DEFLAMIN ISOLATED FROM Lupinus mutabilis SEEDS INHIBITS COLON CANCER CELL INVASION

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INTRODUCTION

The search of food-borne matrix metaloproteinase inhibitors (MMPIs) holds great value due to their effectiveness in reducing cancer development and metastization, in particular the inhibition of the MMP subgroup gelatinases (MMP-2 and MMP-9) that are involved in colorectal cancer (CRC).

Our research group has found a natural MMPI protein (~17KDa) in *Lupinus albus* seeds with anti-cancer and anti-inflammatory activities named deflamin. Since within other legume seeds species, *L.albus* was the one with higher MMPI potential, the search of deflamin in other lupin species is of high interest, specially *L.mutabilis* due to its adaptation to harsh and unique enviromental conditions. Hence our main goal was to attempt to isolate deflamin from *L. mutabilis* and determine its potential against colon cancer cell invasion.

MATERIALS AND METHODS

- Seeds of different *L.mutabilis* varieties were used and deflamin was extracted and isolated through a method developed in our laboratory adapted to this protein's features such as resistance to high temperature and low pH as well as a high water solubility.
- *L. mutabilis* deflamin's polypeptide profile was identified through SDS-PAGE in reductive and non-reductive conditions and compared with *L.albus* deflamin in the same conditions as described.
- Anti-cancer invasion activities were determined in colon adenocarcinoma cells (HT29) using the wound healing assay.

RESULTS

Deflamin Isolation

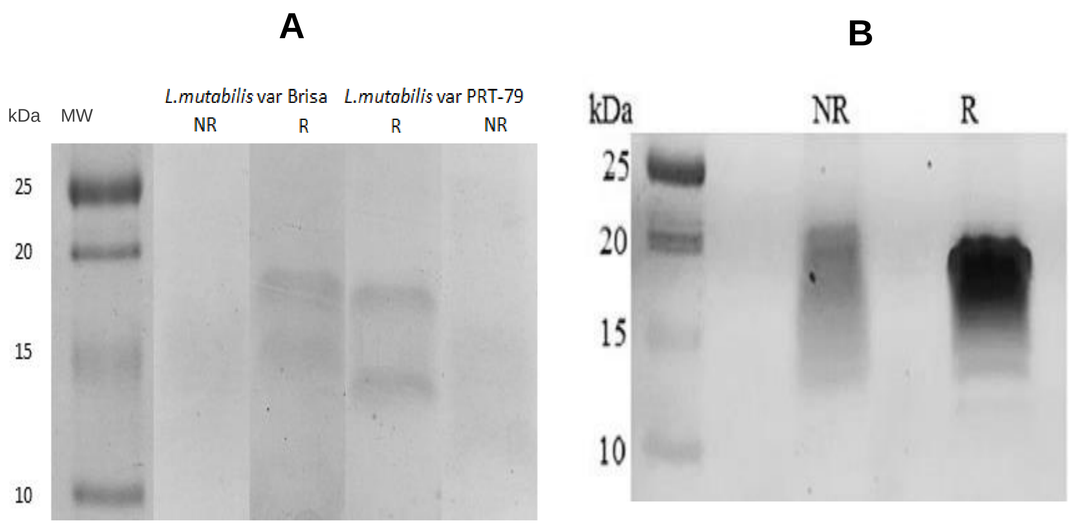

Figure 1 – Polypeptidic profiles *of L.mutabilis* varieties (A) and *L.albus* (B) after deflamin extraction in reductive (R) and non-reductive (NR) conditions through SDS-PAGE gel 17.5 % acrilamide with 100 µg of sample each.

- *Lupinus mutabilis* varieties Brisa and PRT-79 show a similar SDS-PAGE profile to *L.albus* after deflamin extraction.
- Two diferent bands are visible between 20-15kDa in reductive conditions on both varieties and a smear in non-reductive conditions which is characteristic of *L.albus* deflamin (~17kDa).

Cancer cell invasion

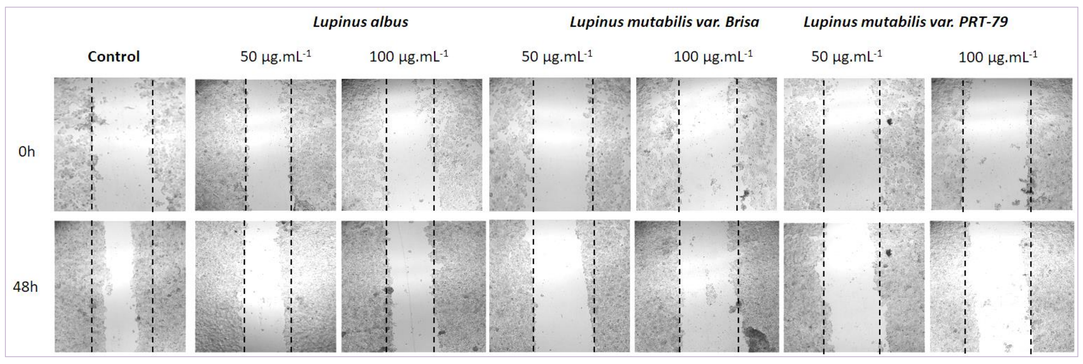

Figure 2- Representative image of HT29 celular invasion between 0-48h through wound healing assay with 50 µg and 100 µg of deflamin from *L.albus* and *L.mutabilis* (Brisa and PRT-79). Control has no proteic inhibitor added.

The wound healing assay allows to test the cancer invason inhibitory potential. *Lupinus albus'* deflamin has shown to inhibit HT29 celular invasion by more than 50% in a dosage-effect relation.

Both *L. mutabilis* varieties Brisa and PRT-79 inhibited HT29 celular invasion, with 50 µg being the concetration with more activity, higher than 100 µg *L.albus* deflamin.

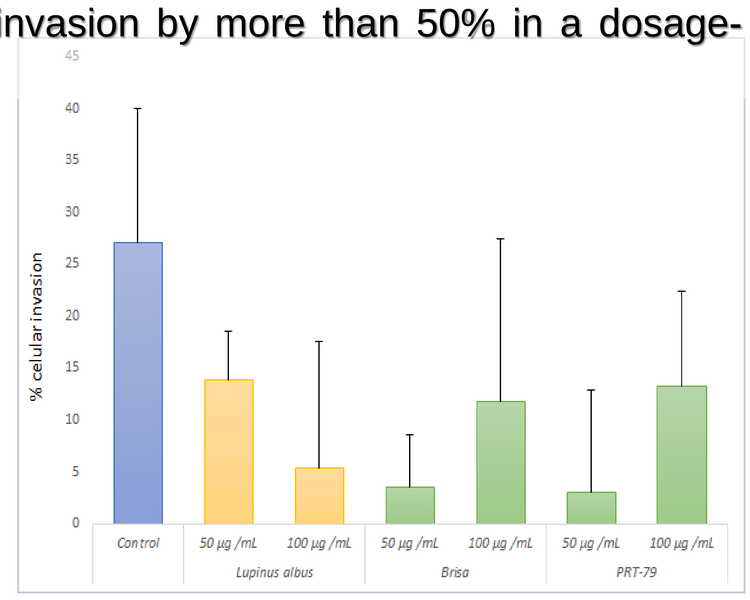

Figure 3 – Percentage of HT29 celular invasion after 48h of exposure to *L.mutabilis* varieties Brisa and PRT-79 and *L.albus* with 50 µg and 100 µg of each proteic inhibitor. The bars represent the average of 3 diferent replicates ± SD.

CONCLUSIONS

- Our results have shown that deflamin might be a common protein within the Lupin genus with not only similar molecular weight but also with anti-cancer invasion activity.
- *Lupinus mutabilis* seems to be a good source of bioactive deflamin, adding more economic value to this species.
- Further steps include sequencing *Lupinus mutabilis* deflamin from different varieties and produce it as recombinant proteins for cancer treatment.

REFERENCES

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