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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии
საქართველოს სამედიცინო სიახლეები

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო ხიახლები – არის უფლებული სამეცნიერო სამედიცინო რევიუზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეცნიელების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რეცენზირდება ინგლისურ ენებზე ქვეყნება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman (Кириллица)**, для текста на грузинском языке следует использовать **AcadNusx**. Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применяющиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи.** Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - <http://www.spinesurgery.ru/files/publish.pdf> и http://www.nlm.nih.gov/bsd/uniform_requirements.html В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or computer-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman (Cyrillic)**, print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf

In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორია საშურალებოდ!

რედაქტორი სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე, დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურნოვან ტექსტებში - **Times New Roman (Кириллицა)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx**. შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სის და რეზიუმების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გამუქდება: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანორმილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოსასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტ-სურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ურნალის დასახელება, გამოცემის ადგილი, წელი, ურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფრჩილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცეზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქტორი იტოვებს უფლებას შეასწოროს სტატიას. ტექსტშე მუშაობა და შეჯრება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქტორი ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდიდად წარდგენილი იყო სხვა რედაქტორიაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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EXPLORING THE MECHANISM OF ACTION OF HEMP SEEDS (CANNABIS SATIVA L.) IN TREATING OSTEOPOROSIS USING NETWORK PHARMACOLOGY

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Abstract.

This study's objective was to elucidate the molecular mechanism of hemp seeds against osteoporosis using a network pharmacology approach. The methodology entailed the retrieval of active components and targets of hemp seeds from the TCMSp database, as well as OP-related targets from GeneCards, TTD, Drugbank, and OMIM databases, followed by identifying their intersection. Network construction and enrichment analyses were performed using Cytoscape and Metascape. Finally, molecular docking was utilized to validate the binding capacity between the core active ingredients and the key targets. As a result, six active components and 96 potential targets of hemp seeds were identified, which intersected with 1,745 OP-related targets to yield 53 common targets. GO enrichment analysis revealed that these targets were primarily involved in biological processes such as inflammatory response, while KEGG analysis highlighted significant pathways including the AGE-RAGE and PI3K-Akt signaling pathways. Notably, a subsequent analysis of the protein-protein interaction (PPI) network topology identified AKT1, TNF, and IL6 as pivotal targets within the therapeutic mechanism. In summary, the present study initially revealed the potential mechanism of OP treatment with hemp seeds and provided a reference for conducting experimental studies.

Keywords. Hemp seeds, osteoporosis, network pharmacology, molecular docking, mechanism of action.

Introduction.

Osteoporosis (OP) is a systemic bone disease characterized by low bone mass, impaired bone microarchitecture, increased bone fragility, and susceptibility to fractures [1]. Osteoporotic fractures pose significant hazards, leading to considerable impairment of the patient's quality of life and elevating the mortality rate [2]. A global estimate suggests that approximately one-third of women and one-fifth of men over the age of 50 years will experience an osteoporotic fracture during their lifetime [3]. While a range of clinical treatments is currently available, issues persist regarding treatment duration, inadequate compliance, and the necessity for combinations of medications, along with severe side effects [4].

Hemp seeds, when utilized as a medicine and food homology (MFH) herb, has been shown to be abundant in nutrients and to serve as a substantial source of natural antioxidants and bioactive compounds [5,6]. Previous studies have reported that hemp seeds improve bone metabolism in humans and in

vitro, upregulating osteoblast differentiation markers while downregulating RANKL [7]. In addition, hemp seed oil has been shown to effectively alleviate joint inflammation and bone destruction [8]. Therefore, it can be hypothesized that hemp seeds may exert therapeutic effects on OP. In this study, we employed network pharmacology techniques to investigate the active components and mechanisms of action of hemp seeds in treating OP, with the aim of providing insights for subsequent in vivo and in vitro biological experiments.

Materials and Methods.

Identification of Active Components and Related Targets:

Using the TCMSp platform (<https://www.tcmsp-e.com/tcmsp.php>, version 2.3, accessed on Oct 2 2025), active ingredients were screened based on the criteria of oral bioavailability (OB) $\geq 30\%$ and drug-like properties (DL) ≥ 0.18 . After matching each component's targets via TCMSp. Following this, the target names were standardized using the Uni-Prot database (<https://www.uniprot.org/>, version 2025_03, accessed on Oct 2 2025) and converted to gene names.

Acquisition of Disease Targets and Intersecting Targets:

Targets of disease were collected by searching databases including GeneCards (<https://www.genecards.org/>, version 5.25, accessed on May 22 2025), Therapeutic Target Database (TTD, <https://db.idrblab.net/ttd/>, accessed on May 22 2025), Online Mendelian Inheritance in Man (OMIM, <https://www.omim.org/>, accessed on May 22 2025), and DrugBank (<https://go.drugbank.com/drugs>, version 5.1.13, accessed on May 22 2025) using "osteoporosis" as the search keyword. To avoid false positives, from all targets retrieved from GeneCards, the top 25% of targets with the highest relevance scores were selected as potential OP-related targets. The consolidation, merging, deduplication and matching to gene names of all targets was completed. The Jvenn platform (<https://jvenn.toulouse.inra.fr/app/index.html>, accessed on Oct 2 2025) was utilized to identify intersection targets between drugs and diseases, generating a Venn diagram.

Construction of Drug-Active Ingredient-Target-Disease Network:

The data pertaining to diseases, drugs, active ingredients, and genes were meticulously organized into Excel files and subsequently imported into Cytoscape 3.10.3 software to construct a visual network diagram.

GO Function and KEGG Pathway Enrichment Analysis:

The intersecting target points were imported into the Metascape database (<https://metascape.org/>, version 3.5, accessed on Oct 4 2025) for GO and KEGG enrichment analysis. The top 20 signaling pathways were selected and imported into the Bioinformatics Online Platform (<https://www.bioinformatics.com.cn/>, accessed on Oct 4 2025) to generate bubble charts.

PPI Network Construction:

The intersecting targets were imported into the STRING database (<https://cn.string-db.org/>, version 12.0, accessed on Oct 4 2025) to generate a PPI network, which was then visualized using Cytoscape 3.10.3 software. Topological parameter analysis was performed to identify key targets.

Molecular Docking:

Key targets and core compounds were screened by PPI and drug-active ingredient-target-disease network diagram analysis. The PDB database (<https://www.rcsb.org>, accessed on Nov 28 2025) provided the crystal structures of the key targets. The PubChem database (<https://pubchem.ncbi.nlm.nih.gov>, accessed on Nov 28 2025) to download component SDF files. The processed protein receptors and corresponding small molecule ligands were imported into AutodockTools 1.5.7 software, and the necessary pre-processing such as hydrogenation was carried out, and molecular docking was carried out by using Autodock Vina to obtain the binding energies of each combination, and the combinations with strong binding ability were visualized by using PyMOL software.

Results.

Active Components of Hemp Seeds and Corresponding Targets:

Hemp seeds contain six active components, including arachidonic acid, sitosterol, stigmasterol, (Z)-3-(4-hydroxy-3-methoxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl] acrylamide, gondoic acid, and luteolin. A total of 96 target proteins were identified for these active components.

OP Targets and Intersecting Targets:

A comprehensive search through GeneCards, TTD, OMIM, and DrugBank databases yielded 7355, 33, 113, and 188 OP-related targets, respectively. The number of targets in GeneCards with relevance scores above the first quartile was 1,834. Subsequent to the processing stage, a total of 1,745 OP disease target genes were definitively identified. Among these, 53 targets intersected with both drugs and diseases (Figure 1).

Construction of the Drug-Active Ingredient-Target-Disease Network:

The drug-active ingredient-target-disease network consists of 104 nodes and 178 edges, primarily comprising 6 active ingredients and 96 target genes, which are distinguished by blue and purple logos, respectively, with green nodes representing hemp seeds (HS) and orange nodes indicating osteoporosis (Figure 2). Analysis based on degree values indicates that the top three active ingredients are luteolin, arachidonic acid, and stigmasterol.

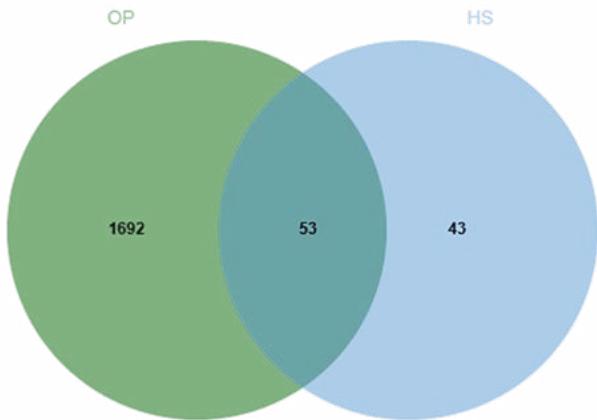


Figure 1. Venn diagram of the intersection of hemp seeds active ingredient targets with OP disease targets. HS: hemp seeds, OP: osteoporosis.

GO Functional and KEGG Pathway Enrichment Analysis:

GO enrichment analysis revealed that the intersecting genes were involved in 872 biological processes (BP), including gland development and regulation of inflammatory response etc., 44 cellular components (CC), including membrane rafts and receptor complexes etc., and 78 molecular functions (MF), such as kinase regulator activity and transcription factor binding (Figure 3). KEGG pathway enrichment analysis identified 161 pathways, primarily involving signaling pathways such as cancer, Lipid and atherosclerosis, AGE-RAGE, PI3K-Akt and TNF (Figure 4).

PPI Network Analysis and Key Target Identification:

The PPI network diagram comprises 51 nodes and 549 edges, with an average degree value of 20.7. Topological analysis revealed the top ten targets by degree value: AKT1, TNF, IL6, TP53, PTGS2, EGFR, MMP9, PPARG, CASP3, and IFNG (Figure 5). The interactions of these targets with other proteins have been determined to play a critical role in the treatment of osteoporosis.

Validation of Molecular Docking:

Molecular docking analysis was performed on the top five key targets (AKT1, TNF, IL6, TP53, PTGS2) and three active components (luteolin, arachidonic acid, stigmasterol), and a heatmap was plotted based on the results (Figure 6). A lower binding energy is widely considered to indicate a more stable interaction [9]. The docking results showed that most active compounds have strong binding affinities to the specified nuclear targets. We selected two combinations with better binding capacities for visualization using PyMOL, and the results showed that stable hydrogen bonds were formed between the receptor and ligand (Figure 7).

Discussion.

The pathogenesis of OP is multifaceted, involving estrogen deficiency, cellular aging, gut microbiota dysbiosis, and inflammation, which collectively contribute to imbalanced bone remodelling and resultant bone loss [10-12]. Consequently, the efficacy of monotherapy frequently falls short of expectations.

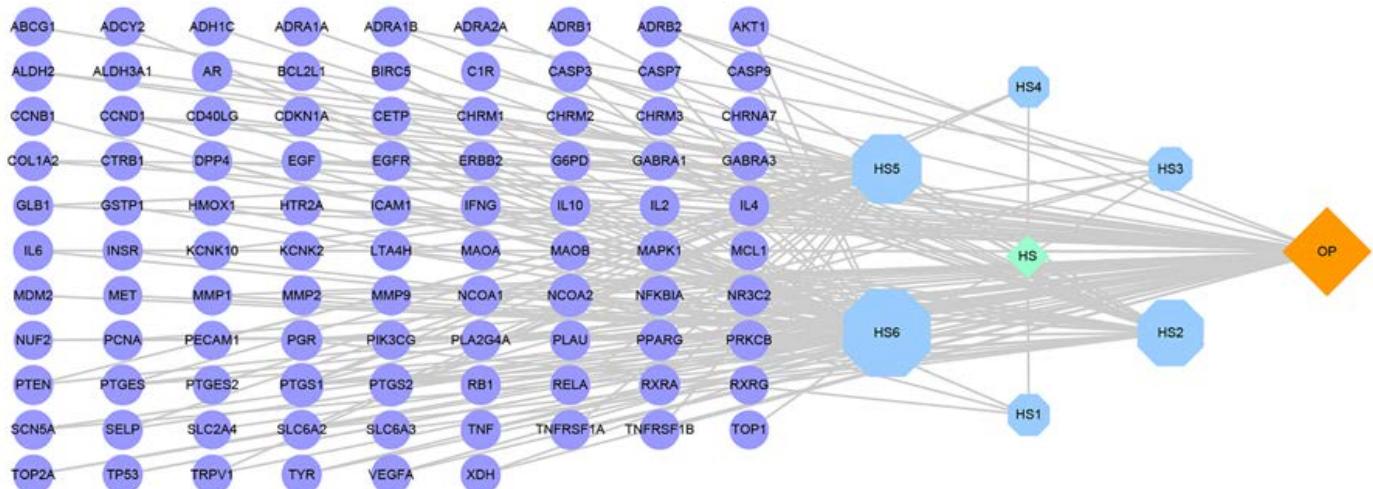


Figure 2. Drug-active ingredient-target-disease network. HS1: sitosterol; HS2: stigmasterol; HS3: (Z)-3-(4-hydroxy-3-methoxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl] acrylamide; HS4: gondoic acid; HS5: arachidonic acid; HS6: luteolin.

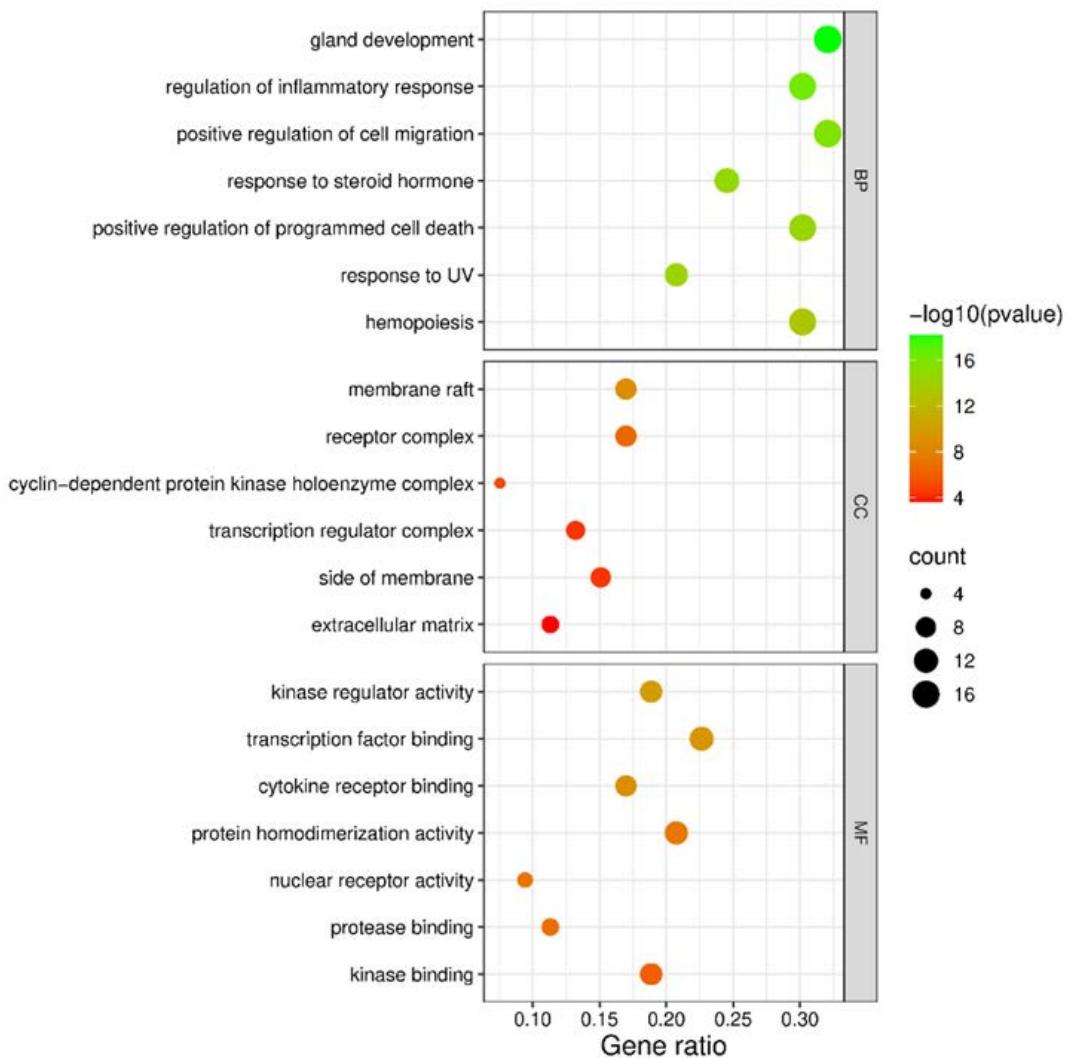


Figure 3. GO enrichment analysis of hemp seeds in the treatment of osteoporosis.

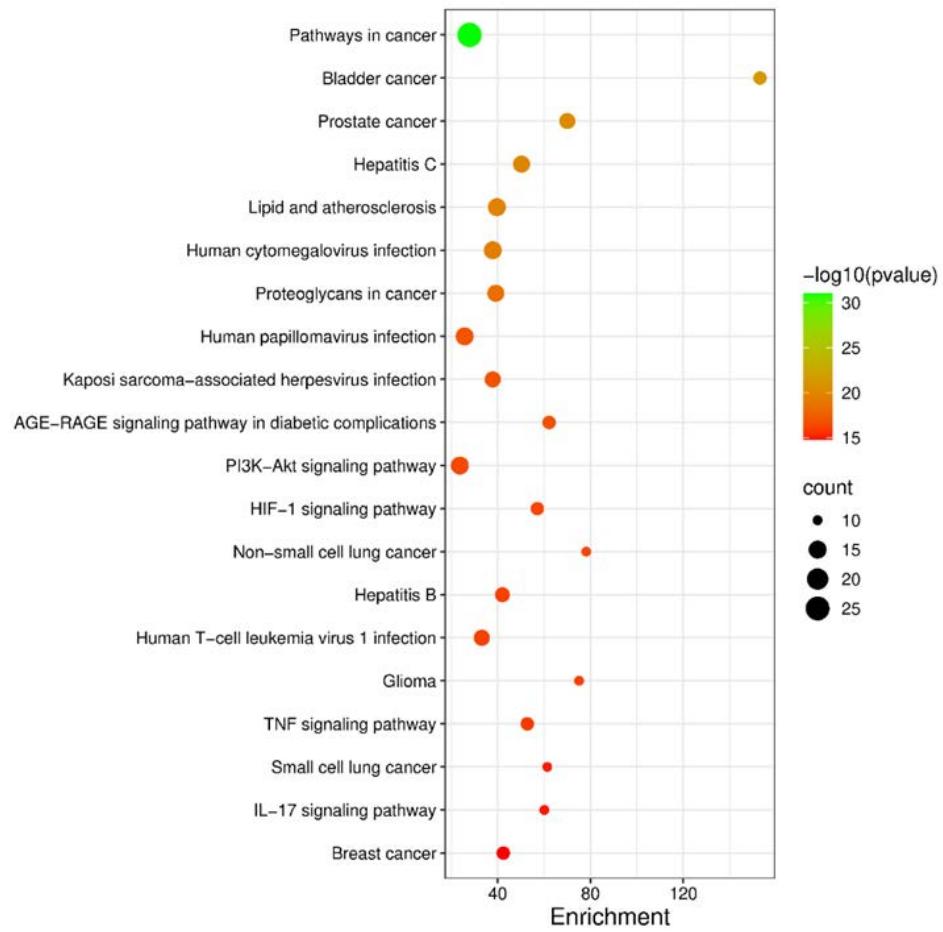


Figure 4. Enrichment analysis of KEGG pathway of hemp seeds in the treatment of osteoporosis.

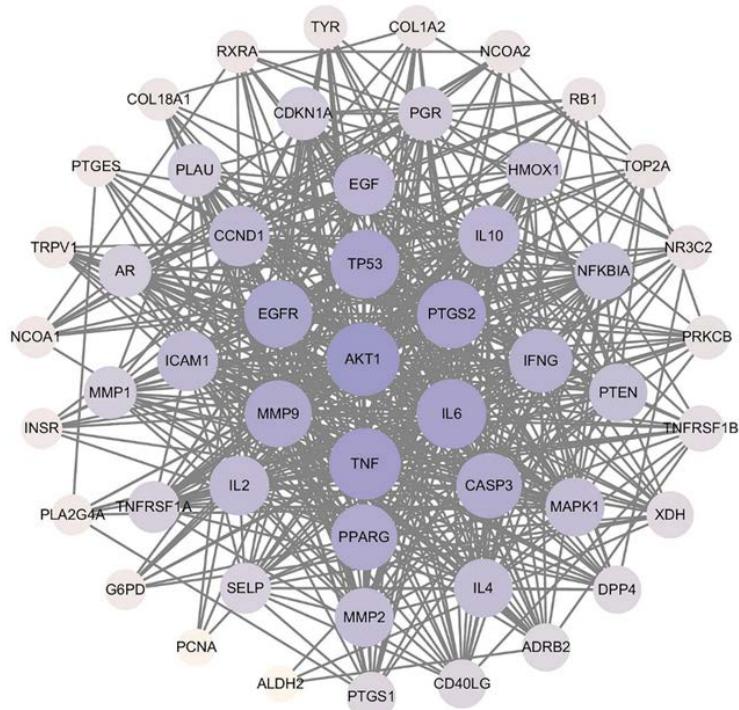


Figure 5. PPI network diagram of the intersection target of hemp seeds prescription and osteoporosis.

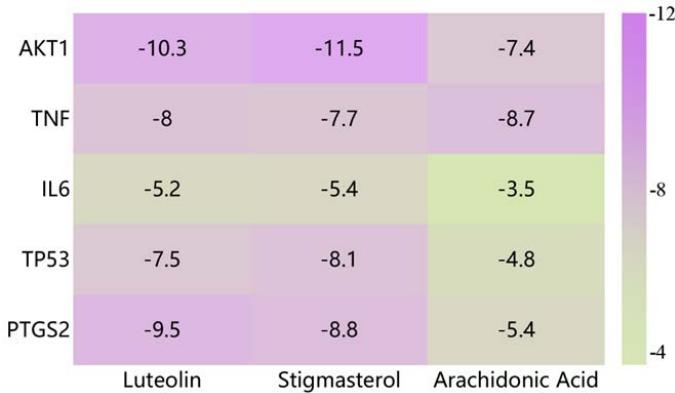


Figure 6. Heat map of the binding energy of molecular docking.

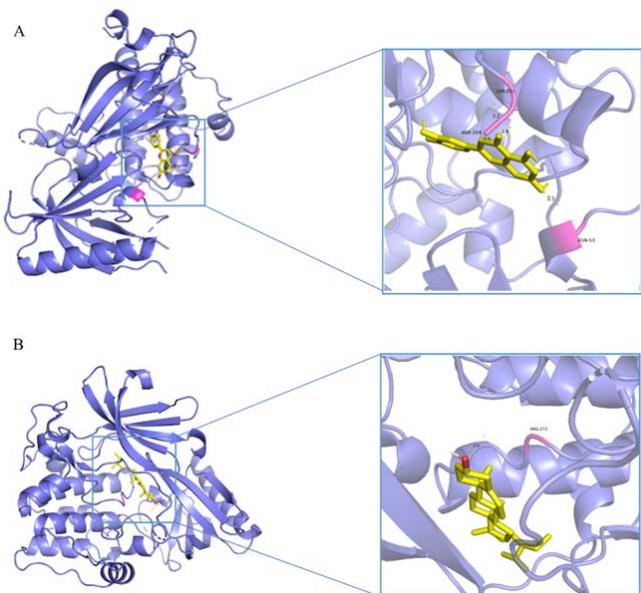


Figure 7. Molecular docking between representative components and core targets. (A) Molecular docking between AKT1 and luteolin; (B) molecular docking between AKT1 and stigmasterol.

Traditional Chinese medicinal materials are distinguished by their multi-component composition, multi-target effects, and systemic regulation, which renders them frequently employed in OP treatment research [13]. As a MFH, hemp seeds possess a diverse pharmacological potential [14]. In addition, research findings suggest that their derivatives may have a beneficial impact on osteoarthritis [7,8]. Nevertheless, the impact of hemp seeds on OP and its underlying mechanisms remain to be fully elucidated. This study employs network pharmacology to demonstrate that hemp seeds may exert effects through active components such as luteolin and stigmasterol to target signaling pathways such as AGE-RAGE and PI3K/Akt. By modulating critical downstream targets such as AKT1, these agents promote bone homeostasis, thus preventing and treating OP.

The present study identified luteolin, arachidonic acid, and stigmasterol as the top three ranked bioactive components in hemp seeds. Luteolin, a flavonoid compound, has been demonstrated to possess anti-inflammatory, antioxidant, antitumor, and metabolic regulatory properties [15]. Zhou et al. discovered that luteolin can inhibit inflammation and oxidative

stress through the AMPK/Nrf2 pathway, thereby protecting osteocyte function [16]. In addition, stigmasterol is a phytosterol that has been demonstrated to possess pharmacological effects, including anticancer, anti-osteoarthritis, anti-inflammatory, anti-diabetic, immunomodulatory, antioxidant, and neuroprotective properties [17]. Xiao et al. shown that stigmasterol has been shown to play a role in preventing OP through regulating the gut microbiota [18]. Arachidonic acid is a polyunsaturated fatty acid, which has been reported to promote osteoclast differentiation through the RANKL pathway and its metabolites, increasing the risk of bone resorption [19]. This appears to contradict our predicted results. However, sitosterol in hemp seed has been demonstrated to modulates osteogenic and adipogenic balance in BMSCs to suppress osteoporosis [20]. Besides, rich cannabinoids in hemp seed, such as $\Delta 9$ -Tetrahydrocannabinol (THC) and Cannabidiol (CBD), have been shown to promote fracture healing and reduce bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines [21,22]. These ingredients, which have been widely confirmed to have anti-osteoporotic effects, can counteract or balance the potential negative effects of arachidonic acid, thereby providing overall bone protection. Furthermore, network pharmacology is characterized by “multi-component-multi-target”, which needs to be combined with subsequent in vitro and in vivo experiments to validate this holistic effect. In summary, we speculate that hemp seeds may influence bone metabolism through anti-inflammatory, antioxidant, gut microbiota-modulating, and metabolic regulatory mechanisms, exerting efficacy in preventing and treating OP.

This study indicates that the key target molecules for hemp seed in preventing and treating OP are AKT1, TNF, and IL6. As is widely recognized, Akt1 functions as a downstream effector molecule of the PI3K/AKT signaling pathway, and it also plays an indispensable role in various cellular processes, including cell death, autophagy, oxidative stress, inflammatory response, and iron death [23]. It has been reported that adipogenic gene expression can be promoted in bone marrow mesenchymal stem cells by activating the PI3K/AKT/Hippo pathway [24]. TNF and IL6 are able to regulate bone metabolism by inducing the recruitment, differentiation and activation of osteoclasts through inflammatory pathways [25]. It is noteworthy that pathways in cancer topped the list in KEGG enrichment analysis, which may stem from the shared nature of inflammatory and metabolic signaling pathways, cancer often involve inflammatory signaling pathways (e.g., TNF, IL-17, NF- κ B, and MAPK signaling pathways), which are prone to be activated under metabolic stress conditions [26,27]. In contrast, in osteoporosis models, shared inflammatory signaling pathways are often activated and directly contribute to increased bone resorption and imbalanced bone reconstitution [28]. These analyses suggest that hemp seeds may influence bone remodelling via pathways including inflammatory signaling cascades, lipid metabolism and the PI3K/Akt signaling pathway.

Conclusion.

In summary, the present study found that hemp seeds may exert anti-OP effects through a combination of mechanisms involving multiple components, targets, and pathways. This study is not without its limitations. Firstly, the data in public databases is

limited and dynamic, so the data collected is incomplete and subject to change. Furthermore, the study did not implement relevant experimental validation. In the future, further *in vivo* and *in vitro* experiments will be carried out to provide a more theoretical foundation for the treatment of OP with hemp seeds.

Authors' contributions.

Conceptualization: Yan Wang, Yulei Xie, Qing Wu. Data curation: Yan Wang, Chong Yin. Investigation: Yan Wang, Chong Yin. Methodology: Yan Wang. Writing-original draft: Yan Wang, Yulei Xie, Qing Wu. Writing-review & editing: Yan Wang, Yulei Xie, Qing Wu.

Conflicts of Interest.

There are no conflicts of interest to declare.

Data availability.

All data included or relevant to the study are available upon request by contact with the corresponding author.

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None

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