

Phytochemical profile, *in Vitro* Cytotoxic, Genotoxic and Antigenotoxic Evaluation of *Cistus Monspelienis* L., Leaf Extract

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S1. Results Supplemental

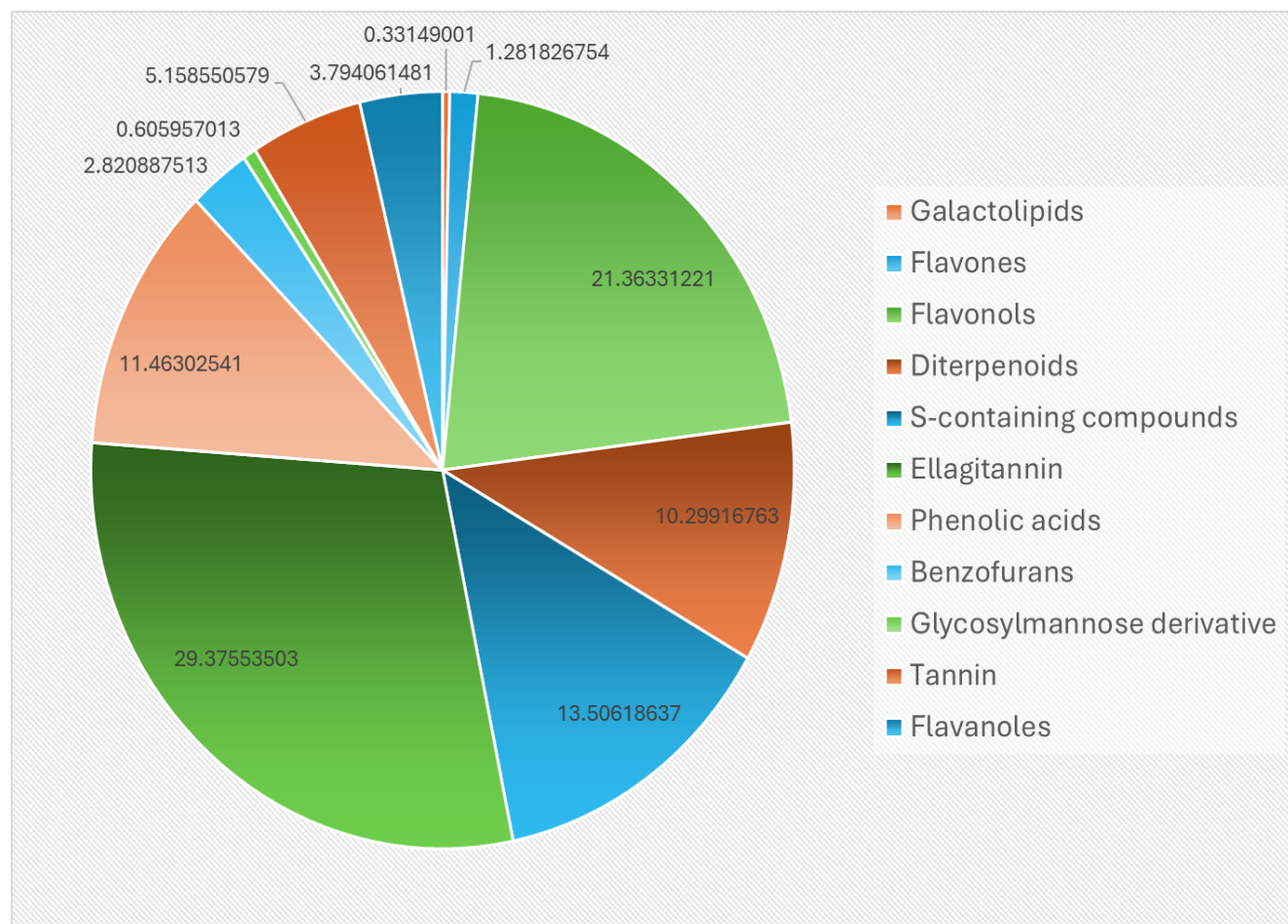


Figure S1: A diagram showing the percentage of the different phytochemicals class in *C. monspeliensis* leaves methanolic extract is presented in in Supplementary documents.

S2. Materials And Methods Supplemental

Nucleus and micronucleus counting strategy in IDEAS software

The ImageStream flow cytometer allows for the quick capture of individual cell images in multiple fluorescence channels. After cell treatment with *C. monspeliensis* extract and MMC, the cells were collected, stained, and then examined. An image capture approach for the acquisition of single cell images for cell samples with concomitant DNA staining using Draq5. The manufacturer's software for the cytometer (IDEAS) was used for the image analysis pipeline.

To filter, identify and score total cells, binucleated cells, mononucleated cells, multinucleated and micronuclei (MN), an optimized analysis template using new features and image masks was developed and created in IDEAS based on previous literature [59-62] with some modification, details analysis step is showing in Fig.2. as following:

"MN_Count_" feature is created using "Spot Count" feature applied to "MN_MASK1" mask. MN_Count_A feature was applied to BNC population deriving from:

"MN_MASK1" mask derives from combining four sub-masks: in particular, "MN_MaskA_Step1" derives from intensity thresholding on the DNA stain channel of 80%; the "MN_MaskA_Step2" derives combining "Spot Mask function" on DNA channel (bright spots between 3-4 pixels) and "MN_MaskA_Step1"; "MN_MaskA_Step3" derives from "Range" function applied to "MN_MaskA_Step2" (2-50 pixels and Aspect Ratio set between 0.4-1.0).

Finally, from "MN_MaskA_Step3" was subtracted the "nuclear mask" created as follows: MN_MaskB_Step3" derives from applying "Range" function to "MN_MaskB_Step2" (set to 250-5000 pixels and Aspect Ratio set between 0.2-1); "MN_MaskB_Step2" derives from applying "Dilate" function to "MN_MaskB_Step1" (dilate set to 1 pixel), while "MN_MaskB_Step1" was produced from "LevelSet" function applied to DNA channel (setting: Middle and 8 pixels).

Boolean logic formula for "MN_MASK1" mask, combined the two masks as follows: MN_MaskA_Step3 and Not MN_MaskB_Step3

where, in details:

MN_MaskA_Step3 = Range (MN_MaskA_Step2, 2-50, 0.4-1)

MN_MaskA_Step2 = MN_MaskA_Step1 and Spot (M05, Ch05, Bright, 4, 3, 1)

MN_MaskA_Step1 = Threshold (M05, Ch05, 80)

MN_MaskB_Step3 = Range (MN_MaskB_Step2, 250-5000, 0.2-1)

MN_MaskB_Step2 = Dilate (MN_MaskB_Step1, 1)

MN_MaskB_Step1 = Level Set (M05, Ch05, Middle, 8)