

Evaluation of the anti-cancer activity of Goji leaves extracts against HT-29-MTX colon cancer cell line

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LYCIUM Chinense.

LYCIUM de la Chine .

Aim and objectives

- The *purpose* of this study is to investigate the anti-tumoral effect of Goji leaves extract on the HT29-MTX colon cancer cell line.
- By examining key cellular events such as cell viability, inflammation, oxidative stress, and apoptosis, this study aimed to elucidate the underlying mechanisms of action of Goji leaves extract in inhibiting colon cancer cell growth.

Aim and objectives

In order to achieve this purpose, several *research questions* have been proposed to be solved during this thesis:

- a) What is the effect of different concentrations of Goji leaves extracts on the viability of HT29-MTX colon cancer cells?
- b) Do the Goji leaves extracts induce oxidative stress in colon cancer cells?
- c) Are any differences between the Goji leaves extracts tested, the method of synthesis being responsible for the anti-tumoral effects?

Colon cancer: brief description

- Colon cancer, is a type of cancer that affects the colon or rectum, begins as a small growth called a *polyp*, which can eventually develop into cancer over time.
- Colon cancer is one of the most common types of cancer worldwide.
- **Risk factors:** a family history of colon cancer or polyps, a sedentary lifestyle, a diet high in red or processed meats, obesity, smoking;
- **Symptoms:** blood in the stool, rectal bleeding, abdominal pain or cramping, unexplained weight loss, weakness or fatigue;
- **Diagnosis:** Colonoscopy, etc;
- **Treatment:** Chemotherapy, Immunotherapy, etc;
- **Prevention:** Regular screenings, Healthy lifestyle choices, Genetic counselling, Engaging in regular physical activity;

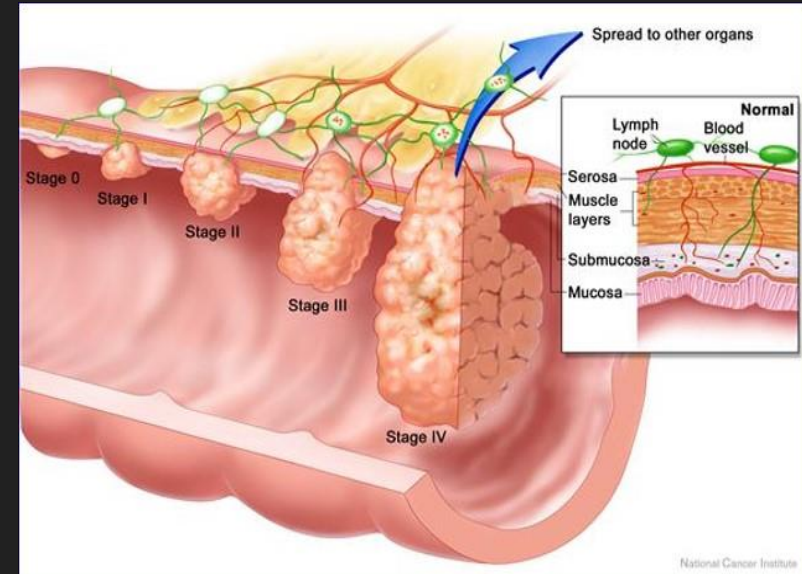


Figure 1. Colorectal cancer stages

(taken from National Cancer Institute).

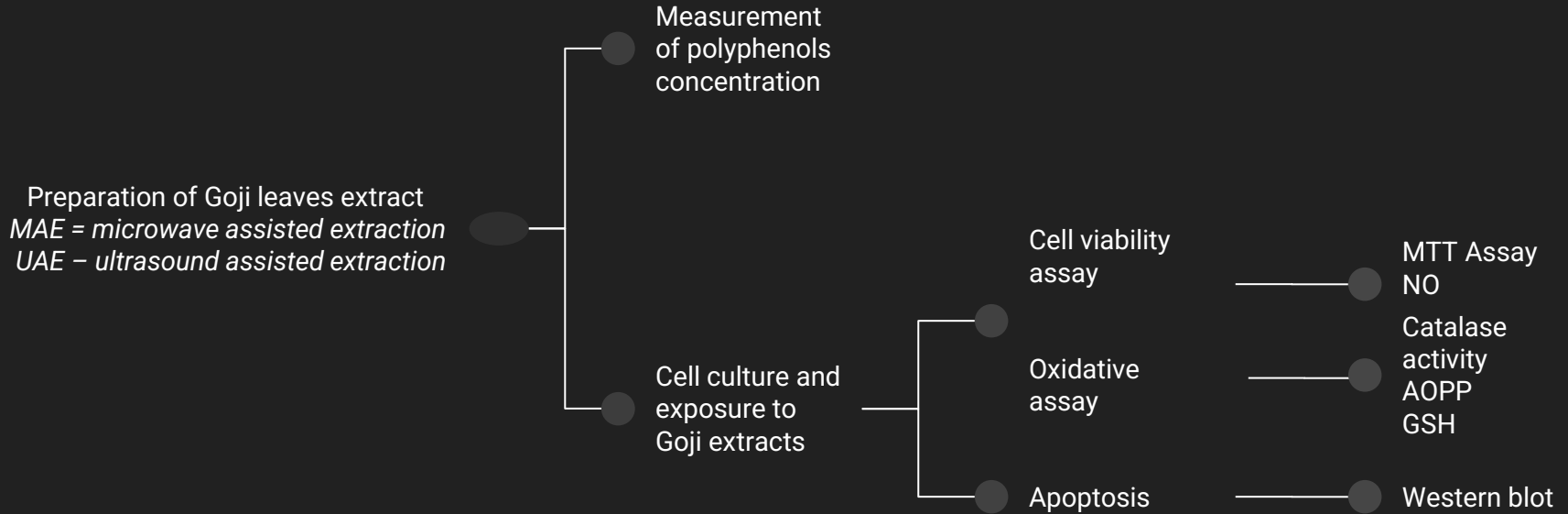
Goji plants & extracts

- Location: China, Mongolia, Asia, etc
- There are two primary species of Goji plants: *Lycium barbarum* and *Lycium chinense*. Both species are used interchangeably and produce similar berries. However, slight variations in taste;
- Health benefits: anti-oxidant properties, immune-boosting potential, potential anti-cancer effects, etc;
- Goji extracts: obtained by water, alcohol extraction, etc;
- Anti-cancer effects: Inhibition of tumor growth, antioxidant and anti-inflammatory actions, etc.



Figure 2. Illustration of different species of Goji plants: *Lycium chinense*

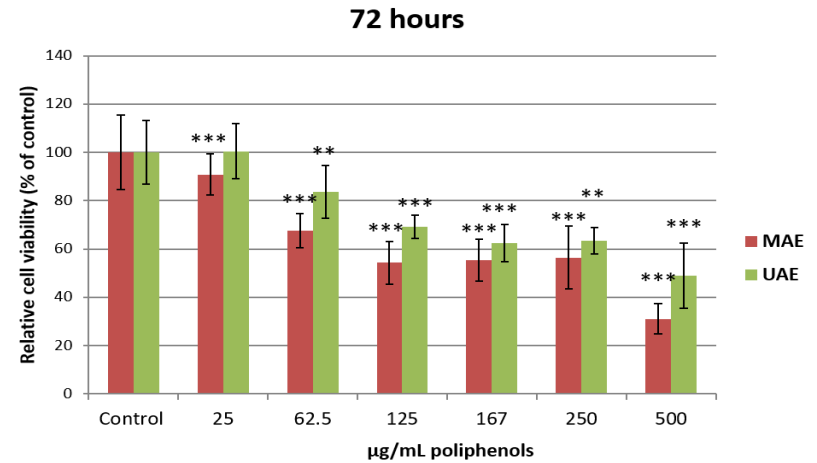
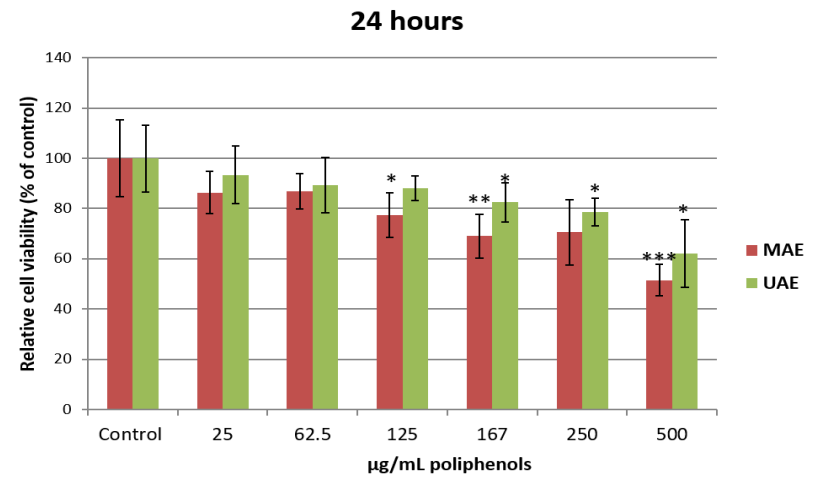
Materials and methods:



RESULTS: *cell viability analysis*

- a decrease in the number of viable cells, which was dependent to the concentration of polyphenols tested, for both types of extracts;
- incubation time (72 hours) resulted in lower cell viability values compared to the first interval,
- only 31% and 49% of cells being live after exposure to the highest concentration of MAE and UAE, respectively.,
- it was noticed that MAE extract induced a higher degree of cell death compared to UAE, as lower values were measured for MAE incubation after both time periods of exposure.

Figure 3. Cell viability after 24 (a) and 72 (b) hours of incubation of HT29-MTX with different polyphenols' concentrations of MAE and UAE extracts.

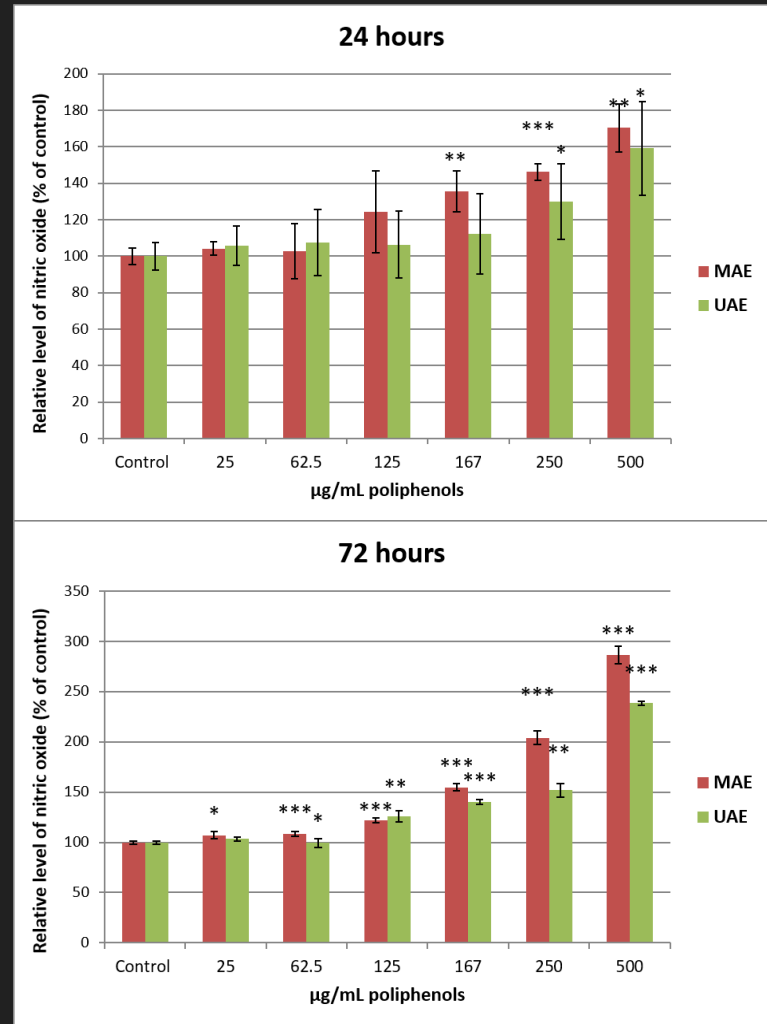


RESULTS: *inflammation analysis*

- There was a NO level increase dependent on the concentration of polyphenols tested, the highest level being recorded for the 500 µg/mL concentration of both types of extracts (MAE and UAE).
- However, the MAE induced a higher level of NO than the UAE extract for both time intervals.
- Moreover, it was noticed a time-dependent increase in the NO level for both extracts.

There is a good correlation between the pattern observed for the cell viability decrease and the that one of NO increase in a concentration-dependent manner, suggesting that both effects, anti-proliferative and inflammatory, were simultaneous induced by the Goji extracts.

Figure 4. Nitric oxide (NO) level after 24 (a) and 72 (b) hours of incubation of HT29-MTX with different polyphenols' concentrations of MAE and UAE extract



Oxidative stress evaluation: catalase activity

- both extracts induced a decrease of catalase activity after both time periods of incubation compared to control;
- there was a slightly lower level after 72 hours compared to that measured after 24 hours in the case of each type of extract;
- the incubation with MAE diminished more the activity of this antioxidant enzyme compared to the other extract (UAE), suggesting that both extracts were able to induce oxidative stress in colon cancer, but MAE had a stronger effect.

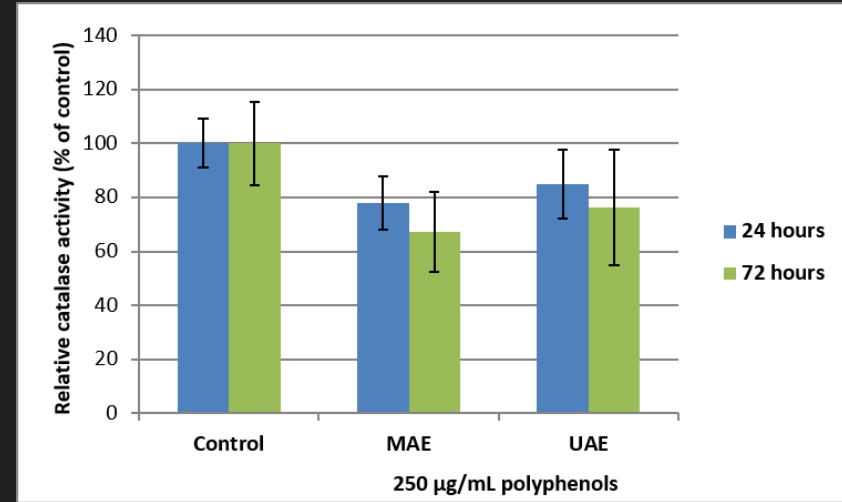


Figure 5. Catalase activity levels after 24 and 72 hours of incubation of HT29-MTX with 250 µg/mL polyphenols' concentrations of MAE and UAE extracts.

Oxidative stress evaluation: protein oxidation

- increase of advanced oxidized protein products (AOPP) was observed after 24 and 72 hours of incubation with Goji extracts compared to control cells.
- It was noticed a time-dependent increase of oxidized proteins, the MAE being able to induce the formation of a higher degree of protein oxidation in comparison with UAE, at same concentration of polyphenols (250 µg/mL).
- This result could be possible grace to the chemical composition of the extract, including polyphenols, which generated reactive oxidative species that attacked the proteins and oxidized them.
- If a protein is oxidized, this will lose its function, and the cellular processes will be affected. This could be one way to limit cancer cell proliferation and to induce their death.

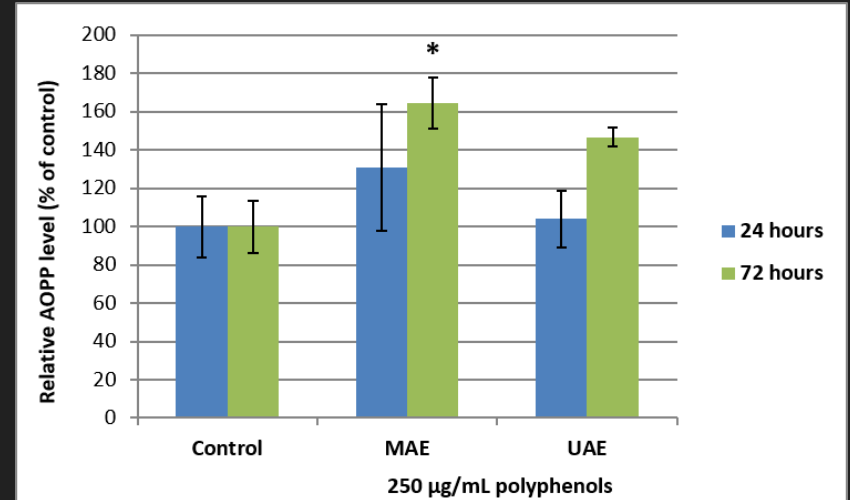


Figure 6. advanced oxidized protein products (AOPP) levels after 24 and 72 hours of incubation of HT29-MTX with 250 µg/mL polyphenols' concentrations of MAE and UAE extracts.

Oxidative stress evaluation: GSH level

- an increase in the case of MAE extract compared to control, suggesting that cancer cells tried to fight with oxidants from Goji extracts. This could be one explanation for the cancer cell that were able to survive and did not die after the exposure to extract.
- In the case of UAE extract, there was a slightly decrease of GSH level after 24 hours, but this increased above control after 72 hours. This could mean that there was a temporary fluctuation in the concentration of GSH due to cell adaptation to extract, explaining also the cancer cell resistance to treatments.

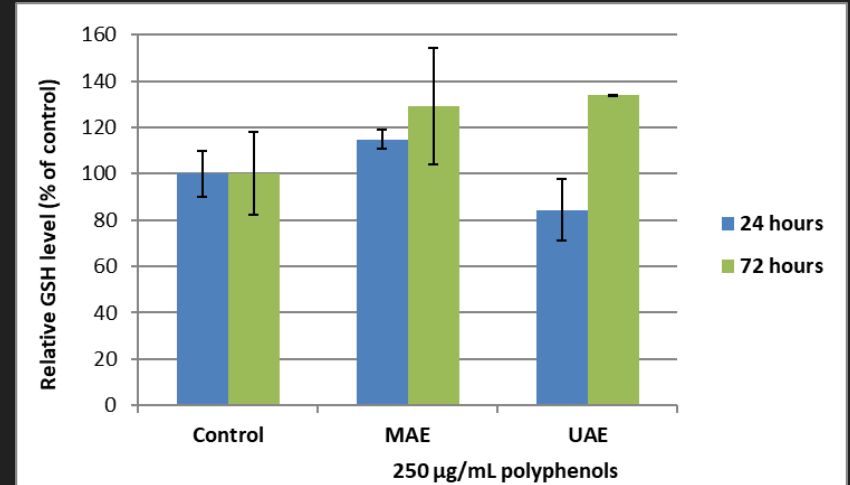


Figure 7. reduced glutathione (GSH) levels after 24 and 72 hours of incubation of HT29-MTX with 250 µg/mL polyphenols' concentrations of MAE and UAE extracts.

Apoptosis evaluation:

- The observed changes in p53 expression suggest a complex interplay between the extracts and cellular processes related to cell viability, inflammation, and oxidative stress.
- Further studies are warranted to elucidate the underlying molecular mechanisms and evaluate the overall impact of these extracts on cellular health.
- Overall, the differential effects of the MAE and UAE extracts from Goji leaves on p53 expression in HT29-MTX cells provide valuable insights into the potential anti-cancer effect induced by these extracts.

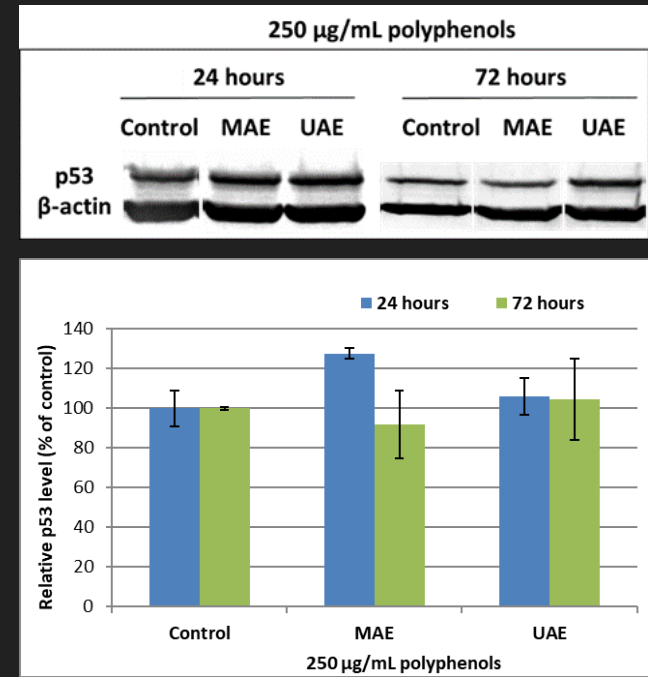


Figure 8. Representative Western blot images and the p53 expression levels obtained after the 24- and 72-hours exposure of HT29-MTX cells to 250 µg/mL polyphenols of MAE and UAE extracts.

Conclusion:

- In summary, the findings suggest that the MAE extract from Goji leaves exhibits potential anticancer activity in colon cancer cells, as evidenced by the upregulation of p53 expression, decreased cell viability, and the induction of oxidative stress and inflammation.
- However, the observed downregulation of p53 expression after 72 hours indicates the complexity of cellular responses over time and highlights the need for further investigations to fully understand the underlying mechanisms.
- These findings provide valuable insights into the potential therapeutic effects of Goji extracts in targeting colon cancer cells and warrant further exploration in the context of cancer treatment and prevention.
- From the results, an anti-proliferative effect was obtained from both extracts of Goji leaves tested, but with a better activity in the case of MAE.

Thanks for your attention!