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Formulation and Evaluation of Natural Antioxidant Cream Containing Ethanolic Extract of Quercetin from Onion (Allium cepa L.) Skin Waste

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Abstract

Brown onion dry outer scales are among the most known sources of free quercetin. Quercetin is only present in other plant tissues in the form of glycosides. Use of antioxidants is a useful measure that will prevent the effects of photoaging of the skin. Quercetin is a natural occurring antioxidant which is essential in the defense of cells against oxidation and inflammation. Due to such characteristics, it has found considerable adoption in skincare products, nutraceutical supplements, and therapeutic uses in pharmaceutical products.

The current paper was done to isolate quercetin in *Allium cepa L.* (alternative name: onion) of Amaryllidaceae family, prepare and test the antioxidant and antimicrobial activity of quercetin topical cream. A procedure of quercetin extraction was expounded. The ethanol extract was prepared and the filtrate obtained dried. Quercetin extract was extracted using the emulsification technique and the extract was formulated into a cream and tested to determine the pH, viscosity, spreadability, stability, antioxidant and antimicrobial. The antioxidant ability of quercetin cream was measured using stable 2, 2 -Diphenyl-1-picryl hydrazyl (DPPH). The disc diffusion method was used to ascertain the zone of inhibition of the formulated cream on the test organisms.

These values were acceptable: pH 5.61+ -0.07 was chosen as good in topical use, viscosity of 16550 + -1.41 mPas (milliPascal second) was considered good, and excellent spreadability. The findings revealed that quercetin cream had high antioxidant property with an IC50 value of 0.004 and 92.3% ± 0.24 radical scavenging activity at a concentration of 0.1mM. Quercetin cream was also investigated in-vitro against four bacterial types in regard to antibacterial activity. It was also

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active against *Staphylococcus aureus* at varying concentrations but was not active against Bacillus cereus *Escherchia coli*, and *Salmonella spp*.Overall, the topical cream made of quercetin extract proved to have significant antioxidant activity and antibacterial activity, which has a promising future application as a component of cosmetic products, especially the anti-aging creams.

Keyword: Quercetin; extraction; antioxidant; cream

Introduction

The extraction of useful bioactive compounds in food waste is in line with the ideals of sustainable and environmentally friendly economic growth. Besides decreasing the size of landfills, this will provide cost-efficient options because of the cheap cost of raw materials and lead to better population health outcomes. Onion being one of the most widely consumed vegetable produces a large amount of household and industrial waste [1]. Onion contains quercetin, a flavonoid antioxidant, and is widely utilized as a natural colorant, food ingredient, and raw material in the manufacture of cosmetics and pharmaceutical products [2]. The amount of quercetin discovered in onion solid wastes varied between 0.05 and 2 g/100 g, depending on the source of the waste. This is a quite high value, which reflects the interest in employing quercetin in onion solid waste [3]. Since most conventional physical and chemical UV filters are limiting and confounding, natural substances have recently received much attention especially flavonoids due to their property of absorbing UV light [4]. Quercetin is one of the most widespread flavonoids in the nature. Quercetin is said to possess anti-aging, anti-inflammatory, anti-cancer and antioxidant properties [5].

Moreover, by scavenging free radicals produced by UV radiation, quercetin has been comprehensively researched as a prospective topical skin photo protectant [6]. Quercetin is one of the flavonoids which has been studied most and was found to have a better ability to eliminate free radicals as opposed to other flavonoids. The ability to provide protection against skin damages caused by reactive oxygen species (ROS) has, therefore, attracted interest in topical use [7]. Along with the aforementioned, the results obtained at this point of time elaborate and demonstrate the potential application of quercetin to bear topical preparations to prevent UVB-induced inflammatory and oxidative skin injuries [8]. Extraction is the most important stage in the manufacturing of

quercetin from plant sources, and it determines the overall process productivity. Because water-ethanol combinations are inexpensive and environmentally safe solvents, they are frequently utilised as extractants [9]. In order to assess quercetin's antibacterial and antioxidant properties, it was taken out of onion scales and made into a topical cream.

Materials and Method

Materials

Sigma Aldrich Co. bought 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) reagent, stearic acid, cetyl alcohol, Span 80, mineral oil, triethanolamine, glycerin, distilled water, ethanol and diethyl ether. The antimicrobial behavior was identified by investigating a panel of different bacterial strains such as *Staphylococcus aureus (Gram-positive), Escherichia coli, Pseudomonas aeruginosa* as well as *Salmonella spp. (Gram-negative).*

Isolation of quercetin

Allium cepa L. dried scales were obtained, washed completely and allowed to dry under air in the dark at room temperature to maintain their organoleptic properties. The dry substance was milled to a coarse powder to improve the penetration of the solvent. One hundred grams of the powdered scales was refluxed with

$700\ mL\ of\ 80\%\ \left(\frac{v}{v}\right)\ pH\ =\ 1.0$ acidified aqueous ethanol during one hour. The extract was filtered and dried under reduced pressure with the help of a rotary evaporator. The residue was dried again in 250 mL of boiling distilled water, and refluxed again. The aqueous layer was then washed with four 100 mL of diethyl ether with a separatory funnel. The organic phase was dried, evaporated under vacuum, resettled in 10 *mL* of distilled water, filtered once more, and dried to remove all water to get the crude quercetin extract [10].

Characterization of isolated quercetin

The FT-IR spectra of isolated quercetin were recorded using a Stuart Shimadzu Corporation (Japan) spectrophotometer with a resolution of 2 cm−1. The spectra varied from 4,000 to 400 cm−1. In a mortar, the sample (1 mg) and potassium bromide (150 mg) were mixed together. Vacuum-pressurizing the mixture produced transparent KBr pellets [11].

Preparation of quercetin cream

The cream was created using emulsification. Briefly, the oil phase (stearic acid, cetyl alcohol, Span 80, and mineral oil) and the water phase (distilled water, triethanolamine, and glycerin) were melted separately in the water bath (Table 1). The water phase was then gradually added to the oil phase in the

hot mortar while stirring constantly until it cooled and formed a cream mass. The isolated quercetin was dissolved in the oil phase.

Determination of pH

The pH meter was initially calibrated with the help of standard buffer solutions. Then, the formulated cream was dispersed in 50 mL of distilled water [12] with 0.5 g of the formulated product accurately weighed. A pH meter was used to determine the PH of the resulting mixture at 25 +0.5 o C.

Spreadability test

To conduct the spreadability test, 0.5 g of the cream was put in a round glass, followed by another glass on top of the cream and left for one minute. The diameter was measured. If the preparation had a 5-7 cm spread, it complied with the standards[13].

Viscosity Determination

The viscosity was determined by pouring the mixture in a 100 mL beaker glass and selecting the appropriate spindle number. This technique was carried out three times to confirm that the values were consistent and accurate, using a Brookfield DV-E viscometer [14].

Table 1: Composition of quercetin cream

Components	Amount (%w/w)
Quercetin extract	2.5%
Stearic acid	7%
Cetyl alcohol	2%
Span 80	5%
Mineral oil	20%
Triethanol amine	2%
Glycerin	5%
Distilled water	Up to 100%

In vitro antioxidant activity study

The antioxidant capacity of quercetin cream in vitro was measured based on its capacity to eliminate the stable free radical DPPH at 517 nm in accordance with the procedure by U. Krings and R. G. Berger [15]. Quercetin and its derivatives were made in 1 mM ethanol stock solutions and allowed to incubate in the dark in order to stabilize. Measurements were taken of absorbance at 517 nm in triplicate using a quartz cuvette. After 30 minutes the percentage DPPH radical scavenging was calculated as indicated in Figure 2.2. A plot of percentage inhibition versus test concentrations was made to ascertain the value of the IC50 used to express the concentration required to inhibit half of the original DPPH radicals [16].

$$\%\,\boldsymbol{DPPH\ remaining} = \frac{\boldsymbol{A\ control\ -\ A\ sample}}{\boldsymbol{A}}\boldsymbol{control} * \mathbf{100}\%$$

In vitro antibacterial activity of quercetin

Quercetin cream has an antibacterial effect determined in vitro by the agar well diffusion technique. Quercetin (0.25, 0.5 and 1mg/mL) was dissolved in DMSO and tested against Bacillus cereus, Escherichia coli, Salmonella spp, and Staphylococcus aureus [17].

Accelerated Stability Testing

The quercetin cream was separated into three parts to test its stability. A single sample was kept at a refrigerator temperature of 8oC. The remaining two were incubated at 25°C +1 and 40 o C + 1 in an incubator at 75 relative humidity. Eight weeks storage conditions were checked every week [18].

Result & Discussion

The Infrared spectra of quercetin

Figure 1 indicates that quercetin has two different absorption bands that are not present in the IR spectra of the compounds that were synthesized. At 1666 cm -1, the C=O stretching at position 4 is conjugated in the 0-system and stabilized primarily through hydrogen bonding with the C5-hydroxyl group and to a lower extent with the C3-hydroxyl group [19]. The second is a general peak between 3200 and 3400 cm -1 due to chelated hydroxyl stretching which is related to strong hydrogen bonding. In the synthesized products, this band, as well as the carbonyl stretching, changes to lower wavenumbers, which attests to stronger intermolecular hydrogen bonds. Also, three bands at 1612, 1562, and 1523 cm -1 indicate C=C stretching in the aromatic ring [10].

High viscosity was observed in the cream with the viscosity reading 16,550 +-1.41 mPas. The pH was 5.61 + -.07, which was in line with the natural pH of the human skin and indicated the non-irritability. There was equal distribution of extracts in all the formulations which was verified through visual and tactile examination.

Antioxidant activity of quercetin

The results indicate that low levels of quercetin cream are powerful as antioxidants [14]. It can be attributed to the fact that the hydroxyl groups of quercetin are electron donating and that quercetin is a planar molecule that can easily delocalize its charges [20].

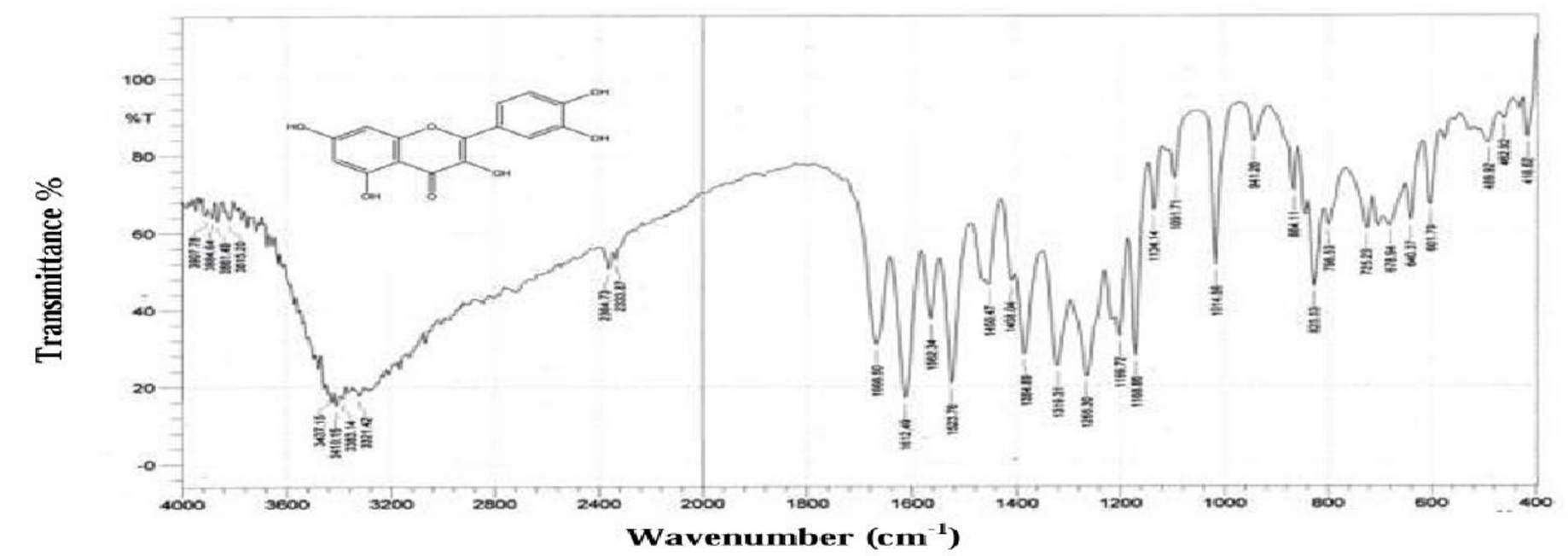

Figure 1: The IR spectrum of quercetin

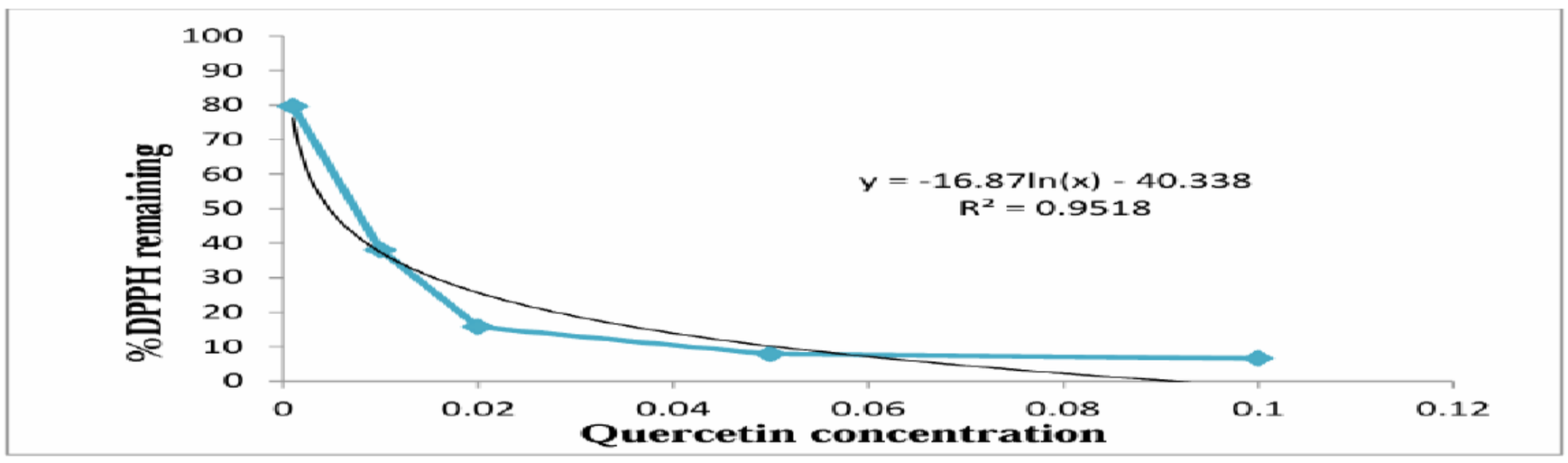

Figure 2: Effect of different concentrations of quercetin cream on the percentage of remaining DPPH radicals

In vitro Antibacterial activity of quercetin

The in vitro antibacterial activity of quercetin was evaluated using two gram-positive bacteria, Bacillus cereus and Staphylococcus aureus, and two gram-negative bacteria, Escherichia coli and Salmonella spp. The specific antibacterial activity results for each strain are summarized in Table 2.

Figure 3: The antimicrobial effects of quercetin cream against Staphylococcus aureus. The labels 1, 2, 3, C are the concentrations of quercetin 1mg/mL, 0.5mg/mL, 0.25mg/mL and the control solvent respectively.

Table 2: Quercetin cream's antibacterial activity against bacterial strains

	Conc. mg/ml	Staph. aureus	B. cereus	E. coli	Salmonella spp.
Quercetin cream	0.25	3	-	-	-
	0.5	7.5	-	-	-
	1	10	-	-	-

In the 8-week accelerated stability, all physiochemical parameters were kept at 8 C with a 0.1 C error at the fridge, 25 C with a 1 C error at the incubator and 40 C with a 1 C error at the incubator. The results of the accelerated stability tests meant that there were no discernible changes to the color of the cream.

Conclusion

The quercetin was isolated in this study using the onion scales and the quercetin was successfully made into a topical cream. Isolated quercetin was identified by the structure through FTIR spectrophotometry method. The antibacterial and antioxidant effects of quercetin cream were studied. The DPPH assay confirmed the important antioxidant ability of quercetin cream. Cream was safe to use on stratum corneum with the pH of a cream and was highly stable in viscosity and spreadability.

Conflict of Interest; Authors declare there is no conflict of interest.

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تركيب وتقييم كريم مضاد للأكسدة طبيعي يحتوي على مستخلص إيثانولي من الكيرسيتين المستخلص من مخلفات قشور البصل(Allium cepa L.)

علا جواد ناجي

فرع الصيدلانيات, كلية الصيدلة, جامعة البصرة

تُعد القشور الخارجية الجافة للبصل البني من أشهر المصادر الغنية بالكيرسيتين الحر، إذ يوجد الكيرسيتين في الأنسجة النباتية الأخرى غالبًا على شكل غليكوسيدات. ويُعد استخدام مضادات الأكسدة وسيلة فعّالة للحد من تأثيرات الشيخوخة الضوئية للجلد. الكيرسيتين هو مضاد أكسدة طبيعي يلعب دورًا مهمًا في حماية الخلايا من الأكسدة والالتهاب. وبسبب هذه الخصائص، أصبح يُستخدم على نطاق واسع في منتجات العناية بالبشرة، والمكملات الغذائية، وكذلك في التطبيقات العلاجية الصيدلانية

أُجريت هذه الدراسة لعزل الكيرسيتين من نبات البصل التابع للفصيلة النرجسية وتحضير كريم موضعي يحتوي على الكيرسيتين ، واختبار فعاليته كمضاد للأكسدة ومضاد للميكروبات. تم شرح طريقة استخلاص الكيرسيتين، حيث تم تحضير مستخلص الإيثانول وتجفيف الراشح الناتج. استُخلص الكيرسيتين باستخدام تقنية الاستحلاب، ثم صُنع منه كريم، وخضع لاختبارات لتحديد درجة الحموضة، واللزوجة، وسهولة الدهن، والثبات، وفعاليته كمضاد للأكسدة ومضاد للميكروبات. قُيست المستقر. استُخدمت (DPPH) فعالية كريم الكيرسيتين كمضاد للأكسدة باستخدام مركب 2،2-ثنائي فينيل-1-بيكرايل هيدرازيل طريقة انتشار القرص لتحديد منطقة التثبيط التي يُحدثها الكريم المُحضر على الكائنات الحية المختبرة

أظهرت النتائج أن القيم كانت مقبولة؛ حيث بلغ الأس الهيدروجيني 5.61 ± 0.07 وهو مناسب للاستخدام الموضعي، وكانت اللزوجة 16550 ± 1.41 ملي باسكال.ثانية، كما أظهر الكريم قابلية انتشار ممتازة. كما بيّنت النتائج أن كريم الكيرسيتين يمتلك حوالي 0.004، وبلغت نسبة تثبيط الجذور الحرة 92.3% ± 0.24 عند IC50 نشاطًا عاليًا كمضاد للأكسدة، حيث بلغت قيمة تركيز 0.1 ملي مولار.

كما تم اختبار النشاط المضاد للبكتيريا للكريم في المختبر ضد أربعة أنواع بكتيرية، حيث أظهر فعالية ضد بكتيريا المكورات Bacillus cereus بتراكيز مختلفة، لكنه لم يُظهر فعالية ضد (Staphylococcus aureus) العنقودية الذهبية Salmonella spp.و Escherichia coliو

بشكل عام، أظهر الكريم الموضعي المحضّر من مستخلص الكيرسيتين نشاطًا ملحوظًا كمضاد للأكسدة ومضاد للبكتيريا، مما يشير إلى إمكانية استخدامه مستقبلاً كمكون فعّال في منتجات التجميل، خصوصًا كريمات مكافحة الشيخوخة

الكلمات المفتاحية: كيرسيتين؛ مستخلص؛ مضاد للأكسدة؛ كريم