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Article

Matrix Metalloproteinase-20 immunolocalization in rat first molar tooth development after treatment with amoxicillin

Samah R. H. AL – Badri[1], and NIDHAL M.S. AL-Janabi [2]*
[1] University of Baghdad, College of Agricultural Engineering, Department of Food Sciences and Biotechnology
[2] University of Baghdad, College of Agricultural Engineering, Department of Food Sciences and Biotechnology
Corresponding author: samah_r2016@yahoo.com
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ABSTRACT

we aimed to apply vaeions solvents and extraction techniques for rice bran amber and jasmine Variety (certified and commercial) to get tricin by using distilled water at boiling point with 70 C^{o} , distilled water with a 70C^{o}, ethanol 80% and methanol 80% by maceration extraction with distilled water with 70 C^{o}showed superiority over the other extraction solvents by depending on the concentration of phenols and total flavonoids as total phenols reached of the amber variety (certified and commercial) were 79.82 ±2.95, 79.17 ±2.57 mg/g respectively and flavonoids 0.71 ±0.08 and 0.79 ±0.08 mg/g respectively, was adopted as the extraction solvent in the assistant extraction methods ,soxhlet ,ultrasounication and microwave, rice bran of the commercial jasmine excelled by possessing the highest of total phenols 78.75 ±2.95, 53.19 ±2.06 and 50.91 ±2.47 mg/g respectively and flavonoids 0.87 ±0.20, 0.79 ±0.14 and 0.63 ±0.15 mg/g respectively, therefore rice bran adopted the commercial jasmine for extraction by thiolysis, base, acid, and the thiolysis was the best with the total of phenols 109.82 ±6.41 mg/g and total flavonoids 1.112 ±0.26 mg/g ,the results of separation were shown on a silica gel 60 column identification was proved by HPLC technology the presence of tricin at highest concentration in the thiolysis extract followed by the base hydrolysis reached 23.487 ±1.07and 12.257 ±0.86 µg/ml respectiveluy and was not found in the acid hydrolysis extract tricin gave an anti-inflammatory activity 92.42 ±4.08 % at a concentration 500 mg/ml.

Keywords: Rice bran, Tricin, Extraction, Solvent, Flavonoids, Anti-inflammatory

INTRODUCTION

Rice bran, a by-product of the rice manufacturing process (the process of bleaching and removing the husk), is (10-12)% of the weight of the grain. It includes the pods, cap and aleurone layer, and embryo. [1] Several studies have indicated the nutritional importance value of rice bran; it contains protein, ash, vitamins, minerals and biologically active substances. It is a good source of dietary fiber, as it contains approximately 21-27% and 1.9% soluble dietary fiber [2].

Tricin(4,5,7-trihydroxy-3,5′-dimethoxy flavone)is found in edible plants such as rice, oats, barley, and wheat [3]. It has many biological activities, including antioxidant [4] anti-inflammatories, antivirals [5] and antihistamines [6] Several studies have shown that tricin inhibits human breast cancer growth and tumor in mice [7] showed that treatment with brown rice extracts containing tricin prevented the proliferation of human breast and colon cancer cells in vitro [8] Tricin has a chemopreventive activity by inhibiting COX type 1 and 2 enzymes and prostaglandins, also inhibits the expression of TNF factor. -α in the colonic mucosa because TNF-α acts as a master switch for synthesizing the -alpha complex Between Inflammation and Cancer [9]. Tricin has chemopreventive activity against inflammatory-associated colorectal cancer [10]

MATERIALS AND METHODS

Preparation of rice bran samples

The certified rice samples for the two varieties (amber and jasmine) were collected from the rice research station in Najaf governorate. Commercial rice bran samples for both of them were collected from the pulverizing sites in the same governorate for the year (2021), after which the crushing process was carried out for the certified rice samples for the two varieties in the General Company for Grain Trade / Quality Control Department / Al Taji, and then chemical tests were conducted in the current study.

Determination of total phenolic compounds

As stated in 11, the total phenolic compounds were estimated for each of the rice bran belonging to the certified and commercial rice varieties of amber and jasmine, as 0.5 ml of the extract (1 mg/ml) was added to 2.5 ml of Folin-Ciocalteu reagent, then 2 ml of Sodium carbonate Na_2CO_3 7.5% and leave the mixture for 30 minutes at room temperature. The absorbance was measured at 760 nm, and the phenolic compounds were calculated depending on the standard curve for gallic acid.

Estimation of total flavonoids

The total flavonoids were estimated, as reported in [12], when mixing 1 ml of aqueous and alcoholic extract (1 mg/ml) in a volumetric flask of 10 ml with 5 ml of distilled water, and 0.3 ml of 5% $NaNO_2$ solution was added to it. After five minutes, 0.6 ml of 5% $AlCl_3$ solution was added. After another five minutes passed, 2 ml of 1 M NaOH solution was added and completed the volume to the mark, as we made some modifications by measuring the absorbance at 415 nm instead of 510 nm, and based on the standard curve of the rutin instead of the catechin.

Aqueous Extract (AaE)

Rice bran was extracted according to the method described by [11], where 2 gm of rice bran was extracted with 100 ml of distilled water at boiling point with a temperature of 70C°, once and again with distilled water using a temperature of 70. C°. Aqueous Extract (AE) was left for 3 hours on the magnetic stirrer, then filtered through filter paper (Whatman No.1), and then concentrated in a rotary evaporator at 60 C°, the concentrated extract was poured into a petri dish and placed in an electric oven at a temperature of 40 C° / 24 hours then the dried powder was scraped from the dishes and collected in a dry bottle and keep in the refrigerator until use.

Alcoholic Extract (AhE)

The extraction was performed according to the method described in (13). where 2 g of rice bran was extracted with 100 ml of ethyl alcohol (80%) (EAE) once and again with methyl alcohol (80%) (MAE) both alone and left for 3 hours on the magnetic stirrer at room temperature, then filtered the extract with Whatman No.1 filter paper, concentrated in a rotary evaporator at 40 C°, then poured the concentrated extract into a Petri dish and placed in the electric oven at a temperature of 40 C° for 24 hours to dry. The dried powder was scraped, collected in dry bottles, and kept refrigerated until use.

Soxhlet Method

Extraction was done with the most efficient solvent, which gave the highest extraction by maceration method (boiling distilled water with 70C^o) according to the method described by [14], taking 2 gm of certified and commercial rice bran belonging to the variety of amber and jasmine with 100 ml of distilled water at boiling point with temperature 70 Co. The mixture is exposed to a temperature of 60-80 C° for 4-6 hours, then the resulting extract is evaporated by a rotary evaporator at a temperature of 40 C°, and the concentrated extract is poured into a Petri dishes and placed in Hot – air oven at 40C^o/24h to dry, the dried powder was scraped and collect into dry bottles and store in the refrigerator until use.

Microwave oven-assisted extraction (MoAE)

The extraction was carried out according to the method described by [15], where 2 gm of rice bran was extracted with 100 ml of the solvent that gave the highest extraction yield in a microwave oven (1450 watts, 50 Hz). The time was set for 10 minutes. Then, the extract was filtered and concentrated in a rotary evaporator at 40C. The concentrate was poured into a Petri dish, dried for 24 hours, scraped, and kept in dried bottles in the refrigerator until use.

UltraSound - assistant extraction (USAE)

The method described by [16] was followed by taking 2 gm of rice bran with 100 ml of solvent, giving the highest extraction (5:1) (h/h). The mixture was subjected to ultrasound for 40 minutes, then centrifuged at 3500 rpm for 10 minutes. The obtained extracts were filtered (Whatman paper No. 41), concentrated in a rotary evaporator at 40 C°, poured into a petri dish and, until dry, scraped and kept in dried bottles in the refrigerator until use.

Extraction by acid hydrolysis

Acid and thiolysis hydrolysis was carried out for the commercial jasmine variety, which gave the highest indicator in qualitative detection, according to [17]. We just weighed. 2 gm of rice bran was added to a volumetric flask of 600 ml capacity containing hydrochloric acid (2 M) at a ratio of 1:12 (weight/volume) respectively and covered with aluminum foil, then put in a water bath at a temperature 100 C^{o} for 45 minutes to conduct the hydrolysis process with stirring every 15 minutes, the mixture is cooled to 25 C°, and filtered under vacuum with filter papers (Whatman No. 1), then using petroleum ether 40-60 C° four times, (25 ml each time) the solvent layer is removed using a separation funnel with a capacity of 500 ml, then Ethyl acetate was used to extract the flavonoids from the aqueous layer and concentrated by arotoay evaporator (40C^{o}).

Extraction by alkaline hydrolysis

The method of [17]. was applied 2 gm of rice bran was added to a volumetric flask of 600 ml capacity containing sodium hydroxide (2g) in a ratio of 1:12 (weight/volume) respectively, then covered with aluminum foil, the flask was placed in a water bath at 100 C° for 45 minutes to perform with stirring every 15 minutes, after which the mixture was cooled to 25 C°, and the mixture was filtered under vacuum using a type pump (BS2208) and a Buechner funnel, with filter papers (Whatman No. 1), using petroleum ether 40-60 C° four times, (25 ml each time), then the solvent ethyl acetate was used to extract the flavonoids the solvent layers were collected.

Thiolysis

The method of [11] was followed with some modification by us using 2-Mercapto Ethanol instead of benzyl mercaptan after dissolving 50 µl of rice bran extract in methanol (5 mg/ml), with 50 µl of mixture (Hydrochloric acid and methanol 3.3: 96.7) were added to 100 micro-

liters of a mixture of 2-Mercapto Ethanol and methanol in proportions (95:5). The mixture was placed in a water bath (40 C^{o}/ 30 min) then left to cool, so that the sample was ready for injection in HPLC system.

Chromatography Column Preparation(CC)
Mix 50 g of silica gel 60 with 100 ml of n-hexane, then pack the mixture into a glass column with dimensions (inner diameter 3 x 56 cm, 400 g), with a degassing process (2 ml/min).

Add the sample
2 ml of the ethyl acetate fraction was obtained using the method of [18]. was added by fractionating (35) ml of aqueous extract of commercial jasmine rice bran (HAE) with (35) ml of ethyl acetate solvent using a separating funnel to the column. It was recovered by adding (130) ml of n-hexane first, then adding (130) ml of ethyl acetate, then 130 ml of methyl alcohol. The fractions were collected at the previously mentioned with a flow rate of 14 ml for each fraction. This process resulted in nine parts of ethyl acetate (from A to I) and then measured by HPLC.

High-Performance Liquid Chromatography HPLC
This method was used to identify tricin in rice bran extracts of commercial jasmine variety (HAE) extract, nine parts of ethyl acetate extract of CC technology, and extracts obtained by acid, base hydrolysis and thiolysis method by adopting the standard compound tricin, and the conditions were followed that It was mentioned by (19). using a non-polar C-18 separation column, the size of the particles is 3 mm micron (50 mm x 4.6 mm) and the mobile phase formic acid: acetic nitrate (55:45 h/h) at a concentration of 1% and it was prepared according to (Suarez *et al*.,2005). with a 1.0 ml/min flow rate at a wavelength of 254 nm. The retention time of the samples was compared with the time of appearance of the standard tricin, and the compounds were estimated according to the following equation:

$$\text{Compound concentration (µg)} = \frac{\text{Form package space}}{\text{Measurement package area}} \text{ x}$$

standard concentration x number of dilution times

Anti-inflammatory activity
The method was adopted by (20; 21). also applied the method described by (22; 9). The basis of the work of the first and second method is protein denaturation; the first method was as follow:
- 0.5 ml of the test solution consisting of 0.45 ml of Bovine Serum albumin (BSA) at a concentration of 5%, 0.05 ml of the extract (HAE) of commercial jasmine rice bran and pure Tricin at a concentration of 250 µg/ml.
- 0.5 ml of a control test solution consisting of 0.45 ml of BSA at a concentration of 5% and 0.05 ml of distilled water.

- 0.5 ml of the control product consisting of 0.45 ml of distilled water and 0.05 ml of the extract (HAE) of commercial jasmine rice bran and pure Tricin at a concentration of 250 µg/ml.
- 0.5 ml of the control solution consisting of 0.45 ml BSA at a concentration of 5% and 0.05 ml of Voltaren at 250 µg/ml.

All solutions mentioned above have their pH adjusted to (6.3) using 1N of HCl. The samples are left at 37 C° for 20 minutes, and then incubated in a water bath at 57 C° for 3 minutes, then cooled, and 2.5 ml of phosphate regulator (0.2M and pH = 6.3) is added to all solutions, and the absorbance is read at a wavelength of 416 nm.

As for the second method, it was similar to the steps of the first method, except for some differences: aspirin was used in place of Voltaren in the comparison solution, incubated in a water bath at a temperature of 51 C° for 20 minutes, the absorbance was read at 660 nm using a spectrometer, and the amount of inhibition was known by applying the following equation: -

Amount of inhibition =[100 - (the absorbance of the test solution - the absorbance of the control product) ÷ (the absorbance of the control solution) x]100.

Statistical Analysis

The Statistical Analysis System- (23) program was used to detect the effect of different factors on study parameters. The least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significantly compare between means in this study.

RESULTS

Quantitative determination of some active compounds in rice bran for tested samples.

Phenolic Compounds

The percentage of these compounds in the rice bran of the certified amber variety was maceration with four types of solvents (boiling distilled water with a temperature of 70 C° (HAE), distilled water with a temperature of 70 C° (AE), ethanol alcohol 80% (EAE), methanol alcohol 80% (MAE)) (79.82 ±2.95, 31.50 ±1.76, 63.25 ±2.92 and 66.15 ±2.86) mg/g respectively. In contrast, the number of phenolic compounds in the rice bran extracts of the jasmine variety certified for the same solvents was (35.85 ±1.85, 20.41 ±0.97, 20.71 ±1.05 and 31.30 ±1.36) mg/g, respectively.

While the amount of phenolic compounds in rice bran extracts belonging to the commercial amber variety of the same solvents above was (79.17 ±2.57, 55.50 ±2.37, 58.93 ±2.47and 60.82 ±2.77) mg/g, respectively, and in rice bran extracts of the commercial jasmine variety for the solvents mentioned above was (81.65 ±3.07, 26.33 ±1.25, 59.70 ±2.61, 63.07 ±2.82) mg/g respectively and extracted by maceration method,

It is noticed from the results of the statistical analysis (Table.1) for the estimation of total phenols that significant differences appeared between the four solvents used in the maceration method, the boiling distilled water solvent with 70 C° distinguished by a signified difference for the jasmine variety was 81.65 ±3.07 mg/ g at the probability level (P≤0.05), and the certified amber variety was the highest with the total amount of phenols 66.15 ±2.86 and 63.25 ±2.92mg / g for the ethanolic and methanol extract, respectively. At the same time, the percentage reached 20.71 ±1.05 and 31.30 ±1.36 mg / g, for certified jasmine variety of ethanolic and methanolic extract represented the lowest concentration among the other types.

	Rice bran variety	**Concentration of phenolic compounds (mg/g)**			
		Aqueous extract		**Alcoholic extract**	
		Distilled water at boiling point + temperature 70C°	Distilled water + temperature 70C°	Ethanol alcohol 80%	**Methanol alcohol 80%**
1	Certified Amber	79.82 ±2.95	31.50 ±1.76	63.25 ±2.92	**66.15 ±2.86**
2	Commercial amber	79.17 ±2.57	55.50 ±2.37	58.93 ±2.47	**60.82 ±2.77**
3	Certified jasmine	35.85 ±1.85	20.41 ±0.97	20.71 ±1.05	**31.30 ±1.36**
4	Commercial jasmine	81.65 ±3.07	26.33 ±1.25	59.70 ±2.61	**63.07 ±2.82**
	LSD value	7.829 *	6.445 *	6.052 *	**7.341 ***
*** (P≤0.05).**					

Table 1. Concentration of Phenolic Compounds in Aqueous and Alcoholic Extracts of Rice bran for Amber and Jasmine variety (certified and commercial) by Maceration method. * Signify difference. NS Non signifies difference.

Depending on the amount of yield, the most appropriate solvent (distilled water at a boiling point with a temperature of 70 C°) was selected to complete the other extraction methods.

It was found that the highest amount of these compounds, phenols and flavonoids in the tested samples of rice bran belonging to the amber and jasmine variety (certified and commercial) was by assist extraction method with soxhlet (SM), followed by ultrasound method (USAE), and the lowest was by microwave method (Mo), and for the rice bran sample Of the commercial jasmine variety the highest portion of these compounds using assist extraction methods was (78.75 ±2.95, 53.19 ±2.06and 50.91 ±2.47) mg / g respectively.

	Rice bran variety	**Concentration of phenolic compounds (mg/g) for assistant extraction methods**		
		Microwave	Soxhlet	**Ultrasound**

1	Certified Amber	44.26 ±2.19	56.27 ±2.55	**46.74 ±2.40**
2	Commercial amber	49.34 ±2.76	74.02 ±3.42	**50.71 ±2.63**
3	Certified jasmine	21.53 ±1.07	30.82 ±1.73	**27.98 ±1.87**
4	Commercial jasmine	50.91 ±2.47	78.75 ±2.95	**53.19 ±2.06**
	LSD value	5.941 *	7.226 *	**5.802 ***
*** (P≤0.05).**				

Table 2. The concentration of phenolic compounds for Rice bran for the variety of amber and jasmine (certified and commercial) with the solvent (distilled water at a boiling point with 70 Cº) by using assistant extraction methods.* Signify difference. NS Non signifies difference.

The steps of selecting the solvent and then the extraction method are the screening stages of the rice variety whose bran was carried out in the current study, so results in (Table 2) lead us to the selection of the commercial jasmine variety, which was superior in its content of total phenols of 50.91 ±2.47, 78.75 ±2.95 and 53.19 ±2.06mg/g for microwave assistant extraction methods. Soxhlet and ultrasound, Where the results of the statistical analysis (Table 2) show that there are significant differences in the assistance of another solvent at the level of probability (P≤0.05). Therefore, the bran of the commercial jasmine variety was filtered to complete the screening by acid-base (Table 3) shows the high content of total phenols by thiolysis method, amounting to 109.82 ±6.41mg/g, the acid method is the lowest in the content of 10.88 ±0.74 mg / g and its median by the primary decomposition is 83.85 ±4.38mg /g.

	Rice bran variety	**The concentration of phenolic compounds (mg/g) in rice bran extract of commercial jasmine variety by different extraction methods.**		
		acid hydrolysis method	alkaline hydrolysis	**Thiolysis**
	Commercial jasmine	10.88 ±0.74	83.85 ±4.38	**109.82 ±6.41**
	LSD value	**11.461 ***		
*** (P≤0.05).**				

Table 3. The concentration of phenolic compounds in rice bran extract of commercial jasmine variety by acid, base and thiolysis hydrolysis * Signify difference. NS Non signifies difference.

Total Flavonoids

The flavonoids were estimated in rice bran extracts of the tested amber and jasmine varieties. (Table .4) there is a significant difference for the above varieties in their content of flavonoids at the probability level (P≤0.05), so it shows the superiority of the aqueous extract (HAE) of the rice bran sample of commercial jasmine over the methanolic extract

for the same sample contained a higher percentage of flavonoids, which reached (0.97 ±0.13 and 0.65 ±0.09) mg/g, respectively, compared to the number of flavonoids for the same sample treated with 80% ethyl alcohol.

	Rice bran variety	Concentration of flavonoid compounds (mg/g)			
		Aqueous extract		**Alcoholic extract**	
		Distilled water at boiling point + temperature 70C°	Distilled water + temperature 70C°	Ethanol alcohol 80%	**Methanol alcohol 80%**
1	Certified Amber	0.71 ±0.08	0.45 ±0.07	0.50 ±0.09	**0.55 ±0.08**
2	Commercial amber	0.79 ±0.08	0.56 ±0.09	0.65 ±0.12	**0.77 ±0.12**
3	Certified jasmine	0.84 ±0.10	0.31 ±0.05	0.49 ±0.08	**0.70 ±0.12**
4	Commercial jasmine	0.97 ±0.13	0.49 ±0.07	0.60 ±0.11	**0.65 ±0.09**
	LSD value	0.206 *	0.163 *	0.142 *	**0.176 ***
*** (P≤0.05).**					

Table 4. Concentration of flavonoids in aqueous and alcoholic extracts of rice bran for the amber and jasmine variety (certified and commercial) by Maceration method * Signify difference. NS Non signifies difference.

It is also noted in (Table .4) that the concentration of flavonoids in all aqueous and alcoholic extracts of rice bran samples of the amber and commercial jasmine varieties differed.

The results indicate that extraction with distilled water at a boiling point with a temperature of 70C° is more suitable for extracting flavonoids than extracting with distilled water at a temperature of 70C°. The commercial amber sample was distinguished by the highest content of flavonoids 0.77 ±0.12mg/ g, followed by certified jasmine, which reached 0.70 ±0.12mg / g for (MAG) extract. In contrast, commercial jasmine is 0.65 ±0.09 mg / g, and the lowest sample certified amber was 0.55 ±0.08mg / g.

Table (5) shows the estimation of flavonoids for the same four tested samples. However, with the assistant extraction methods soxhlet (SM), ultrasound (USAE) and microwave (Mo) by applying the solvent extraction distilled water at a boiling point with 70 C° (HAE), it was found that the highest amount of these compounds in the tested samples of rice bran belonging to the amber and jasmine variety (certified and commercial) was by the soxhlet extraction method (SM), followed by the ultrasound method (USAE).), and the lowest was by microwave method (Mo) and the rice bran sample of the commercial jasmine variety had the highest share of flavonoids (0.87 ±0.20, 0.79 ±0.14 and 0.63 ±0.15) mg/g, respectively with assist extraction methods.

	Rice bran variety	Concentration of flavonoids (mg/g) by assist extraction methods		
		Microwave	Soxhlet	**Ultrasound**
1	Certified Amber	0.51 ±0.07	0.60 ±0.11	**0.53 ±0.07**
2	Commercial amber	0.61 ±0.09	0.76 ±0.15	**0.73 ±0.13**
3	Certified jasmine	0.58 ±0.12	0.66 ±0.11	**0.60 ±0.10**
4	Commercial jasmine	0.63 ±0.15	0.87 ±0.20	**0.79 ±0.14**
	LSD value	0.144 NS	0.162 *	**0.178 ***
*** (P≤0.05).**				

Table 5. Concentration of flavonoids for rice bran of Amber and Jasmine variety (certified and commercial) (distilled water at boiling point with temperature 70C°) assistant extraction methods * Signify difference. NS Non signifies difference.

The rice bran sample of the commercial jasmine variety tested had the highest content of flavonoids according to the data in (Table .5) and the hydrolysis methods of acid, alkaline and thiolysis.extracted it (Table .5) shows that there were significant differences between the method at the probability level (P≤0.05), there were Significant differences between the soxhlet assisted extraction method and ultrasound about the microwave method at the as it was observed (Table 6) probitity (spear) the high concentration of flavonoid compounds by the thiolysis method, and the lowest when extracted by the acid hydrolysis method, while the base analysis was between them, reached (1.112 ±0.26, 0.48 ±0.09 and 0.98 ±0.14) mg / g, respectively.

	Rice bran variety	Concentration of flavonoids (mg/g)		
		acid hydrolysis	alkaline hydrolysis	**thiolysis**
	Commercial jasmine	0.48 ±0.09	0.98 ±0.14	**1.112 ±0.26**
	LSD value	**0.391 ***		
*** (P≤0.05).**				

Table 6. Concentration of flavonoids in rice bran extract of commercial jasmine variety using three hydrolysis methods. * Signify difference.NS Non signifies difference.

Techniques for separation and identification of Tricin Column chromatography (CC) technique

Twenty-seven (27) parts were collected from this stage, and nine (9) parts were collected from the recovery solvent (n-hexane) to wash the column and get rid of unwanted compounds, after which the compound was recovered with ethyl acetate. This is one of the best solvents used in tricin recovery. At this stage, 9 parts were collected (from A to I), after which it was recovered with methyl alcohol and 9 parts were also collected.

High-Performance Liquid Chromatograph (HPLC)

(Table .7) shows the results of separation and identification using high-performance liquid chromatography (HPLC) for samples from tube No. (1) to tube No. (9) containing the ethyl acetate recovery solvent and tube No. (10) containing the extract (HAE) of the dependent rice bran For the commercial jasmine variety, which showed the highest indicators during the quantity chemical estimation, in addition to tube No. (11) containing ethyl acetate extract for the same tested sample obtained by acid hydrolysis and tube No. (12) containing ethyl acetate extract for rice bran for the sample tested above the method of basic hydrolysis, and tube No. (13) containing the extract of the sample tested above by the method of thiolysis.

It was confirmed that the presence of tricin in tubes No (1) to No. (9) containing the recovery solvent ethyl acetate obtained by (CC) method, as well as the presence of the compound in tubes (12 and 13) for the same tested sample by methods of basic and thiolysis, respectively. The ratio of the compound (2.325 ±0.08, 3.447 ±0.17, 4.503 ±0.41, 4.199 ±0.53, 6.20 ±0.56, 7.69 ±0.72, 5.943 ±0.47, 4.811 ±0.27, 3.094 ±0.09, 9.631 ±0.76, 12.257 ±0.86, 23.487 ±1.07) µg/ml respectively, and it is noted that the retention time (Rt) is similar for each of the compounds of these extracts in the above tubes and the tricin standard 2.55 minute.

The presence of tricin was not found in tube (10) prepared by acid hydrolysis.

	Samples	tube number	time detention (RT)/min	compound in extract µg/ml
1	The standard compound Tricin	-	2.550 ±0.06	-
2	Tubes containing extracted (CC) by column chromatography method	1	2.540 ±0.06	2.325 ±0.08
		2	2.560 ±0.08	3.447 ±0.17
		3	2.538 ±0.05	4.503 ±0.41
		4	2.550 ±0.06	4.199 ±0.53
		5	2.538 ±0.09	6.20 ±0.56
		6	2.497 ±0.06	7.69 ±0.72
		7	2.537 ±0.04	5.943 ±0.47
		8	2.525 ±0.06	4.811 ±0.27
		9	2.548 ±0.09	3.094 ±0.09
3	Aqueous extract of rice bran Commercial (Jasmine) (HAE)	10	2.528 ±0.08	9.631 ±0.76
4	Rice bran ethyl acetate extract (Jasmine commercial variety)Prepared by acid hydrolysis	11	-	-
5	rice bran extract (Jasmine commercial variety) Prepared by basic hydrolysis method	12	2.547 ±0.11	12.257 ±0.86
6	rice bran extract(Jasmine commercial variety) Prepared by thiolysis method	13	2.562 ±0.09	23.487 ±1.07
	LSD value	---	0.286 NS	3.071 *
*** (P≤0.05).**				

Table 7. Retention time (RT) of tricin in rice bran extracts of commercial jasmine variety by HPLC technology Signify difference. NS Non signifies difference.

It was also confirmed that tube No. 13 containing rice bran extract of the tested sample obtained by thiolysis method by HPLC is the highest concentration of tricin, then tubes(No. 1 to No. 9) containing the recovery solvent ethyl acetate belonging to the same tested sample obtained by column chromatography method (CC), then tube No. 12 containing the extract of the same sample above obtained by the basal hydrolysis method. In contrast, the tube No. 11 obtained by the acid hydrolysis method did not contain the amount (23.487 ±1.07, 21.106, 12.257 ±0.86, 0) µg/ml, respectively.

Anti-inflammatory activity

(Table .8) shows the percentages of anti-inflammatory activity of the extract (HAE) of commercial jasmine rice bran and pure tricin that were equal when using aspirin and Voltaren, and it was found that the selected extracts are effective in inhibiting Bovine Serum Albomine (BSA) denaturation caused by heat. Pure tricin was observed to have higher activity in inhibiting protein denaturation (BSA) than (HAE) extract. In contrast, the results of the statistical analysis of this activity showed a significant difference between pure tricin and the aqueous extract at the probability level (P≤0.05), Which increased by increasing the concentration of the extract, as the highest inhibition activity of pure tricin reached 92.42 ±4.08 % at a concentration of 500 µg/ml and for (HAE) extract 71.11 ±3.84% at the same concentration. At the same time, it corresponded to 81.51 ±3.52% and 44.01

±2.47% for pure tyrosine and (HAE) extract, respectively, at a Concentration of 250 µg/ml.

Concentration µg L-1	inhibition% (Voltaren 99% and aspirin 96%)	
	(HAE)	Pure Tricin
250	44.01 ±2.47	81.51 ±3.52
500	71.11 ±3.84	92.42 ±4.08
LSD value	6.427 *	5.662 *
*** (P≤0.05).**		

Table 8. Anti-inflammatory percentage of the extract (HAE) of commercial jasmine rice bran and pure Tricin using Voltaren and aspirin as positive control. (voltaren 99% and aspirin 96%)* Signify difference.NS Non signifies difference.

DISCUSSION

Quantitative determination of some active compounds in rice bran for tested samples. Phenolic Compounds. The use of absolute organic solvents is characterized by a decrease in the solubility of some plant compounds, which is the result of strengthening the hydrogen bonds between the compounds capable of forming hydrogen bonds and the proteins in those solutions [24], the step of selecting the extraction solvent for plant models is critical because it will determine the quantity and type of extracted compounds [25] as the extraction efficiency depends on the type of solvent [(26).] Many factors affect the extraction of phenolic compounds, quantitatively and qualitatively: the extraction method, extraction solvents and the size of the particles of the extracted compounds. The time and temperature of extraction, the degree of polarity of the extracted phenolic compounds, as well as the degree of oxidation of the compounds to be extracted [27] The high temperature also stimulates the oxidation of polyphenols [28] It was shown [29] The initial heat treatment at a temperature of 60-50 C° activates the enzyme polyphenol oxidase(PPO), which reduces the concentration of extracted phenols. When using low-pH solvents that stimulate the decomposition of the active compounds during the extraction process [30.]

Total Flavonoids

The decrease in the solubility of polyphenolic compounds and flavonoids using absolute solvents is caused by the strengthening of hydrogen bonds between polyphenols, flavonoids and proteins in these solutions. Therefore, adding water to organic solvents leads to weakening these bonds and extracting a higher amount of phenolic and flavonoid compounds [31]. The extraction solvent was "Important" in determining the quantity and quality of extracted phenolic compounds, [25]. The type of solvent (polarity) used in the extraction process controls the extracted phenolic compounds, [32]. This difference may be attributed to the distinct treatment method [33]

This is what [14] found that the Soxhlet method gives a higher extraction yield due to the presence of the heating process and the volume of the used solvent. They increase the productivity of extracting flavonoids from the dry matter. In addition, the different types and amounts of solvents also affect the yield produced.

Techniques for identification and separation of Tricin by Column chromatography (CC) technique

Phenolic compounds can dissolve in ethyl acetate extract, [34]. This is because it works to decipher the interactions related to the adsorption of aromatic compounds on the used column material [11]

High-Performance Liquid Chromatograph (HPLC)

The acid hydrolysis method works to decompose the tricin compound [16]. In contrast, the thioacidolysis method is considered the most suitable method for releasing tricin from the lignin polymer due to its high efficiency in breaking the tricin- (40 - O - b bond) and its ability to keep the released tricin intact [29] It is practical by cleaving β-O-4 bonds in the lignin polymer found in the plant cell wall.

This study proved that tricin is higher than its percentage in barley, which ranges between (0.32-1.96) mg/kg.

These results are in agreement with what was found by [5] by obtaining seven parts of the recovery solvent (ethyl acetate) (A - G) when passed on a column containing silica gel, with a high concentration of the tricin in the sixth part (F).

[35] Proved that thiolysis is the most effective method, releasing more than 91% of tricin with little dissolution of the compound, because it breaks aryl-ether bonds, which account for about 50% of all bonds between subunits in softwood and 65-80% in hardwood lignin.

Anti-inflammatory activity

Because the tricin is a flavone (5,7,4'-trihydroxy-3',5'-dimethoxy) with anti-inflammatory activity. As an anti-inflammatory for human cancer cell growth, tricin, present in rice, oats, barley, and wheat, suppresses inflammation-associated colon carcinogenesis in mice by blocking the expression of TNF-α [36]

The results of this study agree with the findings [37] that some flavonols and flavones containing two or three double bonds are considered inhibitors of cyclooxygenase-2, which work to stimulate the production of prostaglandins that induce inflammation and pain. Flavonol, flavone, and flavanone or isoflavone are selective COX-2

Inhibitors [38] They also have significant anti-inflammatory activity [12] Therefore, it can be used tricin is an effective anti-inflammatory agent.

CONCLUSION

In this study, we found that the percentage of phenolic and flavonoid compounds in the rice bran of the commercial jasmine variety was higher than that of the amber variety (certified and commercial) and the certified jasmine variety using the extraction method by maceration in distilled water at a boiling point with a temperature of 70 C°, the flavonoid tricin was separated by silica gel column chromatography (60) using n-hexane, ethyl acetate and methanol recovery solvents. With the highest concentration in the thiolysis extract, followed by the basal extract, then in the sixth part of the recovery solvent ethyl acetate, and its absence in the acidolysis extract, pure tricin had higher anti-inflammatory activity than the aqueous extract of the commercial jasmine bran variety compared to aspirin and Voltaren. Use it as a functional supplement for disease prevention and as a food preservative.

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