https://doi.org/10.34883/PI.2024.13.1.014

Ali Mohammed Zaki Al-Jaberi[1], Sabaa Ali Mohammed Al-Fadal[1]✉,
Thukaa Zuhair Abdul-Jalil[2]
[1] College of Pharmacy, University of Basrah, Basrah, Iraq
[2] College of Pharmacy, University of Baghdad, Baghdad, Iraq

Antioxidant Activity and DNA Protective Effects of Rutin Extracted from Ruta chalepensis L. Growing in Iraq: A Research Methodology

Conflict of interest: nothing to declare.
Authors' contribution: visualization, formal analysis, writing original draft, writing review and editing – Ali Mohammed Zaki Al-Jaberi; the plant, material preparation, experiments and data analysis, supervision and validation – Sabaa Ali Mohammed Al-Fadal; data curation, review and editing – Thukaa Zuhair Abdul-Jalil. All authors contributed to the conception and design of the study. All authors read and approved the final manuscript.
The article is published in the author's edition.

Submitted: 07.12.2023
Accepted: 12.02.2024
Contacts: sabaa.mohammed@uobasrah.edu.iq

Abstract

Introduction. Flavonoids, vital plant compounds with established therapeutic benefits, possess a diverse range of pharmacological properties, offering promising avenues for drug discovery. Recent studies highlight their antioxidant role against oxidative stress.
Purpose. To optimize, on the basis of comparative evaluation of different variants of extraction techniques, and to quantify the rutin contained in Ruta chalepensis L. with the subsequent assessment of its performance as an antioxidant agent with DNA-protective activity.
Materials and methods. A mixture of 30% acetonitrile and 70% water (v/v) was used as the mobile phase for isolating rutin by High-Performance Liquid Chromatography (HPLC). Subsequently, five different methods were employed to evaluate the antioxidant activity of rutin: Ferric Reducing Antioxidant Power (FRAP) assay, DPPH free radical scavenging assay, hydroxyl radical scavenging assay, hydrogen peroxide scavenging activity assay, and total antioxidant activity assay. Additionally, the DNA damage protection activity of rutin was investigated using human DNA.
Results. In this study, a reflux method with 80% methanol was used for rutin extraction. High-performance liquid chromatography (HPLC) was used to detect rutin in plant extract, and the percentage of rutin in this extract was 0.0089%. This study elucidates the potential significance of rutin as an antioxidant. The free radical scavenging activity of rutin was determined using various assays. The DPPH assay showed a high free radical scavenging activity for rutin, with an IC50 value of 0.0414 µg/ml. In the hydroxyl radical scavenging assay, the IC50 for rutin was 0.0625 µg/ml, and the IC50 for hydrogen peroxide scavenging activity was 0.027141 µg/ml. The ferric reducing power assay and total antioxidant activity assays also illustrated the antioxidant activity of rutin. Finally, the DNA damage protection assay showed that the best concentrations for DNA protection are 500 µg/ml and 250 µg/ml.

Conclusion. This study shows that rutin is a potent antioxidant that can be used as a dietary supplement or by consuming plants that contain it to help eliminate free radicals.
Keywords: rutin, antioxidant, DNA, reflux, HPLC, Ruta chalepensis L.

Али Мохаммед Заки Аль-Джабери[1], Сабаа Али Мохаммед Аль-Фадаль[1]✉,
Тукаа Зухейр Абдул-Джалил[2]
[1] Фармацевтический колледж, Университет Басры, Басра, Ирак
[2] Фармацевтический колледж, Университет Багдада, Багдад, Ирак

Антиоксидантная активность и ДНК-протекторное действие рутина, выделенного из руты халебской (Ruta chalepensis L.), произрастающей в Ираке: методология исследования

Конфликт интересов: не заявлен.
Вклад авторов: визуализация, формальный анализ, написание чернового варианта, рецензирование и редактирование – Али Мохаммед Заки Аль-Джабери; подготовка растений, материалов, проведение опытов и анализ данных, руководство и валидация – Сабаа Али Мохаммед Аль-Фадаль; курирование, рецензирование и редактирование – Тукаа Зухейр Абдул-Джалил. Все авторы участвовали в разработке концепции и дизайна исследования. Все авторы прочли и одобрили окончательный вариант рукописи.
Статья опубликована в авторской редакции.

Подана: 07.12.2023
Принята: 12.02.2024
Контакты: sabaa.mohammed@uobasrah.edu.iq

Резюме

Введение. Флавоноиды – жизненно важные соединения растительного происхождения с доказанными терапевтическими свойствами, обладающие широким спектром фармакологических свойств, что открывает перспективные возможности для разработки лекарственных средств на их основе. Результаты недавно проведенных исследований свидетельствуют об их антиоксидантной активности в условиях окислительного стресса.
Цель. Оптимизация на основе сравнительной оценки различных вариантов технологий экстракции и количественного определения содержащегося в Ruta chalepensis L. рутина с последующим установлением эффективности его использования в качестве антиоксиданта, способного проявлять к тому же и ДНК-протекторную активность.
Материалы и методы. В качестве подвижной фазы для выделения рутина методом высокоэффективной жидкостной хроматографии (ВЭЖХ) использовали смесь 30% ацетонитрила и 70% воды (% по объему). Для экстракции рутина был применен также метод рефлюкса с использованием 80% метанола. Оценку антиоксидантной активности рутина проводили с применением пяти различных методов: антиоксидантного теста по железовосстанавливающей активности (FRAP), ДФПГ-теста, анализа на удаление гидроксильных радикалов, анализа на удаление перекиси водорода и общего анализа антиоксидантной активности. Кроме того, была исследована протекторная активность рутина в отношении ДНК с использованием человеческой ДНК.

Результаты. Процентное содержание рутина в выделенном с помощью ВЭЖХ экстракте составило 0,0089%. Антиоксидантная активность рутина была оценена с помощью различных методов исследования. ДФПГ-тест показал высокую активность рутина по удалению свободных радикалов, при этом значение IC50 составило 0,0414 мкг/мл (мг/л). В выполненном анализе на удаление гидроксильных радикалов IC50 рутина составила 0,0625 мкг/мл (мг/л), IC50 в отношении перекиси водорода – 0,027141 мкг/мл (мг/л). Антиоксидантный тест по железовосстанавливающей активности и тест на общую антиоксидантную активность также подтвердили достаточно высокую антиоксидантную активность рутина. Кроме того, анализ ДНК-протекторной активности показал, что рутин проявил наиболее выраженную защитную активность в отношении ДНК в концентрациях 500 мкг/мл (мг/л) и 250 мкг/мл (мг/л). Результаты исследования свидетельствуют о потенциальной значимости применения рутина в качестве антиоксиданта.

Заключение. Проведенное исследование свидетельствует о том, что рутин является мощным антиоксидантом, способствующим инактивации свободных радикалов. Представляется возможным использовать его в качестве пищевой добавки или употреблять в пищу содержащие его растения, в особенности пациентами, страдающими разными формами свободнорадикальной (мембранной) патологии.

Ключевые слова: рутин, антиоксидант, ДНК, рефлюкс, ВЭЖХ, Ruta chalepensis L.

■ INTRODUCTION

Reactive oxygen species (ROS) play a crucial role in cellular metabolism. Nevertheless, this advantageous molecule can also have adverse consequences for cellular processes. Both the formation and clearance of this molecule are carefully regulated to maintain a specific and stable amount of reactive oxygen species (ROS). Nevertheless, in specific situations, this equilibrium might be disrupted. The imbalance between cellular formation and the clearance of oxidative stress can lead to the generation of elevated levels of diverse free radical molecules. These molecules can cause detrimental alterations to cellular components, including proteins, DNA, and lipids [1].

Reactive oxygen species (ROS), which are highly reactive molecules, are produced by the biotransformation of molecular oxygen. Multiple forms of reactive oxygen species (ROS) exist, encompassing singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anion radical ($O2^{\cdot-}$) and the exceedingly reactive hydroxyl radical ($^{\cdot}OH$) [2]. The adverse impacts of oxygen are linked to its metabolic conversion into these exceedingly harmful entities. Reactive oxygen species (ROS), which are inherently present in all living cells, are associated with biochemical antioxidants. The primary sources of endogenous reactive oxygen species (ROS) are hydrogen peroxide and superoxide anion. These ROS are naturally generated as byproducts of cellular metabolism, particularly through processes such as the mitochondrial respiratory chain. Moreover, the primary origins of extracellular reactive oxygen species (ROS) encompass ionizing radiation, ultraviolet (UV) radiation, toxins, microbes, and pollution from the environment [3].

The phenomenon of oxidative stress, caused by the detrimental effects of free radicals, has been linked to various degenerative conditions, including osteoarthritis, cancer, diabetes, and cardiovascular illnesses [4]. When the concentration of reactive

oxygen species (ROS) surpasses the capacity of the cell's internal antioxidant defense mechanisms, it is referred to as oxidative stress [5]. The occurrence of elevated levels of free radicals and/or diminished physiological efficacy of antioxidant defenses against free radicals leads to disruptions in the typical redox equilibrium within the cellular milieu. This has the potential to result in harm to all cellular constituents. Cell death can be induced by severe oxidative stress. The classical parameters utilized for the characterization of oxidative stress in biological samples encompass elevated levels of radicals and oxidants, diminished levels of antioxidants, including both lipid-soluble and small molecular water-soluble antioxidants, perturbations in the cellular redox system, and oxidative harm inflicted upon cellular constituents such as proteins, DNA and lipids [6].

The concept of "antioxidant" is frequently employed in scientific literature, and its definition might vary depending on the specific methodologies employed for measuring antioxidant activity. Hence, Halliwell and Gutteridge put forth a proposition outlining the characteristics of an antioxidant as "a substance that exhibits the ability to impede, forestall, or eliminate oxidative harm inflicted upon a specific molecular entity. The substances in question possess a physiological function, as indicated by this definition, which involves protecting cellular components from harm resulting from chemical interactions involving free radicals [7].

Given the numerous detrimental effects of free radicals on vital components inside the human body, it is imperative to conduct comprehensive investigations into novel antioxidant compounds derived from natural sources. Flavonoids have emerged as a prominent subject of scientific investigation due to their status as extensively investigated bioactive antioxidant compounds derived from plants [2].

Ruta chalepensis L. (Rutaceae), commonly known as Rue or Fringed rue, is a wild perennial herbaceous shrub that is widely distributed in the Mediterranean Sea regions. It usually grows on rocky mountain slopes. It has glabrous, alternate pinnatisect leaves with narrow oblong-lanceolate or obovate segments and a cymose inflorescence. It is the only rue species mentioned and covered in the Flora Palaestina (in Iraq, this plant is cultivated from north to south in gardens). It is even mentioned in the Bible under the Greek name Pigam, closely cognate with the Arabic name Figam, which is used as a flavoring for honey, wine, and olive oil. Due to its high content of amino acids, saponins, phenols, flavonoids, alkaloids, and furocoumarins in the leaves and young stems, Ruta chalepensis has pleiotropic pharmacological activities [8].

The aerial parts, fruits, and roots of Ruta chalepensis L. have been subjected to phytochemical analysis, which has indicated the presence of flavonoids. Further analysis using high-performance liquid chromatography (HPLC) has identified seven specific flavonoids in different parts of Ruta chalepensis L., namely catechin hydrate, apigenin, flavone, naringin, luteolin, naphthoresorcinol and kaempferol [9]. According to [10] rutin emerged as the predominant flavonoid in Ruta chalepensis L., with the investigation uncovering the existence of hesperidin, quercetin and epigallocatechin.

Rutin, a flavonol glycoside, is frequently encountered in plants belonging to the Rutaceae family. Its many bioactivities have been widely investigated in numerous studies [11].

■ PURPOSE OF THE STUDY

To optimize, on the basis of comparative evaluation of different variants of extraction techniques, and to quantify the rutin contained in Ruta chalepensis L. with the subsequent assessment of its performance as an antioxidant agent with DNA-protective activity.

■ MATERIALS AND METHODS

Plant Sampling

The aerial components of the Ruta chalepensis L. voucher specimen code (No. 33396) plant were planted in a privately owned orchard located in the Al-FAW area of Basrah. Asst. Prof. Dr. Ula AlMousawi from the Pharmacognosy Department at the Pharmacy College of Basrah University confirmed the authenticity of the plant material. The plant sample was harvested in August 2022, subsequently washed, and subjected to a seven-day shade drying process at room temperature. Following this, the sample was crushed into medium-sized pieces and stored in airtight bottles.

Extraction of Flavonoids from Ruta Chalepensis L.

Hot extraction (reflux) was employed as the extraction method. Time: 3 hours at 50 °C temperature. Ten grams of medium-milled Ruta chalepensis L. was placed in a glass container with 200 mL of diethyl ether and underwent defatting for two hours. The mixture was then filtered using filter paper, and the collected plant material was dried at room temperature for 24 hours. Reflux was used as an extraction method with 250 mL of 80% methanol (solid-liquid ratio 1:25 w/v) [12].

Chromatographic Analysis for the Detection and Isolation of Rutin From Crude Extracts

High Performance Liquid Chromatographic Analysis (HPLC) for the Detection and Isolation of Rutin from Crude Extracts. The HPLC study was conducted utilizing the Sykam HPLC system S 600 series, manufactured in Germany. The system consisted of many components, including the S 1130G quaternary gradient pump, the S 5300 sample injector, the S 3250 UV/VIS detector, and the S 4115 column oven. Prior to the final validation and analysis of real samples, optimization was conducted for the sample preparation, mobile phase, and stationary phase parameters. Preparation of rutin standard solutions was conducted and calibration curves were prepared in the range of 5 to 10 000 µg/mL for rutin standard solution to determine the content of the rutin. The detection process was conducted by eluting the sample using a mobile phase consisting of a mixture of acetonitrile and water in a volumetric ratio of 30% acetonitrile to 70% water. The elution was performed at a flow rate of 1.0 ml/min, and the detection of the analyte was carried out using UV detection at wavelengths of 254 nm and at a temperature of 30 °C. The identification of rutin in a crude extract was achieved by HPLC analysis, where the retention duration of the compound was compared to that of a standards solution. According to the study conducted by [19], the isolation procedure was executed based on the obtained results.

Antioxidant Activity

Ferric Reducing/Antioxidant Power Assay FRAP. The determination of reduced power was conducted following the methodology described by [13], Various concentrations of rutin and ascorbic acid, namely 100, 80, 50, 30, 10, and 5 µg/1 mL, were added to a mixture consisting of 2.5 mL of a 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution and 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6). Subsequently, the reaction mixture was thoroughly vortexed and subjected to incubation at a temperature of 50 °C for a duration of 20 minutes. Following the incubation period, a volume of 2.5 mL of trichloroacetic

acid solution with a concentration of 10% was introduced to the mixture. Subsequently, centrifugation was performed at a speed of 3,000 revolutions per minute for a duration of 10 minutes. The 2.5 mL of supernatant was combined with 2.5 mL of deionized water and 0.5 mL of a 0.1% solution of ferric chloride. The solution of color was measured at a wavelength of 700 nm using a UV spectrophotometer, with the blank serving as a reference and a standard for comparison.

2,2-Diphenyl-1-Picrylhydrazyl Free Radical Scavenging Assay DPPH

The radical scavenging activity of rutin was assessed using the methodology outlined by [14], A mixture of rutin and ascorbic acid at various concentrations (100, 80, 50, 30, 10, and 5 µg/ml) in methanol was combined with 1 ml of a DPPH solution (0.1 mM in methanol) to serve as the source of free radicals. The resulting mixture was then incubated at room temperature for 30 minutes. The measurement of the reduction in solution absorbance at a wavelength of 517 nm was conducted to assess the proton-donating activity of rutin. The reference standard employed in this study was L-ascorbic acid.

The proportion of DPPH radical scavenging activity was determined by employing the subsequent formula:

$$\text{DPPH radical scavenging activity \% AA} = [(A° - A^1) / A^1] \times 100 \quad \text{Equation (1)}$$

where AA stands for antioxidant activity, where A° is the absorbance of the DPPH and A^1 is the absorbance of rutin or the standard sample.

Hydroxyl Radical Scavenging Assay

The determination of the activity of the extracts followed the methodology outlined by [15], The reaction mixture consisted of 1.0 ml of various concentrations of rutin, ascorbic acid, and gallic acid (100, 80, 50, 30, 10, and 5 µg/ml of methanol), 1.0 ml of iron-EDTA solution (0.26% EDTA and 0.13% ferrous ammonium sulfate), 1.0 ml of DMSO (0.85% in 0.1 M phosphate buffer at pH 7.4), 0.5 ml of 0.018% EDTA, and 0.5 ml of 0.22% ascorbic acid. The tubes were securely sealed and subjected to thermal treatment in a water bath maintained at a temperature range of 80–90 °C for a duration of 15 minutes. The reaction was halted by introducing 1.0 ml of TCA (17.5%) that had been cooled to a low temperature using ice. The reaction mixture described above was supplemented with 3.0 mL of Nash reagent, which consists of 75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2.0 mL of acetyl acetone. Distilled water was subsequently added to achieve a final volume of 1 L. The resulting mixture was then incubated at room temperature for a duration of 15 minutes to facilitate the formation of color. The measurement of the intensity of the yellow color produced was conducted at a wavelength of 412 nm, relative to a reagent blank. The standards employed in the study consisted of ascorbic acid and gallic acid.

The proportion of Hydroxyl radical scavenging activity was determined by employing the subsequent formula:

$$\text{Hydroxyl radical scavenging activity inhibition \% AA} = [(A° - A^1) / A^1] \times 100 \quad \text{Equation (2)}$$

where AA stands for antioxidant activity inhibition, where A° is the absorbance of the reagent blank and A^1 is the absorbance of rutin or the standard sample.

Hydrogen Peroxide Scavenging Activity Assay

The assessment of rutin's hydrogen peroxide scavenging ability was conducted based on the methodology proposed by [16]. with several modifications. A 1.0 ml aliquot of a 0.1 mM H_2O_2 solution was combined with 1.0 ml of various concentrations of rutin and ascorbic acid. This mixture was then treated with 2 drops of a 3% ammonium molybdate solution, followed by the addition of 10 ml of a 2 M H_2SO_4 solution and 7.0 ml of a 1.8 M KI solution. The solution containing the mixture was subjected to incubation at ambient temperature for a duration of 5 minutes. Subsequently, titration was performed using a solution of Sodium thiosulfate (NaS_2O_3) with a concentration of 5.09 mM. The titration process continued until the disappearance of the yellow color.

The proportion of Hydrogen peroxide scavenging activity was determined by employing the subsequent formula:

Hydrogen peroxides radical scavenging activity inhibition % $AA = [(A° – A^1) / A^1] \times 100$ Equation (3)

where the variable AA stands for antioxidant activity inhibition, the variable A° represents the volume of NaS_2O_3 solution utilized for titrating the control sample in the presence of hydrogen peroxide, excluding rutin and ascorbic acid. On the other hand, the variable A^1 denotes the volume of NaS_2O_3 solution employed when rutin and ascorbic acid were present.

Determination of Total Antioxidant Activity

The assessment of overall antioxidant activity was conducted using the phosphomolybdenum method described by [17]. A volume of 1.0 mL of the extract was mixed with an equal volume of the standard reagent solution, containing 4 mM ammonium molybdate, 0.6 M sulfuric acid, and 28 mM sodium phosphate. The tubes were sealed and incubated in a thermal block set at 95 °C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm using a reagent blank. The overall antioxidant capacity was expressed in micrograms of ascorbic acid equivalent (AAE) per microgram of rutin.

DNA Damage Protection Assay

The assessment of DNA damage was conducted utilizing genomic DNA obtained from human white blood cells (WBCs). The DNA extraction process was conducted with the FAVORGEN DNA extraction kit, adhering to the guidelines provided by the manufacturer. The study conducted by [18], employed this approach to assess the protective efficacy of rutin. In this study, the genomic DNA of human origin was subjected to photolysis using UV light, while being exposed to hydrogen peroxide (H_2O_2). The subsequent damage inflicted on the DNA was evaluated through the utilization of agarose gel electrophoresis. Four polyethylene microcentrifuge tubes were each supplemented with five microliter aliquots of human genomic DNA, with a concentration of 11.3 ng/µl. Subsequently, the tubes were supplemented with four distinct doses of rutin, namely 500, 250, 100, and 50 µg/ml. A fifth tube was employed as a control for irradiation (CR), devoid of rutin. Each tube was supplemented with a volume of 5 microliters of a 3% hydrogen peroxide (H_2O_2)

solution. Subsequently, the tubes were positioned onto the surface of a UV transilluminator emitting light at a wavelength of 300 nm, and left undisturbed for a duration of 10 minutes at ambient temperature. A sixth tube was designated as a non-irradiated control (CO) and was included in the experiment. This tube contained 1 microliter of human genomic DNA. Thereafter, all the samples were subjected to electrophoresis on a 1% agarose gel and thereafter imaged.

Statistical Analysis

All data are presented as the mean ± standard deviation (SD). T-tests and two-way ANOVAs were performed using the SPSS program to evaluate the results from the DPPH scavenging activity assay, hydrogen peroxide scavenging activity assay, and hydroxyl radical scavenging assay experiments. A p-value less than or equal to 0.05 was considered statistically significant.

■ RESULTS

High Performance Liquid Chromatographic Analysis (HPLC) for the Detection and Isolation of Rutin from Crude Extracts

(Fig. 1–3) depict the reverse-phase high-performance liquid chromatography (HPLC) chromatograms of the rutin standard solution, the mixture of the rutin standard solution and crude extract, and the crude extract alone. The identification of rutin derived from Ruta chalepensis L. was accomplished through a comparative analysis of their retention times in relation to established reference standard. The combination of the rutin standard solution with the crude extract resulted in a distinct peak at 3.313 minutes, which serves as confirmation of the rutin peak's location, as depicted in Fig. 3. The concentration of rutin in the crude extracts was 0.0089%.

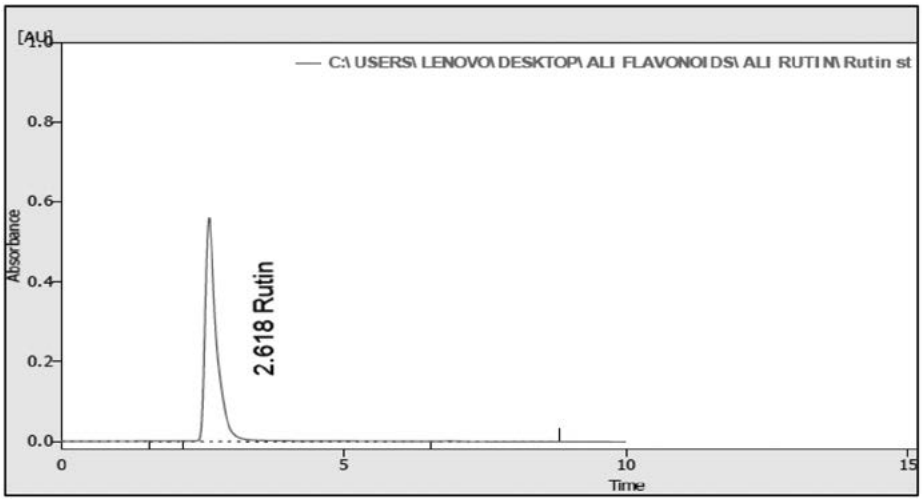

Fig. 1. HPLC chromatogram of rutin standerd

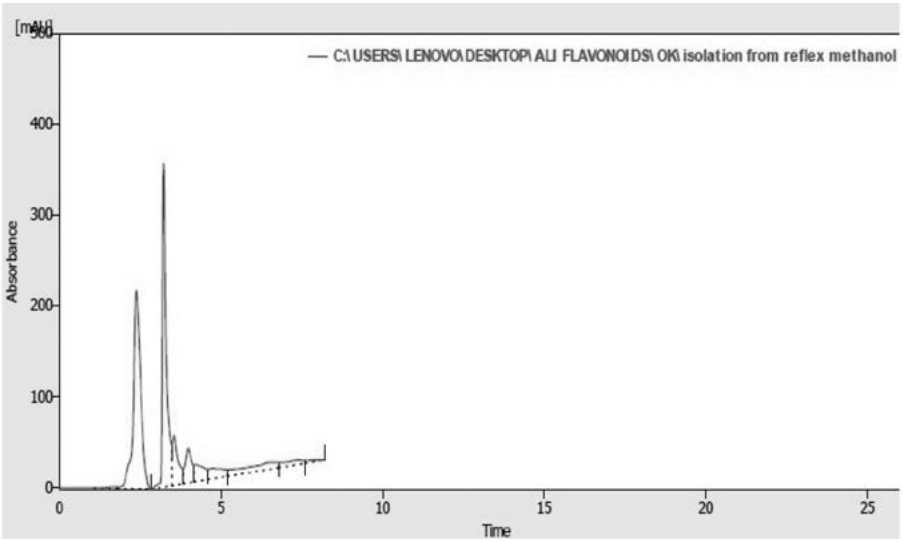

Fig. 2. HPLC chromatogram of crude extract

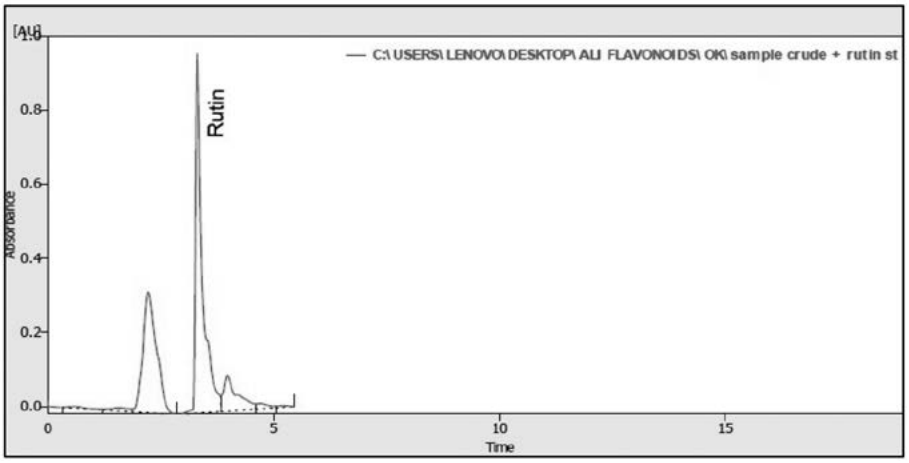

Fig. 3. HPLC chromatogram of mixture of rutin stander solution with crude extract

Ferric Reducing/Antioxidant Power Assay FRAP

The results indicated a general increase in activity as the concentration of rutin was raised, as evidenced by the absorbance at 700 nm. Rutin exhibited concentration-dependent reducing activity and displayed the highest capacity for scavenging free radicals at concentrations of 100 and 80 µg/mL, as illustrated in (Fig. 4).

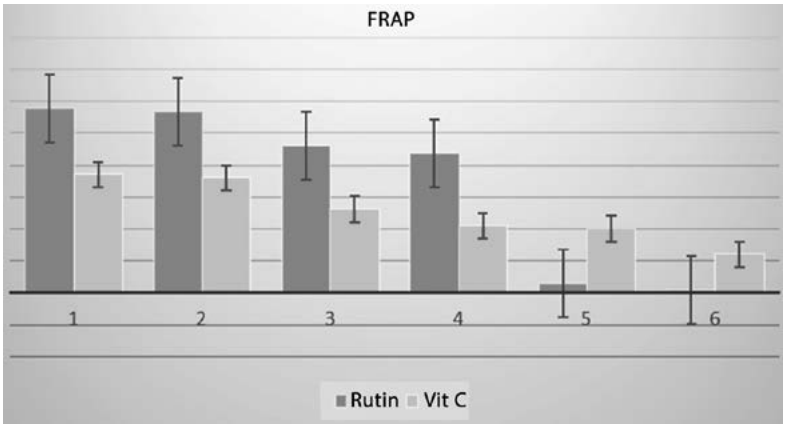

Fig. 4. Ferric Reducing/Antioxidant Power Assay FRAP

2,2-Diphenyl-1-Picrylhydrazyl Free Radical Scavenging Assay DPPH

The quantification of rutin's capacity to scavenge radicals was conducted by measuring the reactivity of DPPH with rutin at a wavelength of 517 nm, as described in previous studies. The capacity of rutin to effectively eliminate free radicals was successfully established, as indicated by The IC50 value for rutin and standard reference vitamin C was found to be 0.0414 µg/ml and 0.0458 µg/ml±0.03493 respectively with p value 0.8345. (Fig. 5) illustrates the comparative percentages of free radical scavenging exhibited by rutin and the reference standard vitamin C at equivalent concentrations of rutin.

Hydroxyl Radical Scavenging Assay

The quantification of rutin's radical-scavenging capacity was conducted by measuring the reactivity of hydroxyl radicals with rutin at a wavelength of 412 nm, as described in

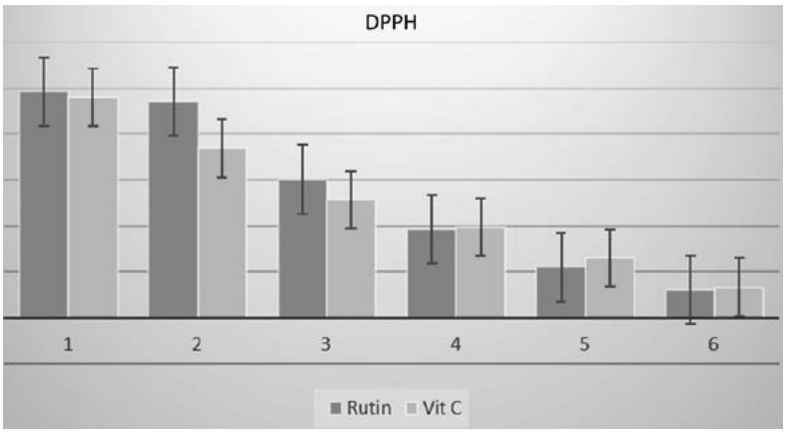

Fig. 5. 2,2-Diphenyl-1-Picrylhydrazyl Free Radical Scavenging Assay DPPH

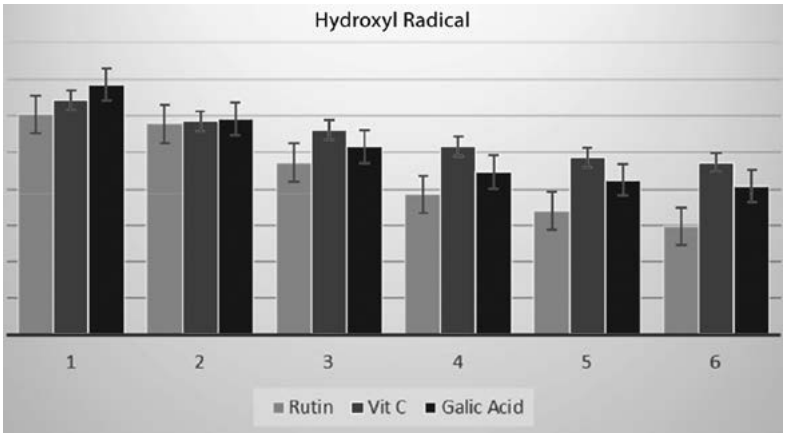

Fig. 6. Hydroxyl radical scavenging assay

previous studies. The study successfully confirmed the capacity of rutin to effectively scavenge free radicals, as evidenced by The IC50 value for rutin and standard reference vitamin C and gallic acid was found to be 0.0625 µg/ml, 0.02 µg/ml and 0.0418 µg/ml ±0.03493 respectively with p value 0.277 (Fig. 6) illustrates the comparative percentages of free radical scavenging by rutin and the reference standard vitamin C, gallic acid, when both are present at equivalent concentrations of rutin.

Hydrogen Peroxide Scavenging Activity Assay

The quantification of rutin's capacity to scavenge hydrogen peroxide was conducted by measuring the reactivity of the hydrogen peroxide radical using titration with sodium thiosulfate (NaS_2O_3), as described in previous studies. The capacity of rutin to effectively eliminate free radicals was experimentally confirmed, with The IC50 value for rutin and

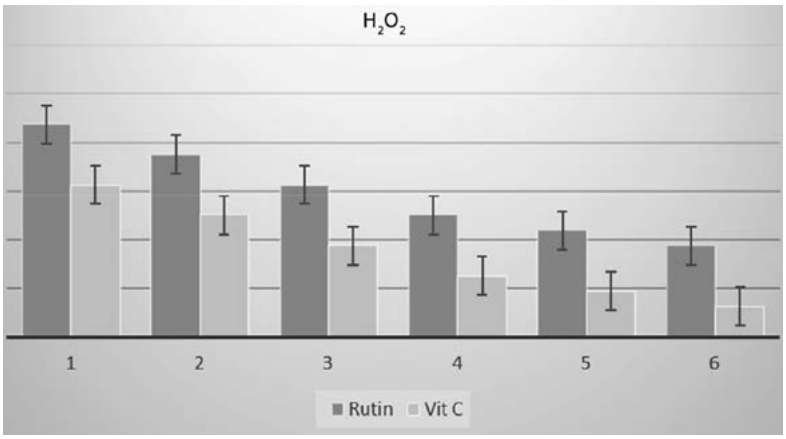

Fig. 7. Hydrogen peroxide scavenging activity assay

standard reference vitamin C was found to be 0.0271 µg/ml and 0.0769 µg/ml ±0.03493 respectively with p value 0.04835. (Fig. 7) illustrates the comparative percentages of free radical scavenging exhibited by rutin and the reference standard vitamin C, both at equivalent concentrations of rutin.

Total Antioxidant Activity

The spectrophotometric measurement of the extracts' overall antioxidant activity was conducted using the phosphor molybdenum method. This approach relies on the subsequent production of Mo (IV) into Mo (V), with a maximum absorption at 695 nm, as previously shown. A positive correlation exists between absorbance levels and the strength of antioxidant activity. (Fig. 8) illustrates the comparative percentages of total antioxidant activity between rutin and the reference standard vitamin C, both at equivalent concentrations of rutin.

The Efficiency of Inhibition the DNA Damage

The data shown in (Fig. 9) demonstrates that rutin exhibits a significant capacity to safeguard human DNA from oxidative stress, particularly at concentrations of 500

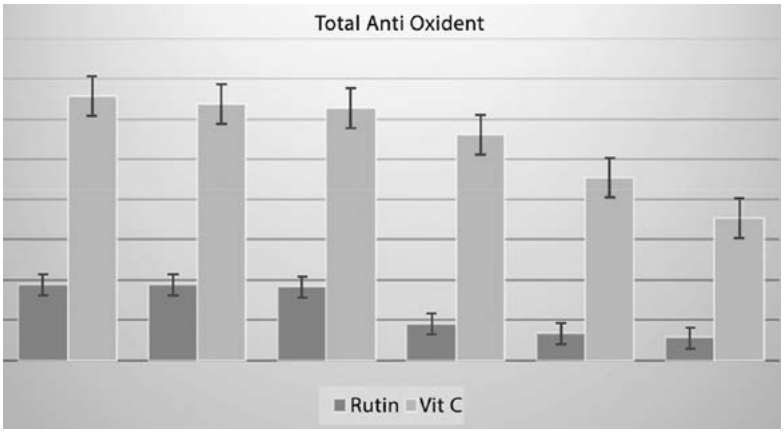

Fig. 8. Total antioxidant activity

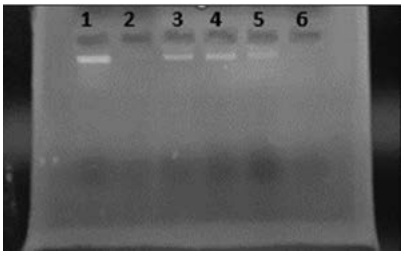

Fig. 9. Ability of rutin to protect DNA from damage caused by UV-light and (3% v/v) H_2O_2 Lane 1: Control (untreated DNA), Lane 2: DNA UV-irradiated and treated with (3% v/v) H_2O_2, Lanes 3–6: Rutin at concentrations of 500, 250, 100, and 50 µg/ml, respectively, with DNA and exposure to UV-light and 3% v/v H_2O_2

and 250 µg/ml. The observed capacity is minimal when the concentrations are between 100 and 50 µg/ml, that can be concluded from the less illuminance in band 5 and the disappearance of band 6. Rutin demonstrates efficient scavenging of hydroxyl radicals and UV radiation, thereby exhibiting the potential to mitigate oxidative DNA damage through the inhibition of reactive oxygen species (ROS) formation.

■ DISCUSSION

Antioxidants can be defined as substances that operate as reductants, participating in redox reactions where oxidants are neutralized through the reduction of one reaction species at the cost of oxidizing the other. According to [20], the introduction of electron-donating compounds resulted in the reduction of Fe^{3+} (ferric) to Fe^{2+} (ferrous). The evaluation of rutin's reducing potential was conducted at concentrations ranging up to 100 µg/mL as in FRAP assay.

The antioxidant activity of the rutin was estimated by using the DPPH method which is one of the most commonly used assays due to its simplicity and high sensitively [21], The DPPH method antioxidant results revealed that rutin has antioxidant potential with an IC50 value of 0.0414 µg/mL, compared to the positive control, vitamin C, which has an IC50 value of 0.0458 µg/ml. DPPH, or 2,2-diphenyl-1-picrylhydrazyl, is a chemically stable nitrogen-centered free radical that is frequently employed in the assessment of the radical scavenging ability exhibited by various compounds or plant extracts. When the stable DPPH radical undergoes reduction by accepting an electron from the antioxidant chemical, the characteristic violet color of the DPPH radical is observed to transition to a yellow color. Substances that possess the capability to facilitate this process can be classified as antioxidants, thereby functioning as radical scavengers [14].

The hydroxyl radical is a particularly reactive oxygen-centered radical that is generated by the reaction between different hydroperoxides and transition metal ions. According to [22], this substance has the ability to target polyunsaturated fatty acids, proteins and DNA, in membranes, and various other biological molecules upon contact. [23] have observed that it can extract hydrogen atoms from membrane lipids, leading to peroxidic reactions of these lipids. and is known to be capable of abstracting hydrogen atoms from membrane lipids. The scavenging ability of rutin against hydroxyl radicals generated in the Fenton reaction system was found to be dependent on its concentration. Hydroxyl radicals were generated through the utilization of ascorbic acid-iron EDTA in an oxidation reaction with dimethyl sulfoxide (DMSO), resulting in the production of formaldehyde. This process offers an easy technique for the detection of hydroxyl radicals through treatment with Nash reagent [15]. This experiment demonstrated that the IC50 of rutin is 0.0625 µg/mL, compared to the reference compounds vitamin C (0.02 µg/mL) and gallic acid (0.0418 µg/mL). Hydrogen peroxide (H_2O_2) holds significant importance due to its capacity to effectively permeate biological membranes. Hydrogen peroxide (H_2O_2) exhibits limited reactivity, although it possesses the potential to induce cellular toxicity due to its capacity to generate hydroxyl radicals within cellular environments [24]. The scavenging of hydrogen peroxide (H_2O_2) by extracts can be linked to the presence of phenolics, which have the ability to donate electrons to H_2O_2, resulting in its neutralization and conversion to water [25]. The findings indicate that all of the rutin samples exhibited significant H_2O_2 scavenging activity, potentially attributed to their antioxidant properties that facilitate the conversion of H_2O_2 into H_2O. Moreover, it was observed that the decomposition of

hydrogen peroxide by rutin was influenced by the concentration of rutin present, The IC50 of rutin in this assay was 0.0271 µg/mL, while that of vitamin C was 0.0769 µg/mL. Flavonoids, including rutin, have the ability to interact with DNA and provide protection against oxidative damage (Fig. 9). The binding mechanisms of antioxidants to the DNA duplex are closely associated with their antioxidant properties [26, 27]. Wild plants have been used for thousands of years in natural therapies, food preservation, pharmaceuticals, alternative medicine, and food. It is essential to scientifically investigate these plants, especially those that have been used in folk medicine, to improve the quality of healthcare offered to human beings. Flavonoids are a type of secondary metabolite [28]. This study demonstrated the importance of rutin, a type of flavonoid, as an antioxidant and for DNA protection.

■ CONCLUSION

Based on the data obtained from the current investigation, it was determined that rutin exhibited notable efficacy as an antioxidant in various in vitro assays, such as reducing power and DPPH radical scavenging. The scavenging of hydroxyl radicals and hydrogen peroxide is being compared to that of common antioxidant chemicals, namely ascorbic acid and gallic acid. The results of the current study indicate that rutin, obtained from Ruta chalepensis L. has the potential to serve as a natural antioxidant. This property makes it a promising candidate for therapeutic applications in biological systems that are exposed to reactions caused by free radicals.

■ REFERENCES

1. Lushchak V.I. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem. Biol. Interact.* 2014;224:164–175. doi: 10.1016/j.cbi.2014.10.016.
2. Banjarnahor S.D.S., Artanti N. Antioxidant properties of flavonoids. *Med. J. Indones.* 2014;23(4):239–244. doi: 10.13181/mji.v23i4.1015.
3. Alfadda A.A., Sallam R.M. Reactive oxygen species in health and disease. *J. Biomed. Biotechnol.* 2012;2012. doi: 10.1155/2012/936486.
4. Birben E., Sahiner U., Sackesen C., Erzurum S., Kalayci O. Oxidative Stress and Antioxidant Defense. *WAO J.* 2012;22(96):9–19. Available at: https://waojournal.biomedcentral.com/track/pdf/10.1097/WOX.0b013e3182439613
5. Sharma P., Jha A.B., Dubey R.S., Pessarakli M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J. Bot.* 2012;2012:1–26. doi: 10.1155/2012/217037.
6. Ghosh N., Das A., Chaffee S., Roy S., Sen C.K. *Reactive oxygen species, oxidative damage and cell death*. Elsevier Inc., 2017. doi: 10.1016/B978-0-12-805417-8.00004-4.
7. Procházková D., Boušová I., Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*. 2011;82(4):513–523. doi: 10.1016/j.fitote.2011.01.018.
8. Jaradat N. et al. Variability of chemical compositions and antimicrobial and antioxidant activities of ruta chalepensis leaf essential oils from three palestinian regions. *Biomed Res. Int.* 2017;2017. doi: 10.1155/2017/2672689.
9. Ouerghemmi I. et al. Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian Ruta chalepensis. *J. Food Drug Anal.* 2017;25(2):350–359. doi: 10.1016/j.jfda.2016.04.001.
10. Loizzo M.R., Falco T., Bonesi M., Sicari V., Tundis R., Bruno M. Ruta chalepensis L. (Rutaceae) leaf extract: chemical composition, antioxidant and hypoglicaemic activities. *Nat. Prod. Res.* 2018;32(5):521–528. doi: 10.1080/14786419.2017.1326491.
11. Al-Majmaie S., Nahar L., Sharples G.P., Wadi K., Sarker S.D. Isolation and antimicrobial activity of rutin and its derivatives from Ruta chalepensis (Rutaceae) growing in Iraq. *Rec. Nat. Prod.* 2019;13(1):64–70. doi: 10.25135/rnp.74.18.03.250.
12. Al-jaberi A.M.Z. et al. HPLC Isolation of Rutin, Hesperidin and Quercetin from Ruta Chalepensis Extract Growing in Iraq. 2023;15(4):606–611.
13. Al-mousawi N. Antioxidant Activity of Rosmarinic Acid Extracted and Purified from Mentha piperita. 2021;76(5):1279–1287. doi: 10.22092/ari.2021.356072.1770.
14. Krings U., Berger R.G. Antioxidant activity of some roasted foods. 2001;72:223–229.
15. Sasikumar V. Evaluation of Free Radical Scavenging Activity of Various Leaf Extracts from Kedrostis foetidissima (Jacq.) Cogn. *Biochem. Anal. Biochem.* 2014;03(02). doi: 10.4172/2161-1009.1000150.
16. Sharma S.K., Singh A.P. In Vitro Antioxidant and Free Radical Scavenging Activity of Nardostachys jatamansi DC. *JAMS J. Acupunct. Meridian Stud.* 2012;5(3):112–118. doi: 10.1016/j.jams.2012.03.002.
17. Prieto P., Pineda M. Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* 1999;269:337–341. doi: 10.1037/a0037168.
18. Abdul-Jabar R.A., Al-Fadal S.A.M., Hameed B.J. The antioxidant and DNA damage protection activity of Hibiscus sabdariffa L. *Plant Cell Biotechnol. Mol. Biol.* 2020;21(65–66):10–23.

19. Šatínský D., Jägerová K., Havlíková L., Solich P. A New and Fast HPLC Method for Determination of Rutin, Troxerutin, Diosmin and Hesperidin in Food Supplements Using Fused-Core Column Technology. *Food Anal. Methods*. 2013;6(5):1353–1360. doi: 10.1007/s12161-012-9551-y.
20. Hung Y.U.N.H.I.N.C., Hang C.H.I.E.N.C., Hao W.E.N.A.N.C., In C.H.W.U.L., Hou S.U.Z.E.C. Antioxidative Activity and Safety of the 50 % Ethanolic Extract from Red Bean Fermented by Bacillus subtilis IMR-NK1. 2002;97:2454–2458,.
21. Jaradat N. et al. Chemical Constituents, Antioxidant, Cyclooxygenase Inhibitor, and Cytotoxic Activities of Teucrium pruinosum Boiss. Essential Oil. *Biomed Res. Int.* 2018;2018. doi: 10.1155/2018/4034689.
22. Aruoma O.I. Free radicals, antioxidants and international nutrition. *Asia Pac. J. Clin. Nutr.* 1999;8(1):53–63. doi: 10.1046/j.1440-6047.1999.00036.x.
23. Jahan I.A., Mostafa M., Nimmi I., Hossain M.H., Ahsan M., Chowdhury J.U. Chemical and antioxidant properties of broccoli growing in Bangladesh. *Dhaka Univ. J. Pharm. Sci.* 2010;9(1):31–37. doi: 10.3329/dujps.v9i1.7427.
24. Gülçin I., Huyut Z., Elmastaş M., Aboul-Enein H.Y. Radical scavenging and antioxidant activity of tannic acid. *Arab. J. Chem.* 2010;3(1):43–53. doi: 10.1016/j.arabjc.2009.12.008.
25. Ebrahimzadeh M.A., Nabavi S.F., Nabavi S.M. Antioxidant Activities of Methanol Extract of Sambucus ebulus L. flower. *Pakistan Journal of Biological Sciences*. 2009;12(5):447–450.
26. Kumar V., Lemos M., Sharma M., Shriram V. Antioxidant and DNA damage protecting activities of Eulophia nuda Lindl. *Free Radicals Antioxidants*. 2013;3(2):55–60. doi: 10.1016/j.fra.2013.07.001.
27. Tiwari P. and Mishra. Role of Flavonoids in DNA Damage and Carcinogenesis Prevention. *J. Carcinog. Mutagen*. 2017;08(04):2–8. doi: 10.4172/2157-2518.1000297.
28. Jaradat N. et al. Phytochemical and Antibacterial Assessment of Rhagadiolus Stellatus Plant in Jerusalem Area – Palestine *Palest. Med. Pharm. J.* 2017;2(1). doi: 10.59049/2790-0231.1028.