Elucidating the immunomodulatory properties of oregano, graviola and cinnamon nanosuspensions against non-small cell lung carcinoma cells

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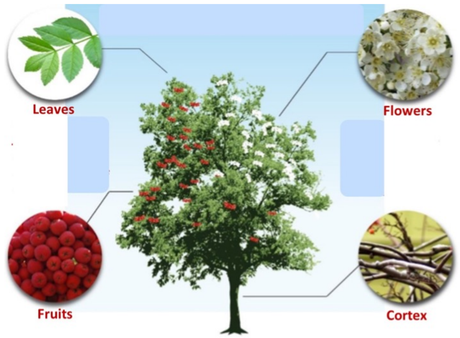
INTRODUCTION

Translational research studies of plants have led to the discovery of many therapeutic drugs available on the market (~50%). Plants contain primary and secondary metabolites. Phytochemicals (>25,000) are the secondary metabolites present in different plant structures. Over-the-counter supplements are the most accessible phytochemical-based medications for the general population, especially those who cannot afford medical insurance. However, many of these supplements are not uniformly prepared to have an effective clinical dose against cancer. Non-small cell lung carcinoma (NSCLC) remains a significant challenge in cancer therapy due to its high acquired resistance to chemotherapy and metastatic potential.



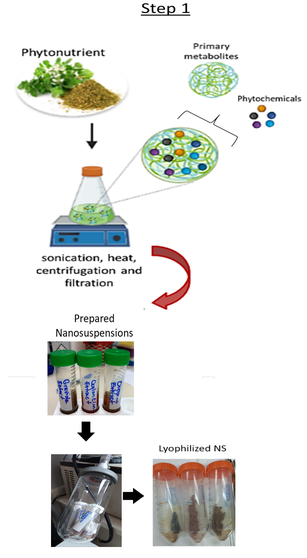
OBJECTIVES

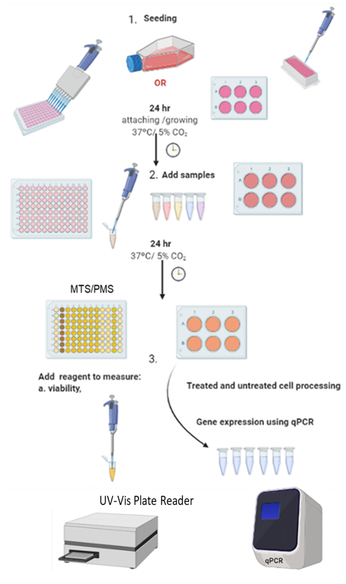
For this project, we selected to study the immunomodulatory properties of three popular phytochemical-rich plants: oregano, cinnamon and graviola as nanosuspensions (NS) by determining the immunomodulation-related biomarkers (TNF-α, PDL1, FOXM1, CXCL11, NFkB1, EGFR NDRG1, IL6 & RAC1) against NSCLC A549 cells using RT-qPCR relative gene expression.

HYPOTHESIS

We hypothesize that these plant-based NS will downregulate EGFR (drives tumor growth), PDL1 (chemoresistance), FOXM1 (metastasis), NFkB1 (tumorigenic inflammation) & RAC1 (cell migration) while upregulating NDRG1 (tumor suppressor) & CXCL11 (anti-tumor inflammation). In the case of the pleiotropic function of TNF-α, we hypothesize that this gene will be upregulated by the phyto-NS.

METHODS





RESULTS

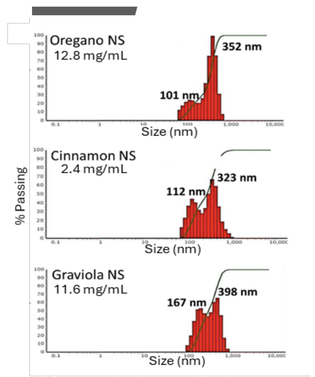


Figure 1. Phyto-NS development and characterization using lyophilization and dynamic light scattering.

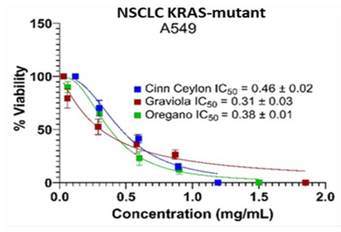
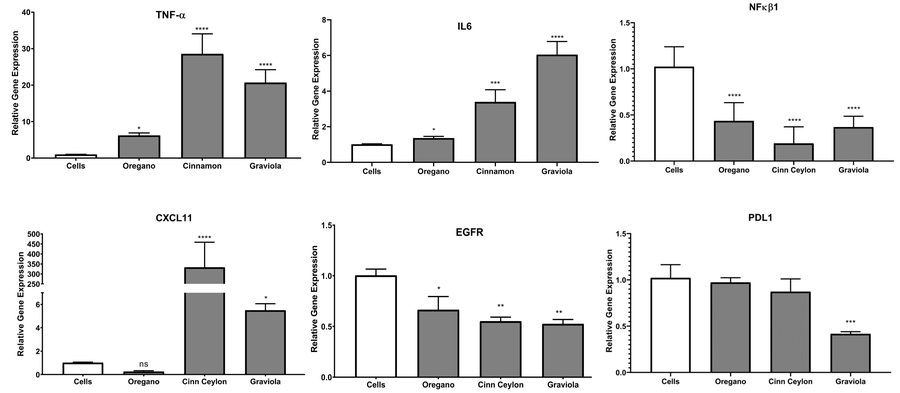


Table 1. IC_{50} results of the Phyto-NS on NSCLC A549 cells in a period of 24 h. This assay was performed in 8 replicates (n=8) of three independent experiments.



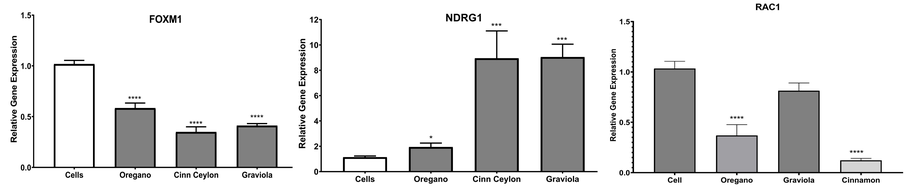


Figure 2. Expression of metastasis and immunoregulatory markers. From RT-qPCR analysis, all phyto-NS downregulated NF-KB1,EGFR, FOXM1, PDL1 & RAC1 genes while upregulating NDRG1, TNF and IL6 genes in NSCLC A549 cells after Phyto-NS incubation using RT-qPCR for 24h. The concentrations used for oregano and graviola was IC50/2 and for cinnamon was IC50/3. GAPDH was used for normalization. This assay was performed in triplicate (n=3) in 3 independent experiments.

CONCLUSIONS

- Plants oregano, graviola, and cinnamon evidently significantly modulate the immune response in NSCLC cells.
- Downregulation of the metastasis-related genes FOXM1 and RAC1 as well as the pro-inflammatory transcription factor NFkB1 by all 3 phyto-NS suggest downregulation of inflammation and a shift toward a less favorable tumor microenvironment.
- Upregulation of the immune response activator CXCL11 by cinnamon and graviola, suggest a potential shift towards a tumor suppressive microenvironment when NSCLC cells are treated with phyto-NS.
- Downregulation of PDL1 expression by graviola highlights the potential of phyto-NS to enhance immune checkpoint blockade therapies and diminish chemoresistance.
- Upregulation of NDRG1 by all 3 phyto-NS suggest reduced rate of metastasis among NSCLC cells treated with these suspensions.
- The upregulation of TNF-α by NSCLC cells treated by all 3 phyto-NS suggests more robust ani-tumorigenic signaling expression when samples were treated with each of the 3 phyto-NS.

FUTURE PROJECTIONS

- As more data is acquired, it is anticipated that an understanding of how these phyto-nanosuspensions modulate immunological pathways related to each of these biomarkers will be achieved. Furthermore, this study will investigate the use of these phyto-NS in an in vivo *Drosophila melanogaster* model to confirm their therapeutic potential against NSCLC.

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ACKNOWLEDGEMENTS

This project was supported by PR INBRE (NIGMS Award Number P20 GM103475-19) and by CAPAC (Award Grant Number# R25CA240120) from the NCI. The content is solely the responsibility of the authors and does not necessarily represent the official views of the sponsoring agencies. These agencies had no role in the design of the study, in the collection, analyses, or interpretation of data.

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