Comparative Analysis on Parasite and Host Bioactive Properties — A *Cytinus hypocistis* (L.) L. Case Study

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Background

Cytinus hypocistis (L.) L. is a rootless, stemless, and leafless holoparasite with a vegetative body reduced to an endophytic system that only grows inside the host [1,2]. Although to date, most studies on plant parasitism were focused on nutrient transfer from host to the parasite and the influence of parasites on host plants, a growing number of studies have documented the transfer of non-nutrient molecules.

Cytinus hypocistis

Halimium lasianthum

The transference of phytohormones, secondary metabolites, RNAs, and proteins suggests that hosts may significantly impact parasite physiology and ecology [3].

Aim

The present work main objective was to perform a comparative study on the bioactive properties of the parasite *C. hypocistis* (L.) L. subsp. *macranthus* Wettst and its host species *Halimium lasianthum* subsp. *alyssoides* (Lam.) Greuter.

Methodology

Extracts: Heat-assisted extraction (95 min at 47°C/74% ethanol)

C. *hypocistis* **(CH)**

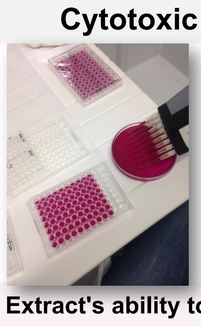
Parasited *H. lasianthum* aerial parts **(PHLAP)**
Parasited *H. lasianthum* root parts **(PHLR)**

Non-parasited *H. lasianthum* aerial parts **(HLAP)**
Non-parasited H. lasianthum root parts **(HLR)**

Cytotoxic and Anti-inflammatory activity

Sulforodamine B

Cytotoxic activity



Tumour cell lines
AGS (gastric adenocarcinoma)
Caco-2 (colorectal adenocarcinoma)
MCF-7 (breast adenocarcinoma)
NCI – H460 (large cell lung cancer)

Non-tumour cell lines
VERO (African green monkey)
PLP2 (porcine liver primary culture)

Extract's ability to inhibit 50% of cell growth

NO Inhibition

Anti-inflammatory activity

Macrophage cells RAW 264.7

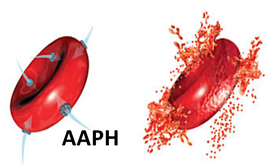
Griess reagent

Extract's ability to inhibit 50% of NO

Antioxidant activity

Oxidative hemolysis inhibition (OxHLIA)

Sheep erythrocytes + Extracts + AAPH



AAPH

AAPH: 2,2′-azobis(2-methylpropionamidine) dihydrochloride

Thiobarbituric acid reactive substances (TBARS)

Pig brain Extracts

Iron sulphate
Ascorbic acid
Malondialdehyde
Thiobarbituric acid

Both assays are used to determine extract's ability to protect cell membranes from lipid peroxidation

Results

Cytotoxic and Anti-inflammatory activity

	CH	PHLAP	PHLR	HLAP	HLR	Positive control
Cell lines	**Cytotoxic activity** (GI_{50}, μg mL^{-1})					Ellipticine**
AGS	20.9 ± 0.9[a]	47.6 ± 0.8[b]	52.7 ± 3.9[c]	23.6 ± 1.1[a]	>400	1.23 ± 0.03
Caco-2	64.1 ± 0.7[c]	41.1 ± 1.1[a]	44.4 ± 1.6[a]	69.7 ± 2.0[d]	55.4 ± 1.2[b]	1.21 ± 0.02
MCF-7	90.1 ± 6.5[c]	53.1 ± 1.9[b]	23.8 ± 0.8[a]	175.4 ± 7.6[d]	50.1 ± 1.2[b]	1.02 ± 0.02
NCI-H460	49.8 ± 3.0[b]	62.4 ± 0.5[c]	19.2 ± 0.4[a]	84.6 ± 4.4[d]	44.0 ± 0.6[b]	1.01 ± 0.01
VERO	286.2 ± 0.8[d]	163.1 ± 10.7[b]	61.1 ± 3.9[a]	158.8 ± 7.1[b]	184 ± 1[c]	1.41 ± 0.06
PLP2	17.9 ± 0.6[a]	42.1 ± 3.4[b]	19.5 ± 2.5[a]	47.6 ± 0.5[c]	20.3 ± 1.5[a]	1.4 ± 0.1
Cell line	**Anti-inflammatory activity** (IC_{50}, μg mL^{-1})					Dexamethasone**
RAW 264.7	75.7 ± 2.4[a]	242.5 ± 14.2[b]	73.1 ± 4.0[a]	223.1 ± 10.8[b]	86.1 ± 4.2[a]	6.3 ± 0.4

The results are presented as mean ± standard deviation and expressed as GI_{50} (extract concentration in μg mL^{-1} responsible for 50% of growth inhibition) or IC_{50} (extract concentration in μg mL^{-1} responsible for 50% inhibition in NO production) values. Different letters correspond to significant differences ($p < 0.05$). **The positive controls (ellipticine and dexamethasone) differ significantly from the plant extracts ($p < 0.05$).

- ✓ **CH** exhibited the best GI_{50} result against **AGS**.
- ✓ The two extracts of the parasited *H. lasianthum* exhibited the best GI_{50} results against Caco-2.
- ✓ **PHLR** extract presented the lowest GI_{50} for **MCF-7** and **NCI-H460**
- ✓ **PLP2 and VERO:** all extracts exhibited cytotoxic effects at **higher concentrations** when compared to **the control.**
- ✓ **CH**, **PHLR**, and **HLR** presented the **best anti-inflammatory activity.**

Antioxidant activity

	OxHLIA (Δ*t* = 60 min) IC_{50}, μg mL^{-1}	TBARS IC_{50}, μg mL^{-1}
CH	7.3 ± 0.3[a]	1.11 ± 0.01[a]
PHLAP	62 ± 2[c]	7.10 ± 0.01[c]
PHLR	307 ± 12[d]	9.5 ± 0.9[d]
HLAP	18 ± 1[ab]	5.7 ± 0.1[b]
HLR	14.0 ± 0.1[ab]	5.3 ± 0.2[b]
Trolox	21.8 ± 0.2[b]	9.1 ± 0.3[d]

The results are presented as mean ± standard deviation and expressed as IC50 values, which correspond to the extract concentration in μg mL^{-1} required to protect 50% of the erythrocyte population from haemolysis for Δt of 60 min or to provide 50% of antioxidant activity during the TBARS assay. Different letters correspond to significant differences ($p < 0.05$).

- ✓ **OxHLIA assay: CH extract** presented the best antioxidant result, with an **IC_{50}** of **7.3 μg mL^{-1}**.
- ✓ **TBARS: CH extract** displayed the **best result,** with an **IC_{50}** of **1.11 μg mL^{-1}**.
- ✓ **CH extracts** exhibited **better results** than the positive control **Trolox.**

Conclusions

To the authors' best knowledge, this is the first report evaluating the cytotoxic, anti-inflammatory, and antioxidant activity of *H. lasianthum*. In absolute terms, the PHLR extract exhibited the lowest GI_{50} for three of the four tumour cell lines. CH was the most antioxidant extract and showed to be the least cytotoxic against the non-tumour cell line VERO. For phenolic profile comparison and bioactivity correlation, further studies on compounds identification will be performed.

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CERTIFICADO DE PARTICIPAÇÃO
PARTICIPATION CERTIFICATE

Certificamos que
We certify that

Ana Rita Santos Silva

participou no **Ciência 2021 - Encontro com a Ciência e Tecnologia em Portugal**, que decorreu de 28 a 30 de junho de 2021 no Centro de Congressos de Lisboa.
participated in the **Ciência 2021 - Science and Technology in Portugal Summit**, from the 28th to the 30th June, 2021, which took place at the Lisbon Congress Centre.

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