

Peer Review Analysis:

The manuscript highlights a significant area of research with promising applications in both cosmetics and therapeutic fields. The synthesis of current knowledge on KGF-2 and the use of plant bioreactors offers a strong foundation for future work. The review is thorough, covering cytokine expression, plant bioreactors, and transdermal delivery systems in detail.

The paper presents a novel synthesis of knowledge on the use of molecular agriculture for producing KGF-2 in plant systems, a promising direction for natural hair care products. While the novelty is clear, the authors could emphasize more explicitly how this approach compares with other biotechnological strategies, particularly in industrial applications.

The review could benefit from greater focus on the limitations of plant-based expression systems, including the challenges of protein purification and environmental variability. Several references are outdated, and the inclusion of more recent studies would enhance the manuscript's relevance and timeliness. There are minor typographical issues that should be addressed to ensure the professionalism of the text.

The arguments are well-developed and supported by relevant studies. However, more attention could be given to explaining the specific advantages of *Arabidopsis* as an expression system for the fusion protein TDP1-KGF-2, particularly in comparison to other plant systems. A more explicit comparison between the use of *Arabidopsis* and other plant bioreactors would add depth to the analysis.

Recommendation: Revise and resubmit with the suggested improvements.

Abstract

The abstract introduces a timely and relevant topic in molecular agriculture with potential commercial and therapeutic applications. With minor revisions to improve clarity, detail, and flow, it would be well-suited for publication in a

scientific journal.

The abstract is well-structured, with a logical flow from the importance of KGF-2, its current limitations, and the proposed solution (fusion proteins for enhanced transdermal delivery). However, the transition between discussing KGF-2's benefits and the introduction of fusion proteins (TDP1-KGF-2) is a bit abrupt. A clearer connection between the limitations of KGF-2's transdermal delivery and the rationale for using fusion proteins would enhance comprehension.

Please consider putting the full name of the acronym KGF-2 at that first moment: keratinocyte growth factor 2 (KGF-2).

The transition between discussing KGF-2's benefits and the introduction of fusion proteins (TDP1-KGF-2) is a bit abrupt. A clearer connection between the limitations of KGF-2's transdermal delivery and the rationale for using fusion proteins would enhance comprehension.

The abstract succinctly describes the scientific problem (low transdermal penetration of KGF-2) and the innovative solution (fusion protein technology), which is an important advancement in the field. However, some additional details would improve the abstract: what are the specific advantages of the Arabidopsis plant system for expressing the fusion protein? How does TDP1 enhance the transdermal penetration of KGF-2? Including a brief mention of the mechanisms or previous successful uses of this approach would strengthen the argument.

The abstract could more explicitly highlight the novelty of the review in comparison to existing literature. Are these approaches already in development, or is this a new concept proposed by the review? More explicitly highlight how this review adds to the existing literature and whether these expression strategies have been previously explored in the context of KGF-2.

Introduction

The passage is mostly clear, but minor issues affect readability. There is no space between one sentence and another, for example, there is no space after the

period in "vaccines.Plant", "proteins.KGF-2"[...]." Additionally, adding conjunctions or better transitions between sentences would improve the flow. The transition between the sentence "[...] Plant bioreactor has many advantages in the expression of cytokines, but there are relatively few studies on the use of plant bioreactor to express fusion proteins." and " KGF-2 can regulate cell proliferation [...]" was abrupt, without connection between the two sentences.

The flow between discussing the advantages of plant bioreactors, the role of KGF-2, and the function of transdermal peptides could be improved. As it stands, the shift from one topic to another feels abrupt. A more logical progression from plant bioreactors to the specific limitations of KGF-2 and how transdermal peptides address these issues would strengthen the coherence.

The citations appear adequate and relevant, although the formatting could be improved for consistency. For example, there is a missing space between the author and the publication year in some citations, e.g., "(Vega-Hernández et al., 2011)" and "(Zhou et al., 2022)(Yang et al., 2002)." These should be corrected to improve professionalism and maintain consistency in referencing style, please, check it in all manuscript. The passage is well-supported by references, but it would benefit from including more recent studies, especially considering that the topic of molecular agriculture and cytokines is a rapidly evolving field. Most of the citations are over a decade old, and recent advancements may provide stronger or updated evidence, please, check it in all manuscript.

While the passage discusses the role of KGF-2 in regulating cell processes such as proliferation, differentiation, and migration, it lacks specificity in terms of how these mechanisms interact with transdermal peptides. Providing more context on how transdermal peptides enhance the function of KGF-2 would make the argument more robust.

REVIEW METHODOLOGY

The methodology section demonstrates a sound and rigorous approach, but it would benefit from additional detail on the specific criteria used to select and

assess the literature, as well as the rationale for the database choices. With these revisions, the methodology will become more transparent and replicable, enhancing the validity of the review.

Overall, the language is clear and professional, but there are minor typographical and grammatical errors. For instance, "Google Escholar" should be corrected to "Google Scholar." Similarly, spaces are missing in some instances, such as "molecular agriculture,molecular agriculture and cytokines" and "KGF-2 and hair growth,transdermal peptide." These small issues should be corrected to improve readability and professionalism.

The search strategy is sound and makes use of well-established academic databases, including PubMed and Web of Science, which ensures that high-quality sources were likely included. However, Baidu Academic Search may not be as universally recognized in the global scientific community, and it may be worth clarifying why this platform was chosen, particularly if relevant literature is only available there.

The use of Boolean search methods is appropriate, but the description of the keywords used is somewhat unclear due to the lack of proper spacing between keywords. Additionally, the authors should mention whether they set any filters or inclusion/exclusion criteria, such as date ranges, language, or relevance, which would help provide more transparency to the review process.

The chosen keywords seem relevant to the topic, but it would be helpful to specify how these keywords were refined and whether different combinations were tested to ensure comprehensive coverage. For example, it would be useful to know whether synonyms or alternate phrases were considered, such as "biomolecular agriculture" or "growth factors in plants" to ensure completeness of the search.

The authors note that PubMed and Google Scholar accounted for 70% of the literature used, but it would strengthen the methodology to explain why these databases were prioritized over others like Web of Science. Were there differences in the availability of relevant papers between the databases?

The statement "relevant reports were acquired, studied, and assessed for suitability to the topic" could be expanded upon. How was the suitability of reports determined? Were there any predefined criteria or protocols to evaluate the relevance and quality of the studies included in the review? Providing more specifics here would give the methodology more rigor.

MOLECULAR FARMING IN CYTOKINE EXPRESSION

The section provides a broad overview of molecular farming, cytokine expression, and plant bioreactors. It effectively highlights key advances in the field and the different plant platforms that have been used.

The breadth of the review is commendable, but it could be more focused. For example, the paragraph begins by addressing molecular farming in general but shifts quickly to cytokine expression and growth factors. There could be a clearer distinction or transition between these themes to maintain a consistent focus.

The term "molecular farming" is clearly explained, but some technical terms such as "post-translation modification" or "oleosin fusion technology" may not be clear to all readers. Brief explanations or references to these techniques would make the section more accessible. Improve transitions between topics and provide brief definitions of specialized terms for a broader audience.

The phrase "a phenomenon commonly observed in can be in prokaryotic system" is awkwardly worded and should be revised for clarity. There are multiple long sentences, which could be broken down for better readability. For example, the sentence starting with "Third, plants have the post-translation modification machinery..." could be simplified for easier. Clean up the formatting and ensure there are no missing spaces or awkwardly worded sentences.

The references appear relevant and well-integrated, though some key citations are relatively old, such as Ma et al. (2003) and Mehta & Fitzpatrick (2007). Given the rapid developments in molecular farming, it would be beneficial to include more recent studies, particularly on technological advancements or newer plant production platforms. Update the citations to include more recent research where possible, especially in areas where technological progress is likely. In this

chapter, authors can find a review of similar subjects with an update of the literature (https://link.springer.com/chapter/10.1007/978-981-97-0176-6_8).

The section provides strong theoretical support for the use of plant bioreactors in cytokine expression, backed by relevant examples like the expression of IL-13, GM-CSF, and bFGF in plant systems. The advantages of plant bioreactors are clearly articulated, particularly regarding cost, scalability, and safety.

The passage highlights well-known advantages of plant bioreactors, but it could benefit from a deeper discussion of the challenges that still remain. For example, the complexity of protein purification in different plant systems or the regulatory hurdles for plant-derived therapeutics could be acknowledged.

Additionally, it would strengthen the passage to mention whether any new trends or emerging technologies are poised to overcome current limitations. Are there any innovations, such as CRISPR-based gene editing in plant bioreactors, that could enhance protein expression and yield? Address challenges and recent innovations in plant bioreactors to provide a more balanced view.

The use of diverse examples (tobacco, soybean, *Arabidopsis*) adds credibility to the argument that plant systems are versatile platforms for protein production. The text could be more concise and focused. At times, it feels as though multiple ideas are being presented without clear transitions between them. While the advantages of plant bioreactors are well explained, the passage lacks discussion on specific limitations or challenges (e.g., the environmental variability of plant growth, regulatory hurdles, or protein yield consistency). Including these would create a more comprehensive overview of the topic.

FUNCTIONS OF KGF-2

Begin this section by briefly outlining KGF-2's general regenerative properties, followed by more specific roles in each of the highlighted areas (e.g., wound healing, eye repair).

KGF-2 in Skin Wound Healing:

Instead of describing the phases of wound healing in general terms, focus more

directly on KGF-2's role in these phases. For instance, highlight how KGF-2's modulation of STAP-2 expression directly influences scar formation.

KGF-2 in Eye Injury Repair:

Use subsections to categorize findings. For example, group studies that focus on epithelial migration together, separate from those on cellular signaling.

KGF-2 in Hair Regeneration:

Emphasize KGF-2's involvement in specific pathways (e.g., Wnt/ β -catenin, Shh) before detailing individual studies, which will contextualize the studies within the broader biological framework.

Provide brief background information on pathways like Wnt/ β -catenin and Shh to make KGF-2's interactions more accessible. Explaining how KGF-2's upregulation of these pathways facilitates hair follicle growth, for instance, would strengthen the link between molecular action and observable outcomes.

STRATEGIES FOR KGF-2 BIOSYNTHESIS

While various expression systems (e.g., prokaryotic and plant-based systems) and fusion strategies are mentioned, the manuscript could enhance the scientific discussion by comparing their efficacy, challenges, and limitations. For instance, a table summarizing each method's advantages and disadvantages would aid readers in understanding the feasibility of each technique.

The section on CPPs would benefit from more detailed mechanistic insights into how peptides like TD1 and TP1 enhance transdermal transport, especially in the context of KGF-2. More explicit explanations on CPPs' mode of action, specifically regarding the potential pathways and interactions with skin cell membranes, could strengthen the manuscript.

Additionally, discussing any potential challenges in applying CPPs with KGF-2, such as peptide degradation rates or immune response considerations, could provide a more balanced view.

The manuscript would benefit from elaborating on the clinical significance and

future applications of KGF-2 in hair growth and skincare, potentially exploring areas like wound healing where improved transdermal delivery of KGF-2 could be groundbreaking. Further, a brief discussion on the stability, bioactivity, and patient outcomes in clinical settings would provide practical relevance.

Minor grammatical errors are present, and some phrases are verbose or ambiguous. For instance, phrases like "the transdermal ability of KGF-2 combined with oil body is also enhanced" could be rephrased for clarity.

Plants extracts for hair growth and care

The evidence provided supports the efficacy of plant extracts in promoting hair growth. The cited studies highlight how plant-derived compounds impact growth factor regulation and cellular proliferation, which are relevant processes for KGF-2's role. However, the manuscript would benefit from a deeper discussion about KGF-2, specifically how it interacts with the signaling pathways involved in hair growth, its unique attributes, and why it represents a promising target for biosynthesis in plant systems.

The manuscript mentions investigating synergistic effects between TD1-KGF-2 and Arabidopsis-derived compounds. However, it lacks data or examples demonstrating these interactions. Results from other works examining the potential enhancement of hair growth-promoting pathways (e.g., upregulation of growth factors) by the fusion protein in conjunction with plant extracts would reinforce the manuscript.

OUTLOOK

The *Outlook* section is well-developed and presents a thoughtful vision for the future of plant-based bioreactors, with an emphasis on the emerging market demand for natural, therapeutic solutions. The proposed exploration of synergistic effects between bioactive compounds and therapeutic proteins is innovative and relevant. However, adding detail to certain areas—particularly on plant model selection, market viability, and technical limitations—would enrich the analysis and provide a more comprehensive roadmap for researchers and industry stakeholders.