

GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 5 (338) Май 2023

ТБИЛИСИ - NEW YORK



ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии
საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press.
Published since 1994. Distributed in NIS, EU and USA.

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 ВИНТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება 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 ВИНТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებშიდან.

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, применявшиеся 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. Корректурa 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.**

ავტორთა საქურაღებოლ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დაიცვათ 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 ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, 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 სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: 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, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგის 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, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფხიხლებში 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 რეცენზია, 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. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

K.S. Altynbekov, N.I. Raspopova, A.A. Abetova. ANALYSIS OF SOCIAL AND DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS WITH PARANOID SCHIZOPHRENIA OF THE KAZAKH ETHNIC GROUP IN THE REPUBLIC OF KAZAKHSTAN.....	6-13
E.A. Karton, F.H. Dzgoeva, M.V. Shestakova, I.G. Ostrovskaya, Taigibov M.H. INVESTIGATION OF THE LEVEL OF MONOSACCHARIDES IN SALIVA OF PATIENTS WITH IMPAIRED CARBOHYDRATE METABOLISM.....	14-18
Seoul-Hee Nam. EVALUATION OF THE ANTI-CARIES EFFECT OF <i>LESPEDEZA CUNEATA</i> EXTRACT AGAINST <i>STREPTOCOCCUS</i> MUTANS.....	19-22
Kudrin AP, Borzykh NA, Roy IV, Rusanov AP, Melenko VI. EVALUATION OF THE EFFECTIVENESS OF PHYSIOTHERAPEUTIC INTERVENTIONS IN THE TREATMENT OF THORACIC PAIN IN PATIENTS WITH THORACIC OSTEOCHONDROSIS.....	23-28
E.Saralidze, I.DiasamiDze, L.Khuchua. THE CHANGES OF EPILEPTOGENIC THRESHOLD IN HIPPOCAMPUS DURING NORMAL SLEEP – WAKING CYCLE.....	29-32
Kucher I, Liabakh A. BIOMECHANICAL COMPARISON OF THREE POSTERIOR MALLEOLUS FRACTURE FIXATION METHODS IN RELATION TO DIFFERENT FRACTURE MORPHOLOGY: A FINITE ELEMENT ANALYSIS.....	33-40
Balytskyy V, Zakharash M, Kuryk O. INFLUENCE OF A VARIETY OF SUTURE MATERIAL ON THE ANAL CANAL WOUNDS HEALING AFTER COMBINED OPERATIONS CONCERNING THE COMBINED ANORECTAL PATHOLOGY WITH USING OF MODERN TECHNOLOGIES.....	41-48
Quanhai Wang, Lianping He, Yuelong Jin, Yan Chen, Yingshui Yao. OLDER FARMERS OR ILLITERATE OLDER ADULTS ARE MORE LIKELY TO FALL: A COMMUNITY-BASED STUDY FROM CHINA.....	49-52
Abeer Abd Al Kareem Swadi, Nihad N. Hilal, Mohammed M. Abdul-Aziz. THE ROLE OF MELATONIN AND VITAMIN D IN IRAQI PREMENOPAUSAL WOMEN OSTEOARTHRITIS PATIENTS.....	53-56
I.S.Rudyk, D.P.Babichev, O.O.Medentseva, S.M.Pyvovar, T.D. Shcherban. COURSE OF POST COVID-19 DISEASE IN HEART FAILURE PATIENTS WITH MODERATELY REDUCED LEFT VENTRICULAR EJECTIONFRACTION.....	57-62
Mohammed H. AL-Shaibani, Maha T. Al-Saffar, Abdulsattar S. Mahmood. THE IMPACT OF ALOE VERA GEL ON REMINERALIZATION OF THE TOOTH AND ITS EFFECT AGAINST ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY.....	63-68
Safaa Hussein Abdullah Al-Oda, Shatha Khudiar Abbas, Khetam Habeeb Rasool. IMPACT OF BLASTOCYSTIS HOMINIS INFECTION ON IMMUNOLOGICAL PARAMETERS IN PATIENTS WITH DIARRHEA: A CROSS-SECTIONALSTUDY.....	69-73
Tereza Azatyan, Lusine Stepanyan. A STUDY OF SPATIAL ORIENTATION AND CONSTRUCTIVE PRAXIS DISORDERS IN NORMALLY DEVELOPING AND MENTALLY RETARDED CHILDREN AGED 8-11.....	74-77
Sh. Kevlishvili, O. Kvlividze, V. Kvirvelia, D.Tananashvili, G. Galdava. SOCIO-ECONOMIC FEATURES OF SEXUALLY TRANSMITTED INFECTIONS AMONG MSM IN GEORGIA.....	78-86
Georgi Tchernev, Simona Kordeva, Valentina Broshtilova, Ilia Lozev. CONGENITAL LYMPHANGIOMA OF THE FOOT MIMICKING MULTIPLE VIRAL WARTS: DERMATOSURGICAL APPROACH WITH SECONDARY WOUND HEALING AND FAVOURABLE FINAL OUTCOME.....	87-90
Fatma S. Abd-Alqader, Entedhar R. Sarhat, Zaidan J. Zaidan. EVALUATION OF THE ROLE OF COENZYME Q 10 IN THE BLOOD OF BREAST CANCER WOMEN.....	91-95
Lezhava T, Kakauridze N, Jokhadze T, Buadze T, Gaiozishvili M, Gargulia Kh, Sigua T. FREQUENCY OF VKORC1 AND CYP2C9 GENES POLYMORPHISM IN ABKHAZIAN POPULATION.....	96-101
Jiangrong Luo, Chunbao Xie, Dan Fan. IS IT MEANINGFUL FOR SERUM MYOGLOBIN IN PATIENTS WITH COVID-19 DECREASED?.....	102-103
Mucha Argjent, Pavlevska Elena, Jovanoska Todorova Biljana, Milenkovik Tatjana, Bitoska Iskra, Jovanovska Mishevaska Sasa. INSULINOMA OF THE TAIL OF THE PANCREAS – A CASE REPORT.....	104-107

Mukola Ankin, Taras Petryk, Igor Zazirnyi, Olena Ibrahimova. SURGICAL TREATMENT OF OLD PELVIC INJURIES.....	108-114
Georgi Tchernev, Valentina Broshtilova. ADVERSE DRUG EVENTS: LICHEN PLANUS OF THE PENIS AFTER INTAKE OF NEBIVOLOL- FIRST REPORTED CASE IN THE WORLDLITERATURE.....	115-116
Borzykh AV, Laksha AM, Borzykh NA, Laksha AA, Shypunov VG. STRATEGY OF RECONSTRUCTIVE AND RESTORATIVE INTERVENTIONS FOR HAND TISSUE DEFECTS.....	117-120
S. Guta, O. Abrahamovych, U. Abrahamovych, L. Tsyhanyk, M. Farmaha. INFECTIOUSNESS OF SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS WITH CYTOMEGALOVIRUS AND EPSTEIN-BARR VIRUS.....	121-125
Wejdan Al-Shakarchi, Yasir Saber, Marwan M. Merkhan, Yasser Fakri Mustafa. ACUTE TOXICITY OF COUMACINES: AN <i>IN VIVO</i> STUDY.....	126-131
Tchernev G, Kordeva S, Lozev I, Cardoso JC, Broshtilova V. SUBUNGUAL HEMATOMA OVERLAPPING WITH SUBUNGUAL LOCATED FOCAL MELANOCYTIC HYPERPLASIA: DERMATOSURGICAL APPROACH AS OPTIMAL TREATMENT CHOICE.....	132-134

EVALUATION OF THE ANTI-CARIES EFFECT OF *LESPEDEZA CUNEATA* EXTRACT AGAINST *STREPTOCOCCUS MUTANS*

Seoul-Hee Nam*.

Department of Dental Hygiene, College of Health Sciences, Kangwon National University, Samcheok-si, 25949, Republic of Korea.

Abstract.

The purpose of this study was to identify the antimicrobial activity of *Lespedeza cuneata* extract, a natural medicine, against a main causative bacterium of dental caries, *Streptococcus mutans* (*S. mutans*).

Lespedeza cuneata purchased from Hwalim Natural Drug Co., Ltd. (Busan, South Korea) was immersed in 70% ethanol for 12 h, and concentrated *Lespedeza cuneata* extract was applied to *S. mutans* diluted to 6×10^5 CFU/mL at the concentrations of 0, 1.25, 2.5, 5, 10, 20, and 40 mg/mL. Then the colony-forming units (CFUs) were checked at 6 and 24 h to evaluate the antimicrobial activity of the extract.

The CFUs and survival rate of *S. mutans* according to the concentration showed a higher mortality rate as the concentration of *Lespedeza cuneata* extract increased. In the time-dependent changes, the minimal inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were 1.25 and 40 mg/mL or more, respectively, at 6 h, but they were 1.25 and 5 mg/mL, respectively, at 24 h.

Therefore, *Lespedeza cuneata* extract is considered an excellent natural antibiotic for the prevention and treatment of dental caries, a typical oral disease, because it has excellent dental caries development suppression and bacteria extermination effects.

Key words. *Lespedeza cuneata*, anti-caries effect, *streptococcus mutans*, oral health.

Introduction.

The oral cavity is a space that maintains a humid state due to the saliva, making it a suitable place for bacteria to reside in; as such, there is a relationship between oral health and pathological condition [1].

Dental plaque is formed by the binding of numerous bacteria in the oral cavity, mainly on the tooth surface [2]. Although the bacterial flora is at a stable state in the healthy condition, the bacterial flora in the pathological condition forms a biofilm in the oral cavity of the human, the host, which causes bad breath, dental caries, gingivitis, and periodontitis [3]. Dental caries, a typical irreversible disease of the oral cavity, is caused by the interaction of the bacteria in the dental plaque, food, and saliva, and *Streptococcus mutans* (*S. mutans*) is known to be the main causative bacterium among the bacteria in the dental plaque [4]. *S. mutans* proliferate by attaching to the tooth surface, and the tooth is demineralized by the acid generated through the acid production process, resulting in dental caries [5]. Therefore, oral disease continues to be a serious health problem worldwide [6].

Although there are many commercially available synthetic compounds, such materials have been reported to be capable of modifying oral microorganisms and exhibiting undesirable side effects, such as vomiting, diarrhea, and dental staining [7]. For this reason, good alternative studies on synthetic compounds have been actively conducted of late. Medicinal plants have

been used as traditional remedies for numerous human diseases for thousands of years and in many parts of the world, and continue to be used as the main medicines in the rural areas of developing countries [8]. To demonstrate the efficacy of natural medicinal plants, studies are actively being conducted for such purpose due to the increasing interest in natural products for their oral microbial inhibitory effect and for the prevention of dental caries associated with oral health [9].

Lespedeza cuneata of genus *Lespedeza* is an herbaceous perennial belonging to the *Leguminosae* family, which is widely distributed in Korea, Japan, and China. It is called “Chinese Lespedeza” in Korea, and is also called by various other names, such as *Sericea Lespedeza*, *Chinese Bushclover*, and *Tiesaozhou* [10]. It has been reported that *Lespedeza cuneata* extract has antioxidant, anti-aging, and antimicrobial effects against skin-resident bacteria from antimicrobial action [11]. Additionally, in the case of *Lespedeza cuneata* ethanol extract, the effects of elastase inhibitory activity and skin regeneration on the surgical wound in animal experiments have been reported [12]. The antimicrobial activity of *Lespedeza cuneata* extract on dental caries, however, an oral disease, has not been actively researched on, and there have in fact been only few studies on such.

Therefore, this study aimed to evaluate the possibility of using *Lespedeza cuneata* ethanol extract as a natural medicine for dental caries through its antimicrobial activity against the typical causative bacteria of dental caries, *S. mutans*.

Materials and methods.

Bacterial strain and growth conditions:

S. mutans (KCTC 3065/ATCC 25175) was used for the experiment after being subcultured in brain heart infusion (BHI; Sigma-Aldrich, St. Louis, MO, USA), which was anaerobically incubated at 37°C for 24 h. For this experiment, *S. mutans* was diluted at a 6×10^5 ratio.

Lespedeza cuneata extraction:

The *Lespedeza cuneata* that was used in this study was purchased from Hwalim Natural Drug Co., Ltd. (Busan, South Korea). After the addition of 70% ethanol 5 times to 100 g crushed *Lespedeza cuneata*, an extract was obtained at 65°C for 12 h. The extract was filtered 3 times using filter paper (Advantec No. 2, Toyo, Japan), and the *Lespedeza cuneata* extract was concentrated using a rotary vacuum evaporator (N-1300E.V.S. EYELA Co., Tokyo, Japan). The concentrated *Lespedeza cuneata* extract was then lyophilized using a freeze dryer (FD5508, Ilshin Lab, Yangju-kun, Kyunggi-do, South Korea). The sample was then dissolved in dimethyl sulfoxide (DMSO) and was stored at -20°C after dilution.

Antimicrobial activity of *Lespedeza cuneata*:

100 μ L of *S. mutans* (6×10^5 CFU/mL) was inoculated into a 24-microwell plate containing *Lespedeza cuneata* extract at each

concentration (0, 1.25, 2.5, 5, 10, 20, and 40 mg/mL). The final volume of each mixture was 1 ml. After the final mixture was kept in a 37°C bacterial incubator for 6 and 24 h, the *S. mutans* in each well were uniformly smeared in an agar medium and then cultured at 37°C for 24 h to check the number of colony-forming units (CFUs) therein. Experiments were repeated three times to observe the reproducibility of the measurements.

Statistical analysis:

Significance analysis was carried out using SPSS (Ver. 21.0 SPSS Inc., Chicago, IL, USA). Student's t-test was conducted to confirm the changes after 6 and 24 h. The difference in each concentration was evaluated through one-way analysis of variance (ANOVA), followed by the Tukey test at the 0.05 significance level.

Results.

Figure 1(A) shows the application of *Lespedeza cuneata* extract after 6 h, and Figure 1(B) shows the application of *Lespedeza cuneata* extract after 24 h. In addition, the survival rate of *S. mutans* calculated by CFUs is shown in Figure 2. As the concentration of *Lespedeza cuneata* extract increased, the bacterial growth inhibition was more pronounced, and in the change over time, high bacterial death was observed at 24 h compared to 6 h.

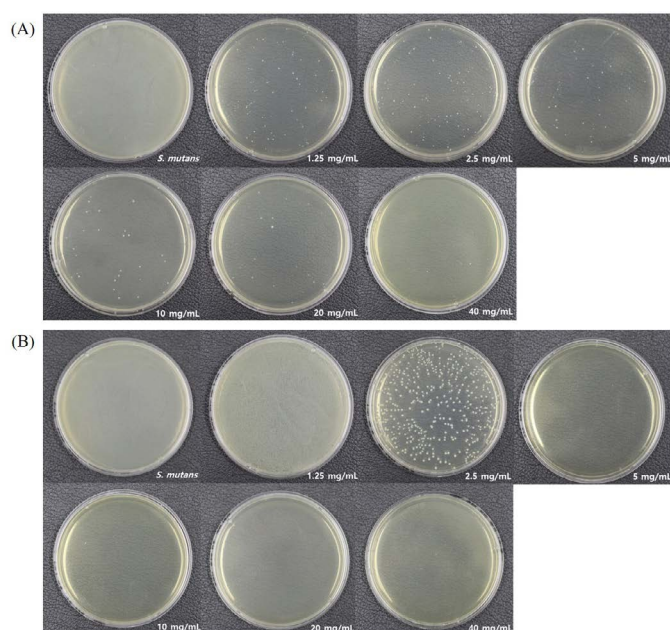


Figure 1. Anticariogenic activity of *Lespedeza cuneata* extract against *S. mutans* (A) after 6 h and (B) 24 h.

As shown in Table 1, the antimicrobial activity of the extract against bacteria over time according to the concentration of the extract was 2.71×10^7 for *S. mutans* (control), 9.10×10^1 for 1.25 mg/mL, 7.00×10^1 for 2.5 mg/mL, 4.80×10^1 for 5 mg/mL, 3.70×10^1 for 10 mg/mL, 3.00×10^1 for 20 mg/mL, and 4.00 for 40 mg/mL at 6 h, showing that as the concentration increased, the growth of the bacteria was inhibited. At 6 h, the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were 1.25 and 40 mg/mL or more, respectively.

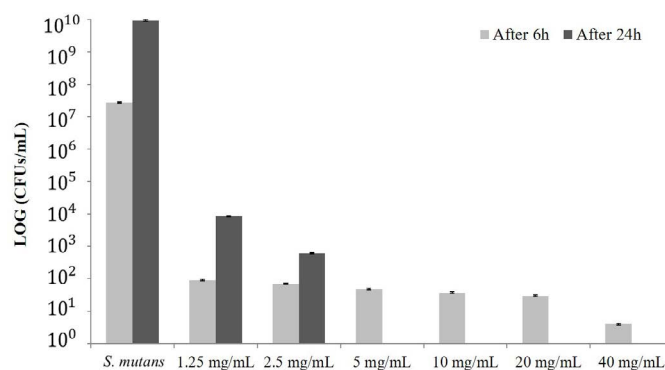


Figure 2. The survival rate of *S. mutans* by *Lespedeza cuneata* extract.

The change at 24 h was 9.53×10^9 for *S. mutans* (control), 8.54×10^3 for 1.25 mg/mL, 6.17×10^2 for 2.5 mg/mL, 0.00 for 5 mg/mL, 0.00 for 10 mg/mL, 0.00 for 20 mg/mL, and 0.00 for 40 mg/mL, showing that a more distinct antimicrobial activity appeared as a remarkable bacteria inhibitory effect with increasing concentration. At 24 h, the MIC and MBC were 1.25 and 5 mg/mL, respectively, indicating that the *S. mutans* bacteria had been completely killed.

Discussion.

Looking at the relationship between oral health and quality of life, if a disability occurs due to oral disease, it affects daily life and may cause severe dysfunction as the age increases [13]. The inconvenience of chewing due to an oral disease directly affects the quality of life by depriving the person of the euphoria that can be derived from eating [14]. The importance of oral-health-related quality of life has been emphasized of late due to the change in its perception, that it should be regarded as part of a person's physical, mental, and social well-being [15].

Although the overall oral and dental health status of people living in industrialized countries has generally improved, the prevalence of dental caries is still up to 90%, and most adults have an oral disease [6]. Dental caries is caused by tooth demineralization due to the acid produced by the proliferation of dental-caries-inducing bacteria and anaerobic bacteria in the dental plaque [16].

S. mutans is a bacterium closely related to dental caries and plays an important role in dental caries biofilm formation [17]. It destroys the tooth enamel by decomposing the carbohydrates contained in food and releasing organic acid, a by-product of the metabolism, to the outside [18].

In the early stage of dental caries, inorganic demineralization and deposition occur reversibly. Therefore, dental caries can be prevented if measures are taken to inhibit demineralization and cause deposition. If the method of increasing acid resistance or promoting teeth remineralization carbohydrate and the method of inhibiting the growth of bacteria-producing acid is used, dental caries can be prevented [19]. In addition, inhibiting the attachment of *S. mutans* on the tooth surface by employing antimicrobial agents against the said bacterium has been reported to be important for preventing dental caries [20].

As the interest in natural products for the prevention of oral diseases has increased, studies on natural products have been

Table 1. Mean±SD and P-value according to CFUs calculation result of *Lespedeza cuneata* extract by concentration and time.

Group	<i>S. mutans</i> (Control)		1.25 mg/mL		2.5 mg/mL		5 mg/mL		10 mg/mL		20 mg/mL		40 mg/mL		ANOVA p-Value
	Mean ±SD	t-test p-Value	Mean ±SD	t-test p-Value	Mean ±SD	t-test p-Value	Mean ±SD	t-test p-Value	Mean ±SD	t-test p-Value	Mean ±SD	t-test p-Value	Mean ±SD	t-test p-Value	
6h	2.71±0.9 10 ^{7a}	0.000*	9.10±0.2 10 ^{1b}	0.000*	7.00±10 ^{1b}	0.000*	4.80±0.2 10 ^{1b}	0.000*	3.70±0.3 10 ^{1b}	0.000*	3.00±0.3 10 ^{1b}	0.004*	4.00±0.5 00 ^b	0.007*	0.000*
24h	9.53±0.3 10 ^{9a}		8.54±0.1 10 ^{3b}		6.17±0.1 10 ^{2b}		0.00±00 ^b		0.00±00 ^b		0.00±00 ^b		0.00±00 ^b		0.00±00 ^b

*The significant difference for 6h and 24h comparison by student t-test ($p < 0.05$).

*The significant difference among the four groups in one-way ANOVA. Different letters (a and b) by the presented statistically significant result of the post hoc Tukey HSD ($p < 0.05$)

actively conducted for their oral microorganism inhibitory effect [21]. Synthetic compounds suppress the growth of microorganisms in the oral cavity, but there is a problem in that they cause side effects like resistance to antibiotics during long-term use. Therefore, there is a growing interest in research using natural products as a good alternative to synthetic compounds [22].

This study aimed to determine if *Lespedeza cuneata* extract has antimicrobial activity by applying it to *S. mutans*, and to evaluate and develop a natural antimicrobial agent effective for dental caries prevention and treatment as an alternative to the conventional synthetic compounds. The antimicrobial activity against *S. mutans* was evident as the concentration of the extract increased.

Helichrysum italicum ethanol extract has been reported to show antimicrobial activity against *S. mutans*, *Streptococcus sanguis* (*S. sanguis*) and *Streptococcus sobrinus* (*S. sobrinus*) [23], and *Forsythiae Fructus* extract showed a high antimicrobial action against the dental-caries-inducing bacteria *Streptococcus intermedius* (*S. intermedius*) and *Streptococcus gordonii* (*S. gordonii*) [24]. Additionally, the study conducted by extracting and using horseradish reported that horseradish has an inhibitory effect against *S. mutans* [25]. The antimicrobial activity of *Glycyrrhiza uralensis* extract against *S. mutans* but also had *S. mutans* but also had a killing effect on it, showing a distinct antimicrobial activity [26].

In this study, as the concentration of *Lespedeza cuneata* extract increased, its antimicrobial activity *Lespbecame* evident. As a result of the measurement of CFUs over time at 6 and 24 h, it was confirmed that a definite antimicrobial activity was shown from the low concentration as the application time of *Lespedeza cuneata* extract increased. The extract inhibited the *Lespedeza cuneata* of *S. mutans* from a 1.25 mg/mL concentration, confirming that there was antimicrobial activity from of extract. At the concentration of 5 mg/mL after 24 h, a remarkable antimicrobial activity, by which the *S. mutans* bacteria were completely killed and new bacteria did not grow, was shown. This suggests that the antimicrobial activity of *Lespedeza cuneata* extract was maximized over time by reacting to *S. mutans*.

Based on the results of this study, *Lespedeza cuneata* extract was found to inhibit the growth of *S. mutans* at a low concentration, and to have a definite antimicrobial activity as the concentration increases. Thus, it will contribute to dental caries prevention and oral health promotion. Future studies will evaluate the effect from in vivo to clinical trials.

Conclusion.

For *S. mutans*, a typical causative bacterium of dental caries, *Lespedeza cuneata* extract showed a clear antimicrobial activity

with increasing concentration, and definite bacterial killing over time. At a 1.25 mg/mL concentration of the extract, the growth of *S. mutans* was inhibited, and from a 5 mg/mL concentration, the *S. mutans* bacteria were completely killed. Therefore, *Lespedeza cuneata* extract is suitable as a natural extract substitute for the conventional synthetic antimicrobial agents with side effect problems and can help much in preventing dental caries.

REFERENCES

- Zaura E, Keijsers B J, Huse S M, et al. Defining the healthy "core microbiome" of oral microbial communities. *BMC Microbiol.* 2009;15:259-270.
- Kolenbrander PE, London J. Adhere today, here tomorrow: oral bacterial adherence. *J Bacteriol.* 1993;175:3247-3252.
- Kroes I, Lepp PW, Relman DA. Bacterial diversity within the human subgingival crevice. *Proc Natl Acad Sci U S A.* 1999;96:14547-1452.
- Loesche WJ, Rowan J, Straffon LH, et al. Association of *Streptococcus mutans* with human dental decay. *Infect Immun.* 1975;11:1252-1260.
- Hamada S, Koga T, Ooshima T. Virulence factors of *Streptococcus mutans* and dental caries prevention. *J Dent Res.* 1984;63:407-411.
- Petersen PE, Bourgeois D, Ogawa H, et al. The global burden of oral diseases and risks to oral health. *Bull World Health Organ.* 2005;83:661-669.
- Park KM, You JS, Lee HY, et al. An antibacterial agent from the root bark of *Morus alba* against oral pathogens. *J Ethnopharmacol.* 2003;84:181-185.
- Chitme HR, Chandra M, Kaushik S. Studies on antidiarrheal activity of *Calotropis gigantea* R. Br. in experimental animals. *J Pharm Pharm Sci.* 2004;7:70-75.
- Pai MR, Acharya LD, Udupa N. Evaluation of antiplaque activity of *Azadirachta indica* leaf extract gel-a 6-week clinical study. *J Ethnopharmacol.* 2004;90:99-103.
- Kim YH, Ryu SN. Antioxidant activity of methanol extract from aerial parts in *Lespedeza cuneata* G. Don. *Korean J Crop Sci.* 2008;53:121-123.
- Lee HJ, Lim GN, Park MA, et al. Antibacterial and antioxidative activity of *Lespedeza cuneata* G. Don extracts. *Korean J Microbiol Biotechnol.* 2011;39:63-69.
- Kim DI, Kim HJ. Regeneration effects of *Lespedeza cuneata* ethanol extract on experimental open wound in rat. *J Korean Soc Food Sci Nutr.* 2014;43:516-521.
- Locker M, Miller Y. Subjectively reported oral health status in an adult population. *Community Dent Oral Epidemiol.* 1994;22:425-430.
- Gillbert GH, Foerster U, Duncan RP. Satisfaction with

- chewing ability in a diverse sample of dentate adults. *J Oral Rehabil.* 1998;25:15-27.
15. Mariño R, Schofield M, Wright C, et al. Self-reported and clinically determined oral health status predictors for quality of life in dentate older migrant adults. *Community Dent Oral Epidemiol.* 2008;36:85-94.
16. Gibbons RJ, Van Houte J. Bacterial adherence in oral microbial ecology. *Annual Review of Microbiology.* 1975;29:19-44.
17. Rosenbloom RG, Tinanoff N. Salivary *Streptococcus mutans* levels in patients before, during, and after orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 1991;100:35-37.
18. Koga T, Askawa H, Okahashi N, et al. Sucrose-dependent cell adherence and cariogenicity of serotype c *Streptococcus mutans*. 1986;132:2873-2883.
19. Song G, Luo Q, Qin J, et al. Effects of oxymatrine on proliferation and apoptosis in human hepatoma cell. *Colloids Surf B Biointerfaces.* 2006;48:1-5.
20. Katsura H, Tsukiyama RI, Suzuki A, et al. In vitro antimicrobial activities of bakuchiol against oral microorganisms. *Antimicrob Agents Chemother.* 2001;45:3009-3013.
21. Pai MR, Acharya LD, Udupa N. Evaluation of antiplaque activity of *Azadirachta indica* leaf extract gel-a 6-week clinical study. *J Ethnopharmacol.* 2004;90:99-103.
22. Kwak DJ. Antibacterial activities of *Phellodendri cortex* on the *Streptococcus mutans*. *J Korean Soc Hyg Sci.* 2004;10:99-107.
23. Nostro A, Cannatelli MA, Crisafi G, et al. Modifications of hydrophobicity, in vitro adherence and cellular aggregation, *Streptococcus mutans* by *Helichrysum italicum* extract. *Lett Appl Microbiol.* 2004;38:423-427.
24. Choi JO, Choi YR, Nam SH. Antibacterial effect of *Forsythiae fructus* extract in dental caries. *BIOMED RES-INDIA.* 2019;30:465-468.
25. Masuda H, Inoue T, Kobayashi Y, Masuda H, et al. Anticaries effect of Wasabi components: oriental foods and herbs. *ACS symposium series: Oxford University Press;* 2003:142-153.
26. Choi YS, Kim JY, Nah HR, et al. Anti-caries effect of the *Glycyrrhiza uralensis* extract. *BIOMED RES-INDIA.* 2017;28:8271-8275.