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COMMENTS on PhD Thesis "Quantum Chemical Studies of Selected Compounds Determined as Active Components of Traditional Chinese Medicine"

by

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and completed under the supervision of Professor Szczepan Roszak

The submitted thesis has been completed in the Department of Advanced Materials Engineering and Modeling, Faculty of Chemistry, Wrocław University of Science and Technology under the supervision of Professor Szczepan Roszak.

Accepting the task of reviewing a thesis from Professor Roszak team and dealing with traditional Chinese medicine (TCM) was a kind of challenge and curiosity from my side. Should I expect a discussion about cold and hot plants and components leading to a dynamic balance? Is there somewhere hidden theoretical chemistry and molecular modeling?

However, while reading the thesis I was nicely surprised. The author, a PhD student from China turned out to be well experienced not only in explanation of historical approach leading to TCM and contemporary modification of patient treatment combining tradition and modern science in a kind of symbiosis. What was more surprising to me, the Author could show a direct link between Chinese medicine and suffering of nearly half of China population from smog, caused mainly by PM2.5 dust particles. As a remedy for the nation health problems commonly used active compounds included in selected plants are proposed. Among the active components are flavones and various free radical scavengers. A real shock to me was the use of DFT calculations for gluing very tiny particles into larger ones, capable to remove by electrofilters, called electrostatic precipitator (ESP). In this case the Author performed

modeling H-bond interactions of particle fragments with water and organic moieties (for example pectins).

Interestingly, the whole thesis combines experiment and theory. The first one is mainly based on NMR studies (one-dimensional, 1D, and 2D ^{1}H and ^{13}C spectra) of selected active components from Chinese rhubarb (Rheum palmatum) and Radix puerariae. In addition, in cooperation with a Chinese team the Author studied experimentally the removal of dust particles flue gas as an exhausts of electrical plants burning coal.

The Thesis is prepared in a traditional form – a monograph starting with literature studies and own "experimental part". It starts with three abstracts – in English, Polish and Chinese followed by two pages of content. From the Content we can notice a clear division of work into six chapters. The Introduction (5pages) explains the problem of toxic smog in China and natural remedies used to cure (or just prevent) its harmful effects. At this point the reader learns about chemical formulas of several active compounds capable to scavenge harmful free radicals. Among them are emodin, puerarin, daidzein and genistin. In my opinion the Thesis is written very well and is not too long or boring with too many unnecessary details.

The First Chapter is fairly short (pages 16-25) and gives a nice overview of traditional Chinese medicine. A very short Chapter two (pages 26-27) explains the role of antioxidants and presents vitamin E and melatonin. Chapter four is indeed very short – just less than one page. However, it defines the aim of work: "reliable studies of chemical properties of active components of Rheum Palmatum and Radix puerariae." In addition, experimental studies (NMR) of previously mentioned compounds are supported by theoretical modeling of magnetic parameters.

The real science starts from Chapter four (pages 29-35). In this chapter the Author presents in a very concise and nice form theoretical methods and computational details of her studies. Here she talks about solvent effect via PCM model and calculation of NMR shieldings and chemical shifts. Using Gaussian 2016 program package she mainly depends on DFT calculations functionals (B3LYP as traditionally used and reliable one, M06-2X as better describing long-range effects and PBE0, as used in material sciences. Two basis sets are used – Pople's 6-311G** and Dunning aug-cc-pVTZ.

Chapter five (pages 36-38) describes a unique NMR experiment directly providing nuclear magnetic shieldings without using TMS and referring to chemical shift. It is worth mentioning some very specific NMR studies leading to direct nuclear shielding measurements in solutions employing Helium-3 resonance as a universal, primary reference and next deuterated solvents. These studies (without TMS as reference) were performed at Warsaw University in

cooperation with Professor Karol Jackowski, who is a "creator of such an approach together with Michał Jaszuński". It is worth mentioning that the NMR experiment is very well described.

Chapter six is significantly longer (pages 39-86) and covers results and discussions. The last part of text contains Conclusions (two pages) and two pages of references (134 positions from the last 30-40 years). Interestingly, no references to some ancient Chinese Medicine texts was given. On pages 100-102 are listed Author's scientific achievements. This part I analyzed with mixed fillings. The Author starts with listing conference contributions without giving co-author names. Here the reader is left guessing. It would be nice to divide the fragment into original papers (7 published) and two submitted. Besides, it would be nice to include impact factors of these journals. Thus, I checked IF values for these journals: 2.011; 3.230; 1.78; 3.230; 2.936. It appears that the total IF for these journals is 17.788. The two submitted papers are supposed to appear (if accepted) in journals lower ranked (1.507 and 1.545 adding to 3.052. These numbers alone support a very high quality research published by the Author.

Final part of the Thesis contains a kind of Supplement (called Appendix). In the Appendix are presented proton and carbon NMR spectra of the studied biologically active molecules. It is difficult for the reader to go back to the beginning of thesis and compare chemical formula with a spectrum. Thus, I am missing here chemical formulas and signal assignment presented on 16 pages without numbers. At the very end of the thesis are included copies of three papers, showing Author's scientific publication activity. The first paper reports on NMR spectra of daidzein and puerarin (paper published in Journal of Molecular Structure). Unfortunately, the last page with conclusions and references is missing. The second paper, published in Fuel, reports on experimental and DFT studies of PM2.5 removal by chemical agglomeration. The third paper (Energy & Fuels) reports on SO3 reduction in flue gas using a selected chemical agent.

At this point I should admit that I am very impressed with an idea of using DFT modeling to control the amount of harmful particles in flue gas. This way of scientific thinking and practical engineering shows a very application-oriented person, capable of solving difficult problems of daily life.

Now I will shift my attention from a general form of the Thesis to more detailed discussion of the obtained results and the Author's discussion included in Chapter 6 (pages 39-88).

Selected molecular properties of the reference molecule (dantron) and active compounds isolated from Chinese rhubarb (emodin, chrysophanol and physcion) are reported in chapter 6.1.1.1. Thus, as result of B3LYP/aug-cc-pVTZ optimization selected carbon-carbon and

carbon-oxygen bonds are analyzed. There is a clear impact of H-bonding leading to longer C=O bond in anthraquinone molecule. Besides, stabilization of planar geometry by pi-orbitals is demonstrated. In case of the corresponding radicals the C9=O1 bond is shorter by about 0.03 Å.

Similar data are contained in Table 4 in case of compounds present in Radix puerariae. Next, the Author studies oxidative properties of selected compounds. The author stresses delocalization of pi-electrons over the whole neutral molecule. However, in case of radicals formed by H removal the electron is delocalized over the half of anthraquinone ring. Thus, s special distribution of electron spin density is nicely illustrated in Table 5. From the bond dissociation energy (BDE) an antioxitative properties of the studied compounds are derived. In fact, the lowest BDE corresponds to the H atom abstraction from the C3-OH position of emodin. These studies allowed the Author to select the most probable reaction pathway: single-step hydrogen atom transfer (HAT). In page 58 interesting correlations between 13C and 1H NMR chemical shifts and BDE of phenolic O-H bond dissociation enthalpies and HAT energies are presented in Table 9.

Chapter 6.3 describes NMR studies of the selected compounds. Interestingly, a correlation between BDE and σ^+ parameter in case of 4-X-phenols. As an electronic descriptor in QASAR/QSPR modeling is proposed chemical shift of carbon bonded to phenolic OH group. DFT predicted nuclear magnetic shielding parameters calculated with three density functionals (B3LYP, M06-2X and PBE0) in the gas phase and chloroform using PCM approach are gathered in Table 10 and compared with experimental emodin data. Theoretical values were obtained with two basis sets: aug-cc-pVTZ and 6-311G(2d,2p). In this case I would prefer separate RMS values for carbon and proton data. However, it is obvious that the quality of NMR shieldings depends on the used density functional: PBE0 > B3LYP >> M06-2X. This result is very pleasing to me. Several years ago we reported on very bad performance of Truhlar's density functionals for prediction of NMR parameters in the complete basis set limit. Figures 19 and 20 show fairly good linear correlation between experimental and predicted proton and carbon nuclear magnetic shielding constants (and chemical shifts) for emodin. As expected, RMS values decrease to a reasonable level (1.5 – 2.6 ppm) in case of chemical shifts. In this case systematic errors cancel and RMS of 1.6, 2.6 and 1.6 is calculated with B3LYP, M06-2X and PBE0 basis sets. Probably as an accidental error cancellation, solvent effect improves the results by about 0.1 ppm but a better quality basis set (aug-cc-pVTZ) does not warrant improved results. Similar data were presented for daidzein. A linear correlation of daidzein chemical shifts in Figure 21goes through the origin

of coordinates and shows better agreement of theory with experiment. Similar data were gathered for puerarin in tables and figures.

In Chapter 6.4 are presented very interesting experiments related to anticancer activity of the studied compounds. These studies were based on intercalating emodin into two base-pairs system of DNA. This way a noncovalent complex, stabilized by a network of H-bonds, was formed. In the next step the Author compared the interaction energy for emodin, daunorubicin and mitoxantrone with DNA model (-50.9, -37.6 and -30.7 kcal/mol) and indicated stronger activity of the former compound. In addition, the Author performed modeling studies on interaction between daidzein/genistein and tankyrase inhibitor (TNKS2).

Conclusions of Chapter 6 are valid for the entire Thesis. As expected, here the Author summarizes her efforts leading to theoretical and experimental characteristics of selected biologically active components of Chinese rhubarb and Radix puerariae as part of modern approach to Traditional Chinese Medicine. In particular, structures, electron density distribution and experimental NMR spectra, supported by GIAO-DFT calculations were obtained. In addition, antioxidant and anticancer properties of several plant components were demonstrated. The reported results could broaden our understanding about antioxidants and give some hints about controlled design of more efficient drugs.

The approach demonstrated by the Author and the obtained results indicate a well experienced person in both theoretical studies and experiment. These features are necessary for a future independent scientist.

As a reviewer I should also point out some mistakes and problems with the Thesis. However, the general shape of the "book" shows no cracks. I have already mentioned some small mistakes or fragments I would like to see better explained. For me, as a reader, the most serious problem was lack of formulas supplied with NMR spectra in the Annex. However, I am fully aware that such small mistakes happen and do not affect the entire work.

Among the most important scientific achievements reported in the Thesis are:

1. DFT modeling of molecular and electronic properties of selected compounds isolated from plants.
2. Experimental characteristics of selected compounds in solution with signal assignment supported by DFT calculations.
3. Demonstration of antioxidant properties of the studied compounds.
4. Demonstration of anti-cancer properties of the studied compounds.

In conclusion, the Thesis is a very nice piece of scientific report containing elements of an interdisciplinary approach. Thus, it solves an important scientific task and fulfills traditional and official requirements of Polish Law Regulations[1]. **Therefore, I have a pleasure to propose the Thesis prepared by Yang Yi for subsequent stages of procedure to Staff Ensemble, Faculty of Chemistry, Wrocław University of Science and Technology[1].**
In addition, taking into account the interdisciplinary approach, represented by the Author, I propose to accept it as a distinguished work.

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[1] Podsumowując stwierdzam, iż rozprawa doktorska Pani Yang Yi stanowi oryginalne rozwiązanie problemu naukowego i w pełni spełnia wymogi art. 13 ustawy z dnia 14 marca 2003 roku „O stopniach naukowych i tytule naukowym oraz o stopniach w zakresie sztuki" (**Dz. U. z 2003 r. nr 65 poz. 595 ze zm. w Dz. U. z 2005 r., nr 164, poz. 1365**) i ustawy z dnia 18 marca 2011 r. o zmianie ustawy — Prawo o szkolnictwie wyższym, ustawy o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki oraz o zmianie niektórych innych ustaw **Dz. U. z 2011 r. nr 84 poz. 455** oraz **Dz.U. 2016 poz. 882** (Obwieszczenie Marszałka Sejmu Rzeczypospolitej Polskiej z dnia 3 czerwca 2016 r. w sprawie ogłoszenia jednolitego tekstu ustawy o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuk) oraz **ROZPORZĄDZENIA MINISTRA NAUKI I SZKOLNICTWA WYŻSZEGO z dnia 19 stycznia 2018 r.** w sprawie szczegółowego trybu i warunków przeprowadzania czynności w przewodzie doktorskim, w postępowaniu habilitacyjnym oraz w postępowaniu o nadanie tytułu profesora, **§ 6.**
Ponadto, z uwagi na interdyscyplinarny charakter pracy Pani Yang Yi proponuję jej wyróżnienie.