Biomedical Applications of *Inula* species

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MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR
POPH POTENCIAL HUMANO
COMPETE PROGRAMA OPERACIONAL FACTORES DE COMPETITIVIDADE
QREN QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL PORTUGAL 2007.2013

Introduction: genus *Inula*

- The genus *Inula* (from Asteraceae family) comprises more than one hundred species.

Egyptian/Assyrian pharmacotherapy: used to treat neoplasm, wound, freckles and dandruff

Traditional Chinese medicines: used as expectorant, antitussive, diaphoretic, antiemetic, and bactericide

Ayurvedic and Tibetan traditional medicines: to treat bronchitis, diabetes, fever, hypertension and inflammations

Moroccan folk medicine as antihelmintic, diuretic, anti-anemic and anti-rheumatic

Comercial applications of *Inula* species

I. japonica Thunb. on "Huang-Lian-Shang-Qing" tablets treating headache, toothache, tinnitus, and constipation

I. helenium L. on the antiulcerous drug Ventrofit

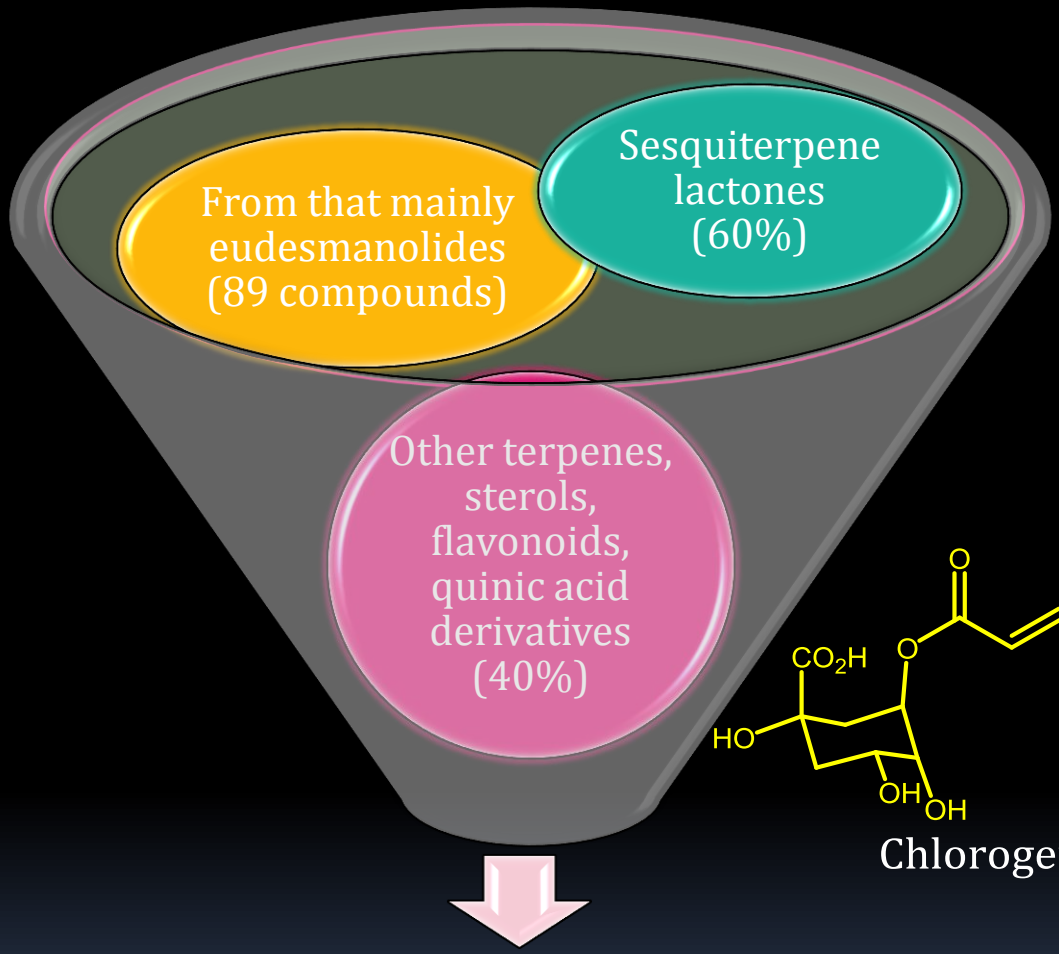
Herbal preparations/ supplements with *Inula* species

I. helenium L. and *I. racemosa* Hook.f. are cited in the European pharmacopoeias as Radix Helenii (Netherlands) and Rhizoma Helenii (Germany)

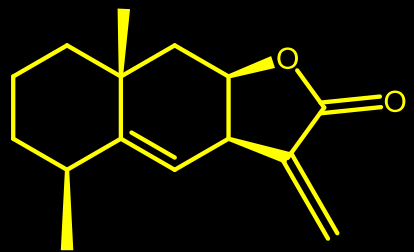
I. helenium L. on Pancreophyt, Relaxing Balm® and Syrup of smokers®

Pinto *et al.*, *J. Ethnopharmacol.* **2014**, 154, 286

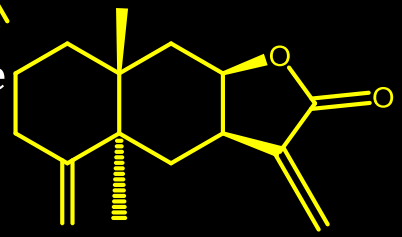
Secondary metabolites in *Inula* genus



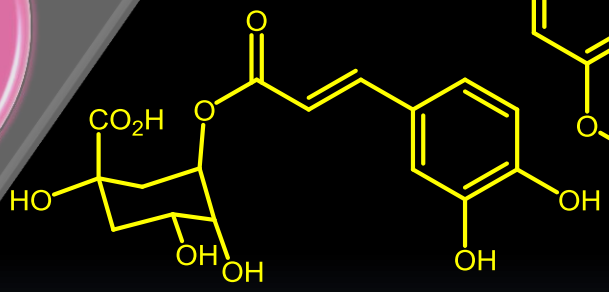
Nearly 400 secondary metabolites, including more than 100 new natural products.



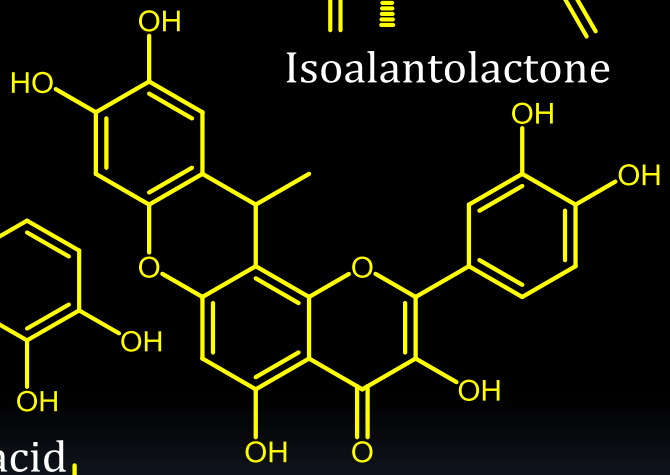
Alantolactone



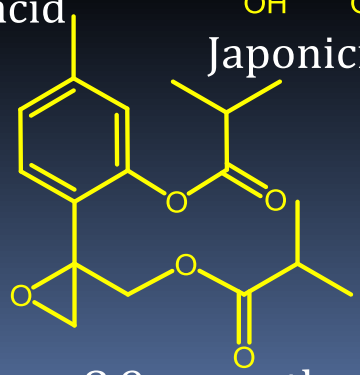
Isoalantolactone



Chlorogenic acid



Japonicin A



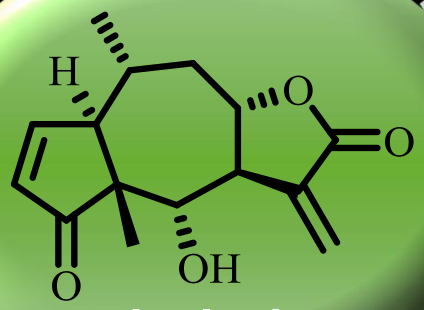
10-Isobutyryloxy-8,9-epoxythymol isobutyrate

Pinto *et al.*, *Chem. Biodivers.* **2014**, *in press*

Bioactive secondary metabolites

Isolated from petroleum ether extract of *I. helianthus-aquatica* leaves and flowers

Anti-inflammatory activity by NO inhibition in LPS-stimulated RAW264.7 cells (IC_{50}=0.9 µM, aminoguanidine 7.9 µM)][2]



Bigelovin

Inhibitory activity against tumor cell line U-937 IC_{50}= 0.45µM[1]

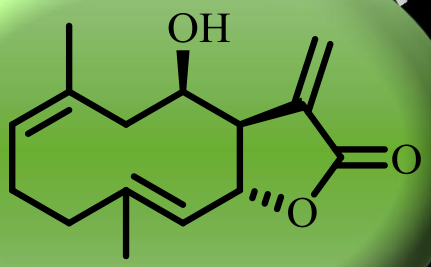
Inhibitory mechanism involves induction of apoptosis and cell cycle arrest at G0/G1 phase[1]

[1]Zeng *et al.*, *Phytother. Res.* **2009**, 23, 885
[2]Qin *et al.*, *J. Nat. Prod.* **2011**, 74, 1881

Bioactive secondary metabolites

Isolated from aerial parts of *I. japonica* and *I. britannica var. chinensis.*

Anti-inflammatory activity by NO inhibition in LPS-stimulated RAW264.7 cells (IC_{50}=2.39 μM, parthenolide 2.45 μM).



Eupatolide

Inhibitory activity on the LPS-induced NF-*k*B activation (IC_{50} = 1.54 μM, parthenolide IC_{50} 2.97 μM).[1]

Inhibited the production of NO and PGE2 as well as iNOS and COX-2 protein expression in LPS-stimulated RAW 264.7 cells.

Jin *et al.*, *Planta Medica*, 2006, 72, 40

The "Deadly Sins" in herbal research

- How correct is the plant identification?

Examples of poor practice :

1. *Inula linariifolia* Turcz. (the correct spelling is *Inula lineariifolia* Turcz.)
2. *Inula verbascifolia* subsp. *methanea* (it is not an accepted name. It is a synonym of *Inula methanaea* Hausskn.)
3. *Inula royleana* (could be *I. royleana* DC. an accepted name, or *I. royleana* C.B.Clarke a synonym of *Inula racemosa* Hook.f.)
4. *Inula crithmoides* L. (the correct taxonomic identification is *Limbarda crithmoides* (L.) Dumort.)

The Plant List or IPNI or Global Compositae online database

The "Deadly Sins" in herbal research

- How active is the extract?

1. *in vitro* assay ⇒ show cytotoxic or antiproliferative effect
2. NCI assigns a significant cytotoxic effect if $IC_{50} < 30$ µg/mL.

…"sources of anticancer compounds"… $IC_{50} > 54$ µg/mL.[1]

…"show anticancer effect" … $IC_{50} > 200$ µg/mL.[2]

Unacceptable

[1]Merghoub *et al.*, *J. Med. Plants Res.* **2009**, 3, 1045
[2]Mazzio *et al.*, *Phytother. Res.* **2009**, 23, 385

The "Deadly Sins" in herbal research

- How active is the extract?

> "Almost all substances exhibit antioxidant effects depending on the concentration tested"

Methanolic and chloroformic extracts of *I. verbascifolia* (Willd.) Hausskn. :

DPPH activity EC_{50} [illegible] and 23.75 mg/mL.[3]

Unacceptable

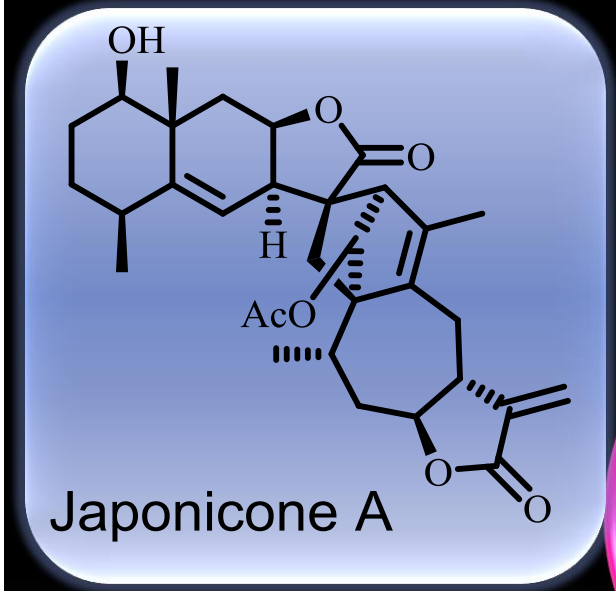
EC_{50}(quercetin) = 1.80 µg/mL

EC_{50}(BHT) = 31.0 µg/mL

Jukic *et al.*, *Nat. Prod. Res.* **2012**, 26, 1703

The "Deadly Sins" in herbal research

- How to ensure the results highest impact



Potent, compared with…??? IC_{50} for a reference drug

Statistical analysis?... At least mean ±SD

Potent cytotoxicity against LOVO, MDA-MB-435 and CEM cells lines

(IC_{50}= 0.256, 0.198 and 0.001 µg/mL, respectively)

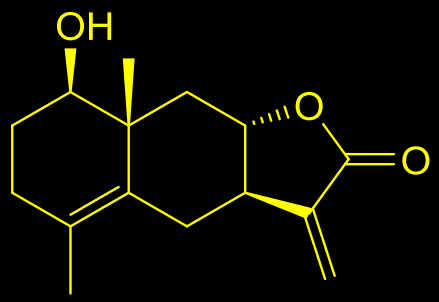
CEM tumor cell line...which? CCRF-CEM? CEM/C2? CEM/SS

Selectivity…?

Activity against non-tumoral cells???

Qin *et al.*, *Bioorg. Med. Chem. Lett.*, **2009**, 19, 710

The "Deadly Sins" in herbal research



8-*epi*-ivangustin

ivangustin

Other less common "deadly sins"	Examples
Compound name	Britanin or Britannin)
Compound structure	8-*epi*-ivangustin
Part of the plant analysed	*I. oculus-christi* with amoebicidal activity

Pinto *et al.*, *J. Ethnopharmacol.* **2014,** 154, 286
Pinto *et al.*, *Chem Biodivers.* **2014,** *in press*

Conclusions

From the 16 *Inula* species used in folk medicine, only 7 had their pharmacological potential validated by *in vitro* or *in vivo* bioassays.

I. royleana and *I. obtusifolia* species have ethnomedicinal use and should undoubtedly be the focus of future research

I. oculus-christi and *I. ensifolia* are species for future phytochemical studies, because their extracts showed very interesting activities

Toxicological evaluation and clinical trials must be done

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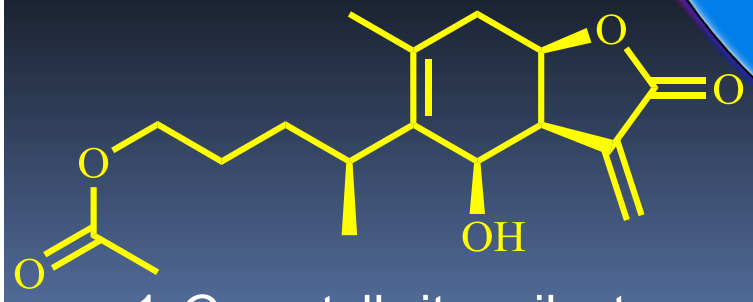
Conclusions

The *in vivo* assay is essential to validate or ascertain their medicinal potentials

The sesquiterpene lactones should be tested against *Plasmodium falciparum*

1-*O*-ABL is the most studied one as anti-inflammatory

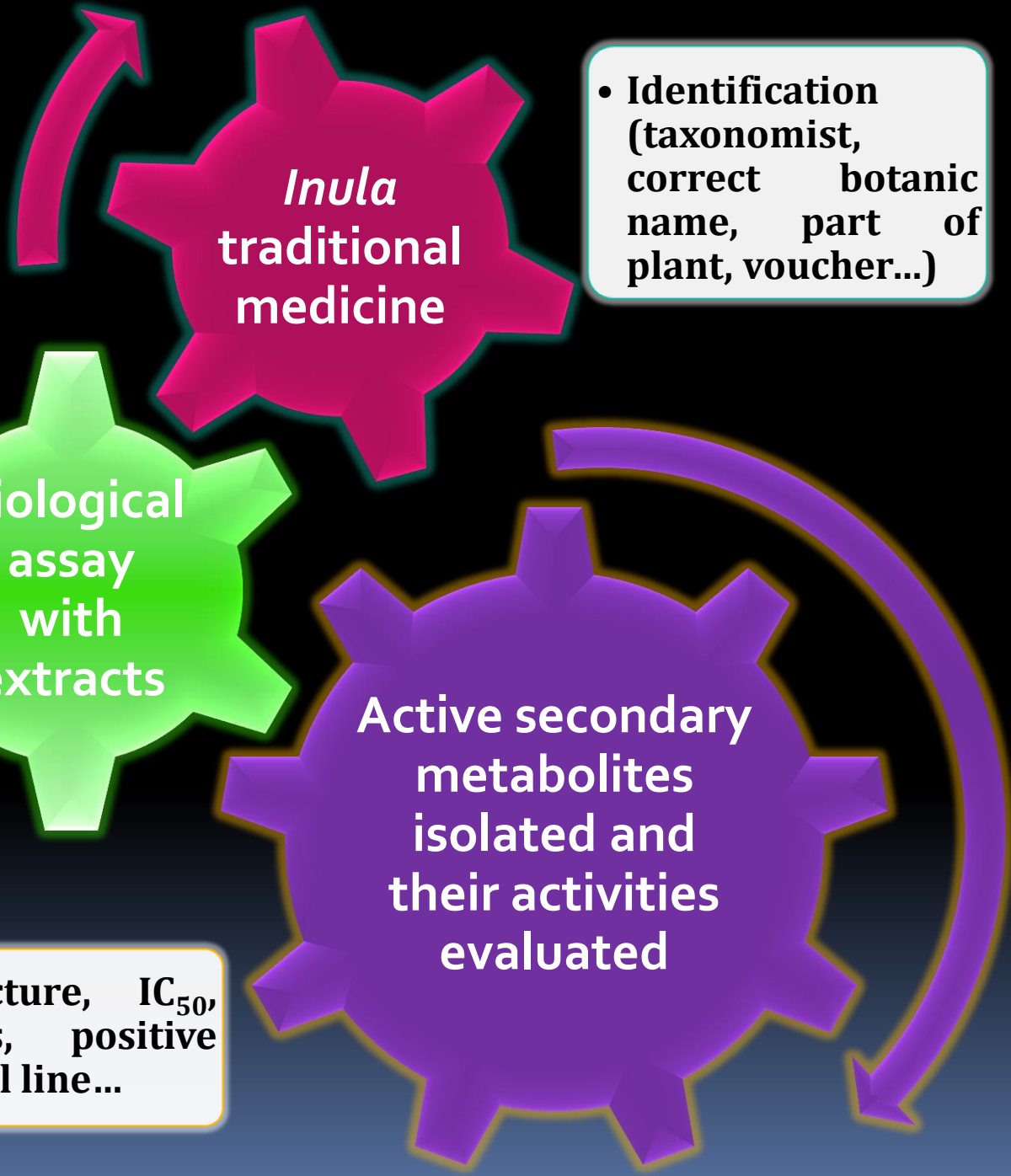
Metabolites from *Inula* species



1-*O*-acetylbritannilactone

Pinto *et al.*, *J. Ethnopharmacol.* **2014**, 154, 286

Conclusions



- Identification (taxonomist, correct botanic name, part of plant, voucher…)
- Tested concentration not too high, drug reference, curve dose-response
- Exact chemical structure, IC_{50}, statistical parameters, positive control, non-tumoral cell line…

Pinto *et al.*, *J. Ethnopharmacol.* **2014**, 154, 286

Acknowledgments

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https://www.facebook.com/josemgpereira.photography

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