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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии
საქართველოს სამედიცინო სიახლე

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.

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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 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, 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.

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HPLC METHOD FOR THE QUANTIFICATION OF SOME ACTIVE FLAVONOIDS IN ETHYL ACETATE EXTRACT OF LEAVES OF BUTEA MONOSPERMA LINN

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Abstract.

Aim: The aim of the present investigation is to study HPLC process to evaluate Some Active Flavonoids in Ethyl Acetate Extract of Leaves of *Butea monosperma* Linn.

Material and methods: Using a soxhlation device, the leaves of *Butea monosperma* Linn. were extracted in stages. Each powdered batch (500g) was extracted in stages with polarity-graded solvents such as petroleum ether (Pet. Et) (60-80°), chloroform (CHCl₃), ethyl acetate (EtOAc) using a soxhlet extractor. Alkaloids, flavonoids, glycosides, tannins, phenols, and steroids, among other chemical families of components, were identified through qualitative phytochemical screenings of each extract. To make a 10 g/ml stock, standard phenolic markers like quercetin, rutin, catechin, gallic acid, and chlorogenic acid were dissolved in methanol. Phytoconstituents were separated and identified from extracts using various solvents and combinations of solvents, which were chosen after consulting the literature.

Results and Discussion: Preliminary phytochemical screening showed the revealed that the leaves contain steroid, triterpenoids, fatty acid and alkaloids. While the ethyl acetate extract found to contain therapeutically important phytoconstituents such as steroids, triterpenoids, saponins, flavonoids, and tannins. Bioactive extracts of *Butea monosperma* were found to include flavonoids and phenolic substances. In ethyl acetate extract, various flavonoids and phenolic compounds were discovered.

Conclusion: This is a preliminary report on the identification of phytochemical and HPLC evaluation of ethyl acetate extract of leaves of *Butea monosperma* Linn. and to unravel the mechanisms driving bioactive qualities and the existence of putative synergy among these substances, more research is needed on the isolation and characterization of individual Flavonoids or phenolic compounds.

Key words. HPLC method, quantification of some active flavonoids, ethyl acetate extract, *Butea monosperma* Linn., quercetin, rutin, catechin.

Introduction.

Nature continues to fulfill its role as man's primary source of healing. Functional nutrition has been shown to be important in reducing the risk of some chronic diseases, according to research in preventive medicine. The human body's natural defence mechanism may be insufficient to counteract the harm produced by oxidative stress [1]. Quercetin and other flavonoids are structured to operate as potent antioxidants. This has been demonstrated in vitro numerous times. Quercetin, as a prominent flavonoid ingredient, is able to prevent various chronic degenerative illnesses [2]. Synthetic antioxidants are connected to a variety of negative side effects, such as liver

damage and mutagenesis, according to growing scientific data [3]. As a result, there has been a surge in interest in natural goods as antioxidants, which restrict free radical reactions and protect the human body from illnesses like cancer and diabetes. Plants and other herbal extracts, Polyphenolic substances (such as gallic acid, ellagic acid, pelargonidin, and sitosterol) might have high antioxidant capabilities, according to recent study [4].

In conclusion, high-performance liquid chromatography (HPLC) is a versatile analytical procedure that takes cheaper equipment and less experience. The investigation aims to develop a rapid, sensitive, and accurate analytical process for assessing flavonoids in plant extract bioactive extracts and their pharmaceutical dosage form, which could be used to examine various plant extract samples and formulations on a regular basis [5]. The goal of this research is to figure out how much flavonoids are in *Butea monosperma* Linn extract.

Materials and Methods.

Extraction of plant materials:

The leaves of *Butea monosperma* Linn. are subjected for successive extraction using soxhlation apparatus. Each powdered batch (500g) was extracted in stages with solvents of varying polarity, such as petroleum ether (Pet. Et) (60-80°), chloroform (CHCl₃), ethyl acetate (EtOAc) using a soxhlet extractor. The extraction was carried out till to exhaust. The extract was dried under vacuum and processed for the further HPLC analysis [6].

Preliminary phytochemical evaluation:

The existence of main bioactive phytochemicals or components (alkaloids, flavonoids, glycosides, tannins, phenols, and steroids) was investigated employing qualitative screens of each extract [7].

Marker based estimation of extracts by HPLC:

Agilent liquid chromatography system series 1200 with quaternary pump, Rheodyne injector with fixed loop of 20 l, and UV detector is employed. At room temperature, the separation was performed using a Waters Symmetry C-18 Column (250 X 4.6mm, particle size 5m) followed by an ODS guard column (10m, 10mm X 5mm ID) [8].

Preparation of sample solution: The bioactive extract i.e. EtOAc was dissolved in respective solvents to get 1 mg/ml as a reference solution [9].

Preparation of standard solutions: Standard phenolic and flavonoids markers (quercetin, rutin, catechin, gallic acid, and chlorogenic acid) are dissolved in methanol to make a 10 g/ml stock [10].

Chromatographic conditions:

Phytoconstituents were separated and identified from extracts using various solvents and combinations of solvents, which were chosen after consulting the literature. The best possible combination of mobile phase is employed for each marker [11].

HPLC specifications for analysis of extract.

Specifications for HPLC analysis of ethyl acetate extract (Table 1).

Table 1. Specifications for HPLC analysis of ethyl acetate extract.

Sr. no	Active Phytochemicals	Mobile phase (v/v)	Flow rate (ml)	Standard Rt (Min)	Detection (nm)
1	Gallic acid	Methanol+	0.6	2.47	280
2	Chlorogenic acid	(polar solvent)	0.6	4.2	280
3	Catechin		0.6	5.0	280
4	Rutin	Water (55:45)	0.6	5.8	280
5	Quercetin	pH-2.0 with OPA	0.6	11.02	280

Results and Discussion.

Preliminary phytochemical screening showed the revealed that the leaves contain steroid, triterpenoids, fatty acid and alkaloids. While the ethyl acetate extract found to contain therapeutically important phytoconstituents such as steroids, triterpenoids, saponins, flavonoids, and tannins (Table 2). Polyphenols have a hydroxyl group in their structure, as well as the potential to transfer electrons, making them a powerful antioxidant. The qualitative chemical test clearly revealed that the potent antioxidant phytoconstituents i.e. flavonoids, tannins were highly accumulated in ethyl acetate extract [12,13].

Table 2. Phytochemical analysis of different extracts of leaves.

Tests	Leaves Extracts of <i>Butea monosperma</i> Linn.		
	Petroleum ether	Chloroform	Ethyl acetate
Alkaloid	-	+	+
Glycoside	-	-	-
Saponins	-	-	+
Steroid	+	+	+
Triterpenoids	+	+	+
Tannins and Phenolic	-	-	++
Flavonoid	-	+	+++
Test for carbohydrates	-	-	-
Proteins	-	+	-
Fats and Lipids	+	+	-

(-): Not present, (+): present in less quantity, (++) : Moderate presence, (+++): Strong Presence.

Marked Based Estimation of *Butea monosperma* by HPLC:

Bioactive extracts of *Butea monosperma* were found to include flavonoids and phenolic substances. In ethyl acetate extract, presence of different phenolic (gallic acid, chlorogenic acid) and flavonoids i.e. quercetin, rutin, catechin were discovered. These are well documented for their different pharmacological activities i.e. antioxidant, anti-inflammatory, immunomodulatory, antimicrobial and anticancer activity (Table 3) [14-16].

Table 3. HPLC analysis of *Butea monosperma* leaves extracts.

Sr. No	Phytoconstituents	Rt (min)	<i>Butea monosperma</i> Linn. Ethyl acetate Extract
1	Quercetin	11.02	11.2
2	Rutin	5.8	5.87
3	Catechin	5.0	-
4	Gallic acid	2.47	-
5	Chlorogenic acid	4.2	4.09

(-) absent

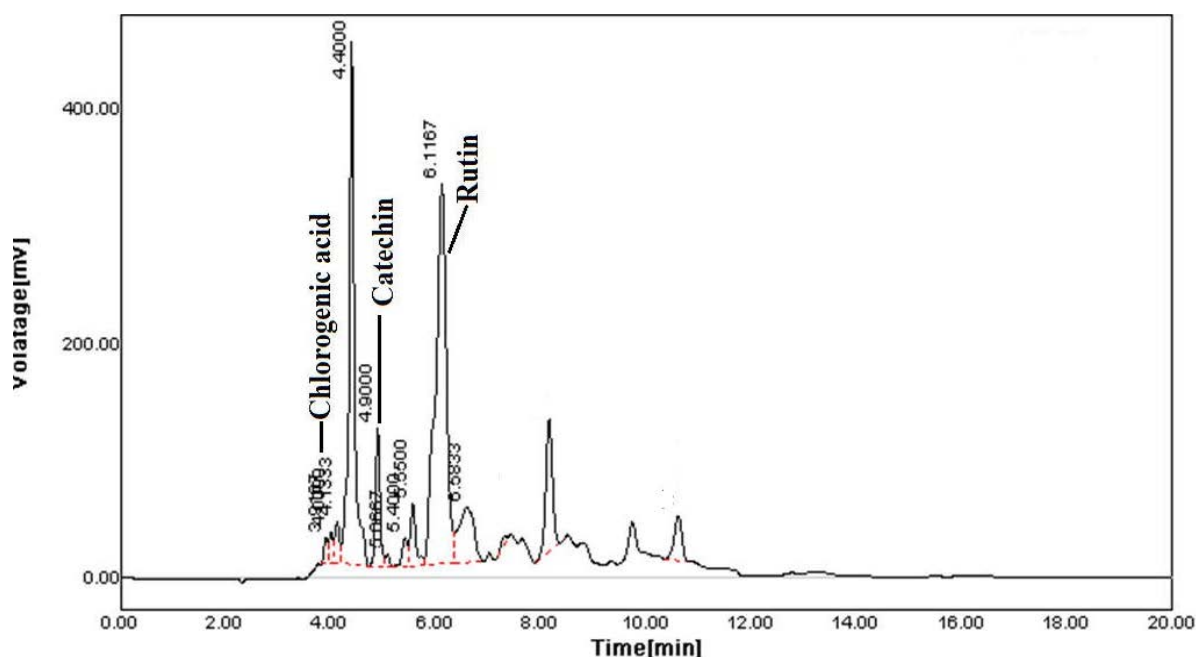


Figure 1. Chromatogram of leaves ethyl acetate extract for presence of different flavonoids.

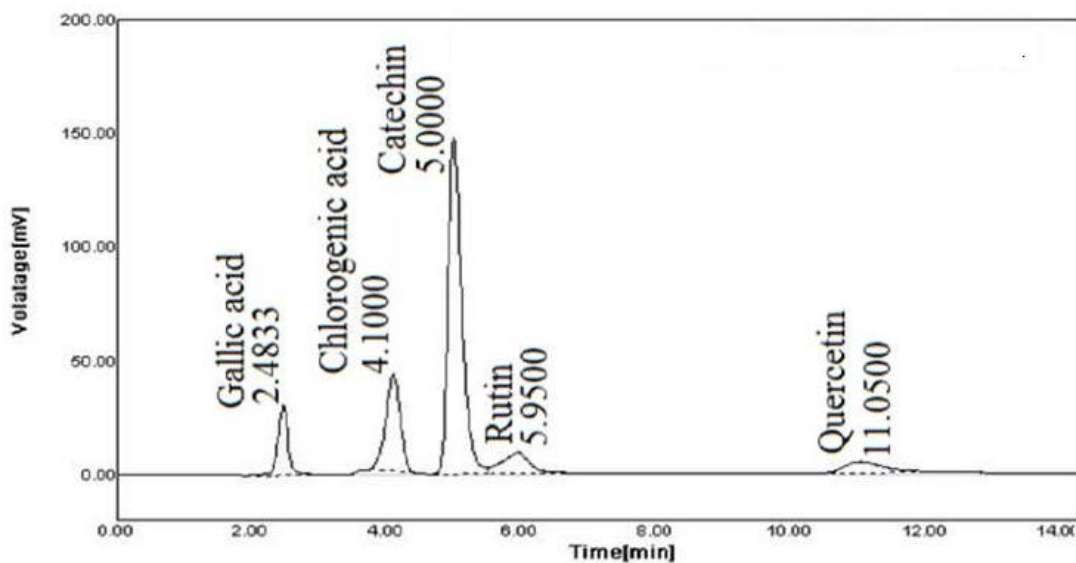


Figure 2. Chromatogram of standard different flavonoids.

With the emergence of new terminologies like nutraceuticals, food supplements, botanicals, dietary supplements, multifunctional foods, etc., the market of natural substances has intensely changed. But to maintain their sustain use; there is also a need of adequate analytical tool which will ensure the quality and purity of these herbal products [17,18]. These analytical tools should be such that they can withstand with the complexity of natural product mixture. As a result, it is now possible to develop acceptable procedures for quality analysis and standardisation of herbal medicines in order to preserve as much uniformity as possible in the plant extract [19,20]. High-performance liquid chromatography can be used to standardise and control the quality of raw materials, extracts, and final herbal remedies, among other things, alone or in combination (Figures 1 and 2).

Conclusion.

Butea monosperma Linn. grows abundantly in tropical and subtropical places under a variety of climatic circumstances, and they can be mass-produced as value-added products on a huge scale. As a result, the findings may provide a scientific foundation for the usage of plant species in commercial products. This is a preliminary study on the phytochemical identification and HPLC analysis of an ethyl acetate extract of *Butea monosperma* Linn leaves. Further research is needed to determine the processes driving the bioactive qualities of specific Flavonoids or phenolic compounds, as well as the existence of probable synergies, if any, among these molecules.

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