To Whom It May Concern:

Košice, August 15th, 2022

Re: Evaluation of the doctoral thesis entitled **Developing novel tools towards understanding the hypericin biosynthesis in *Hypericum perforatum* L.** submitted by **Matam Pradeep**

Despite the enormous effort of several scientific teams to elucidate the individual steps of the biosynthesis of a significant secondary metabolite – hypericin produced in the plant kingdom exclusively by some representatives of the genus *Hypericum*, the current state of knowledge of the regulation of the key steps of biosynthesis is not at a level that would allow its production by an alternative biotechnological way.

The submitted dissertation thesis has the ambition to contribute to the mosaic of knowledge with several original results. The thesis is presented as a set of two complex scientific papers published in the journal Industrial Crops & Products, which are briefly introduced and concluded taking into account the key findings.

The first paper is aimed at simultaneous determination of anthraquinones including bisanthraquinones and naphthodianthrones in extracts of *H. perforatum* shoot cultures. Along with optimization of the extraction, detection and quantification procedures, the authors identified in *H. perforatum* samples four skyrin derivatives for the first time. The significance of the second article is, first of all, in developing a new tool for irreversible dark gland and hypericin biosynthesis inhibition which may reveal some crucial aspects of hypericin biosynthesis regulation.

Given that the published results have undergone a revision process by the authorities of the scientific community, I will comment on the introductory parts of the dissertation and the prospects for further research in the field.

In Summary, p. 2, is the following statement: “*The traditional use of extracts … as antidepressants is largely attributed to the presence of hypericin”.* According to the state of the art, multiple bioactive compounds contribute to the antidepressant activity with potential synergism. Despite the results on MAO inhibition, receptor-binding or re-uptake of neurotransmitters are ambiguous, they favour hyperforin as the main active principle along with flavonoids and partially hypericin.

In Introduction, p. 6, hypericin is also present is some other organisms like *Dermocybe austroveneta*, crinoids or fungal endophytes. In plant kingdom, it is exclusively present only in some *Hypericum* species.

On the bottom of the same page you mention problems in the application of metabolic engineering and synthetic biology approaches. However, recently published paper of Wu et al. (2021) provides a way of hypericin production without detail knowledge on all biosynthetic steps “*in planta”.* Will you, please, comment on it?

In Introduction, p. 7, the cited paper of Soták et al. 2016b did not deal with placental tissue.

In addition, I have following questions to the dissertation topic:

1. You have concluded that HpPOCP, HpOKS and HpPKS2 are the enzymes with a substantial role in hypericin biosynthesis. Which enzyme is, according to your opinion, responsible for the dimerization reaction?

2. It is known that some bisanthraquinones are present in *Hypericum* spp. in a glycosylated form. What role could they play in the process of biosynthesis?

3. Many biosynthetic genes coding for bisanthraquinones in microorganisms, for instance in fungal endophytes, are grouped in clusters. Do you expect similar arrangements in the plant genomes?

4. Do you thing that production of transgenic plants (*Hypericum* or model?) expressing (or overexpressing) the *PKS, OKS* and *POCPs* genes would bring more understanding to the hypericin biosynthesis?

5. You have successfully inhibited dark gland development and hypericin biosynthesis by glyphosate. Which of the candidate biosynthetic genes in light of forward genetics could be inactivated by the treatment?

**Conclusion:** Based on the submitted dissertation and significance of the published results I recommend awarding the applicant the title of ***“Philosophie Doctor”*** (PhD.).

Professor Dr. Eva Čellárová, DSc.