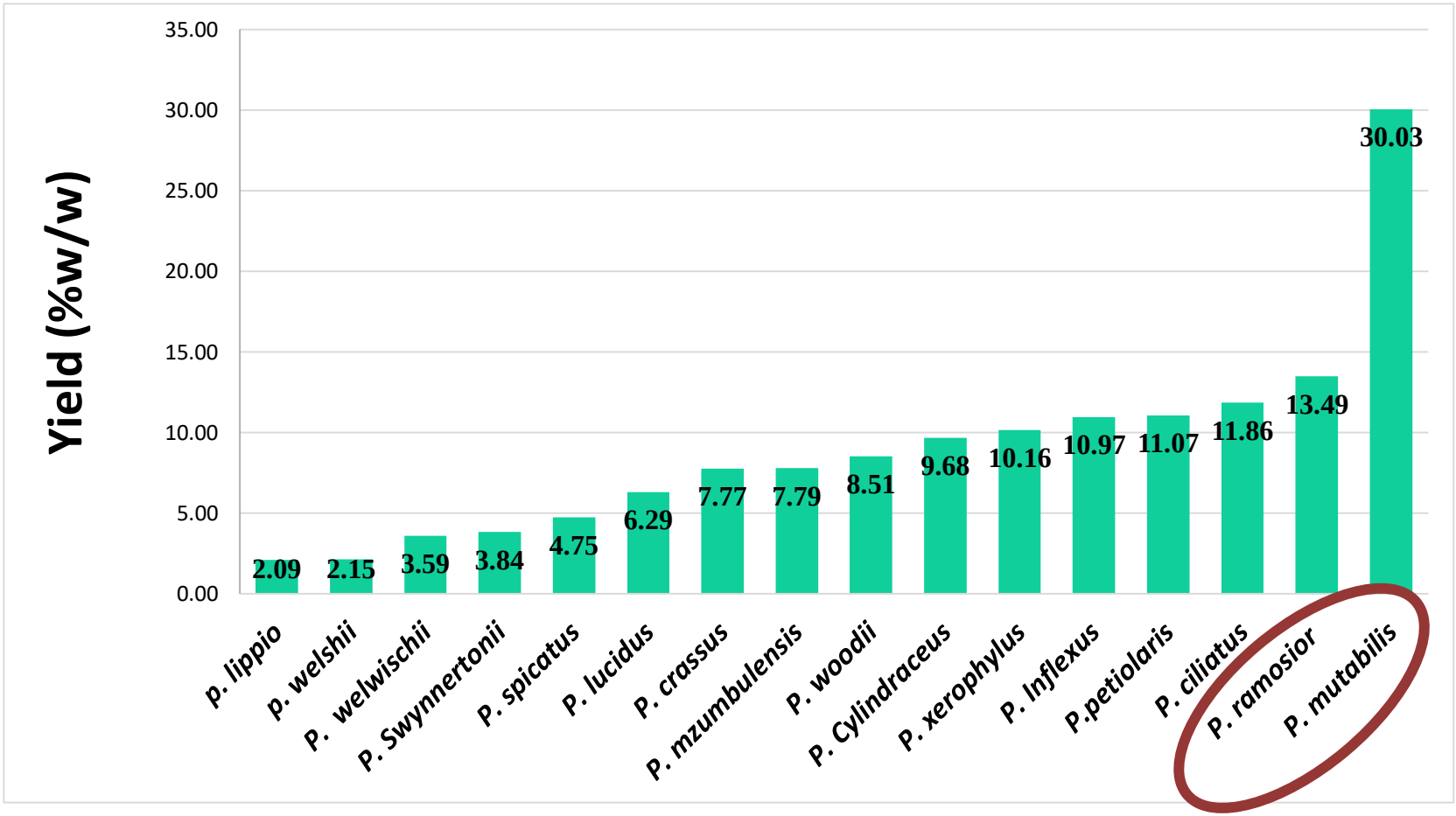
(10 % w/v)
acetone

Sonication

Extract Concentration
(< 40° C)

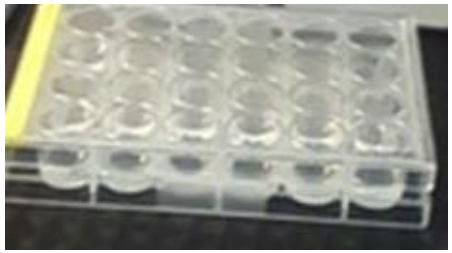
Extracts
10 mg/mL
DMSO

Extraction yield (%)



General Toxicity (*Artemia salina* assay)

Grow for 48 h



Expose to the extract for 24 h at 10ppm of extract

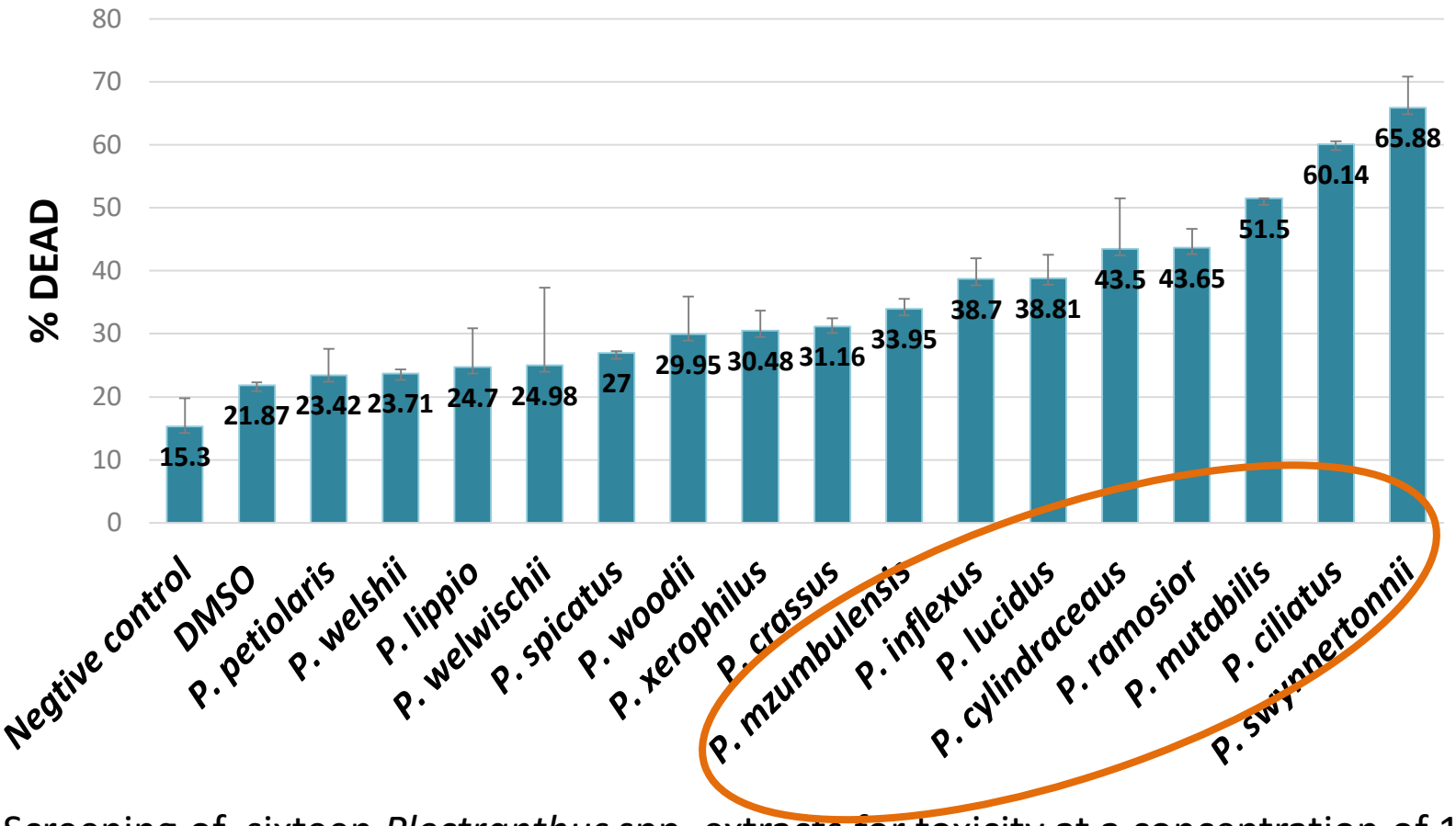
The number dead is counted after 24h

[redacted]

% Dead calculated

Rimpiläinen *et al*, *ACS Omega.* 2018; **Ntungwe *et al*.,** J Biomed Biopharm Res. 2017

Artemia salina assay



Screening of sixteen *Plectranthus* spp. extracts for toxicity at a concentration of 10 ppm using the *Artemia salina* test (lethal concentration, %)

Artemia salina assay (LC_{50})

LC_{50} values (µg/mL) for the most active extracts using the *Artemia salina* test

Extracts	LC_{50} (µg/mL)
P. swynnertonnii	**0.036**
P. cylindraceaus	**0.504**
P. ciliatus	**0.984**
P. ramosior	**0.88**
P. mutabilis	**0.55**
P. lucidus	1.053
P. inflexus	0.986

Antitumor Activity/ sulforhodamine B assay

Cancer cell lines:
-colon colorectal carcinoma (**HCT116**),
-human breast adenocarcinoma (**MCF-7**)
-lung cancer carcinoma (**NCI-H460**)

IC_{50} (µg/mL) of five most toxic acetonic extracts

	HCT116	MCF-7	H460
P. mutabilis	28.00 ± 2.00	35.00 ± 1.00	36.00 ± 2.00
P. swynnertonii	7.95 ± 0.35	15.05 ± 0.02	13.50 ± 0.50
P. ramosior	3.45 ± 0.35	2.9 ± 0.10	3.00 ± 0.10
P. ciliatus	2.25 ± 0.75	6.70 ± 0.30	6.45 ± 0.05
P. cylindraceus	10.25 ± 0.75	12.00 ± 1.00	12.50 ± 0.50
Doxorrubicine	-	0.02 ± 0.08	0.006 ± 0.001

Epole *et al,* 7α-Acetoxy-6β-hydroxyroyleanone from *Plectranthus ramosior*: a screening study for anticancer lead molecules, 2019, In submission.

P. mutabilis: Bio-guided isolation

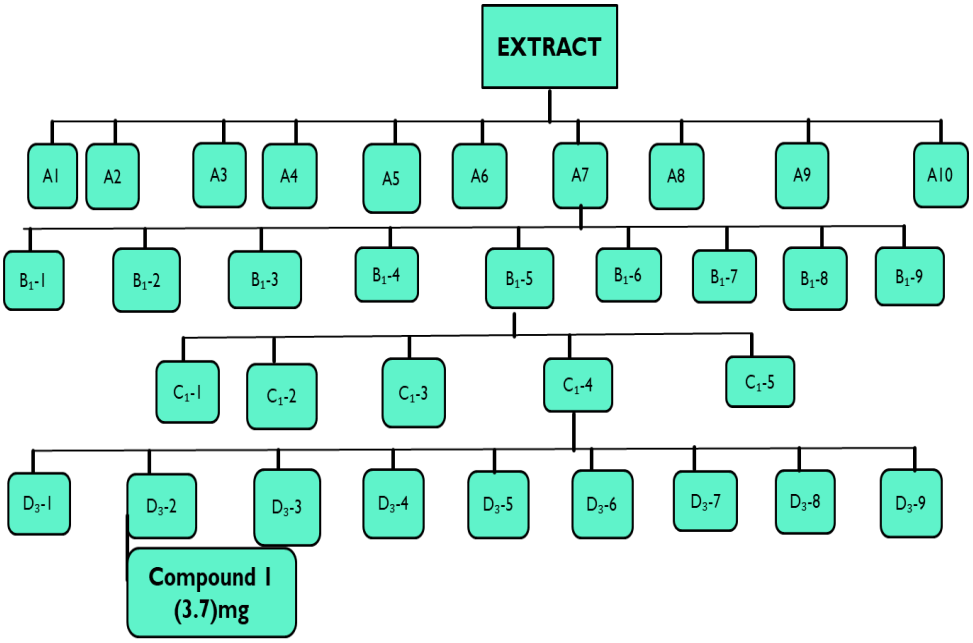
Flash chromatography

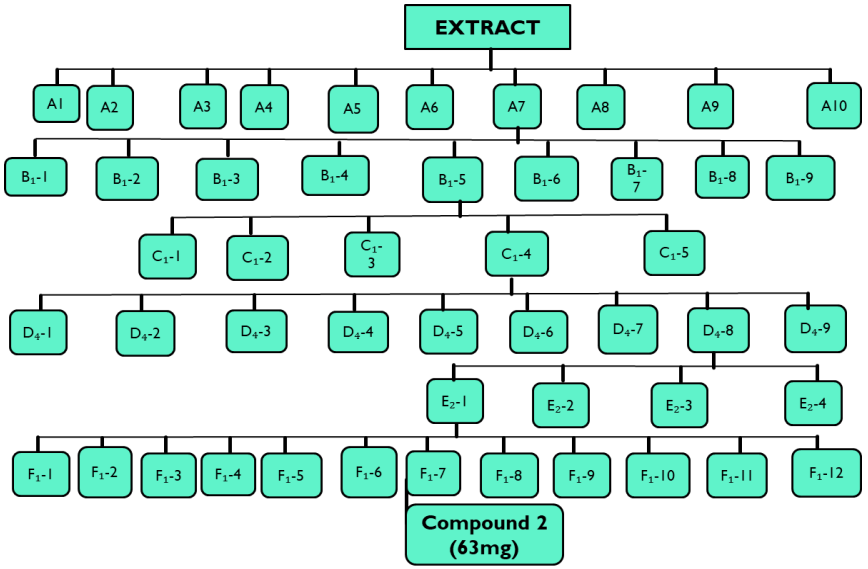
Semi-Preparative HPLC

Normal and Reverse chromatography

- Silica (9385)
- Polyamid (26520-025)
- C-18 Column (Kinetex 5u XB-C18 100ª, 250-21.2mm)
- Solvent: increasing polarity

Isolation of two Abietane diterpenoids





Flash chromatigarphy

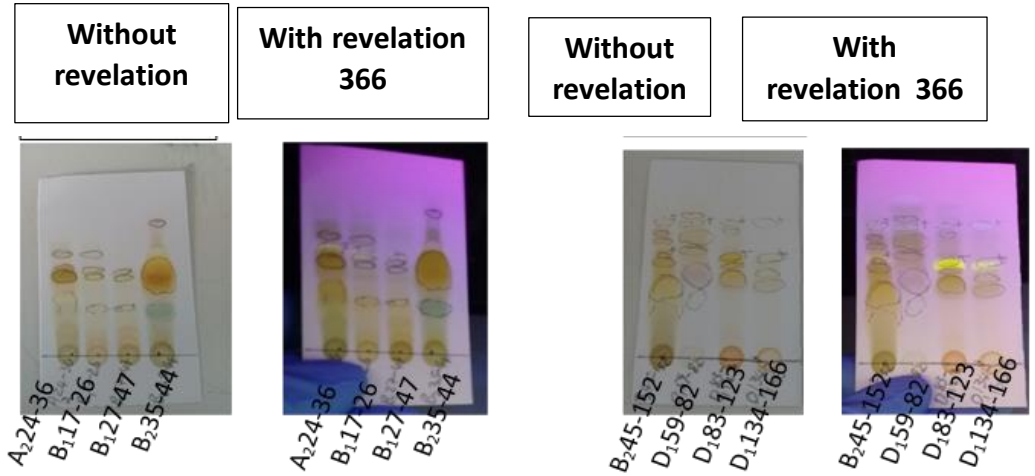
- Polyamid, 100g
- Eluent: 0:12% MeOH in DCM

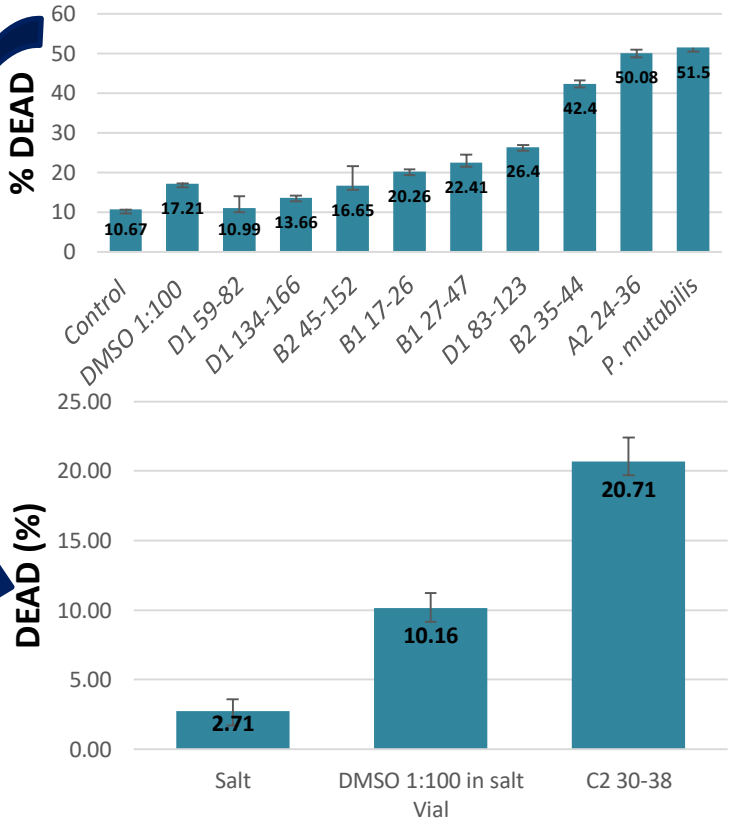
Semi-preparative HPLC

- 53% aqueous acetonitrile
- C18 column (kinetex 5u XB-C18 100A, 250 x 21.2mm)

Biological activity: Column fractions

General toxicity –*Artemia salina* model

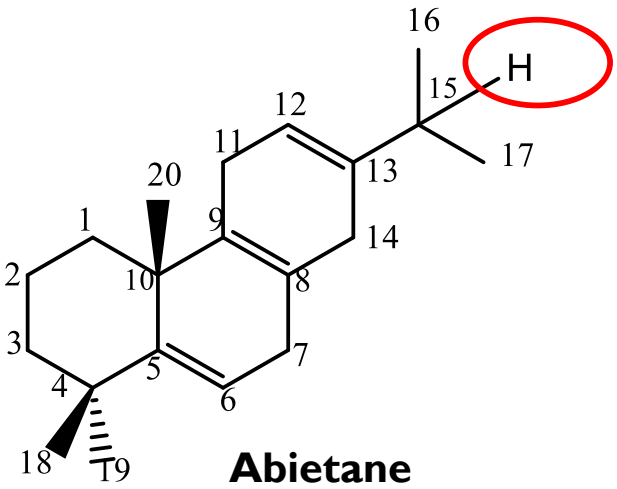




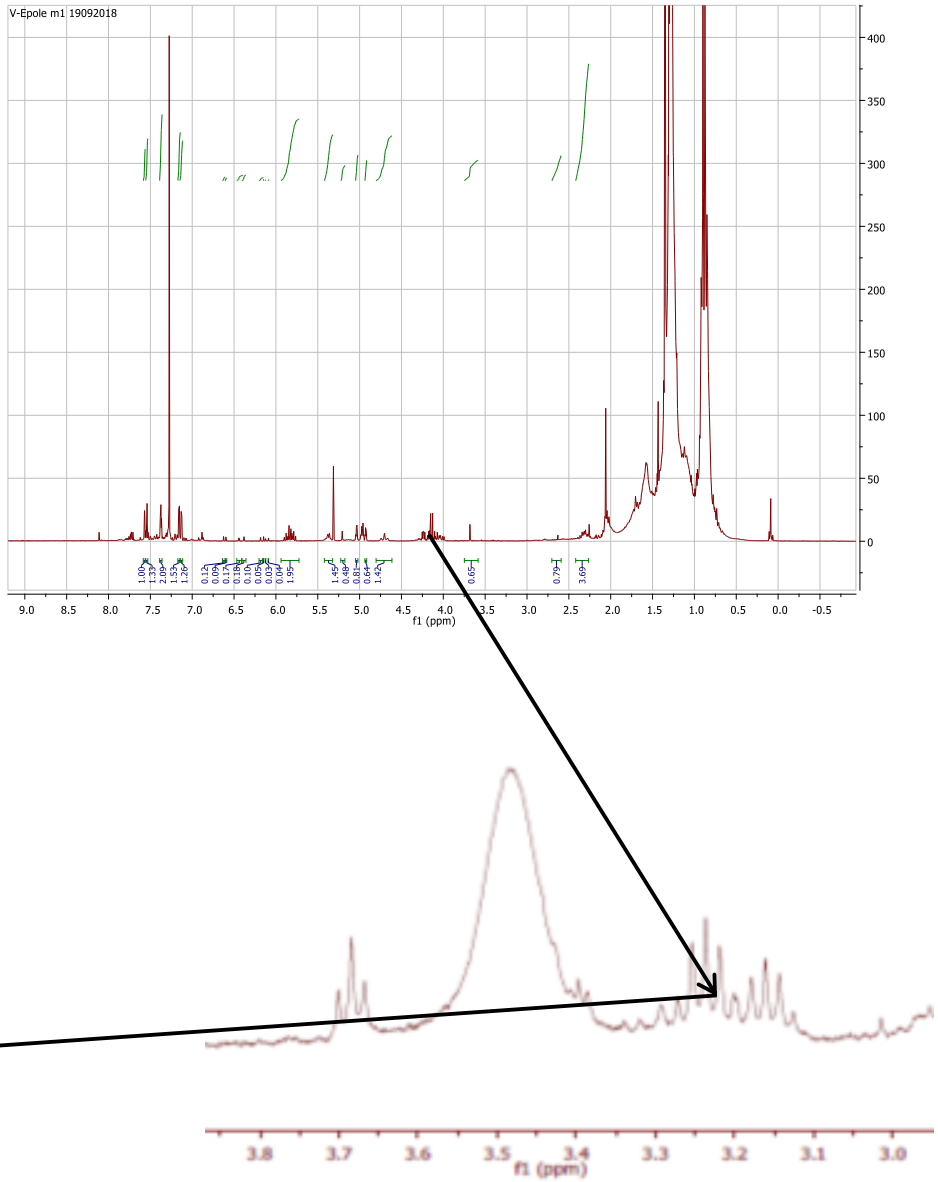
Screening of the *P. mutabilis* column fractions for general toxicity at a concentration of 10 ppm using the *Artemia salina* test

Results and discussion

^{1}H-NMR column fraction

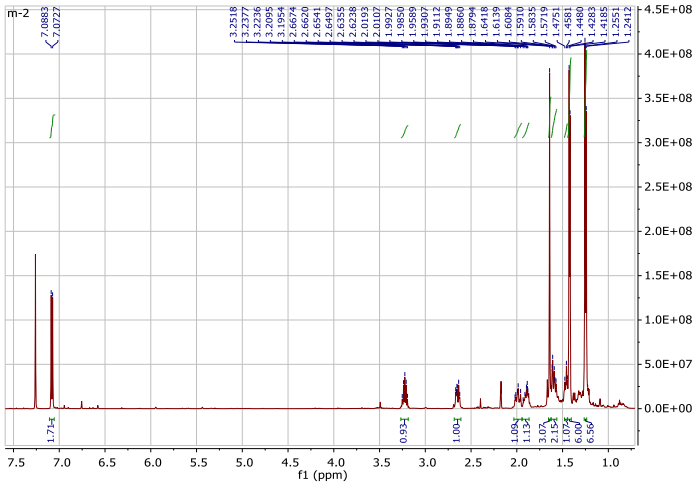


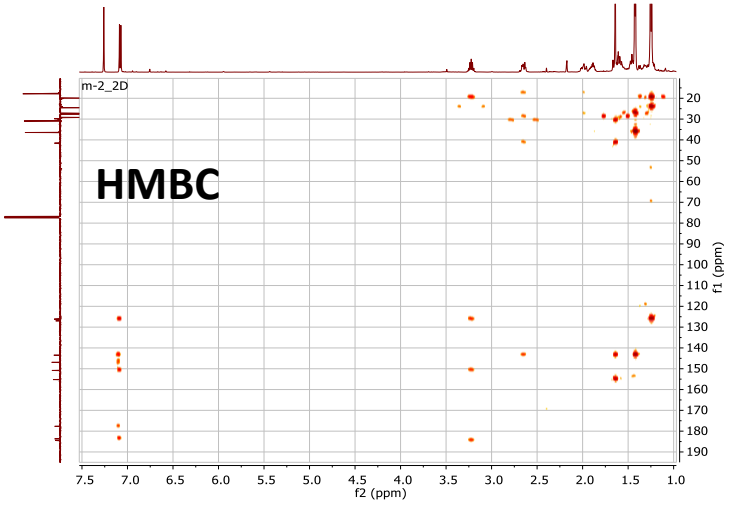
Abietane skeleton



Characteristic for Abietane compound

Characterization of isolated compounds

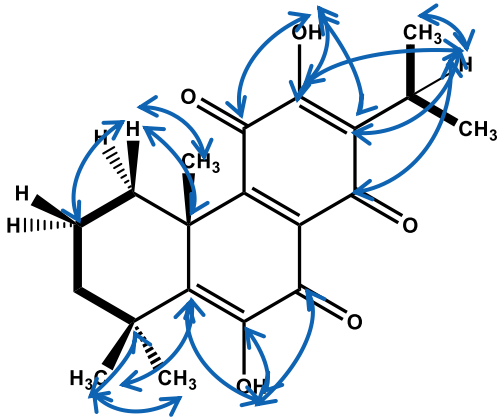




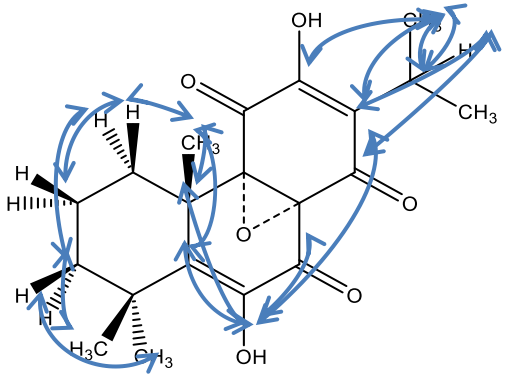
Structure elucidated

1D and 2D NMR

- ^{1}H, ^{13}C, HMBC, HSQC, COSY, NOESY
- Available Literature



Coleon U quinone (1)



8α,9α-Epoxycoleon U quinone (2)

Coleon U quinone (1) cytotoxic study

Coleon U quinone: Cytotoxicity study, IC50 (µg/mL) Colo 205, Colo 320 cell lines.

	Colo 205	Colo 320	MRC-5
Compound **1**	75.17	45.71	105.958
Cisplatin	31.94 ± 2.17	4.81 ± 0.68	12.41 ± 0.367

Human colonic adenocarcinoma cell lines Colo 205 doxorubicin-sensitive (ATCC-CCL-222), Colo 320/MDR-LRP multidrug resistant overexpressing ABCB1 (MDR1)-LRP (ATCC-CCL-220.1), MRC-5 human embryonal lung fibroblast cell lines (ATCC CCL-171)

- **Moderate cytotoxicity with selectivity towards the resistant cell lines**
- **Not a substrate for Pgp**

Flow cytometric assay

Effect of Coleon U quinone (1) on reversal of multidrug resistance (MDR) on human ABCB1 gene-transfected Human colonic adenocarcinoma cell lines (Colo 320/MDR-LRP)

Samples	conc. µg/ml	FSC	SSC	FL-1	FAR
Compound 1	5	1822	857	2.26	0.41
	10	1847	891	1.95	0.36
	15	1781	920	1.83	0.34
Verapamil	10	1843	900	26.2	4.80
DMSO	1.50%	1833	882	3.41	0.63

- FSC: Forward scatter count of cells in the samples (cell size ratio);
- SSC: side scatter count of cells in the samples;
- FL-1: mean fluorescence intensity of the cells.
- FAR: fluorescence activity ratio

- Compound **1** does not inhibit the ABCB1 gene at all concentrations tested
- Intracellular accumulation of the ABCB1 substrate rhodamine 123.

Conclusions

- *P. mutabilis*: a source of abietane diterpenoid
  - Coleon U quinone (**1**)
  - 8α,9α-Epoxycoleon U quinone (**2**)

- Coleon U quinone
  - Moderate cytotoxicity in Human cancer and normal cell lines
  - selectivity towards the resistance cancer cell lines
  - not a substrate for P-gp
- Further studies ongoing

Acknowledgments

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STRATAGEM
CA17104

Short Term Scientific Mission (STSM),
COST ACTION: CA17104: New diagnostic and therapeutic tools against multidrug resistant

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