



Referee: Dr. Claudio Ferrante, Department of Pharmacy, Botanic Garden “Giardino dei Semplici”, “G. d’Annunzio” University, Chieti-Pescara (Italy).

Ph.D. Candidate: Aleksandra Maria Juszcak

Title of the Thesis: Phytochemical and biological activities of *Jasione montana* L. (Campanulaceae)


The present dissertation is focused on a multidirectional evaluation of the aboveground parts of *Jasione montana* L. (Sheep’s bit scabious).

Particularly, the candidate explored the potential applications of extracts, at different polarities, in different biological models, including melanoma and fibroblast cell lines.

The approach is rigorous and the candidate conducted a reliable investigation of the phytocomplex, with qualitative and quantitative determinations of phytochemicals, especially flavonoids, finding luteolin and its derivatives as prominent flavonoids, in the phytocomplex.

The results of the studies conducted by Dr. Juszcak are extensively described into two articles published in high impact factor journals with peer review.

Specifically, the first article presented by Dr. Juszcak is titled “In vitro anticancer potential of *Jasione montana* and its main components against human amelanotic melanoma cells”. The article has been published in 2021 in “International Journal of Molecular Sciences”, which has a 2020 IF of 5.924, and is ranked in the first quartile, for the subject category “Biochemistry and molecular biology”. This is consistent with the thematic of the PhD course in pharmaceutical sciences, as declared by the candidate. In this publication Dr. Juszcak is present as first author and at the end of the manuscript it is declared that participated actively at different stages of the study, including: conceptualization, methodology, writing original draft, and project administration; thus, indicating excellent skills in the coordination of a scientific study.

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Specifically, the present study is structured as a multidirectional investigation touching the field of different fields deeply involved in the characterization of the potential applications of herbal extracts. It is clear the pharmacognosy description of the plant material employed, with clear indication of the harvesting area and the voucher number.

Additionally, the methods for the preparations of the extracts which have been analyzed with different techniques: LC-ESI-MS, TLC, ¹H-NMR and ¹³C-NMR.

Although the quantitative analysis of the phytochemicals has not been included in the present study, Dr. Juszcak and co-authors carried out a rigorous and appreciable pharmacological evaluations of the anti-cancer properties of the extracts from *J. montana*.

Indeed, the extracts were tested against the human amelanotic melanoma cell line C32, and accurate evaluation of cytotoxic effects by the extracts and the impact on cell cycle were included in the article.

It is sensitive to note, and also to appreciate, that Dr. Juszcak did not limit the pharmacological study to the only description of the extracts' effects, but she also compared the effects by extracts with different and isolated phytochemicals, among which luteolin was the most potent, and a reference cytotoxic drug, namely vinblastine sulfate.

This is index of an accurate planning of the study, which is not always included in the scientific literature dealing with herbal extracts and which also agree with guidelines of high impact factor pharmacology journals that strongly recommend the inclusion of isolated phytochemicals and reference drugs for investigating the pharmacological effects of herbal extracts.

As a concluding remarks about the methodology followed by Dr. Juszcak there is to mention the huge amount of supplementary data included in the manuscript which makes easy to validate the results and permit their replication.

Whereas the anticancer activity in the skin, together with the prominent flavonoid component of the phytocomplex, makes rational the following steps of the PhD project of Dr. Juszcak, that is the evaluation of the protective effects of *J. montana* extracts in fibroblast cell lines; thus opening the scenario for the comments about the second publication presented by the candidate.

Specifically, the second article published by Dr. Juszczak is entitled “Wound healing properties of *Jasione montana* extracts and their main secondary metabolites”.

The article has been very recently published in the journal “Frontiers in Pharmacology”, section Ethnopharmacology. The journal is of high impact factor (IF 2020: 5.811) and it is ranked in the first quartile for the subject category “Pharmacology and Pharmacy”. Also the choice of this journal is consistent with the thematic of the PhD project of the candidate.

In this publication, Dr. Juszczak is present as first author and at the end of the manuscript it is declared that participated actively at different stages of the study, including: conceptualization, methodology, writing original draft, and project administration

In this article the candidate improved her knowledge about the phytochemical composition and the pharmacological properties of the extracts from *J. montana*.

Regarding the phytochemical investigation, it is sensitive to highlight that Dr. Juszczak has filled the gap related to the lack of quantitative determination of phenolic compounds, in the extracts. The quantitative analysis was rigorous and focused on luteolin and derivatives. Supplementary materials were added for permitting the replication of the study, although the eventual inclusion of the chromatogram would be appreciated.

Additionally, the candidate also explored the intrinsic properties of the extracts, with determination of scavenging/reducing and enzyme inhibition properties. The enzyme inhibition effects were tested against the elastase; thus, further supporting the investigation of protective effects, in the skin, through the use of normal human dermal fibroblast cell line (OCS-201-012).

In fibroblasts, the candidate evaluated the effects of the extracts in spontaneous cell migration, through the wound healing model, and the extracts were effective in stimulating the cell migration at sub-toxic concentrations, consistent with results presented in the previous article published in “International Journal of Molecular Sciences”.

The complexity of the investigation and the strict accordance of the studies conducted with different biological models further corroborate high expertise and rigor that makes Dr. Juszczak worthy in defending her thesis.

It is also sensitively appreciated the quality of writing and the huge amount of data presented for each of the original articles which are the core of the experimental part of the present PhD project. It is of great importance the level of internationalization of the present studies which included mobility of the candidate to different foreign laboratories, among which the one of Prof. Marijana Zovko Koncic, at the Department of Pharmacognosy of the Faculty of Pharmacy and Biochemistry, University of Zagreb (Croatia). Prof. Zovko Koncic is also co-supervisor of the present thesis dissertation. The expertises that the candidate has developed during the international mobility have been brilliantly applied to the studies proposed. This is surely one key target that is expected from a PhD student, which improves the value and significance of the PhD project.

However, there are some critical points which have to be discussed during the thesis defending. Specifically, the candidate should discuss the mechanism of action of the extracts, and also discuss about antioxidant effects which were only marginally explored via colorimetric assays.

And in this regard my questions for the candidate are the following:

-Did the candidate propose a specific mechanism of action as regards the effects of the employed herbal extracts?

-In alternative, what kind of experimental approach can be carried out for improving the mechanism of action, with particular regards to antioxidant effects, as well?

-And finally, did the candidate carry out in silico studies for predicting innovative targets underlying the observed effects?

The candidate also presented other two papers, namely review articles which are integral part of the dissertation thesis.

The two review papers are focused on luteolin, the main compound present in the *J. montana* extracts.

The thematic discussed in the two review articles are consistent with the PhD project of the candidate.

The first review article was published in 2019, in the journal *Biomolecules*. In that year, the impact factor of *Biomolecules* was 4.082, and the journal was ranked in the second quartile for the subject category “Biochemistry and molecular biology”, which is consistent with the thematic of the PhD project of the candidate. In this article, titled “Recent trends in the application of chromatographic techniques in the analysis of luteolin and its derivatives”, the candidate is present as first author and according to the author contribution statement reported at the end of the manuscript, she played an active role in all phases of manuscript preparation and publication.

Similar considerations can be done as regards the second review article published by the candidate and entitled: “Skin cancer, including related pathways and therapy and the role of luteolin derivatives as potential therapeutics”. The article has been published in 2021 in the prestigious journal “*Medicinal Research Review*”, which has a 2020 impact factor of 12.994 and is ranked in the first quartile (excellence zone) for both the subject categories of medicinal chemistry and pharmacology and pharmacy.

Also in this publication Dr. Juszczak is listed as first author and the candidate contributed to all stages of manuscript preparation.

Furthermore, the candidates published other two scientific articles in the journal *Molecules* and *South African Journal of Botany*. In these papers, whose topics are not consistent with the PhD thesis project, the candidate is present as co-author.

It is also important to highlight that the candidate delivered five oral presentations at international congresses about the results of her PhD, and was also principal investigator of the scientific projects with grants and identification numbers reported.

Finally, but not for importance, the candidate also reported the indication of integrative didactic activities conducted in the field of pharmacy and cosmetology subject at the Medical University of Bialystok, which further contribute to the academic growth of the candidate, whose scientific profile is of international level.

Based on the above data, I support the application to the Council of the College of Pharmaceutical Sciences Discipline of the Medical University of Bialystok for admission of Ms. Aleksandra Maria Juszcak to further stages of her doctoral dissertation.

Chieti, 12-07-2022

Dr. Claudio Ferrante

A handwritten signature in black ink, appearing to read "Claudio Ferrante". The signature is written in a cursive style with a large, stylized initial 'C'.