In vitro Evaluation of *Jasione montana* and Its Main Flavonoids on Human Melanoma Cells

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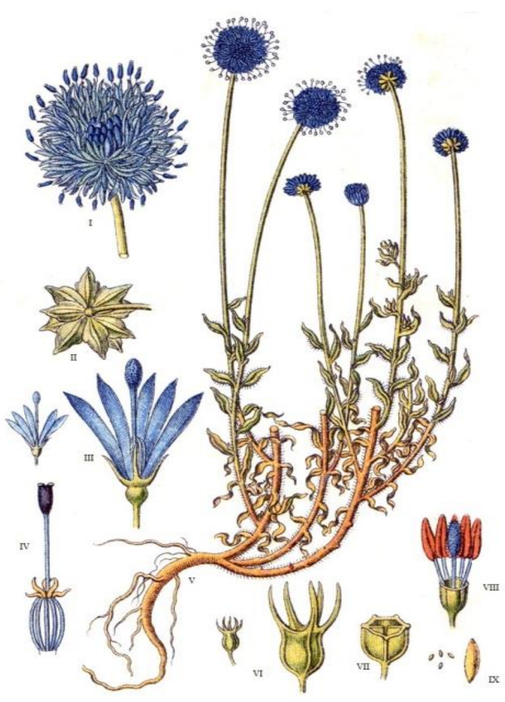
INTRODUCTION

Jasione montana L. (Campanulaceae) is traditionally used in Belarusian folk medicine in cases of restless sleep of children. However, considering the fact that there are limited reports about aboveground parts of *J. montana*, its phytochemical composition and biological activity have to be exhaustively assessed. In the previous reports the main constitutes of *J. montana* as luteolin (JM7), luteolin 7-*O*-glucoside (JM8), and luteolin 7-*O*-sambubioside (JM9) were confirmed.

AIM

Taking these aspects into consideration, the biological potential of H_2O (JM1), 50% MeOH (JM2), MeOH (JM3) Et_2O (JM4), EtOAc (JM5), *n*-BuOH (JM6) extracts, and isolated compounds (flavones) has been assessed using human melanoma cells (CRL-1585).



MATERIALS AND METHODS

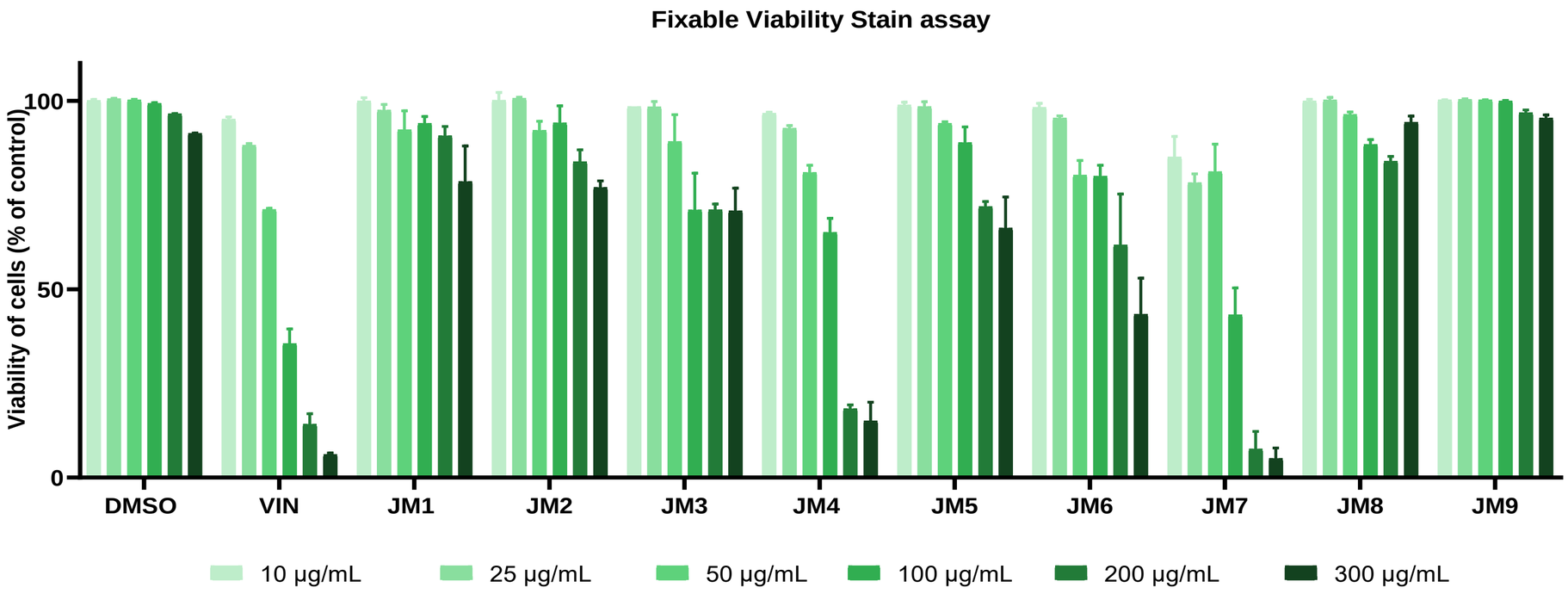
The Fixable Viability Stain assay was performed to characterize the level of viability cells under treatment with extracts (JM1 – JM6) and isolated compounds (JM7 – JM9) and vinblastine (10 – 300 µg/mL). Cytotoxicity was evaluated by BD Horizon™ Fixable Viability Stain 520 (FVS520) under a flow cytometer (BD FACSCanto II flow cytometer, San Jose, CA). Additionally, to characterize the mode of cell death provoked by JM4 fraction (25-100 µg/mL) and, JM7 (25 µg/mL), flow cytometry assessment of Annexin V binding was carried out under a flow cytometer (BD FACSCanto II flow cytometer, San Jose, CA) using Apoptosis Detection Kit II (BD Pharmingen, San Diego, CA). Moreover, the dysfunction of the mitochondrial membrane potential (MMP) after treated with JM4 (25-100 µg/mL), JM7 (25 µg/mL) was performed by lipophilic cationic probe 5,5',6,6'-tetrachloro-1,1',3,3'-tetrarthylbenzimidazol-carbocyanine iodide (JC-1 MitoScreen kit; BD Biosciences).

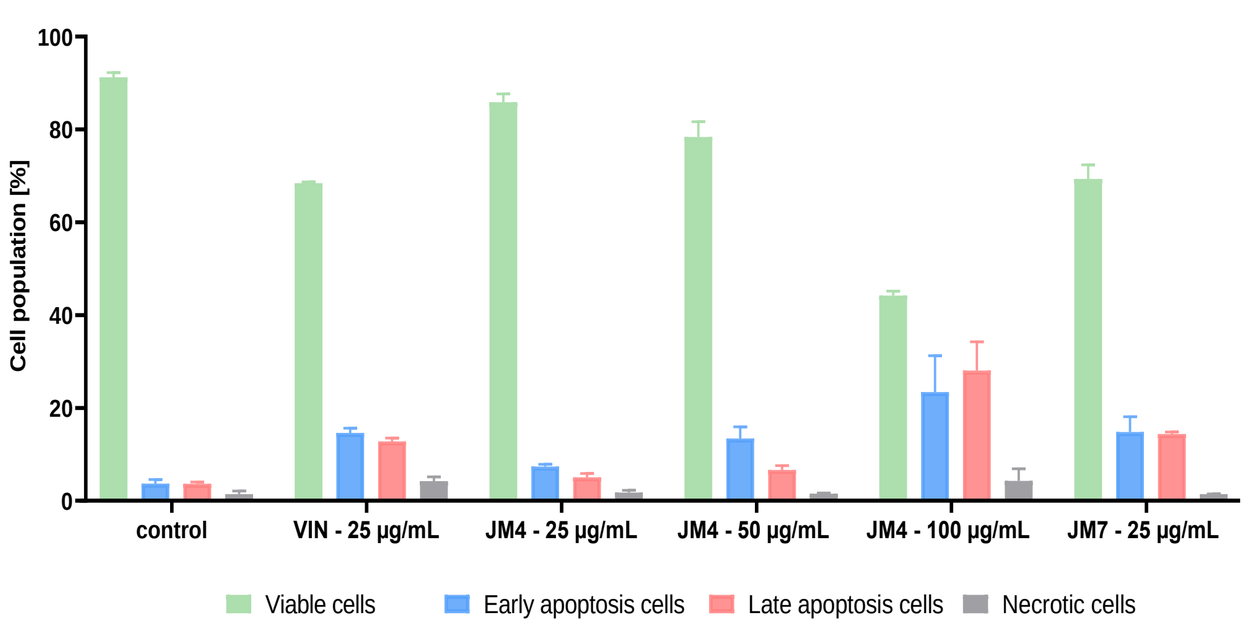
RESULTS

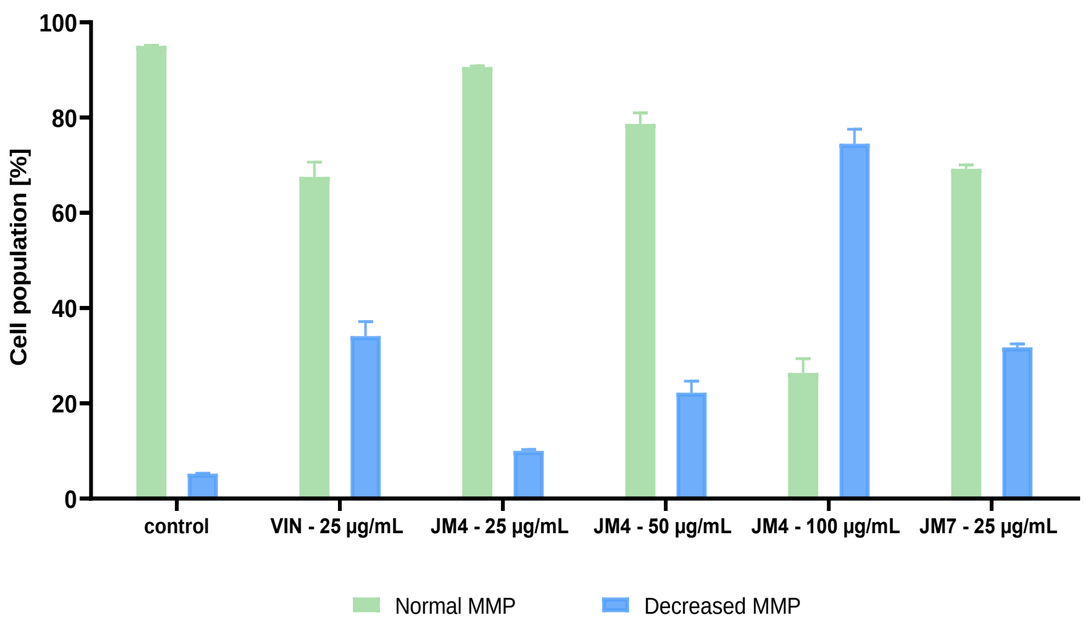
As a result of Fixable Viability Stain assay, the JM4 fraction and JM7 (300 µg/mL) displayed strong (dose-dependent) inhibition of CRL-1585 cells (the following viability of CRL-1585: JM4, 11.5%; JM7, 2.3%), which was similar to vinblastine sulphate (positive control). The obtained results suggest that JM4 fraction and JM7 compound were the most active on human melanoma cells.

Flow cytometry assessment of Annexin V binding analysis proved that the tested compounds significantly induced programmed cell death in CRL-1585 cells in comparison with the control (untreated cells), where we detected 44.2% viable cells and 51.5% apoptosis in the case of JM4 (100 µg/mL), and 69.3% viable cells and 29.2% apoptosis in JM7 (25 µg/mL). At the same time, it should be noted that in the case of vinblastine (25 µg/mL), the number of apoptotic cells was at a similar range as in the case of JM7. However, in the case of vinblastine sulphate, the number of cells involved by necrosis was twice higher.

We found that the decrease in MMP caused by JM7 was similar to that caused by vinblastine sulphate.







CONCLUSION

Further studies of the phytochemical and biological assays are currently in progress. Our results indicated that different extracts/fractions or their main compounds can be a promising component of preparations with anticancer properties.

ACKNOWLEDGEMENTS

The work was funded by the project № POWR.03.02.00-00-I051/16 from European Union funds, POWER 2014-2020, grant № 05/IMSD/G/2019.