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Evaluation of doctoral thesis

Aleksandra Maria Juszczak has prepared her doctoral thesis (entitled: Phytochemical analysis and biological activities of *Jasione montana* L. (Campanulaceae)) under the supervision of Dr. Michał Tomczyk, Medical University of Bialystok, Poland and Dr. Marijana Zovko Končić, University of Zagreb, Croatia. The topic of the dissertation is the phytochemical analysis and assessment of certain biological activities of *Jasione montana*.

The thesis is based on four publications, all of which were published in highly ranked journals. Three papers were published in Q1 ranked journals (International Journal of Molecular Sciences, Biomolecules, Frontiers in Pharmacology) and one paper in a Q2 journal (Medicinal research Reviews). The candidate is the first author of all these papers. Two articles are original research papers, whereas two other articles are very comprehensive research papers on topics closely related to the focus of the thesis (chromatography of luteolin and its derivatives, the potential role of luteolin in skin cancer treatment). Summarizing the background literature and publishing in review papers is a very smart practice, which is encouraging and useful for PhD students, especially if the PhD thesis does not contain extensive literature summary. The quality of the papers that are the basis of this dissertation is a prerequisite of a successful doctoral work.

This thesis is a good example of a well-designed scientific research, which combines the competences of supervisors and the possibilities of two pharmacognosy departments of two European countries. The starting point of the work is the lack of scientifically based information on a plant that has been traditionally used in folk medicine. *Jasione montana* has been used as a sedative in Belorussian folk medicine, however, its effects have not been studied so far. From a phytochemical point of view the plant is practically undiscovered, only some flavonoids have been described from the herb.

The goal of the doctoral work was adjusted to the possibilities and expertise of the two departments. Instead of analyzing the bioactivity observed in folk medicine, the starting point

was the comprehensive phytochemical analysis of the plant. Such data were missing, and the expertise of the supervisors guaranteed a high-quality project in this field.

And indeed, the phytochemical studies resulted in two precious manuscripts. In the first one, the qualitative analysis of water, water-methanol and methanol extracts and selected fractions of *Jasione montana* extracts are described. The methodology used here meets the contemporary scientific requirements. In the paper, the isolation of certain flavonoids (luteolin 7-*O*-sambubioside, luteolin 7-*O*-glucoside and luteolin) is also presented, confirming that the candidate gained experience in preparative phytochemical work during her studies. One further paper reports the quantitative analysis of the isolated compounds and different extracts using a newly developed and validated analytical method. This subproject was supported by the literature search that was done in order to prepare the review paper on luteolin analytics and I am convinced that the analytical experiments performed by the candidate also contributed to the quality of the review manuscript.

The successful isolation of the three flavonoids resulted in amounts sufficient for biological analyses. The literature overview of the biological activities of luteolin and its derivative points out that these compounds might be valuable for dermatological use. Two activities have been studied in detail, the potential anticancer and wound healing effects. A major virtue of this work is that it really goes into the details of these activities, analyzing the potential mechanisms behind the observed effects. Preliminary experiments confirmed, that extracts and compounds of *Jasione montana* affect cell viability of human amelanotic melanoma cells. Based on this observation, detailed research confirmed that only two extracts and one compound, luteolin exert cytotoxic activity. Further, it was observed that the proapoptotic effect plays major role in these activities in case of one of the extracts and luteolin and induction of autophagy might also play role in case of the extract. Cell cycle arrest was also detected as a possible mechanism of action. The overall effect might also be related to the effect on caspases. This part of the work is very comprehensive and evaluation of the potential anticancer effect of *Jasione montana* extracts and flavonoids points out the possible use of complex extracts and the flavonoid luteolin in therapeutic settings. Of course, this is only the first, but very important step towards this goal.

One further possible utilization of *Jasione montana* extracts and metabolites is the use in wound healing. Speeding up the wound healing process is challenging. Certain plant extracts (e.g., *Allium cepa*) have scientifically established role in this area, so the analysis of further potential plants looks promising. The flavonoid content of *Jasione montana* might be a good basis for

wound healing effect, since the confirmed antioxidant capacity might play a role. More specific effects of extracts and luteolin derivatives have also been detected. The elastase inhibitory activity, the increase of collagen content of fibroblasts (though the latter contradictory), the beneficial activity on granulation tissue formation, together with the anti-inflammatory effects of certain extracts and of the flavonoid luteolin make further analysis of wound healing promising.

I have the following questions to the candidate:

1. What would be the next steps in the development of *Jasione* extracts to potential wound-healing products?
2. Was the analytical method validated for all the three compounds (including compound 9)? If not, what was the reason for this?
3. Are there any plans to study the sedative effect of this plant observed in folk medicine?

Conclusions

The scientific value of the work summarized in the thesis entitled Phytochemical analysis and biological activities of *Jasione montana* L. (Campanulaceae) is indisputable. The quality of the work meets not only the objective standards, but also other (formal, grammatic, stylistic) aspects. Overall, I claim that the thesis fully satisfies the standards for a doctoral thesis, hence I recommend allowing it to move forward with the PhD doctoral degree process.

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