PhD School of Veterinary Sciences

XXXI cycle **Curriculum: Basic Sciences**

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Cytotoxic effects of *Artemisia annua* L. and artemisinin on D-17 canine osteosarcoma cell line: an *in vitro* study

- *Artemisia annua* L. is a medical herb that has been used for centuries in Chinese Traditional Medicine.
- *A. annua* contains the sesquiterpene lactone **artemisinin**, which has been found to be particularly active against malaria.
- Recently, several researches have been focused on the possibility of using extracts of this plant in anticancer treatments.

In veterinary medicine, canine osteosarcoma represents a highly aggressive tumor that often leads to therapeutic failure due to lung metastasis and development of chemo-resistance, highlighting the urgent need of novel therapies.

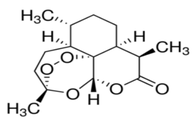
AIMS

Pure artemisinin and a commercial **hydroalcoholic extract** obtained from *A. annua* dried leaves **have been tested on canine osteosarcoma cell line (D-17) with the aim to evaluate their cytotoxicity and anti-proliferative effects**. The last aim was to optimize an analytical protocol for the determination of intracellular iron in D-17 cells.

MATERIALS & METHODS

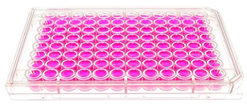
Hydroalcoholic extract

or



Pure Artemisinin

Treatment for 24 h



D-17 canine osteosarcoma cell line

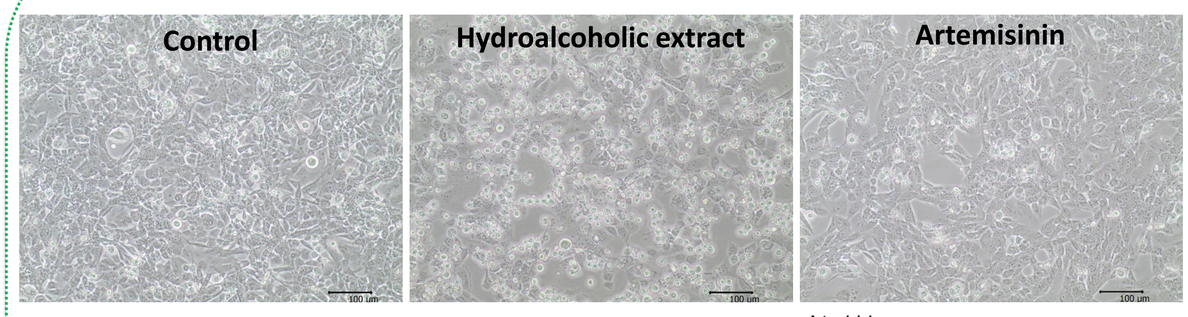
MTT Assay to obtain IC_{50}

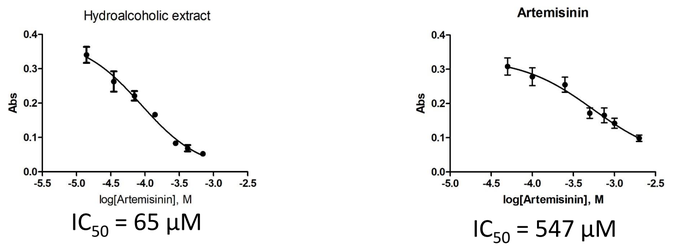
Cell Cycle Analysis by flow cytometry

Atomic Absorption Spectrometry for iron analysis

RESULTS

D-17 canine osteosarcoma cell line

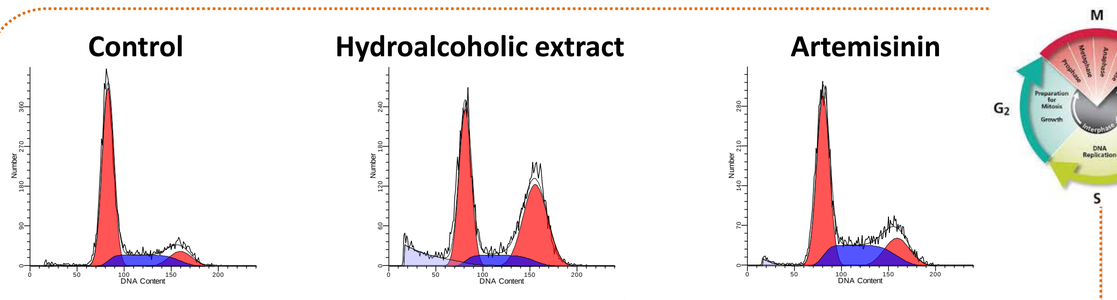




IC_{50} = 65 µM

IC_{50} = 547 µM

Artemisinin and hydroalcoholic extract exerted cytotoxic effect in a dose-dependent manner.



Pure artemisinin caused an increase of cells in S phase, while hydroalcoholic extract induced an evident block in G2/M.

Fe	
	ng/million D-17 cells
Control	45±6
Hydroalcoholic extract	32,3±3,2
Artemisinin	34,9±5,1

A decrease of iron concentration in D-17 cells treated with pure artemisinin and hydroalcoholic extract compared to untreated cells was measured.

CONCLUSION

Though preliminary, these data indicated that hydroalcoholic extract showed, *in vitro*, much more potent cytotoxic activity than artemisinin, supporting the synergistic effect of the phytocomplex. Better characterization of the phytoextract, especially regarding the accurate quantification of artemisinin and other active compounds are in progress.

Biochemical characterization of algae used as herbal and nutritional supplements

Spirulina maxima
green-blue microalgae, belonging to *Cianobacterya*
important source of proteins, essential elements, vitamins and antioxidant

Ascophyllum nodosum
brown algae (*Pheophyta*) of the *Fucaceae* family
utilyzed in veterinary medicine to reduce plaque and halitosis

Gracilaria spp.
red algae (*Rhodophyta*)
notable for its economic importance as an agarophyte

Ulva spp.
green macroalgae (*Chlorophyta*) of the *Ulvaceae* family
Used in cosmetic treatments and thalassotherapy

AIM

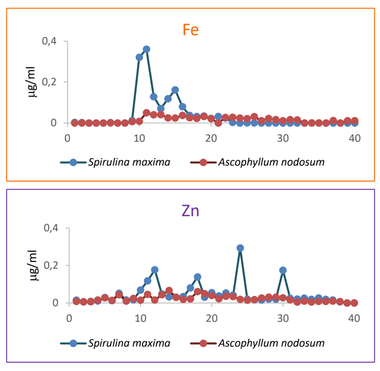
The aim of this preliminary research **was to analyse trace metal concentrations and to isolate metalloproteins in samples from different micro** (e.g. *Spirulina spp.*) **and macroalgae** belonging to the divisions *Rhodophyta*, *Pheophyta* and *Chlorophyta*.

RESULTS

Metals	*Spirulina Maxima*	*Ascophyllum nodosum*	*Gracilaria spp*	*Ulva spp*
Fe	517,936	423,459	3415,8	1168,3
Zn	16,822	23,175	20,41	8,565
Mn	21,664	19,923	5479,3	68,043
Cu	0,654	1,767	6,404	3,843
Al	21,16	67,289	1459,1	436,011
As	0,559	31,003	10,195	4,564
Cd	0,013	0,343	0,118	0,094
Pb	0,13	0,205	5,611	2,017
Hg	nd	0,031	0,025	0,007

Metal concentrations expressed as mg/Kg dry weight.

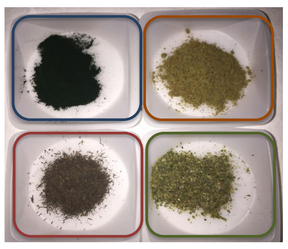
Essential trace elements Toxic trace elements



Trace metals profiles after gel filtration chromatography on Sephadex G-75

MATERIALS & METHODS

Protein extraction



Homogenization and centrifugation in Tris HCl 20 mM, pH 8

Protein separation

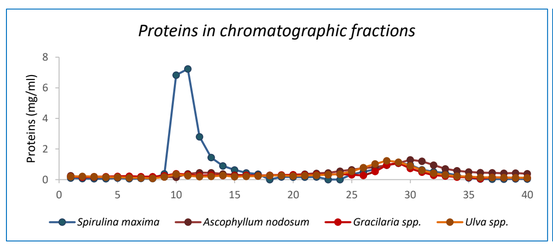
Gel-filtration chromatography (Sephadex G75)

Chromatographic fractions

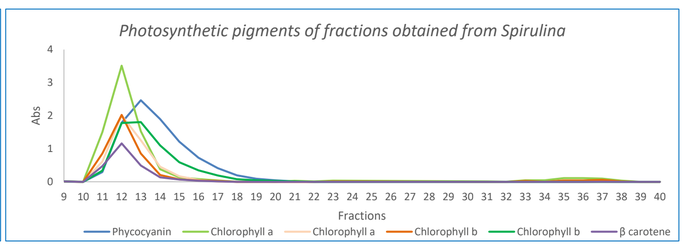
Analytical methods

Uv Spectrometry for protein quantification at 280 nm

AAS and ICP-MS for trace element analysis



Profiles of total proteins and other uv-absorbing molecules after gel filtration chromatography on Sephadex G-75



Different photosynthetic pigments occur among the algae: chlorophylls, carotenoids and phycobilins. In *Spirulina*, the phycocyanin peak is overlapping with the peak of high molecular weight proteins.

CONCLUSION

These preliminary data showed great variability in the chemical composition in micro and macro algae, especially regarding trace metals and proteins. In particular, *Spirulina* contained high concentrations of trace metals and proteins. However, toxic traces metals, e. g. Pb, Cd and As, have been found in the algae examined. Given the growing interest in algae, further studies are needed to deepen the knowledge regarding their bioavailability, thus allowing a targeted use in the field of nutrition.

Papers:

- Martina Bertocchi, Gloria Isani, Federica Medici, Giulia Andreani, Irvin Tubon Usca, Paola Roncada, Monica Forni, Chiara Bernardini; *Anti-inflammatory activity of Boswellia serrata extracts: an in vitro study on porcine Aortic Endothelial Cells* «OXIDATIVE MEDICINE AND CELLULAR LONGEVITY», 2018 *In Press*
- Martina Bertocchi, Federica Medici, *Caratterizzazione, attività antiinfiammatoria in vitro e in vivo di estratti di Boswellia serrata Roxb. e applicazioni in medicina veterinaria* «NATURAL 1», 2018, pp. 58 – 67.
- Beghelli, Daniela; Isani, Gloria; Roncada, Paola; Andreani, Giulia; Bistoni, Onelia; Bertocchi, Martina; Lupidi, Giulio; Alunno, Alessia, *Antioxidant and Ex Vivo Immune System Regulatory Properties of Boswellia serrata Extracts*, «OXIDATIVE MEDICINE AND CELLULAR LONGEVITY», 2017, pp. 1 – 10
- Bernardini Chiara; Zannoni Augusta; Bertocchi Martina; Bianchi Francesca; Salaroli Roberta; Botelho Giuliana; Bacci Maria Laura; Ventrella Vittoria; Forni Monica, *Deleterious effects of tributyltin on porcine vascular stem cells physiology*, «COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY. C. TOXICOLOGY & PHARMACOLOGY», 2016, 185-186, pp. 38 - 44

Oral comunications:

- Martina Bertocchi, Chiara Bernardini, Giulia Andreani, Giovanna Farruggia, Concettina Cappadone, Monica Forni, Isani Gloria; ***S**tudio preliminare in vitro dell'attività di Artemisia annua L.* «PIANTE MEDICINALI», 2018 *In Press* [XXVI Congresso di Fitoterapia, Fiuggi 26-28 maggio 2018]
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Posters & Abstracts (2017-2018):

- Federica Medici, Martina Bertocchi, Enea Ferlizza, Giorgio Fedrizzi, Giulia Andreani, Gloria Isani; Caratterizzazione biochimica dell'alga bruna *Ascophyllum nodosum* e valutazione della sua attivita' sulla salute del cavo orale nel cane «PIANTE MEDICINALI», *In Press* [atti di: XXVI Congresso di Fitoterapia, Fiuggi 26-28 maggio 2018]
- Martina, Bertocchi; Chiara, Bernardini; Alba Chiara Abbruciati, ; Giulia, Andreani; Giovanna, Farruggia; Concettina, Cappadone; Monica, Forni; Isani, Gloria, *Cytotoxic effects of Artemisia annua L. and Artemisinin on D-17 Canine Osteosarcoma cell line: an in vitro study*, [atti di: VI SYRP: S.I.Fit. Young Researchers Project, Republic of San Marino, 20 November 2017]
- Giulia Andreani, Martina Bertocchi, Alba Chiara Abbruciati, Enea Ferlizza, Augusta Zannoni, Monica Forni, Cinzia Benazzi, Gloria Isani, *Trace metal concentrations in laying hens fed a feed supplemented with anti-inflammatory phytoextracts,* [atti di: Scientific Meeting AISETOV 2017, Reggio nell'Emilia 20 ottobre 2017]
- Federica, Medici; Martina, Bertocchi; Enea, Ferlizza; Giulia, Andreani; Gloria, Isani, *Trace metals in Algae used as herbal and nutritional supplements*, [atti di: VI SYRP: S.I.Fit. Young Researchers Project, Republic of San Marino, 20 November 2017]
- Chiara, Bernardini; Augusta, Zannoni; Martina, Bertocchi; Irvin, Tubon; Monica, Forni, *In vitro proangiogenic effect of vascular wall progenitor cell secretome*, [atti di: ABCD Congress, Bologna, Italy, 21-23 September 2017]
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- Bertocchi, Martina; Medici, Federica; Bernardini, Chiara; Roncada, Paola; Forni, Monica; Andreani, Giulia; Isani, Gloria, *Characterization of Boswellia serrata extracts and evaluation of their effects on porcine Aortic Endothelial Cells*, «REVIEWS ON CLINICAL PHARMACOLOGY AND DRUG THERAPY», 2017, 15, pp. 42 - 42 [atti di: The 21th International Congress Phytopharm 2017, 2-5 July 2017 Graz, Austria]
- Isani, Gloria; Andreani, Giulia; Ferlizza, Enea; Scozzoli, Maurizio; Bertocchi, Martina; Zannoni, Augusta; Forni, Monica; Benazzi, Cinzia, *Attività antinfiammatoria di un mangime complementare addizionato con estratto secco di Boswellia serrata e Salix alba in galline ovaiole*, «PIANTE MEDICINALI», 2017, 16, pp. 64 - 65 [atti di: XXV Congresso di Fitoterapia, Arezzo 26-28 maggio 2017]