MUNI
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Evaluation review of dissertation thesis

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Workplace: Department of Pharmacognosy and Pharmaceutical Botany

Name of student: Viriyanata Wijaya, MSc.

Title of dissertation: Alkaloids of Dicranostigma franchetianum (Prain) Fedde and their selected biological activity

Reviewer: prof. PharmDr. Karel Šmejkal, Ph.D.

Workplace of reviewer: Department of Natural Drugs, Faculty of Pharmacy, MU

Text of review:

Viriyanata Wijaya's dissertation is written on the topic of isolation, structural analysis and testing of biological active natural substances. It is focused on the analysis of alkaloids obtained from plants of the *Dicranostigma franchetianum* (Prain) Fedde. At his workplace, he isolated a series of alkaloids typical of *Dicranostigma* plants, what is a typical member of Papaveraceae family. Classical chromatographical techniques based on separation of liquid-liquid extraction pre-purified material utilized flash chromatography on silica, chromatography on alumina, and preparative TLC. Additional crystallization process was used to obtain pure compounds. In total, 21 alkaloids were obtained, and identified based on MS and NMR analysis. These substances were later evaluated for biological activity towards enzymes of interest in the treatment of Alzheimer's disease, as well as for the evaluation of potential antimycobacterial activity.

Isolation of natural substances from plant material is a very traditional pharmacognostic discipline. It has long provided a description of thousands of structurally diverse compounds that serve as drugs or as models for the synthesis of more active compounds. At present, the discipline may be somewhat underestimated because it requires patience, the results are often not fantastic, and the result is suitable for publication often only when needs to be combined with extensive biological activity testing, which makes the outcomes of the whole process more attractive. The work performed by the author is a typical representative of the phytochemical analysis described here. He isolated a series of

substances in an amount sufficient both for testing biological activity. Tests of activity in connection with Alzheimer's disease show some interesting activity, with greatest potential displayed by berberin and palmatin against HAChE, 6-ethoxydihydrochelerithrin and chelerythrin against hBChE. Some anti-POP activity was observed for sanguinarin. The results of bioactivity assays were supported by docking studies. The important is ability of these compounds to act in brain, therefore their ability to cross BBB was assayed and several isolated alkaloids showed the potential to cross it. Furthermore, the antimycobacterial activity of tested compounds was low to moderate. The results of studies are original and sound reasonable. I understand that could be sometimes complicated to publish the isolation of known compounds together with not "star-shining" bioactivity. The author was the first author or participated in the publication of a series of works, an overview of which is given. The works are published in renowned and quality magazines and there is no need to doubt their quality.

Graphically, the work is at a standard level. Part of the work is also an overview of used literature.

The main problem which according to me decreases the value of the presented work is this theoretical overview, which suffers from many problems, including the utilization of work with scientific literature. The information is sometimes mis-interpreted or incorrectly cited, the information is difficult to find and confirm.

The second problem is rising from the identification of compounds isolated, several times here I see assignation of optical rotation +/-, but also for compounds which do not display chirality. Like for protopin (in text written the optical rotation was measured, and after is written it was not measured). For some compounds, like DF-06, it would be nice to see absolute or relative configuration at chiral centers. Laudanosine (DF-16 has chiral center, but it is stated that does not have and the optical rotation is not measured. Could you please explain? Furhter, when signals for NMR are just listed without assigning, I had no chance to control and evaluate.

I have many formal comments which are listed below, and their presence is making the text bit worser. The work also rises several questions.

Formal comments:

Abbreviations: H_2O, EtOH, H_2SO_4, HCl etc. are not abbreviations; STORR S-*reticuline* epimerase, better explain than like this. Units do not have to be explained. The list of abbreviations is quite long, some abbreviations are excessive and sometimes complicate the reading – like Mtb for *M. tuberculosis.*

Page 13: The knowledge of traditional medicine knowledge

Paclitaxel is not a good example of alkaloid

Leucemia x leukaemia

Among the listed subgroups, protoberberines are the largest group: They constitute 25% of all elucidated structures of 14 isoquinoline alkaloids, making them the most widespread secondary metabolites containing nitrogen among natural products [11-13]. – I did not find this info in cited literature.

I was not able to find fulltexts of several Chinese journal papers. Like this one: The effect of Dicranostigma Leptopodum (Maxim) Fedde (DLF) extraction on suppressing oxidative hemolysis of erythrocytes and its mechanism Q Zhao, Y Han, YP Du, TP Wang, Q Wang - Journal of Lanzhou University Medical …, 2006

Page 16: Literature 3 is not giving the cited information, the same 9

Part touching Papaveraceae was obtained from Wiki

Chemical constituents of the tribe Chelidonieae – not found in 35

Page: 18 a 19: Not uniform S/R in italics

Page 22: I would prefere to write morphinane, not morphine type alkaloid

Page 23: Details of chromatography excessive

Page 28: up-regulating the expression of peroxisome proliferator activated receptor-γ coactivator 1α

Page 31: Genes should be written in italics. The font of table sis relatively low, little bit difficult to read.

After 1st appearance in the text, the name of plant should be acronym of genus + species.

Anti-inflammatory x anti-inflammatory

Essential oil prepared by supercritical extraction from the same plant showed bactericidal effects with minimum inhibitory concentration (MIC) against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa with values of 0.25 g/ml, 0.30 g/ml, and 0.26 g/ml, respectively [58]. Are the units correct?

Page 37: which catalysts were used?

Page 38: I do not think the word syrup is appropriate in this context – better thick solution

Uniform marks for priming the position of atoms in molecule and assignation of positions in NMR should be used.

Page 51: why the protopin is assigned +-, when you say it has no chiral center and the optical rotation was not measured? Furthermore, you say the the structure was assigned based on optical rotation experiment. Also for other compounds. For compounds the proton and carbon signals could be assigned to specific carbons and protons, the numbering of the structures is present…

Page 59 laudanosine *has got a chiral centre.*

Questions

1) Page 13: What do you mean by semisynthetic process in living organism?
2) It is quite simplification to say only alkaloids, phenolics, terpenes, and *literature source 4* is quite strange for this information. Could you explain in more details?
3) What is meant by distribution in context of subclassification of tyrosin derived alkaloids?
4) Page 16: Why you discuss vinblastin, paclitaxel, and atropin here with Papaveraceae?
5) Page 24: Other alkaloids present at higher concentrations in this plant are protopine (31), chelidonine (16), and stylopine (22) [25, 52]. Could you please specify the concentration of isolated alkaloids more deeply?
6) Page 28: For example, intragastric administration of 50 mg/kg of BBR (23) once daily for 14 days demonstrated a pronounced improvement in spatial memory deficits in a rat AD model [77], which was also proved in a study with streptozocin-diabetic rats at a dose of 100 mg/kg. Could you please specify connection of AD model on rats and diabetic rats?
7) Page 29: Consistent with this result, sanguinarine (19) demonstrated high inhibition activity against hAChE in another study, which was proved with IC50 1.85 µg/ml as equal to 5.57 µM determined using the HPLC-DAD method [94]. How is this connected with HPLC, could you explain?
8) Touching various biological activity, could you evaluate the most interesting or most promising from point of view of concentration of compounds and possibility of reaching these in vivo / in clinics?
9) Page 35: An alkaloidal extract of *D. leptopodum* was studied for its antibacterial activity. This extract showed promising activity against Klebsiella pneumoniae with an IC50 value of 1.389 mg/ml. In addition, the extract has been able to destruct the cell wall, increase membrane permeability, and inhibit bacterial growth Is this activity really promising? What is the common activity of extracts to be further tested or used for isolation of active compounds?

Conclusion

The student has demonstrated creative skills in the field of research, the work meets the requirements of standard dissertation in the field. I recommend the thesis for defence before the commission for state doctoral examinations and defence of dissertations and obtain degree Ph.D. after successful defense.

Datum: 8/09/2023

Signature: Karel Šmejkal