IN VITRO PRODUCTION OF SECONDARY METABOLITES BY *CAPPARIS SPINOSA* CALLUS CULTURE

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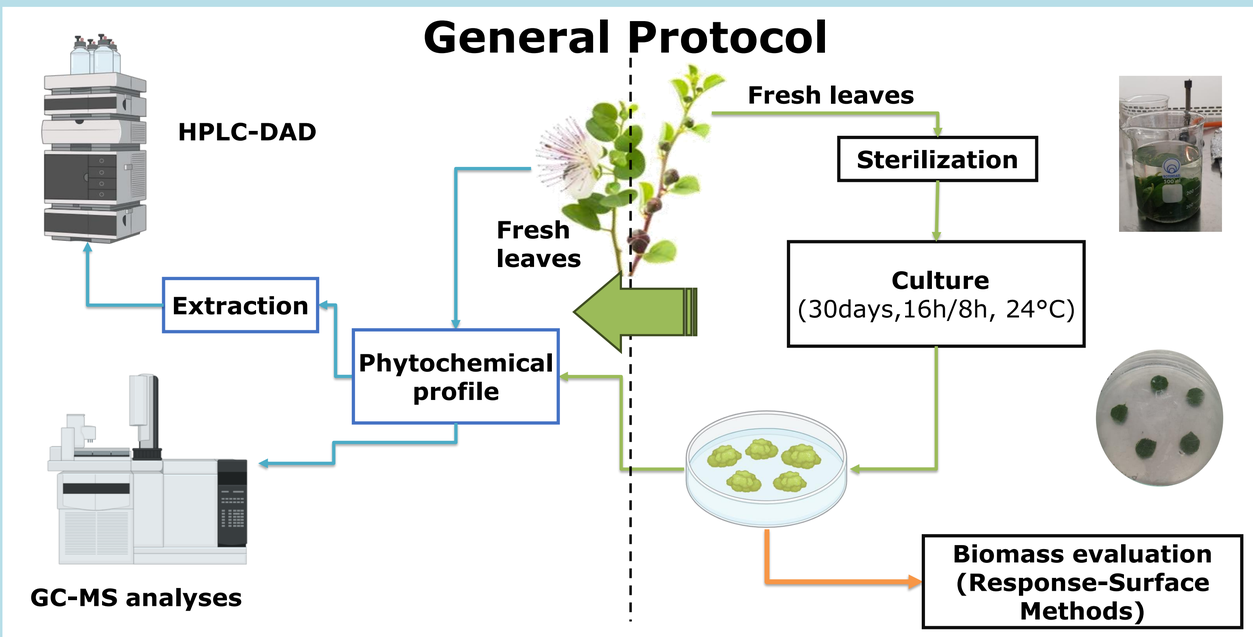
1 Introduction

Caper (*Capparis spinosa L.*) is a well-recognized medicinal plant in the Mediterranean region which holds significant interest due to its medicinal, pharmacological, and culinary properties. Up to now, numerous bioactive phytochemical compounds have been isolated and identified from different parts of this plant (aerial part, roots, seeds), making it a valuable source of molecules for the pharmaceutical industry (Sun et al., 2023). Plant metabolites are also increasingly used for agronomic purposes as they can display interesting properties, and notably insecticidal and herbicidal activities. However, the extensive use of these metabolites has prompted the need to enhance their production. The utilization of biotechnological tools and notably of *in vitro* production systems presents economic and environmental benefits for the sustainable production of these metabolites (Mohaddab et al., 2022). Specifically, callus culture emerges as a prevalent technique for the generation of biologically active compounds derived from medicinal plants (Li et al., 2022).

While the production of volatile and non-volatile *C. spinosa* phytochemical compounds by callus culture has not been explored yet, it could avoid the complete harvesting of plant material and has a high potential for ensuring the sustainable production of these secondary metabolites. In this context, the objective of this study was to induce callus using *C. spinosa* leaf explants and a matrix containing two cytokinins (kinetin "KIN" and 6-benzylaminopurine "BAP") along with two auxins (1-naphthaleneacetic acid "NAA" and 2,4-dichlorophenoxyacetic acid "2,4-D"). We therefore conducted a comprehensive phytochemical investigation and established correlations between different hormonal concentrations applied in the culture media and the volatile and non-volatile components produced by the callus.

Keywords: *Capparis spinosa*, callogenesis, phytohormones, secondary metabolites.

2 Methods



Callus induction in *C. spinosa* leaves was performed using MS medium. After thorough disinfection, the leaves were treated with various hormonal combinations: [BAP; NAA], [BAP; 2,4-D], [KIN; NAA], and [KIN; 2,4-D]. We utilized a total of 1,500 explants. For the identification of secondary metabolites, we employed a dual approach, using both HPLC-DAD for polyphenols and GC-MS for volatile components.

3 Results

Response-Surface Methods (RSM)

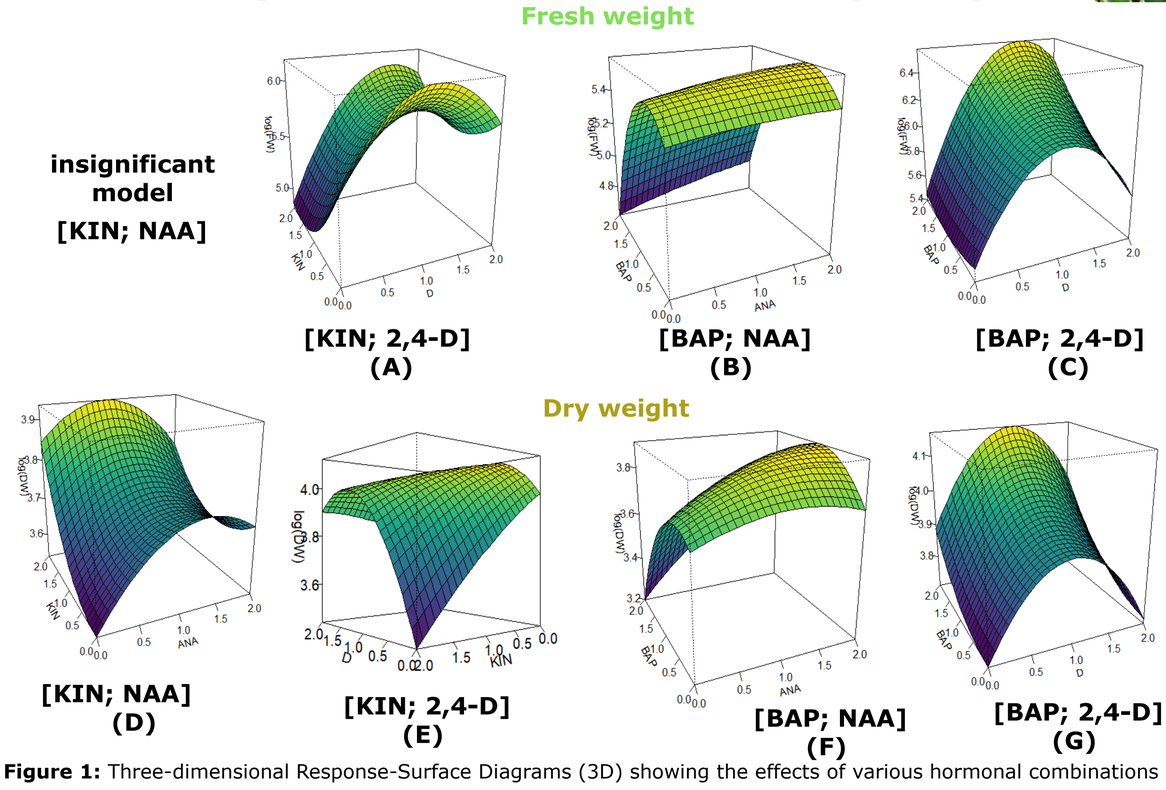


Figure 1: Three-dimensional Response-Surface Diagrams (3D) showing the effects of various hormonal combinations on the fresh (A,B and C) and dry (D,E,F and G) weight of *C. spinosa* callus.

Figure 1 shows the effect of different hormone combinations on callus induction. The application of 2,4-D in combination with KIN or BAP produced a maximum response in terms of both fresh and dry weight. The optimum concentrations observed (in mg/L) were as follows: A = [0; 1], C = [2; 1.5], E = [0; 0.5], and G = [2; 1], whereas [KIN, NAA] had no significant impact on callus formation.

Phytochemical analyses

GC-MS analyses (HS-SPME-GC-MS)

A total of 197 compounds have been accurately identified. These compounds encompass diversity of chemical families, including sulfuric compounds, terpenes, aldehydes, as well as pyrroles (fig.2).

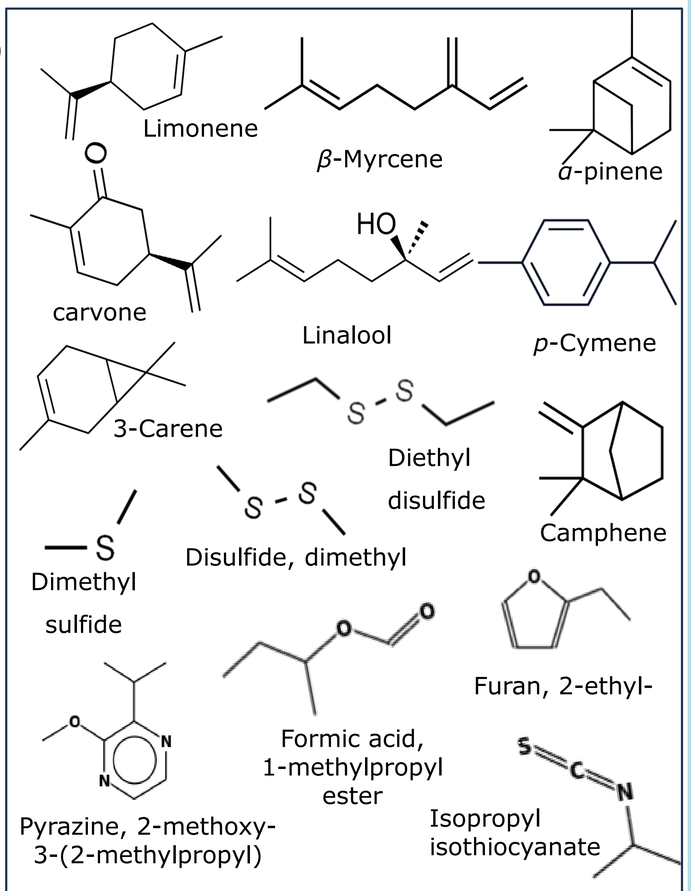
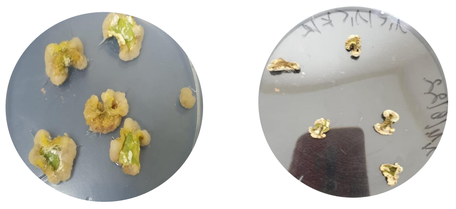


Figure 2: Chemical structures of volatile compounds detected in callus using HS-SPME-GC-MS.



Identification of Polyphenols:

The HPLC-DAD analysis revealed the presence of several major compounds (Fig. 3 and 4). By using standards, we were able to identify rutin and kaempferol 3-*O*-rutinoside among these peaks. But, in order to identify the remaining peaks, an LC-MS-MS analysis will have to be conducted.

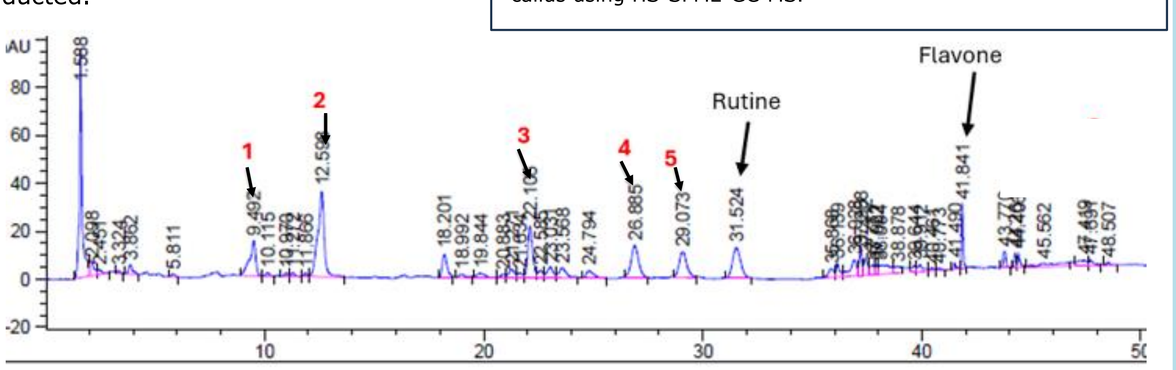


Figure 3: Chromatogram obtained via HPLC-DAD without phytohormones, Flavone was the internal standard.

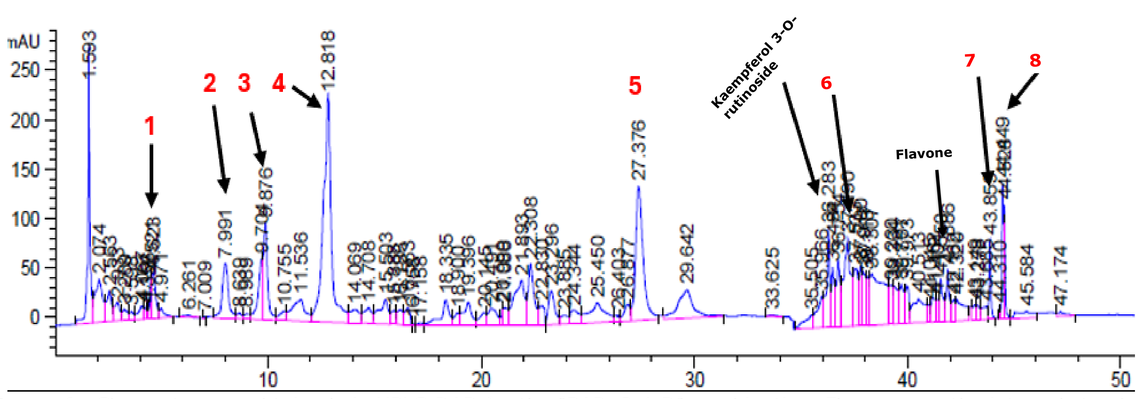


Figure 4 : Chromatogram obtained via HPLC-DAD for the [BAP ;2,4-D] combination, Flavone was the internal standard.

4 Conclusion

This study holds significant importance in the context of the sustainable production of secondary metabolites from *C. spinosa*, which is essential for the valorization of these compounds. The secondary molecules of *C. spinosa* could be utilized for diverse applications, including pharmaceuticals and agronomic purposes. Plant botanicals are increasingly favored as substitutes for synthetic chemical pesticides. Additionally, our study lays the groundwork for transitioning to suspension culture and scaling up cultivation on a larger scale. Nevertheless, further investigations are necessary to fully understand the biosynthetic pathways and epigenetic mechanisms that govern the production of secondary metabolites, aiming to achieve targeted production with a consistently high yield of the desired compounds.

References

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