

Peer Review File

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Reviewer A

The short article provides an insight on the application of Chinese herbs in the treatment of diseases. In addition to the traditional use of dried herbs, the use of fresh herbs affords the formation of plant-derived lipid nano particles, which can enhance the bioavailability of active components in a specific organ. The elucidation of the structural characteristic of these lipid nanoparticles is instrumental to the development of novel drug delivery vehicle.

Comment: The article was well written and organized. Nevertheless, it would be useful for reader if the author can explain why plant-derived nanoparticles cannot be formed by using dried herbs.

Reply: I thank the reviewer for encouraging comments. Plant-derived lipid nanoparticles may not be obtained in dried herbs because the drying of the fresh plant causes increased intracellular partition of amphiphilic substances into the lipid, which changes the membrane lipid profile [1] and may affect the self-assembly ability of the membrane lipids. I have added this statement in the revised manuscript (Page 4, lines 131-134)

Changes in the text: (Page 4, lines 131-134) Maintaining the freshness is crucial to produce the FHDLNs as the dehydration of fresh plant increases the intracellular partition of amphiphilic compounds into the lipid phase, which changes the membrane lipid composition and may disable the self-assembly capability of membrane lipids into nanoparticles.

Ref: [1] Elena A. Golovina, Folkert A. Hoekstra, and Marcus A. Hemminga. Drying Increases Intracellular Partitioning of Amphiphilic Substances into the Lipid Phase, Impact on Membrane Permeability and Significance for Desiccation Tolerance, Plant Physiol. (1998) 118: 975–986.*

Reviewer B

This is a short, interesting and concise overview on medicinal plant derived nanoparticles.

Comment 1: In this short overview no evidence is provided for any therapeutic benefits (or disadvantages of these nanoparticles). In fact it is not even clear whether the four medicinal plants are actually developed into a product which complies with the definition of nanoparticles, or are these simply medicinal plant preparations?

Reply 1: I thank the reviewer for raising this concern and sorry for rendering the statements that may confuse the readers. Indeed, these emerged fresh herb-derived nanoparticle researches are in their early stages; I now have clarified these points in the revised manuscript (modified in the title and subtitles).

Changes in the text:

The title "Plant-derived lipid nanoparticles: a new potential therapeutic approach for

traditional Chinese medicine's fresh herbs"

Subtitles:

(Lines 50-51): Fresh herb-derived lipid nanoparticles are becoming new therapeutic approaches in preclinical studies

(Lines 73-74): Fresh ginger-derived nanoparticles target the colon and reduce colitis in animal models

(Line 123): Future of fresh herb-derived nanotherapeutics: Can we put new wine in old bottles?

Comment 2: So, is it old wine sold under a new label? I would really like to see a more critical assessment of what nanoparticles can(not) contribute to healthcare. It would be good to have a more balanced assessment including the limitations.

Reply 2: It is not old wine sold under a new label. I sincerely appreciate the reviewer's suggestion and have added the limitation of the fresh herb-derived nanoparticles in the last section of the revised manuscript (see page 4, lines 138-143).

Changes in the text: (Page 4, lines 138-143) However, the current technical limitation of FHDLNs is the lack of a sterile technique to ensure the stability of the lipid nanostructure and the loaded macromolecule cargos (such as siRNA or mRNA). It is also necessary to establish a quality control method of the fresh herbal material and simplify the preparation process that can decrease the batch-to-batch variation of produced FHDLNs.

Comment 3: I suggest to add the full Latin binomials of the species, to make the MS more visible in international databases (e.g. <http://mpns.kew.org/mpns-portal/> or <http://www.plantsoftheworldonline.org/>) and the full species name including authorities and family needs to be included as well as the drug name, if one has been assigned in a pharmacopeia (see Rivera,D., et al., What is in a name? The need for accurate scientific nomenclature for plants. *Journal of Ethnopharmacology* (2013), 152: 393–402. <http://dx.doi.org/10.1016/j.jep.2013.12.022i>). Here an example of how it should be presented: *Salvia miltiorrhiza* Bunge [Lamiaceae; *Salviae miltiorrhizae* radix et rhizoma]

Reply 3: I completely agree and appreciate the reviewer's insight. I have included full Latin binomials of the species in the revised manuscript.

(Lines 27-29) Lily [*Lilium Lanci folium* Thunb; *Lilium brownii* F. E. Brown var. *viridulum* Baker; *Lilium pumilum* DC.] *Rehmannia* [*Rehmannia glutinosa* Libosch.]

(Line 33) *Artemisia* [*Artemisia annua* L.]

(Lines 41-43) lotus [*Nelumbo nucifera* Gaertn.] leaves, fresh Argy wormwood [*Artemisia argyi* Levl. Et Vant.] leaves, fresh cypress [*Platycladus orientalis* (L.) Franco] leaves

(Line 44) grape [*Vitis vinifera* L.]

(Line 53) garlic [*Allium sativum* L.]