

Lipid Peroxidation and Nitric Oxide Scavenging Activity of the triclin extracted from rice bran

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Abstract

Back ground: Rice bran is an industrial waste resulting from the grain milling process. Consisting of husk, aleurone, and fractions. It contains many nutrients, including soluble and insoluble fiber, vitamins, minerals, fats, proteins, and phytochemicals such as γ -oryzanol, tocopherols, tocotrienols and triclin which has health-beneficial properties.

Objective: The aim of research to estimate antioxidant activity of the aqueous extract of rice bran of the commercial jasmine variety and the triclin compound purified from it, by the method of nitric oxide suppression activity and the effectiveness of inhibiting lipid peroxidation, and the effect of the temperatures (63, 72, 72, 85, 100, 121, 121, and 138) C° and the pH (4, 5, 6, 7, 8) in the activity.

Materials and methods: Samples of rice bran of the commercial jasmine variety were collected from Al-Tahbeesh sites in Al-Najaf Al-Ashraf for the year (2021). The aqueous extract was prepared using the maceration in boiling distilled water with a temperature of 70 C°, and the crystals of triclin were isolated by using a silica gel column (60) and solvents recovery n-hexane, ethyl acetate and methanol, respectively. This process resulted in nine parts (A-I) of the ethyl acetate solvent, concentrated in a rotary evaporator and left in the refrigerator 48 hours with the addition of chloroform to it. Needle-shaped yellow crystals were obtained, which were used to estimate antioxidant activity.

Results: The effectiveness of purified triclin as an ability to inhibit nitric oxide was higher than that of the aqueous extract of rice bran and ascorbic acid at percentages of (97.82, 72.99 and 77.20)% respectively at a concentration of 400 μ g / ml, the highest concentration used, also triclin was superior in inhibiting lipid peroxidation of 98.31% over the aqueous extract of rice bran of the commercial jasmine variety 81.28% and ascorbic acid 93.10 at the same concentration, purified triclin showed stability towards temperatures at times (30 min, 15 sec, 30 min, 1 min, 30 min, 5 min, 15 min and 2 sec) higher than that of the aqueous extract in both methods, as the effectiveness ratios of triclin by nitric oxide suppression method reached (64.25, 53.87, 42.78, 47.44, 38.48, 45.74, 39.04 and 36.13)% respectively, and by the lipid peroxide suppression method (90.73, 85.06, 63.32, 77.50, 32.27, 76.65, 35.06 and 25.30) % respectively, the two methods of effectiveness were used to estimate the stability of the aqueous extract of rice bran and the triclin towards different pH. The effectiveness of triclin was higher than the aqueous extract of rice bran at all tested numbers, and the highest effectiveness of triclin at pH (pH7) with the method of inhibiting nitric oxide and inhibiting lipid peroxidation was (46.56 and 90.92)% respectively.

Conclusion: purified triclin higher antioxidant activity than the aqueous extract using both methods, and showed higher stability towards changes in temperature and during different time periods, and higher stability towards a change in pH, especially in neutral conditions compared to acidic conditions and basal.

Key words: rice bran, triclin, antioxidant activity, flavonoids, Part of the doctoral dissertation of the first researcher.

Introduction

Rice (*Oryza sativa*) is one of the major cereal crops, as well as a staple of the diet of most of the world's population, especially Asian countries (1). It is one of the most important dietary foods (about 60% of the world's population) (2). Approximately 600 million tons are harvested annually worldwide (3). Often, rice is eaten in cooked form to obtain various kinds of nutrients, as well as to supplement the calories ingested by humans (4). Unpeeled rice contains approximately 70% of the rice yield (endosperm) as the ingredient the main product, although there are some non-consumable parts of the rice produced such as rice husk (20%), rice bran (8%) and rice germ (2%) (5).

Rice bran is one of the industrial wastes resulting from the grain milling process, consisting of the husk, aleurone and fractions (6). Bran contains more important nutrients soluble and insoluble fibers, vitamins, minerals, fats and proteins (7). Rice bran (both soft and not too fine) is a good source of bioactivity because it contains phytochemicals such as γ -oryzanol, tocopherols and tocotrienols, which have beneficial properties for health and antioxidant activity (8).

Flavonoids are Poly phenol secondary metabolites (9). Tricin is a flavonoid compound (5,7,4'-trihydroxy-3',5'-dimethoxy flavone) that has low toxicity, antioxidant efficacy, antibacterial and antibacterial properties, insecticide, anticancer, anti-allergic, and anti for HIV, anti-inflammatory and other biological activities, the interest in triclin is growing due to its activity. Most amounts of triclin is found in wheat and rice, barley husks, bamboo, palm trees, and sugar cane. It is also found in the seeds and fruits (10). The ethanolic extracts of triclin from bamboo and oat husks have anti-inflammatory, anti-obesity effect and inhibit adipocyte differentiation by inhibiting aggregation by 69% (11).

Flavonoids possess antioxidant activity, as they are polyphenol compounds that have a high tendency to undergo oxidation and reduction and work to get rid of free oxygen radicals (12). They have a number of biological agents such as antioxidant, antifungal and liver toxicity (13). Natural products play important role in drug discovery and studied these compounds have led to development many potent antioxidant agents (14). Antioxidants play an essential role in the oxidizing of oxidative stress in body cells caused by free radicals (15).

Materials and Methods

Preparation of rice bran samples

Rice bran samples of commercial jasmine variety were collected from Al-Tahbeesh sites in Al-Najaf Al-Ashraf Governorate for the year (2021).

Extraction

Maceration Method (Aqueous Extract)

Extraction for rice bran of the commercial jasmine variety was employed according to the method described by (16-17), where 2 gm of rice bran was extracted with 100 ml of distilled water at a boiling point with a temperature of 70 °C Hot Aqueous Extract (HAE), and left for 3 hours on the magnetic stirrer, then filtered through filter paper (Whatman No.1), and concentrated by a rotary evaporator at 60 °C. The concentrated extract was poured into a Petri dish and placed in the electric oven at 40 °C / 24 hours to dry, the dried powder was scraped of and collected in dry bottles to kept in the refrigerator until use.

Isolation of triclin crystals

Tricin was isolated from rice bran of commercial jasmine variety according to the method described by (18), 100 ml of distilled water was added to commercial jasmine variety (2 g) at a temperature of 70 °C with leaving it for 3 hours on a mixer. The extract was fractionated using a separating funnel by taking (35) ml of the previously prepared aqueous extract, with (35) ml of ethyl acetate solvent, then taking 2 ml of the obtained ethyl acetate fraction. It was removed from the separating funnel and passed over a silica gel 60 column (silica reagent, 40-50 μ m) with dimensions (inner diameter 3 x 56 cm, 400 g), with degassing, and washing the column with solvents n-hexane, ethyl acetate and methanol over this process yielded nine parts (A-I) of the ethyl acetate. The nine parts were collected and concentrated in a rotary evaporator to a quarter of their original volume at 40°C and left in the

refrigerator for 48 hours in order to obtain the precipitated tricin crystals. Then chloroform was added to the concentrated extract to obtain yellow crystals.

Antioxidant Activity

Nitric Oxide Scavenging Activity

The inhibition of nitric oxide was carried out based on the Greiss method (19), by taking 2 ml of sodium nitroprusside at a concentration 10 mM and 5.0 ml of phosphate solution (pH 7.4), mixed with 0.5 ml of bran aqueous extract. Commercial jasmine rice extracts (HAE) and purified tricin with different concentrations (50-400) µg/ml separately, was incubated at a temperature of 25°C for 150 minutes. As for the control treatment, the extract was replaced with water. After incubation, 2 ml of the mixture was taken. 2 ml of Greiss reagent was added and incubated at room temperature for 30 min. The absorbance was read at a wavelength of 540 nm, and ascorbic acid was used for comparison. The percentage of inhibition of nitric oxide was calculated as in the following equation:

$$\text{Nitric Oxide inhibition \%} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Lipid Peroxidation Scavenging Activity

The method (20), was followed. 0.5 ml of 10% egg yolk homogenizer was taken (10 ml of yolk homogenizer completed with distilled water to 100 ml) and added to 0.1 ml of aqueous extract of commercial (HAE) and purified tricin at a concentration of (50-400) µg/ml separately, the volume is supplemented to 1 ml with distilled water, then 0.05 ml of FeSO₄ at a concentration of 0.07 M was added, and the mixture was incubated at room temperature for 30 minutes to stimulate lipid oxidation. Then 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of TBA at a concentration of 0.8% (w/v) were added, prepared in 1.1% (w/v) dodecylsodium sulfate and 0.05 ml of 20% TCA (weight / volume) and the addition is sequentially. The resulting mixture was mixed with the mixture and heated at a temperature of 95 C° for 60 minutes, then cooled. 5 ml of n-butanol was added and centrifuged at 3000 rpm for 10 minutes, separating the top organic layer and reading it at a wavelength of 532 nm. Ascorbic acid was used for comparison. The percentage of inhibition was measured by applying the following equation:

$$\text{Inhibition of lipid peroxidation \%} = \frac{(1 - \text{Absorbance of the sampel})}{\text{Absorbance of the control}} \times 100$$

Testing the effect of temperature and pH in rice bran extract and purified tricin

With a few changes, the method described in Risalat (21), was used in the current study.

Temperature

Separately, (0.04) gm of commercial jasmine rice bran aqueous extract powder of (HAE) and purified tricin were dissolved in (100) ml, of distilled water to obtain final concentration (400) µg/ml . The tubes containing these extracts were kept at different temperatures and different time (63 C° / 30 min, 72 C° /15 sec, 72 C° /30 min, 85 C° /1 min, 100 C° /30 min, 121 C° /5 min, 121 C° /15 min, and 138 C° /2 sec) respectively. The test of antioxidant activity was carried out for each extract to evaluate the highest activity at certain temperature.

pH

Separately (0.04) g of commercial jasmine rice bran extract powder (HAE) and purified tricin were dissolved in (100) ml of phosphate buffer solution with pH (7,6,5,4 and 8). The tubes containing the extracts were kept in the refrigerator for 15 days. The antibacterial and antioxidants activities were estimated for each extract.

Results

Nitric Oxide Scavenging Activity

Table (1) shows that at a concentration of 400 µg/ml, the purified tricin molecule has the highest percentage suppression of nitric oxide 97.82%, followed by ascorbic acid 77.02%, and aqueous extract of rice bran (HAE) 72.99%.

Table (1): The inhibition rates of nitric oxide activity of rice bran extract (HAE) and purified tricin

Sample	Concentration µg/ml				
	50	100	200	300	400
HAE	43.76%	51.48%	55.19%	58.60%	72.99%
Purified tricin	50.44%	63.35%	73.59%	81.49%	97.82%
Ascorbic acid	48.96%	55.48%	63.35%	75.07%	77.02%

Lipid Peroxidation Scavenging Activity

Table (2) shows the percentage of lipid peroxidation inhibition activity. At the concentration of 400 µg/ml of purified tricin highest rate of inhibition 98.31% in comparison with ascorbic acid 93.10% and aqueous extract of rice bran (HAE), 81.28%.

Table (2): The inhibition rates of lipid peroxidation activity of rice bran extract (HAE) and purified tricin

Sample	Concentration µg/ml				
	50	100	200	300	400
HAE	16.54%	21.26%	73.25%	75.99%	81.28%
Purified tricin	25.31%	59.54%	76.74%	82.70%	98.31%
Ascorbic acid	17.76%	29.11%	74.72%	77.12%	93.10%

Testing the effect of temperature and pH on the antioxidant activity of aqueous extract of (HAE) and purified tricin

Effect of heat treatments on antioxidant activity

Nitric Oxide Scavenging Activity

Table (3) shows the results obtained by testing the antioxidant activity by inhibition of nitric oxide, after exposing the tested samples to the Purified tricin and the aqueous extract of the rice bran (HAE) to different temperatures.

Table (3): The inhibition rates of nitric oxide activity of rice bran extract (HAE) and purified tricin (at a concentration 400 µg/ml) at varying temperatures over various time intervals

Sample	Nitric oxide activity %							
	Temperature(Co)/time							
	63Co/ 30 min	72Co/ 15 sec	72Co/ 30 min	85Co/ 1 min	100Co/ 30 min	121Co/ 5 min	121Co/ 15 min	138Co/ 2 sec
HAE	47.13%	44.95%	39.82%	43.63%	35.15%	41.00%	37.18%	33.92%
Purified tricin	64.25%	53.87%	42.78%	47.44%	38.48%	45.74%	39.04%	36.13%

The highest retention of antioxidant activity was seen in the aqueous extract (HAE) and purified tricin 47.13% and 64.25% respectively, when exposed to 63C° for 30 min. On the other hand. The lowest antioxidant activities 33.92% and 36.13% respectively, were observed for HAE and purified tricin at 138C°/ 2 sec .At the same temperatures a shorter exposure time resulted in higher antioxidant activity for both HAE and purified tricin. For example, HAE had 44.95% at 15 sec and 39.82% at 30 min at 72C°, while purified tricin recorded 53.87% at 15 sec and 42.78% at 30 min. This trend was repeated at 121 C°.

Lipid Peroxidation Scavenging Activity

Table (4) shows the results of the lipid peroxidation inhibition efficacy test for the aqueous extract (HAE) and purified triclin at different temperatures .

The tested samples had the best retention of antioxidant activity for aqueous extract (HAE) and purified triclin 80.13% and 90.73% respectively, When exposed to 63C° for 30 min. The lowest antioxidant activities 19.65% and 25.30% respectively, was seen for HAE and purified triclin at 138C°/ 2 sec . Shorter time at the same temperatures showed higher antioxidant activity for both HAE and purified triclin. For example, HAE had 66.25% at 15 sec and 36.01% at 30 min at 72C° , while purified triclin recorded 85.06% at 15 sec and 63.32% at 30 min . At 121 C° , the same pattern was observed.

Table (4): The inhibition rates of lipid peroxidation activity of rice bran extract (HAE) and purified triclin (at a concentration 400 µg/ml) at varying temperatures over various time intervals

Sample	Lipid peroxidation activity %								
	Temperature(Co)/time								
	63Co/ 30 min	72Co/ 15 sec	72Co/ 30 min	85Co/ 1 min	100Co/ 30 min	121Co/ 5 min	121Co/ 15 min	138Co/ 2 sec	
HAE	80.13%	66.25%	36.01%	47.16%	30.62%	38.18%	33.14%	19.65%	
Purified triclin	90.73%	85.06%	63.32%	77.50%	32.27%	76.65%	35.06%	25.30%	

Effect of pH on antioxidant activity

Nitric Oxide Scavenging Activity

Table (5) shows the results of the nitric oxide inhibiting activity test for the purified triclin and the aqueous extract (HAE) at a concentration of 400 µg/ml for each of the tested samples. At acidic pH levels 4,5 and 6 there was a decline in the antioxidation activity of purified triclin after 15 days of incubation to 40.93%, 41.47% and 43.69% respectively. The antioxidant activity of purified triclin was 97.82% without treatment. Similarly, the aqueous extract (HAE) showed a decrease to 35.96%, 38.84% and 40.45%, respectively, after being 72.99% without treatment. At a neutral pH of 7, a slight decrease in the antioxidant activity was observed , with 46.56% and 45.28% for purified triclin and HAE respectively. At basic pH of 8, a sharp in antioxidant activity was seen after 15 days of refrigerated incubation with HAE at 25.96%.

Table (5): The inhibition rates of nitric oxide activity of rice bran extract (HAE) and purified triclin (at a concentration 400 µg/ml) at different pH

Sample	Nitric oxide activity %				
	pH				
	4	5	6	7	8
HAE	35.96%	38.84%	40.45%	45.28%	25.96%
Purified triclin	40.93%	41.47%	43.69%	46.56%	35.01%

Lipid Peroxidation Scavenging Activity

The results in Table 6 indicate that purified triclin was the most tested sample and effectively Prevented lipid peroxidation at pH ranging from 4 to 7 especially, at pH 7, the compound showed its highest efficacy with a retention of 90.92%, after being at 98.31%.However , there was a steep decrease in its antioxidant activity at pH 8, which was 17.67.

Table (6): The inhibition rates of lipid peroxidation activity of rice bran extract (HAE) and purified triclin (at a concentration 400 µg/ml) at different pH

Sample	Lipid peroxidation activity %				
	pH				
	4	5	6	7	8
HAE	21.07%	45.55%	76.27%	80.56%	11.34%
Purified triclin	62.38%	64.27%	80.24%	90.92%	17.67%

Discussion

Nitric Oxide (NO) is a free radical produced by mammalian cells. It participates in the regulation of various physiological processes and is important in inflammatory processes, but its excess production is directly toxic to tissues, causing damage to blood vessels and other diseases (19). This toxicity increases when it interact with the superoxide radical ($-O_2$) to form the second reactive compound peroxynitrite anion ($ONOO^-$). We conclude from the results that purified triclin has an antioxidant activity by inhibiting the nitric oxide radical, it can be utilized ad used in food applications. It prevents the formation of nitrite through the process of "radical generation" (N) by direct competition with oxygen. In addition (20). Triclin can be a promising compound as a preservative applied in canned meat products to reduce the nitrates added to it for the purpose of preservation and reduce its risks. However due to the fact that synthetic antioxidant may constitute a potential health hazard for consumers (21).

Triclin structure is composed of two phenyl rings and a heterocyclic ring (22). It has health benefits as an antioxidant that can inhibit fatty acid oxidation, and has a gentle effect on vitamin E in the membrane of red blood cells (23-24).

Where triclin isolated from sorghum showed a strong activity in removing free radicals along with the activity of anti-lipid peroxidation (25), and (26) found that the phenolic extract of sugarcane juice containing triclin (10%) of the total polyphenol content, has a strong effect in inhibiting fat oxidation in mice, which indicates the possibility of using it for its beneficial health effects and therapeutic applications. The simple phenolic compound is one of the secondary metabolites produced by the plant, as it consists of one aromatic ring (C6) attached to it by one or more hydroxyl groups (OH), and these groups act against the free radicals formed as a result of oxidation and thus prevent the formation of free radicals by linking with them and thus inhibiting their effectiveness (27).

The decrease in antioxidant activity could be attributed to the reduction of active compounds in the samples due to the high temperature and extended exposure time. The aqueous extract (HAE) contains phenolic compounds and flavonoids, especially that triclin is a flavonoid that belongs to phenolic compounds. The reduction in antioxidant activity is mainly attributed to the decrease in total phenolic content as a result of increasing temperature. This decrease in efficacy could be due to the loss of antioxidant compounds as heat exposure increases, as indicated in (28). The decrease in the antioxidant activity of the tested samples, purified triclin and aqueous extract of rice bran (HAE), upon a change in pH (4,5,6 and 8), is due to the dissolution of the antioxidant compounds under acidic and basic conditions (29), but in the neutral conditions, a slight decrease in the antioxidant activity was observed during the incubation period, and that the reason for this is due to the ability to reduce the antioxidant compounds in the neutral conditions as mentioned in (28), and the results of tests samples indicate the stability of the activity. The anti-oxidation of purified triclin and aqueous extract (HAE) under moderate conditions is better than that of acidic and alkaline conditions. The effectiveness of antioxidants in food depends not only on the location and number of hydroxyl groups, but also on other factors such as the site capable of interacting with other food components and environmental conditions such as pH (30-31).

Conclusion

The results of this study indicated that purified triclin from rice bran of commercial jasmine variety had higher antioxidant activity than the aqueous extract using both nitric oxide scavenging activity and lipid peroxide scavenging activity, methods. Additionally, it demonstrated greater stability with changes in temperature and across

different time periods, as well as stability towards pH change, with optimal performance in neutral conditions compared to acidic and basic conditions.

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دراسة الفعالية المضادة للأكسدة للمركب الفلافونويدي Tricin المستخلص من نخالة رز الياسمين التجاري

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الخلاصة

خلفية عن الموضوع : نخالة رز من المخلفات الصناعية الناتجة من عملية طحن الحبوب، تتكون من القشرة والأليورون و الكسور، تحتوي على العديد من العناصر الغذائية منها الألياف القابلة للذوبان وغير القابلة للذوبان ، والفيتامينات ، المعادن والدهون والبروتينات و المواد الكيميائية النباتية مثل Y-oryzanol و tocopherols و tocotrienols و tricin التي لها خصائص مفيدة للصحة. الهدف من البحث : تقدير الفعالية المضادة للأكسدة للمستخلص المائي لنخالة الرز صنف الياسمين التجاري و مركب التريسين المنقى منها بطريقة فعالية كبح أوكسيد النيتريك و فعالية كبح بيروكسيد الدهون و التعرف على تأثير درجات الحرارة (138, 121, 100, 85, 72, 63) م° عند الاوقات (30 دقيقة, 15 ثانية, 30 دقيقة, 1 دقيقة, 30 دقيقة, 5 دقيقة, 15 دقيقة و 2 ثانية)، ورقم الهيدروجين (4 ، 5 ، 6 ، 7 ، 8) في الفعالية . المواد وطرق العمل : جمعت عينات نخالة الرز لصنف الياسمين التجاري من مواقع التهبيش في محافظة النجف الاشرف لعام (2021)، حضر المستخلص المائي النقع في الماء المقطر المغلي مع حرارة 70 م° ، عزلت بلورات التريسين باستخدام عامود هلام السيلكا جل (60) ومذيبات الاسترداد- هكسان و خللات الاثيل و الميثانول أسفرت هذه العملية عن تسع اجزاء (A-I) لمذيب خللات الاثيل ، ركزت بالمبخار الدوار وتركت في التلاجة 48 ساعة مع اضافة الكلوروفورم وتم الحصول على بلورات صفراء إبرية الشكل ، استخدمت في تقدير الفعالية المضادة للأكسدة. النتائج : كانت فعالية التريسين المنقى كقابلية كبح أوكسيد النيتريك أعلى من المستخلص المائي لنخالة الرز و حامض الاسكوربيك بنسب بلغت (97.82 ، 72.99 و 77.20) % على التوالي عند تركيز 400 مايكروغرام / مل اعلى تركيز مستخدم، وتفوق التريسين ايضا بفعالية كبح بيروكسيد الدهون البالغة 98.31% على المستخلص المائي لنخالة الرز 81.28% و حامض الاسكوربيك 93.10 عند التركيز نفسه ، وظهر التريسين المنقى ثباتيه تجاه درجات الحرارة عند الاوقات المختبرة ، اعلى من المستخلص المائي في كلا الطريقتين اذ بلغت نسب الفعالية للتريسين بطريقة كبح أوكسيد النيتريك (64.25 ، 53.87 ، 42.78 ، 47.44 ، 38.48 ، 45.74 ، 39.04 و 36.13) % على التوالي ، و بطريقة كبح بيروكسيد الدهون (90.73 ، 85.06، 63.32، 77.50 ، 32.27 ، 76.65، 35.06 و 25.30) % على التوالي ، اذ اعتمدت طريقتي الفعالية في تقدير ثباتيه المستخلص المائي لنخالة الرز و مركب التريسين تجاه الارقام الهيدروجينية المختلفة فكانت فعالية التريسين اعلى من المستخلص المائي لنخالة الرز عند جميع الارقام المختبرة وبلغت اعلى فعالية للتريسين عند الرقم الهيدروجين (pH7) مع طريقة كبح اوكسيد النيتريك و كبح بيروكسيد الدهون (46.56 و 90.92) % على التوالي.

الاستنتاج والمناقشة: التريسين المنقى كان له نشاط مضاد للأكسدة اعلى من المستخلص المائي باستخدام كلا الطريقتين، كما أظهر ثباتاً اعلى تجاه التغيرات في درجة الحرارة خلال فترات زمنية مختلفة ، واستقراراً اعلى تجاه التغيير بالرقم الهيدروجيني ، وخاصة في الظروف المتعادلة مقارنة بالظروف الحامضية والقاعدية.

الكلمات المفتاحية : نخالة الرز ، تريسين ، فعالية المضاد للأكسدة ، الفلافونويدات

