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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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 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 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, 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. 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 compu-ter-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 საყურადღებოდ!

რედაქციაში 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 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 შეღებვის 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 ფჩხილებში 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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ANTICANCER ACTIVITY OF PHLORETIN COMPOUND PURIFIED FROM IRAQI *MALUS DOMESTICA* L. (APPLE) LEAVES

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Abstract.

The present study was dealing with a Polyphenolic compound known as Phloretin. Phloretin (Ph), a dihydrochalcone, was determined qualitatively and quantitatively in different aerial parts for Iraqi *Malus domestica* (apple), cv." Ibrahimi" included leaves, petioles, stems, fruit pulp, and peels extracts. Leaves represented a rich source of Ph, which was separated and purified by preparative HPLC. The chemical structure of the isolated Phloretin (Ph2) was confirmed using various analytical characterization techniques: TLC, HPLC, FTIR, Melting point, CHN elemental analyses, 1H-NMR, and 13C-NMR). The scavenging efficacy of Ph2 by DPPH assay was employed. Cytotoxic effect was assessed by MTT assay against cancer cell lines including (Hep G2/ human hepatocyte carcinoma, A549/ human lung adenocarcinoma, SW480 / human colon cancer cell, and AGS /adenocarcinoma of the stomach), beside the non-cancerous cell line (HEK 293). About 1.404 g Ph2 was obtained from 18.146 g apple leaves (7.7%). The DPPH and MTT assay results demonstrated that the purified Ph2 possessed potent antioxidant activity with significant anticancer effects on all cancer cell lines. Data suggested that purified Ph2 from Iraqi apple leaves has potential antioxidant, cytotoxicity, which may benefit in human health.

Key words. *Malus domestica*, Apple, Dihydrochalcone, Phloretin, Hep G2, A549.

Introduction.

Cancer ranks as the second most prevalent cause of mortality globally, following cardiovascular disorders [1]. Cancer, a complex heterogeneous disease process, poses a significant health challenge for human [2]. Despite significant advancements in medical research, medicinal chemistry, and cell biology, the incidence and mortality rates of cancer continue to rise globally, often deemed incurable [3]. There is expected to be a significant increase in the worldwide cancer rate between 2020 and 2040, with an estimated 28.4 million new cases of cancer in 2040 [4]. Important lifestyle variables that contribute to the development of cancer include, but are not restricted to, the ingestion of carcinogens through diets, solar radiation exposure, smoking, consuming alcohol, and the absence of physical exercise. Several of these are subject to modification, indicating that cancer can be prevented by adopting a suitable lifestyle [5]. Approximately fifty years ago, Michael B. Sporn introduced the notion of cancer prevention and created the term "chemoprevention." This term refers to using non-toxic chemicals to block or reverse the development of tumorigenic processes [6].

Chemotherapy (pharmacotherapy) is one of the most widely used anticancer treatments globally, which is based on compounds including platinum-containing compounds such as cisplatin, carboplatin, and oxaliplatin [7]. Almost 50% of cancer patients receive treatment with cisplatin [8]. Regrettably, cisplatin has several disadvantages, which include causing damaging effects on the patient's normal tissues (nephrotoxicity, ototoxicity, neurotoxicity, gastrointestinal toxicity, hepatotoxicity, cardiotoxicity, and hematological toxicity) and the development of cisplatin resistance by cancer cells during treatment [9]. As a result, it greatly diminishes the quality of life for patients. It necessitates a decrease in the dosage of this drug or possibly its entire cessation, thus reducing the efficacy of anticancer therapy [10].

Moreover, the heterogeneous nature of cancer presents a formidable obstacle in developing effective anticancer therapies. This often results in chemotherapy failure or increased resistance to the treatment, ultimately leading to cancer recurrence [11]. Plant-based therapies are frequently employed in traditional medicines [12]. Certain medicinal plants effectively treat and prevent highly lethal cancers [13,14]. Drugs containing plant secondary metabolites as the primary active ingredients have been used for a long time to treat human diseases [15]. Dihydrochalcones belong to the flavonoid family, characterized by a simple C6-C3-C6 skeletal structure consisting of only two aromatic rings and a saturated chain C3; this group of flavonoids does not possess a heterocyclic C ring. The A-rings are formed from the acetate pathway, while the B-rings are derived from the shikimate pathway [16]. So far, over 200 types of dihydrochalcone have been found [17]. Around 30 plant families, such as Rosaceae, Asteraceae, Fabaceae, and Fagaceae, have been discovered to possess natural dihydrochalcone compounds, primarily in the form of glycosylated compounds instead of aglycones and It's an interesting fact that many naturally occurring dihydrochalcones act as sweeteners [18]. The *Malus* genus, including the species domestic (apple) stands out among these plants due to its exceptionally high dihydrochalcone concentration. The main dihydrochalcone compounds include phloretin, phlorizin, phloretin 20-O-xyloglucoside, 3-hydroxyphloretin, 3-hydroxyphlorizin, trilobatin, and sieboldin [19,20].

Phloretin, [2',4',6'-Trihydroxy-3-(4-hydroxyphenyl)-propiophenone] (Figure 1) and called (phloretin or dihydro naringenin) is a Dihydrochalcone flavonoid is predominant in the leaves, bark, and fruit of apple trees and apple-derived products [21], mainly in green and immature apples [22]. Phloretin consists of two aromatic rings (A and B) with hydroxy groups located at positions 2, 4, 4', and 6 [23]. Considerable attention has recently been given to phloretin, a flavonoid among 6000, due to its biological activities [24] and the health benefits include antioxidant [25], antibacterial [26], antidiabetic [27], anti-inflammatory [28], antiviral[29], antithrombotic [30], estrogenic [31], immunomodulation [32], neuroprotective [33],

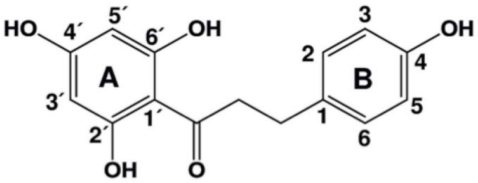


Figure 1. *Chemical structure of phloretin.*

anticancer [6], hepatoprotective activity [20], cardioprotective activity, antimicrobial activity, anti-allergic [34] properties, prevents photodamage caused by UV light on the skin [24], and the possibility of using phloretin and derivatives in dermoid-cosmetic preparations [35,36]. The extraction of phloretin from natural plant materials is crucial for industrial manufacturing. The presence of plant material containing the desired component can reduce the overall cost of processing. Over the last 25 years, researchers have conducted more than 150 studies on the effects of phloretin on tumors and cancers. Notably, half of these studies were published between 2014 and 2023, indicating a recent surge of scientific interest in this dihydrochalcone. This growing interest can be attributed to the compound's essential antitumor activity, making it a promising study area for future research. Apple parts, particularly apple peel, leaves, bark, and apple pomace, are an economical and abundant reservoir of essential polyphenols. This work describes the isolation, structure, and identification of phloretin from Iraqi M. domestica leaves. Subsequently, the antioxidant activity, the cytotoxicity of four cancer cell lines, and the comparison between phloretin and traditional anticancer chemotherapy drug (cisplatin) to obtain more profound insight and provide evidence into the bioactivity of the phloretin from Iraqi *M. domestica*. Until now, there have been no previous reports of phloretin being isolated from apples grown in Iraq.

Materials and Methods.

Plant material collection and preparation of samples:

Aerial parts: Early in the winter of 2022, in November, Iraqi *M. domestica* aerial parts (leaves, petioles, and stems) were collected from a private orchard in Diyala Governorate, the Ala'bara region, Iraq, "Ibrahimi "cultivar. The identification of the plant was made by senior Taxonomist Dr. Sukina Abbas (University of Baghdad Herbarium / Department of Biology-College of Sciences- University of Baghdad); the aerial parts, healthy and fresh, were cleaned before being air-dried for 15 days (for leaves), 20 days (for petioles), and 25 days (for stems) at room temperature, with air circulation, and shade. The dried materials were finely powdered using a two-step process, including manual grinding followed by electrical grinding, and utilized for extraction.

Fruits: The apple Fruits of the "Ibrahimi" cultivar were obtained from a privately owned orchard located in the Diyala Governorate, that is, in the Ala'bara district of Iraq, in June of the year 2023. The same trees from which the aerial parts were collected. The peels were separated from the pulp using a hand steel peeler, and the seeds were manually separated. The fruit was divided into peels, pulp, and seeds. The two components (pulp and peels) were fragmented into smaller pieces to facilitate direct extraction to avoid browning while the seeds were discarded (Figure 2).

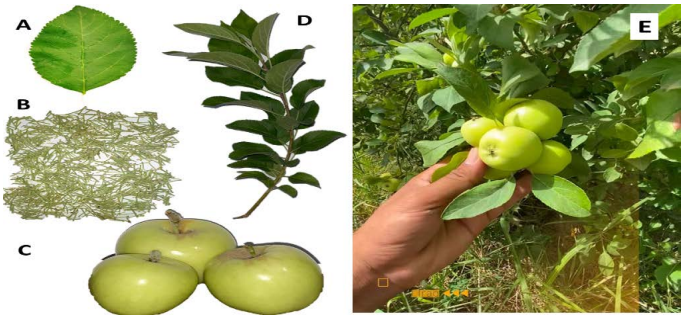


Figure 2. *Photo of Iraqi M. domestica plant: (A) Leaf, (B) Petioles, (C)Ripe Fruits(D) Branch/stem with leaves, and (E) Tree with leaves and fruits.*

Chemicals and Materials: All chemicals and reagents used were of high-quality grade and from Sigma-Alidrich Company. A pale-yellow powder was purchased from Hyper Chem (LOFT49 City Pioneer Zone, Jiru Road, Gongshu District, Hangzhou, 310015, China). Molecular weight 274.27 g/mol, Molecular Formula ($C_{15}H_{14}O_5$), purity 98.176 %, and CAS NO. (60-82-2).

Extraction Procedure: Fifty grams of dried aerial parts, consisting of leaves, petioles, and stems, and fresh fruit parts, including pulp and Peels, were individually subjected to triple cold maceration using 500ml of 70% ethanol for 24 hours, with occasional stirring and then filtered. The marc was macerated twice more in 70% ethanol for 24 hours each time (the maceration method was repeated up to three times, and the filtrate was mixed). The filtrate was subjected to a final drying process using a rotary evaporator at a temperature of 40°C. This resulted in the formation of dense concentrate dried extracts, which were subsequently weighed and kept at a temperature of 4°C until they were ready for use. Marc was ejected [37,38].

Qualitative analysis of phloretin in Ethanolic extracts by Thin Layer Chromatography (TLC): The ethanolic extracts of aerial parts (leaves, petioles, stems, peels, and pulp) of Iraqi *M. domestica* were identified qualitatively in their phloretin content done by TLC technique, using premade silica gel GF254nm plates. The solvent system (mobile phase) consists of toluene, Ethyl acetate, Formic acid, and methanol (ratio 55: 30: 10: 5) for separating extract components [39]. About 100 ml of the solvent system was placed into a glass tank. A drop from extracts and standard was applied to the TLC plates using capillary tubes; after developing the technique, the dry plates were examined by UV light at 254 nm and 366 nm. A pencil marked the separated spots, and the retardation factor (Rf value) for each constituent visualized as a fluorescent spot under UV light was calculated [40].

$$Rf\ value = \frac{Distance\ moved\ by\ spot}{Distance\ moved\ by\ the\ solvent}$$

Identification and Quantification of phloretin by HPLC Method: The Standard solution of phloretin was prepared in methanol at the concentration of 1 mg/ml (for TLC, HPLC, and preparative HPLC Procedure), protected from light, and stored

at 4 °C between uses. The phloretin standard symbolized as Ph. The high-efficiency liquid chromatographic HPLC examination was carried out at the Ministry of Science and Technology/Baghdad – Iraq, the instrument origin was Shimadzu, Japan. Phloretin.

HPLC analysis was conducted to identify phloretin in ethanolic extracts (Leaves, petioles, stems, peels, and pulp) of Iraqi *M. domestica*. Quantitative estimation was done using liquid chromatography (equipment SHIMADZU, Japan); column was ODS-C18 (250 mm× 4.6 mm I.d.), 5µm particle size, the flow rate was 1.0 ml/min, room temperature, column pressure was 8.9, the injection volume was 20 µl, and the mobile phase was Methanol 70% (A) and 0.1% Acetic acid 30% (B), and detection wavelength was UV-Vis at λ 288nm.

Isolation and purification of proposed phloretin from the crude leaves extract by preparative HPLC (PHPLC): A Phloretin was isolated from the ethanolic extract of the leaves performed according to standard conditions [41] by HPLC equipment (Shykam, Germany), the column was ODS-C18 (250 mm× 4.6 mm I.d.), 5µm particle size, the flow rate was 0.8 ml/min, column temperature (25±1°C), the Injection Volume was 0.1 ml, and the mobile phase was = Acetonitrile 30% (A), water 70% (B), and Phosphoric acid 0.08% (C), filtered and degassed before use, and detection wavelength was UV-Vis at λ 288nm, at the Ministry of Science and Technology, the Department of Environmental and Water Research. A fraction collector collects the target peak from the time of its appearance until its near or almost end. The proposed phloretin was subjected to a final drying process using a rotary evaporator at a temperature of 40°C. This resulted in the formation of powder concentrate dried proposed phloretin, which was subsequently weighed and kept at a temperature of 4°C until ready for use. The identification and purity of the target compound (proposed phloretin) were checked using chromatographic (TLC, HPLC), spectroscopic (FT-IR), Melting point, CHN analysis, 1H NMR and 13C NMR spectra. The isolated phloretin symbolized as Ph2.

Structural Identification and characterization of the proposed phloretin (Ph2):

The proposed phloretin (Ph2) was identified by using different identification methods as follows (Figure 3).

Identification by TLC: TLC was performed for (Ph2) by utilizing the same mobile phase (Toluene, Ethyl acetate, Formic acid, and Methanol) by comparing their Rf values with the standard, using the same method discussed previously in previous step.

Identification by HPLC: The purity of the (Ph2) was checked using HPLC and the same conditions previously discussed in previous step.

Melting point: The melting point of the proposed phloretin was determined by using the electrothermal melting point equipment conducted at the Ministry of Science and Technology, the Department of Environmental and Water Research.

Identification by Fourier Transforms Infrared (FT-IR) Spectroscopy: The functional groups in the isolated proposed phloretin were detected using Fourier transform infrared spectroscopy (FTIR) with (BRUKER apparatus, USA). The KBr technique was employed to turn the sample into pellets for analysis. (FTIR) analysis is a spectroscopic technique used to determine the absorption of a substance inside the infrared region, specifically within the wavelength range of 400-4000 cm^{-1}. The absorbance of infrared light energy at different wavelengths was evaluated for the samples to ascertain the molecular composition and structure of the materials [42]. The analysis was conducted in the Ministry of Science and Technology under the Environmental and Water Research Department.

CHN Analysis: The CHN analysis technique can accurately measure the elemental content of carbon, hydrogen, and nitrogen in the (Ph2) sample. The PERKIN-ELMER 2400 CHN analyzer was employed for the analysis. Oxygen percentage is determined as the difference between the total percentage and the sum of the obtained amount of (C) and (H) [43].

Nuclear magnetic resonance (NMR): NMR investigations were conducted using) the Bruker-500 (instrument (Bruker Corporation, Fällanden, Switzerland). The proton (^{1}H NMR)

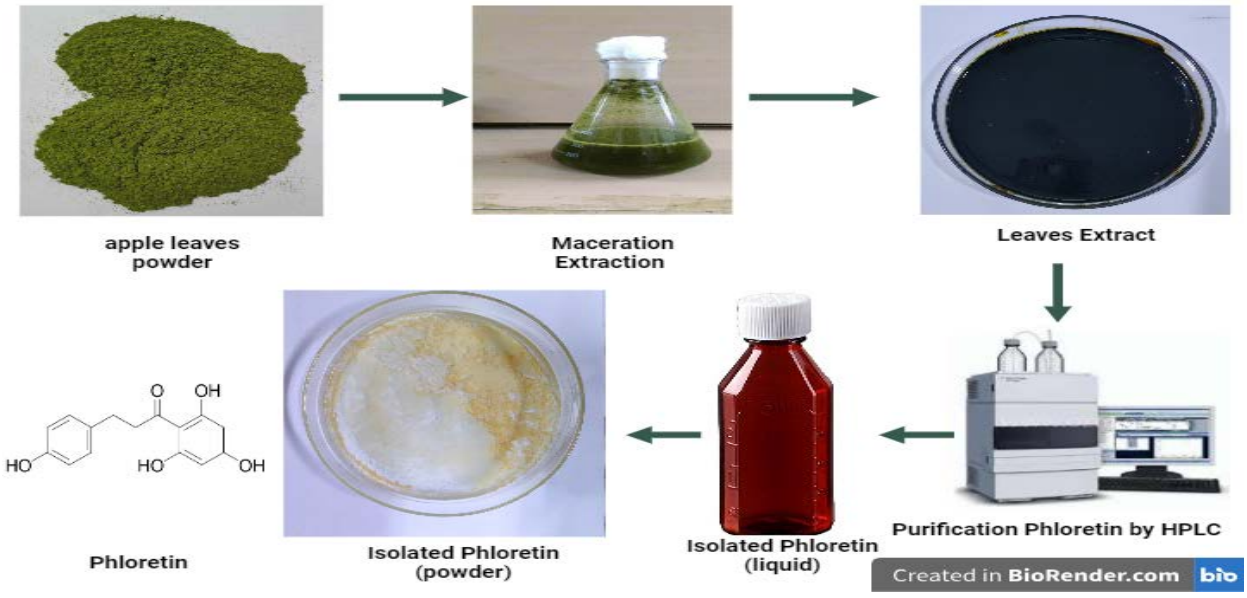


Figure 3. *Purification phloretin procedure from Iraqi apple leaves.*

and carbon (^{13}C NMR) spectra were recorded. The dissolution of the compound was carried out in deuterated dimethyl sulfoxide (DMSO).

Estimation of Antioxidant Activity by (DPPH) Reagents: The DPPH (2,2′-diphenyl-1-picrylhydrazyl) free radical scavenging activity assay was conducted following the protocol outlined by Brand-Williams et al. [44] with minor adjustments. A solution of DPPH was produced using methanol as the solvent at a concentration of 0.1 mM. The sample (pure isolated phloretin/Ph2) was prepared using methanol at 2 mg/ml. A 2 ml aliquot was obtained from the sample and combined with 2 ml of a prepared DPPH solution. The resulting mixture was then incubated in darkness at room temperature for 30 minutes. The absorbance was measured at a wavelength of 517 nm utilizing a UV spectrophotometer. The antioxidant activity of the sample was performed using the ascorbic acid calibration curve. The percentage of radical scavenging was computed employing the following formula.

$$\text{Percentage of radical scavenging} = \left[\frac{A0 - A1}{A0}\right] * 100$$

Where A0= Absorbance of DPPH solution, and A1= Absorbance of the sample.

Cytotoxicity assay of isolated phloretin of Iraqi *M. domestica* by MTT Assay: To evaluate the toxicity of the isolated phloretin obtained from the leaves of Iraqi *M. domestica* in vitro, the MTT assay was conducted as follow [45].

Cell culture: The cancer cell lines (Hep G2/ human hepatocyte carcinoma, A549/ human lung adenocarcinoma, SW480 / human colon cancer cell, and AGS /adenocarcinoma of the stomach) and normal cell line/Non-cancerous (HEK 293/ human embryonic kidney cell line) were incubated in a humid environment at 37°C CO_2 incubator in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 µg/mL of streptomycin, and 100 U/mL of penicillin [46].

MTT assay: Hep G2, A549, SW480, AGS, and HEK 293 cell lines were initially seeded at a density of 1×10^4 cells per well. Subsequently, the plates were carefully transferred to a CO_2 incubator overnight. The following day, the medium was replaced with a fresh medium that contained varying concentrations (500, 250, 125, 62.5, 31.25, and 15.625) µg/ml of the isolated phloretin Ph2, as well as control samples. Additionally, cisplatin was employed as the positive control. The cells were then incubated for 24 hours. Subsequently, the medium was extracted and replaced with 100 µl of an MTT solution with a concentration of 0.5 mg/ml. The cells were then incubated for 4 h at a temperature of 37°C. Subsequently, 50 µl dimethyl sulfoxide (DMSO) was added to each well. The plate was then incubated at 37°C for 15 minutes with continuous agitation. Finally, absorbance was measured using a microplate reader set at a wavelength of 570 nm [47]. The experiment was conducted in triplicate. Determining the proportion of viable cells involved a comparison of the absorbance values obtained from cells treated with isolated phloretin and cisplatin to those of untreated control cells. Cell viability percentage was calculated using the formulae described by Al-Shammari [48].

$$Percent\ cell\ viabilty = \frac{Average\ absorbance\ of\ treated\ cells}{Average\ absorbance\ of\ untreated\ cells} \times 100$$

Results and Discussion.

Thin layer chromatography for crud ethanolic extract: TLC of ethanolic extracts for aerial and fruit parts were done using the solvent system to show a spot of phloretin, with the same Rf values similar to the phloretin standard, as illustrated in Figure (4). The R_f values are presented in Table (1).

Phloretin Concentration in Different Apple Parts Detected by HPLC: The concentration of Ph quantified by HPLC in the apple aerial parts and fruit (Table 2 and Figure 5). Highly varied concentration ranges of Ph were found in the results; the highest concentration of Ph detected was in the peels at 1.30 mg /g, while the lowest was in the petioles at 0.07 mg/g. Phloretin is predominantly present in the peels of the apple 80-420 mg/kg and pulp 16-20 mg/kg. The yield of this compound recovered through extraction can vary significantly between different apple cultivars and is also influenced by the extraction procedure [36].

According to TLC and HPLC results, the presence of (Ph) was documented in the extracts of *M. domestica* by comparison with Rf and Rt values of phloretin standard. (Ph) in the extract of leaves isolated by PHPLC, the PHPLC chromatogram of the extract showed different peaks that represent other compounds according to their retention times; one of these peaks, phloretin, had a retention time of 6.18 min, which was identified by comparison with phloretin standard with a retention time of 6.15 min. The target peak was collected by a fraction's collector from the time of its appearance until its descendants (Figure 6A, 6B and 6C). The proposed phloretin yield (Figure 6D).

Polyphenols, including dihydrochalcones, are purified through traditional techniques such as Extraction by using organic solvents, precipitation, crystallization, and chromatography, which enhance the concentration of extracted polyphenols by eliminating residual solid particles and unwanted compounds [49]. The chromatographic technique is regarded as a highly efficient purification technique, providing high purity [50]. HPLC is a commonly employed method for effectively separating bioactive compounds and achieving a high recovery rate [51] the principle of separation is based on the bonds that exist between each constituent of the sample and the chromatographic phases (mobile and stationary phases). The selection of phases is contingent upon the characteristics of the sample (polarity, structure, and solubility) and the desired compound to be isolated [52,53].

Identification and characterization of the proposed phloretin.

Further analysis was conducted using the following techniques to validate the isolated phloretin's identity.

Identification of proposed Phloretin by TLC: TLC of proposed phloretin (Ph2) was done using the solvent system and showed spots of phloretin, with the same Rf value similar to that of phloretin standard as illustrated in Figure (6A and 6B). (Ph2) had an Rf 0.466, which was identified by comparison with the phloretin standard spot.

Identification of proposed Phloretin by HPLC: The identification of the proposed phloretin (Ph2) was carried out by HPLC, comparing its HPLC retention time with that of the standard. Peak (Ph2) had a retention time of 6.11 min, compared with phloretin standard with a retention time of 6.15 min. (Figure 6).

Table 1. *Retardation factor(Rf)values of the extracts for aerial,fruit parts and standard.*

Plant part	R_f value of phloretin standard	R_f value of extracts
Leaves	0.441	0.441
Petioles	0.441	0.441
Stems	0.441	0.441
pulp	0.510	0.510
Peels	0.510	0.510

Table 2. *The retention time and Concentration of phloretin identified by the HPLC analysis in the different apple parts.*

Phloretin	leaves	petioles	stems	Peels	Pulp
Ret. Time for Standard	3.541	3.541	3.541	3.571	3.571
Ret. Time for extract	3.496	3.425	3.530	3.455	3.552
Concentration (mg /g)	0.34	0.07	0.15	1.75	1.46

Table 3. *The melting point of isolated purified phloretin compared to reference melting point.*

Isolated compound	Tested Melting Point °C	Reference Melting Point °C	Reference
Phloretin	264	263.5	[54]

Table 4. *CHN/O analysis results of proposed phloretin(Ph2).*

Compound	C %	H %	N %	O % *
proposed phloretin (Ph2)/Found	65.13	5.02	0.00	29.85
Phloretin standard / Calculated	65.63	5.10	—	29.17
*O% was determined by subtraction of C % and H % from the total composition. / -- = absent				

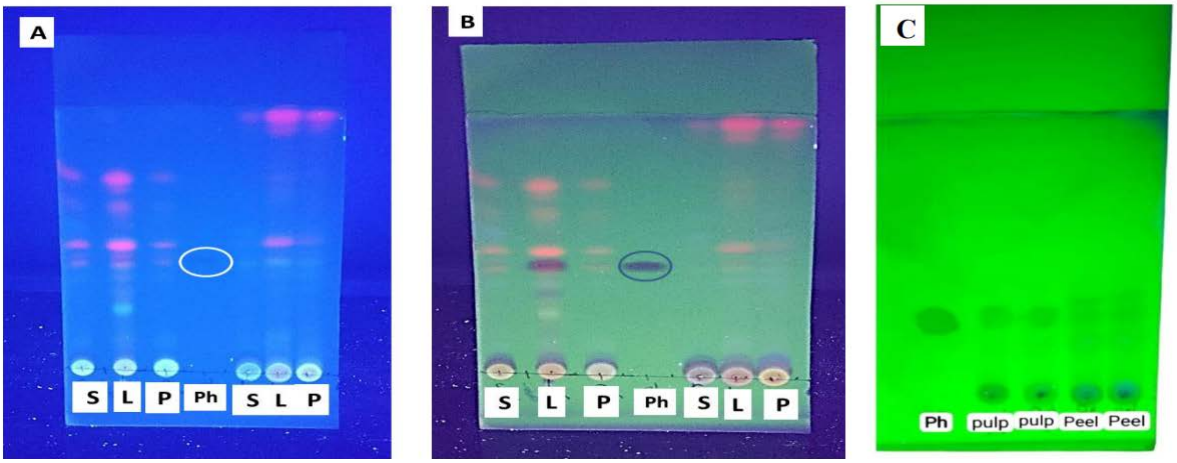


Figure 4. *TLC chromatogram obtained by extracts (L= Leaves, S= Stems, and P= Petioles) with phloretin standard(Ph) under UV light, A: 366 nm and B:254 nm, C: pulp and peels with phloretin standard(Ph) under UV light 254 nm.*

Identification of proposed Phloretin by Melting point.

Table 3 compares the melting point of the isolated purified phloretin (Ph2) and the reference melting point.

Identification of proposed Phloretin by FTIR: The identification technique involves matching the bands found in the measured FTIR spectra with those reported in reference literature. The FT-IR spectrum (Figure 7) displays the unique characteristics of Phloretin. The observed peaks at 3525, 3410, and 3315 cm^{-1} correspond to the OH groups found in the phenolic compound. Additionally, the stretching vibration of the aromatic rings is evident at 3032 cm^{-1}. The band observed at 1753 cm^{-1} is assigned to the carbonyl group (C=O). The stretching vibrations observed at 1668 and 1456 cm^{-1} confirm the C=C in the aromatic rings, thus confirming the skeleton of Phloretin.

Furthermore, the C-O carbonyl group stretching is visible at 1319 cm^{-1}. The vibrations for C-OH on the aromatic rings are also noticeable at 752 and 682 cm^{-1}. This finding is consistent with the data presented in previous literature [55,56].

Identification of proposed Phloretin by CHN analysis:

The CHN analysis technique can accurately measure the elemental content of carbon, hydrogen, and nitrogen in the proposed phloretin sample. The PERKIN-ELMER 2400 CHN analyzer was employed for the analysis. Table 4 displays the percentages of (C), (H), and (N) constituents within the examined sample. The findings indicate a strong agreement between the theoretical data (Calculated data) and the experimental data (Found) of Ph).

Identification of proposed Phloretin by NMR analysis:

Proposed Phloretin (Ph2) compound was extracted and purified from the leaves of *M. domestica*, ^{1}H NMR and ^{13}C NMR were used to identify this compound.

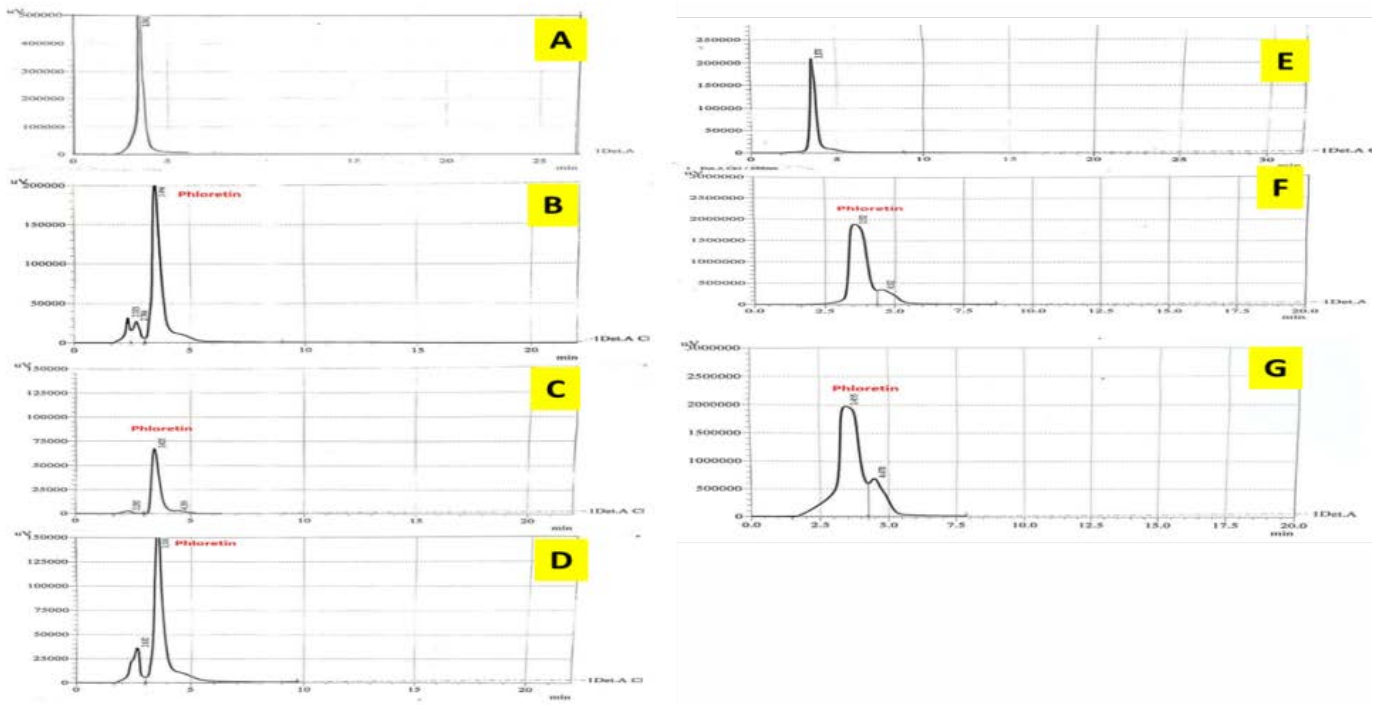
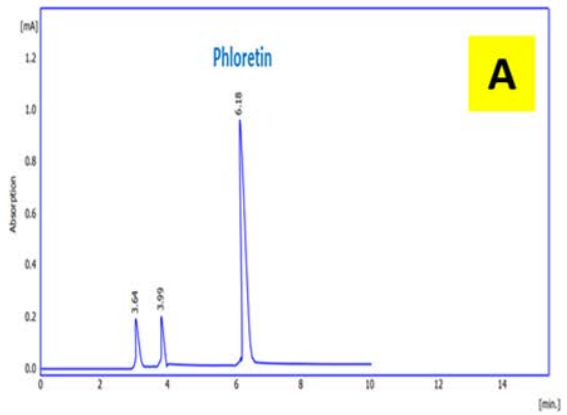
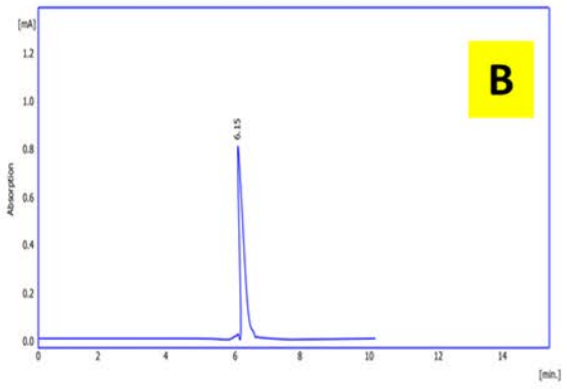
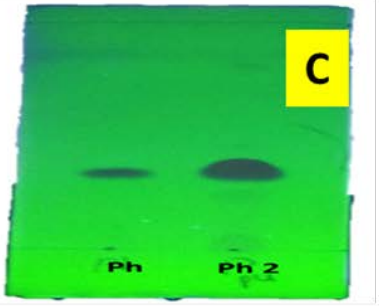


Figure 5. *HPLC chromatogram of (A)phloretin Standard, (B) leaves, (C) petioles, and (D) stems, (E) Phloretin standard, (F) Pulp, and (G) Peels.*







Amount of Proposed phloretin in the plant leaves	
Weight of leaves powder (g)	50
Yield	18.146
Weight of proposed phloretin powder (g)	1.404

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Figure 6. *PHPLC chromatogram for (A) leaves extract matched with (B) phloretin standard. (C) TLC chromatogram obtained by proposed phloretin (Ph2) and phloretin standard (Ph) under UV light 254 nm. (D) Amount of Proposed phloretin in the plant leaves.*

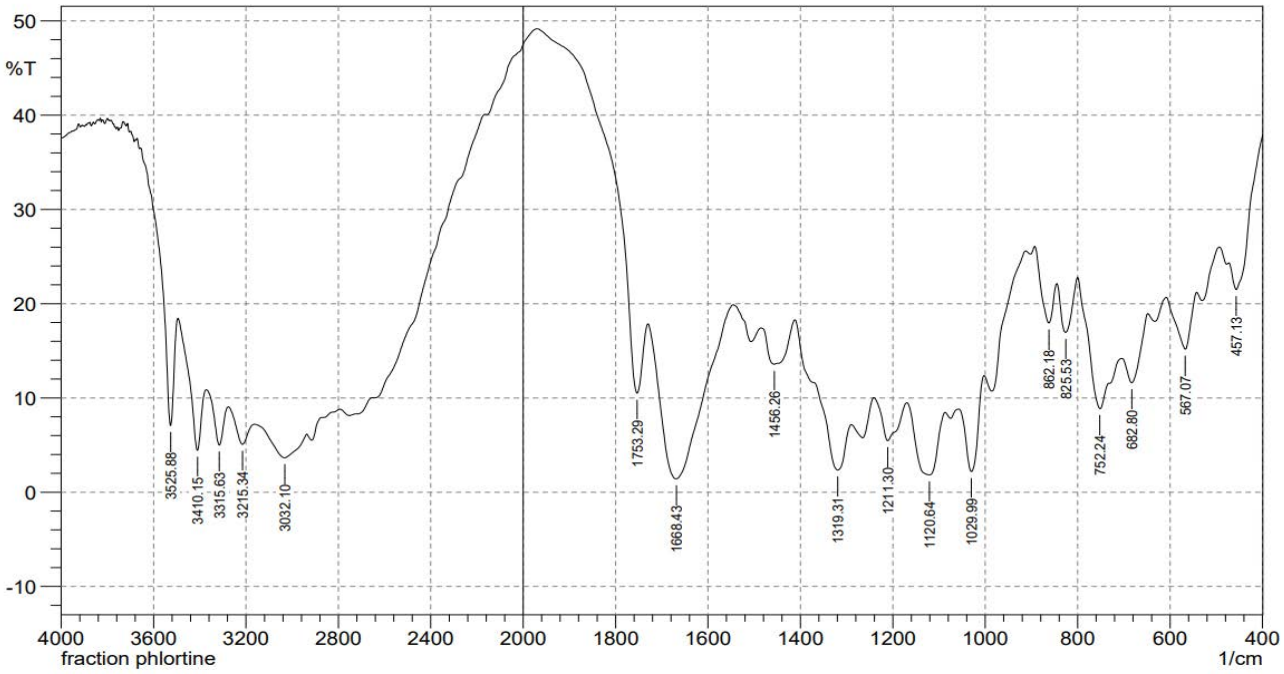

Figure 7. *FT-IR spectra of isolated phloretin(Ph2).*

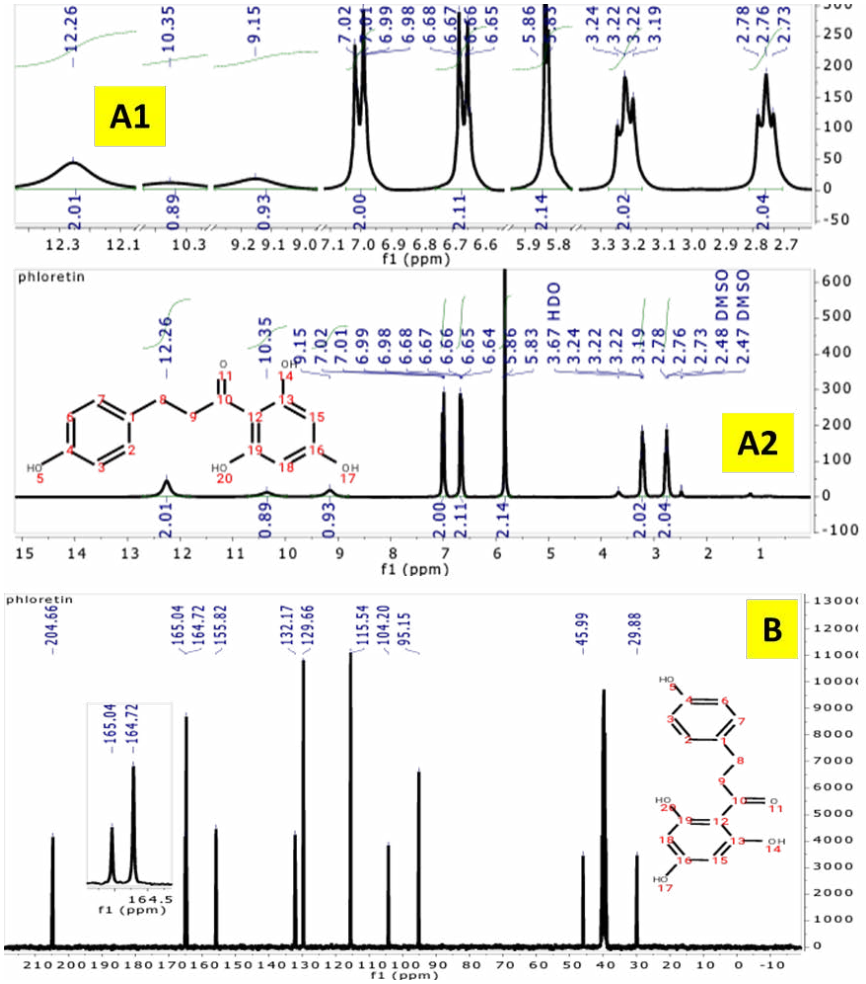

Figure 8. *Identification of proposed Phloretin by NMR analysis (A1 and A2) [1]H-NMR of phloretin (B) [13]C-NMR of phloretin.*

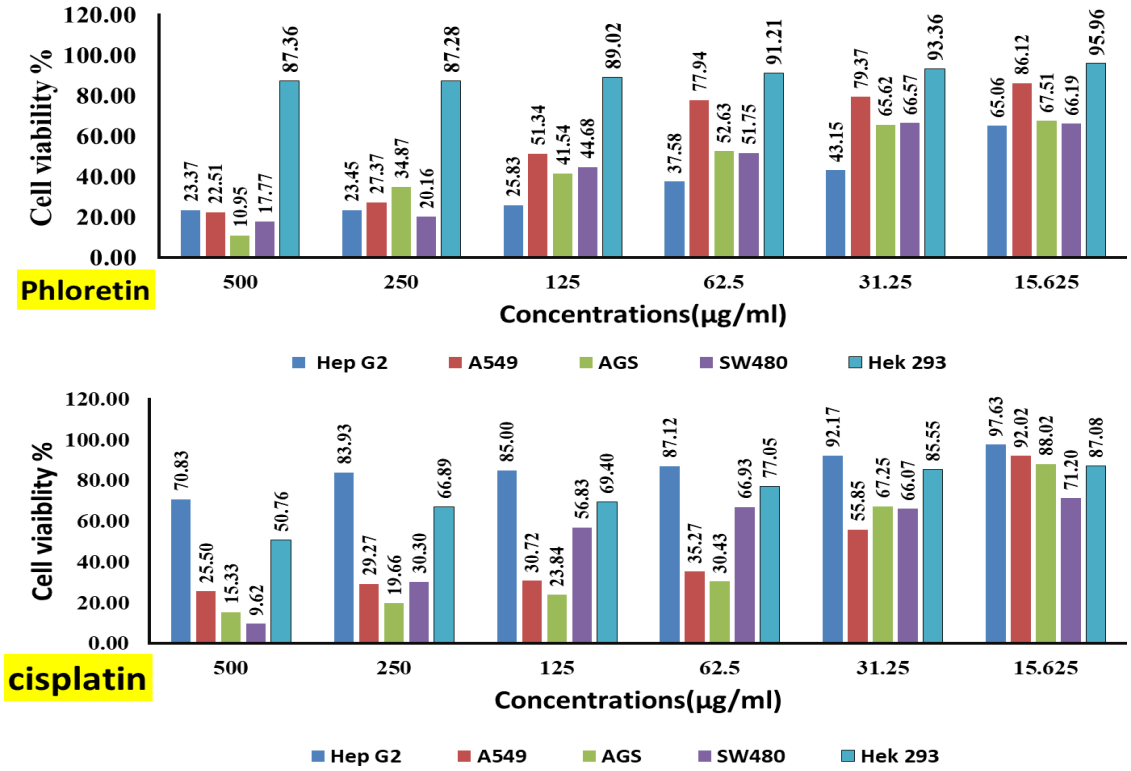

Figure 9. *The percentage of cell viability per different doses of the isolated phloretin of M.domestica leaves versus Cisplatin on different cell lines.*

^{1}H-NMR of phloretin:

The spectrum (Figure 8A) shows that the isolated compound is pure as it only gives the characteristic signals for this compound. The spectrum showed three singlet signals at 12.26, 10.35 and 9.15 ppm, which attributed to the protons of (OH 14 and 20), OH17 and OH6, respectively. The spectrum also showed two doublet signals at 7.01 and 6.66 ppm of the protons (H2 and H7) and (H3 and H5), respectively, with J^{3} equal 8.54 and J^{4} equal 2.65, respectively. Furthermore, the spectrum showed a singlet signal at 5.83 ppm, which could be assigned to the proton of H15 and H18 in addition to two virtual triplet signals at 3.22 and 2.76 ppm of H5 and H6, respectively, with J^{3} equal 8[57].

^{13}C-NMR of phloretin:

The spectrum (Figure 8B) showed the following signals at 204.66, 165.04, 164.72, 155.82, 132.17, 129.66, 115.54, 104.20, 95.15, 45.99 and 29.88 ppm of C10 of C=O group, (C13,19), C16, C4, C1, (C2, 7), (C3,6), C12, (C15, 18), C9 and C8, respectively [57,58]. Based on all these results, the target compound (Ph2) structure was identified as phloretin (Ph).

Antioxidant activity for the pure isolated phloretin (Ph2):

DPPH assay proved that the pure isolated phloretin (Ph2) could scavenge activity record of 51.8%. This in vitro assay indicates that *M. domestica* has a significant source of antioxidants, which might help prevent the progress of various oxidative stresses. A wide range of pharmacological impacts of Phloretin is produced by a carbonyl group, hydroxyl groups, and two phenol aromatic rings (A and B rings), and the presence of sugar residues attached to the molecule in their chemical structure [3]. Many pre-clinical studies have indicated that Phloretin has significant antioxidant activity attributed to its flavonoid structure with four O-H bonds [59]. The antioxidant capacity of compounds can be evaluated based on their bond dissociation enthalpy (BDE/ is known as a part of the endothermic process and indicates the energy required to break a bond into two isolated molecules or atoms) and ionization potentials (IPs/ refer to the energy required to deprive an electron of its quiescent molecules or atoms) according to the density functional theory (DFT) [60]. IPs provide information on the efficiency of singlet-oxygen scavenging and quenching of free radicals, and molecules with a low BDE have better antioxidant activity and break bonds easily; the process of removing an electron from Phloretin in water, to destroy singlet oxygen and scavenge free radicals, requires minimal energy consumption, allowing it to exhibit incredible antioxidant activity [61]. Phloretin can inhibit lipid peroxidation and scavenge peroxynitrite radicals due to the (2,6-dihydroxyacetone) moiety in its structure. It has been determined that the hydroxyl group at the (20 position) of Phloretin is an essential pharmacophore for radical scavenging and lipid peroxidation activities [6]. Additionally, studies to understand the molecular mechanisms of Phloretin's antioxidant capacity have shown that cellular levels of antioxidant enzymes, such as heme oxygenase-1 (HO-1) and glutathione (GSH), are restored by Phloretin due to its significant impact on the redox-regulated transcription factors such as extracellular signal-regulated and nuclear factor erythroid related factor-2 (Nrf2) pathways [3]. As a result, Phloretin can neutralize charged radicals and reactive oxygen species [19,62].

Cytotoxic Effects of Phloretin (Ph2) on Four Cancer Cell Lines:

In vitro, the cytotoxicity potential of Ph2 was evaluated in Hep G2, A549, SW480, AGS, and HEK 293 cell lines (Figure

9). The cytotoxicity impact on cell growth was examined at different concentrations (15.625 – 500 µg/ml), and the results measured the ability of ph2-induced inhibition. The results show no cytotoxic effects towards Ph2 treated Noncancerous cell line of HEK-293. In Hep G2, A549, SW480, and AGS cells, the percentage of viability of cancerous cells decreased with the increasing concentrations of Ph2. It shows that the cytotoxic effect of the Ph2 against cancer cell lines was stronger than the non-cancerous cell line. In vitro, the cytotoxicity potential of Cisplatin was evaluated in cancer and non-cancer cell lines (Figure 9). Our data shows that different cells had different sensitivity to the inhibition effect of the Ph2.

Phloretin exhibits many anticancer characteristics, including anti-inflammatory, anti-proliferative, anti-invasive, cell-cycle arrest, pro-apoptotic, antimetastatic, and antiangiogenic effect [63]. The double bond between positions (α and β) on the three carbon atoms bridge between (A and B) rings. This former makes Phloretin a highly flexible molecule that effectively binds with biological macromolecules. These interactions and activation/block of intracellular signalling pathways lead to striking biological properties, including anticancer activity.64 Phloretin has exhibited the ability to modify several protein functions, reversing cellular transformation and abnormal signalling. In addition, research has shown that Phloretin can protect against oxidative DNA damage in human colon cancer cells (Caco-2 and HT-29) by replenishing cellular glutathione (GSH) levels [6,65]. Another study documented that Phloretin inhibited COLO 205 colon cancer cell (COLO 205) proliferation by arresting the cell cycle through p53 and suppressing glucose transporter activity [66].

Previous studies have indicated significant anticancer properties of phloretin, demonstrating its ability to inhibit the malignant progress in various preclinical models of oral cancer, lung cancer, esophageal cancer, colon cancer, gastric cancer, liver cancer, cervical cancer, breast cancer, and prostate cancer [56,67,68]. In doing so, phloretin can modulate many different intracellular signalling pathways, attack several molecular targets, regulate cell proliferation, and cell growth, stimulate cytoprotective enzymes, inhibit cytotoxic systems, scavenge free radicals, induce apoptotic cell death, inhibit type 2 glucose transporter (glucose deprivation).

As a result of the presence of four hydroxyls groups (OH) in its structure can hinder the trans-membrane transport of saccharide resulting in apoptosis of malignant cells. Zhang et al. reported that phloretin can inhibit tumor formation and cause tumor cell apoptosis [69]. These pathways can vary depending on the cell model and experimental conditions [70].

Qin et al. reported that the position and number of hydroxyl groups attached to the A-ring and the position and nature of the carbohydrate units in the glycosides could influence flavonoids' medicinal uses [18]. Phloretin purified from Crabapple Leaves showed the strongest anticancer activity among the derivatives due to its small molecular mass and some glycoside moiety is bound to the phloretin structure decreases the anticancer activity of phloretin, such as (trilobatin and Phlorizin), the obtained result is similar to our exploration. Similarly, Kai Xü et al., purified phloretin from the bark of an apple tree [71].

Finally, phloretin's various sources and pharmacological activities have led to new drug development derived from apples.

Conclusion.

Apples are the most extensively cultivated and consumed fruit globally. Phloretin is a crucial component of apple products, known for their abundance of essential biomolecules. These biomolecules play a vital role in promoting human health due to their potent anti-inflammatory, antiradical, and anticancer properties. The purification of phloretin from apple tree parts is of great interest to enhance the value of natural raw materials and obtain extracts with a higher concentration of phloretin. TLC, PHPLC, FTIR, CHN, and Melting point analysis confirmed the presence and purity of phloretin. Further, the findings of the cytotoxic study against human cancer cell lines by MTT assay exposed that Ph2 is a potential anticancer drug compared to the standard chemotherapy Cisplatin. These experimental and pre-clinical results are the base for advancing a prospective natural drug.

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