Author's response to reviews

Title: Biomedical potential of genetically modified flax seeds overexpressing glucosyl transferase gene.

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Author's response to reviews: see over

29.10.2012, Malbork

COVER LETTER FOR SUBMISSION OF MANUSCRIPT

Dear Editor-in-Chief of

BMC Complementary and Alternative Medicine

I am enclosing herewith the revised manuscript entitled "The biomedical potential of genetically modified flax seeds overexpressing glucosyl transferase gene" by myself, Anna Kulma, Karolina Bazela and Jan Szopa for consideration of publication in BMC Complementary and Alternative Medicine.

The authors have addressed the comments in the revised manuscript and the detailed answers to the reviewer's comments are enclosed below.

Please consider the present form of manuscript for publication in BMC Complementary and Alternative Medicine. In case of any questions please do not hesitate to contact me anytime.

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Response to the reviewers' concerns:

Compulsory Revisions

The authors must address the issue of lack of proper controls. Specifically there is no head to head comparison with extracts of non-transgenic flax seed.

I kindly remind that the subject of the present manuscript in not the comparison between control flax (Linola) and modified flax (GT4), which was the subject of the previous study by Lorenc- Kukuła et al. 2009. The present manuscript comprises determination of biomedical potential only of modified GT4 seeds, because the previous comparison of modified GT flax with the control Linola flax indicated that GT4 flax has much better

qualities than non- transgenic flax. Especially the greatest achievement was the significant improvement of GT flax resistance and thus productivity and also the increased levels of different phenylpropanoids. These two qualities make GT flax the better source of such a metabolites, mainly because its cultivation is economically profitable. For these reason GT4 modified fax has been chosen as an object for further studies, presented in this manuscript. Moreover the previous preliminary research on the cell cycle of fibroblasts treated with seedcake extracts of Linola seeds and GT4 seeds indicated that the percentage of proliferating fibroblast was higher for GT4 preparation treated cells than for Linola preparation treated cells (data not shown). Therefore, the further experiments were performed with use of GT4 preparations.

Moreover, according to the literature, the a priori assumption could be made, that plants that exhibit the increased resistance and elevated levels of different secondary metabolites i.e.phenylpropanoids are more profitable to use for putative applications.

The comparison of composition of the extract is not based on comparison of extracts derived from GT and the non-GT flax seed parent grown at the same location in the same year. The growing conditions have a significant influence the accumulation of phenylpropanoid metabolites including SDG.

For the purpose of this study, the composition of the extract derived from transgenic GT flax and control Linola flax is based on the seeds grown at the same location and in the same year. According to the scientific and agricultural standards the control and transgenic lines were cultivated within the same area (Lower Silesia, Poland) with respect of all necessary distances. Even when the area of cultivation of GT4 and Linola was performed in different region of Poland (North of Poland), the obtained data of phenylpropanoid content of transgenic and non- transgenic lines always indicated the increased amount of these metabolites in modified GT4 flax and also when the obtained results were an average.

Each generation of GT flax is always compared with the control flax Linola. While comparing the phenylpropanoid levels, the same procedure (cultivation location, year, extraction protocol, identification by UPLC, etc...) is held. According to our observation the elevated level of phenylpropanoids in GT flax occurs yearly in comparison to the control,

so the modification effect continues and the stoichiometry between individual metabolites does not change as well. Thus we suggest that at least in the analyzed period the stability of compounds production in transgenic flax is acceptable.

Since all assays are done with diluted extracts, all experiments must include similar non-GT flax seed extracts other wise the authors can make no claims as to the usefulness or potential of the GT flax lines.

As the GT flax exhibit the significant increased resistance to its pathogen *Fusarium culmorum* and *Fusarium oxysporum* in comparison to the controls plants, its usefulness for any application is reasonable. Again, the authors emphasize the application potential of GT flax due to its unusual resistance and the elevated level of phenylpropanoids.

The Data presented does not support the Title of the manuscript or the conclusions.

The authors suggest, that the title of the manuscript remains related to the presented results. Nevertheless, we are open for reviewer suggestions.

Quality of written English: Not suitable for publication unless extensively edited.

The manuscript was edited by native speaker professional text editor, moreover the specialist of scientific background.