OPTIMAL CONDITIONS FOR EXTRACTION PHENOLIC COMPOUNDS AND FLAVONOIDS FROM MILLET BRAN AND STUDYING SOME OF THEIR BIOLOGICAL ACTIVITY

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ABSTRACT

The aim of this research is to determine the optimal conditions for extracting phenols and flavonoids from the bran of Iraqi proso millet and Ukrainian foxtail millet, whole and defatted, and studying some of their biological characteristics. Petroleum ether, methyl alcohol (60% and 80%), distilled water, and ethyl alcohol at concentrations of 60, 80, 90, and 99% were used as extraction solutions. The flavonoid content was estimated using the Rutin standard curve, and the phenolic content was determined using the Folin-Ciocateau method based on the gallic acid standard curve. The results showed that the concentration of phenolic compounds and flavonoids in proso millet bran extracts were (770.56, 865.38, 1059.94, 883.16, 722.17, 726.12, 587.79, 400.69) µg/g and (188.26, 196.26, 353.60, 671.38, 96.94, 93.60, 117.60, 300.60) µg /g, respectively, and in Ukrainian millet bran extracts (889.08, 1034.80, 1182.00, 1331.17, 1008.19, 990.42, 592.85, 581.43) µg /g and (302.94, 484.26, 758.94, 940.32, 188.26, 97.60, 217.60, 554.94) µg/g respectively. The highest phenolic and flavonoids compounds concentrations were achieved when ethyl alcohol (99%) as extraction solution was used for Ukrainian millet. Therefore, this treatment was adopted in conducting the free radical scavenging test, at different concentrations (0.125, 0.250, 0.500, 0.750, 0.800) mg/ml, as RSA were (24.68, 40.18, 67.56, 81.98, 89.55%), respectively, compared to ascorbic acid at the same concentrations, which were (40.14, 57.34, 73.46, 89.93, 99.55%).

Keywords: extraction, antioxidant, millet bran, total flavonoid, antibacterial, good health
***part of M.S.c. of the 1st author**

الظروف المثلى لاستخلاص المركبات الفينولية والفلافونويدات من نخالة الدخن ودراسة بعض أنشطتها البيولوجية

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المستخلص

هدف البحث الحالي الى تحديد الظروف المثلى لاستخلاص الفينولات و الفلافونويدات من نخالة دخن البروسو العراقي ودخن ذيل الثعلب الاوكراني المنشأ الكامل والمزال منه الدهن و دراسة خصائصها الحيوية. تمثلت محاليل الاستخلاص المستخدمة بالكحول الأثيلي بتراكيز 60 و80 و90 و99% والكحول المثيلي 60% و 80% والماء المقطر والبتروليوم ايثر. قدر المحتوى الفلافونويدي بالاعتماد على المنحنى القياسي لمركب الروتن Rutin والمحتوى الفينولي بطريقة Folin–Ciocateau اعتمادا على المنحنى القياسي لحامض الكاليك. اظهرت النتائج أن تركيز المركبات الفينولية والفلافونويدية في مستخلصات نخالة دخن البروسو كانت بواقع (770.56 ، 865.38، 1059.94، 883.16، 722.17 ، 726.12 ، 587.79 ، 400.69) مكغم/غم و (188.26 ، 196.26 ، 353.60 ، 671.38 ، 96.94 ، 93.60 ، 117.60 ، 300.60) مكغم/غم على التوالي وفي مستخلصات نخالة الدخن الاوكراني (889.08، 1034.80 ، 1182.00 ، 1331.17 ، 1008.19 ، 990.42 ، 592.85 ، 581.43) مكغم/غم و (302.94 ، 484.26 ، 758.94 ، 940.32 ، 188.26 ، 97.60 ، 217.60 ، 554.94) مكغم/غم على التوالي. كان اعلى تركيز في المركبات الفينولية و الفلافونويدية في معاملة الاستخلاص بالكحول الاثيلي بتركيز 99% في الدخن الاوكراني وعليه أعتمدت هذه المعاملة في اجراء فحص كبح الجذور الحرة إذ استعملت التراكيز (0.125 ، 0.250 ، 0.500 ، 0.750 ، 0.800)ملغم /مل وقد كانت الفعالية الكبحية فيها بواقع (24.68 ، 40.18 ، 67.56 ، 81.98 ، 89.55) % على التوالي مقارنة مع حامض الاسكوربك عند نفس التراكيز (40.14 ، 57.34 ، 73.46، 89.93 ، 99.55) %.

الكلمات المفتاحية: الاستخلاص، مضاد الأكسدة، نخالة الدخن، المحتوى الفلافونويدي الكلي، مضاد للبكتريا، صحة جيدة
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INTRODUCTION

Grain crops are the mainstay of the human diet, as they topped the list of global agricultural production due to their suitability to climate and soil conditions (42), as well as the ease of handling them in terms of their service, care, and ease of storage and transportation. They are also an essential source of energy and protein (22,13), but its production quantities do not cover global needs (31). Alternative grains to wheat must be explored in order to confront food insecurity (21). Millet is considered the best candidate for this (31), as its full potential has not been exploited. It is the most resistant to drought and pests (7) and its chemical composition is superior in some of its components to other major grains. In addition, its proteins are free of gluten, which makes it a good food source for patients suffering from digestive disorders (25,16). As it is known bran is a by-product of the grain milling process and is an important product rich in nutrients such as proteins, Omega-3, and phenolic compounds. Millet grains contain an abundance of phytochemicals, especially phenolic compounds (32) represented by phenolic acids and tannins. Flavonoids are also found in good quantities, as well as phytic acid salts (phytates), which all act as antioxidants. They have been shown to act as reducing agents, free radical scavengers, metal chelating agents, and can have beneficial health effects to prevent and delay the occurrence of non-communicable diseases. Phenolic content varies between millet varieties and parts of the grain itself. Phenolic compounds are defined chemically as a substance consisting of one or more aromatic rings with hydroxyl substituents, including their functional derivatives. The plant produces them as secondary metabolites (3) that it needs for the purposes of growth and defending itself against diseases. The antioxidant capacity of natural phenols is important for foods, especially functional foods, medicinal foods, and nutritional supplements, as multiple phenols are associated with a wide range of physiological properties, including anti-inflammatory, antimicrobial, antioxidant, and powerful radical scavengers (37). The ability of phenolic compounds antioxidant activity depends on the position of the aromatic ring and the number and location of hydroxyl groups on this ring (18). Flavonoids are a type of natural phenolic organic compounds found in plants .In terms of chemical structure, flavonoids have phenolic and pyrene rings in their structure. It has many subclasses (2, 4), such as flavonols, flavones, flavanones, chalcones, and anthocyanidins (47) Flavonoids have a wide range of pharmacological activities that include antioxidant (41), antidiabetic, and antimicrobial. They are anti-inflammatory, anti-mutagenic, and anti-cancer, and their effects on human health are often attributed to their potential ability to act by reducing the steady-state concentration of free radicals in biological elements of systems and thus providing antioxidant protection (24, 33, 34).The purpose of this study was to determine the optimal extraction conditions for phenolic and flavonoid compounds from the bran of local proso and foxtail millet, besides determine their biological activity against some bacteria species and as an antioxidant agent.

MATERIALS AND METHODS

Materials: Ukrainian millet, foxtail type, and Iraqi millet, Proso type, were obtained from Iraqi local markets - Baghdad. Methods described by (8) were followed for millet bran isolation. Cleaning was done well to remove all foreign materials broken and immature grains. The grains were washed after soaking for 15 minutes, and dried in the shade (hot air 40-45°C) for 24 hours.

Isolating and crushing the bran of experimental millet: Bran isolation under taken according to (1) where millet grains were ground using a German Brabender quad grinder (Department of Food Sciences/College of Agricultural Engineering Sciences), after conditioning the grains for 24 hours its moisture raised to 12% (26). The isolated bran was crushed using a Cia-Tronic home coffee grinder, then the powder was passed through a sieve (150 microns), stored in tightly sealed plastic bags in the freezer (-18 °C) until subsequent tests were conducted.

Chemical composition analyses of millet bran flour: The chemical composition (moisture, ash, protein, fat, and fiber) were analyzed using the established procedures

outlined in A.A.C.C. with the symbols (19-44), (30-25), (46-11), and (01-8). The total carbohydrate content was determined by subtracting the sum of these approximated elements from 100.

Defatting of millet bran: Fat was extracted from millet bran using hexane in a cold process according to (24)., and the traditional method (Soxhlet) according to A.O.A.C. (14).

Extraction of phenolic compounds:

With minor adjustments, the procedure outlined by (20) was used to extract phenolic components from both whole and defatted millet bran. The extraction solutions included distilled water, petroleum ether (100%), methyl alcohol (60 and 80%), ethyl alcohol (60, 80, 90, and 99%). The mixing ratio was 8:1 (w/v). Fifty gram of millet bran in 500 ml containers was combined with 400 ml of each solvent separately. The mixture was thoroughly mixed and incubated in a water bath at 60°C for 30 minutes, with stirrings every two minutes. For ten minutes, the tubes were centrifuged at 3500 x g. The solvent layer containing the phenolic extract was separated and transferred to clean, weighed and pre-marked tubes, then stored in refrigeration until tests were carried out.

Phenolic compounds determination

The method described by (8) was followed in determining the total phenolic content of millet bran using the Folin-Ciocalteu assay at 1 N concentration, using the standard curve for gallic acid at a concentration of 200 (µg/ml) (stock solution).

Qualitative detection of the active compounds in the crude extract of millet bran powder: The active compounds (alkaloids, flavonoids, terpenes, tannins and saponins) were detected according to Ali *et al*., method (6). Each active compound was detected by two different specific qualitative reagents (Table1).

Table 1. The types of detections and the reagents that used in the current study

No.	Type of detection	Type of reagents
1	Alkaloids	A- Mayer reagent
2		B- Wagner reagent
3	Flavonoids	A- Magnesium crystals and 1% HCl
4		B- H2SO4 reagent
5	Terpenes	A- Chloroform and H2SO4
6		B- Anace aldehyde reagent
7	Tannins	A- FeCl3 reagent
8		B- Lead acetate reagent
9	Saponins	A- Foam reagent
10		B- HgCl2 reagent

Purification of flavonoids: The crude extract was concentrated by rotary evaporator to 60 ml and transferred to a separating funnel; 30 ml of distilled water and 300 ml of ethyl acetate was added, and Shaked several times. Finally, the organic layer was separated from the aqueous layer and dried using a rotary evaporator at 40 °C. The dry matter, which represents the flavonoids, was collected.

Determination of flavonoids: The method described by (29) was followed. Based on the standard curve of Rutin at a concentration of 2 µg/g.

Determination of antioxidant activity: DPPH Radical-Scavenging Activity (RSA)

The RSA was measured according to (29,28) with some modulations. First, one ml sample (1 mg/ml) was mixed with 1 ml DPPH solution (0.1 M) was prepared by dissolving 0.0039 g in an appropriate amount of ethyl alcohol 95 % and then transferred to a dark volumetric flask µl of sterile extract through (0.45 µm) Millipore filters. The plates were incubated at 37°C for 24 hours. The diameter of the clear zone surrounding the disc and free of growth was measured, the diameter of which is directly proportional to the inhibitory activity of the extract (4).

Statistical analysis: The statistical program Statistical Analysis System - SAS (2018) (38) was used to analyze the data to study the effect of different parameters on the studied traits according to a completely randomized design (CRD), and the significant differences between the means were compared with the least

significant difference test (Least Significant Difference-LSD).

RESULTSE AND DISCUSSION

Chemical composition of millet bran flour

Table (2) shows the percentages of the chemical components represented by moisture, protein, oil, ash, fiber, and carbohydrates for millet bran powder under study (Proso millet and Ukrainian millet). The protein content was (7.9, 8.1%) for proso and foxtail millet, respectively, these values are lower than (30) finding (10.80) and (35) finding (9.91). The percentages of oil extracted from the experimented samples (2.8, 3.0%) were lower than that shown by (34) for same varieties in this study, which were 3.46 and 3.79%, respectively. As for the ash percentages, they were (4.8, 4.5%), these values were higher than the values reported in above mentioned studies, as they were (2.97, 3.23) %, respectively. As listed in Table (2) The moisture percentages were (7.8, 7.9%), which were lower than (35) finding for proso and foxtail millet (12.97, 12.13%). This is due to keeping the grains under hot climate in Iraq. The percentages of total fiber in the experimental millet samples were higher (7.1, 7.6%) than what was found by (35), being (5.2, 6.7%), respectively, for the same varieties. Similarly, the percentages of carbohydrates in the varieties under study were higher (69.6, 68.9%) than in (35), being (63.75, 62.41) %, respectively. All chemical components, specifically protein, are mainly affected by genetic factors (variety and type) , the prevailing climatic and agricultural conditions (environmental conditions) during the growth stage (12).

Table 2. chemical composition analysis of Proso and foxtail millet).

Chemical Composition	Dry matter	Moisture	Ash	Fiber	Protein	Fat	CHO.
foxtail millet powder bran	88.38	7.90	4.50	7.60	8.10	3.00	68.90
Proso millet powder bran	92.76	7.80	4.80	7.10	7.90	2.80	69.60

Qualitative and quantitative assay of the active compounds in alcoholic and aqueous extracts of proso and foxtail millet bran

The qualitative detection of some active compounds in the experimental millet bran extracts showed that the foxtail millet bran contained alkaloids, terpenes, saponins, tannins and flavonoids, (table 3). The results are consistent with Al -Mhyawi (8) who mentioned to the presence of terpenes, saponins, tannins and flavonoids in the extracts of alcoholic and aqueous extracts of proso and foxtail millet bran. Table (4) shows the concentration of phenolics compounds in the alcoholic and aqueous extracts of proso and foxtail millet bran, based on gallic acid equivalents. These compounds were extracted using (ethyl alcohol at a concentration of 60, 80, 90, 99% and methyl alcohol at a concentration of 60, 80%, petroleum ether 100%, and distilled water). The results indicate that the concentration of phenolics compounds in the alcoholic extracts were higher as the aqueous extract and the petroleum ether extract, as compare to their concentrations reached (889.08, 1034.80, 1182.00, 1331.17, 1008.19, 990.42, 587.85, 581.43 (μg/g), respectively, in foxtail millet bran, respectively, while for proso millet bran were (770.56, 865.38, 883.16, 1059.94, 722.17, 726.12, 592.79, 400.69) μg/g, respectively. It has been noticed that there were significant differences between foxtail bran extract content of active compound and proso bran extract.

Table 3. Qualitative detection of active compounds for alcoholic and aqueous extracts of proso and foxtail millet bran

Active compounds	Methanol extract 80%		Ethanol extract 99%		Water extract		Petroleum ether extract	
	Reagent A*	Reagent B*	Reagent A*	Reagent B*	Reagent A*	Reagent B*	Reagent A*	Reagent B*
Alkaloid	(+) White precipitate	(+) Brown precipitate	(+) White precipitate	(+)Brown precipitate	(+) White precipitate	(+)Brown precipitate	(+) White precipitate	(+)Brown precipitate
Saponins	(+) Thick foam	(+) White precipitate	(+) Thick foam	(+) White precipitate	(+)thick foam	(+) White precipitate	(+)thick foam	(+) White precipitate
Terpenes	(+) Brown - reddish	(+)Brown precipitate	(+)Brown - reddish	(+)Brown precipitate	(+)Brown – reddish	(+)Brown precipitate	(+)Brown – reddish	(+)Brown precipitate
Tannins	(+) Green - bluish	(+)Yellow precipitate	(+) Green - bluish	(+)Yellow precipitate	(+) Green – bluish	(+)Yellow precipitate	(+) Green – bluish	(+)Yellow precipitate
Flavonoid	(+)Red-orange	(+) Red	(+)Red-orange	(+) Red	(+)Red-orange	(+) Red	(+)Red-orange	(+) Red

Table 4. the concentration of the active phenolic compounds in the alcoholic and aqueous extracts of proso millet bran and foxtail

NO	Extraction treatment	Concentration of the active compounds (μg/g) foxtail millet bran	Concentration of the active compounds (μg/g) proso millet bran	L. S .D.
1	P1	889.08 [b]	770.56 [b]	136.03 *
2	P2	1034.80 [d]	865.38 [bc]	107.53 *
3	P3	1182.00 [c]	883.16 [b]	122.94 *
4	P4	1331.17 [a]	1059.94 [a]	117.62 *
5	P5	1008.19 [c]	722.17 [c]	134.87 *
6	P6	990.42 [cd]	726.12 [c]	122.64 *
7	P7	592.85 [e]	587.79 [d]	71.05 NS
8	P8	581.43 [f]	400.69 [e]	98.78 *
L. S. D.		144.02 * .(P≤0.05) *	127.64 *	---

P1, P2, P3, P4: Ethyl alcohol at concentrations of 60, 80, 90 and 99%. P5, P6: Methyl alcohol at concentrations of 60 and 80%. P7: Petroleum ether 100%. P8: Distilled water

Al-Mahyawi (8) reported that the concentration of phenolic compounds in the aqueous extract of bran of proso millet and foxtail was (166.01 and 185.07) μg/g and in the 70% ethyl alcohol extract were (208.97 and 329.88) μg/g, respectively, which is lower than what was found in this study for the same two extracts. Both Al-Mhyawi (8) and Al-Morshedy (10) confirmed that phenolic compounds are concentrated in the outer layers of the grain, and their concentrations vary with the extraction solution with green tea leaves and pomegranate peels resulted in higher percentages of phenolic compounds.

Quantification of phenolic components in extracts from defatted millet bran

Table (5) shows the phytochemical content represented by phenolic compounds extracted with ethyl alcohol (99%) and calculated on the of gallic acid equivalents at 60 °C for different treatments (whole foxtail millet bran (TP), bran defatted by cold method (CP), and defatted by Soxhlet (SP). The results indicated that the (TP) treatment recorded the highest phenolic compounds concentration, followed by the (CP) treatment, and the lowest value recorded by (SP) treatment being (1331.17, 758.24, 267.848) μg/g, respectively. Al-Mhyawi (8) underlined the significance of millet lipids and their high phenol content by pointing out that the removal of fat from millet resulted in lower percentages of phenolic compounds. Ethyl alcohol (70%) extract of whole-grain proso millet flour and whole-grain millet bran flour, phenolic compound concentrations were (208.97, 401.77) μg/g, respectively. This confirms the high concentration of phenolic compounds in the millet bran, which contains the highest percentage of fat, in addition to the effect of the extraction temperature in enhancing the phenolic compounds release into the extraction solution. This is consistent with the results of this study, as the concentration of phenolic compounds in (TP) treatment was higher than (CP) and (SP) treatments.

Table 5. Concentrations of phenolic compounds in bran of whole and defatted foxtail millet bran powder

No	Treatment	Concentrations of phenolic compounds (μg/g)
1	TP	1331.17
2	CP	758.24
3	SP	267.848
	L.S.D.	32.967 *
		.(P≤0.05) *

TP=alcoholic extraction of whole millet, CP=alcoholic extraction of cold-defatted millet, SP=alcoholic extraction of Soxhlet-defatted millet

Identification of flavonoids in millet bran aqueous and alcoholic extracts: Table (6) shows that foxtail millet bran samples achieved the highest concentrations in all extracts compared to proso millet bran extracts, and that the differences among them were significant. Similarly, the 99% ethyl alcohol extract recorded the highest concentration compared to the rest extracts. Issoufou, *et al*., (27) mentioned that the flavonoids concentration in walnut bark extracts using different extraction solutions chloroform, ethyl acetate, 80% ethanol, and methyl alcohol were (3.50, 32.81, 13.59, 11.48) µg/g, respectively. It was obvious that ethyl alcohol (80%) gave the highest concentration compared to other extracts, and this is consistent with the results obtained in this study. Meanwhile, Cai and Tang (19) stated that ethyl alcohol (80%) extracts from aloe vera skin gave the highest percentage of flavonoids (5.1 mg/g). However, the flavonoids concentration was decreased as the ethyl alcohol concentration increased, in contrast to what was observed in this study.

Table 6. Concentration of flavonoid in the alcoholic and aqueous extracts of proso and foxtail millet bran

Extraction treatment	Concentration of flavonoid foxtail millet bran (µg/g)	Concentration of flavonoid (µg/g) proso millet bran	L. S. D.
F1	302.94 de	188.26 c	98.37 *
F2	484.26 c	196.26 b	107.64 *
F3	758.94 b	353.60 c	159.02 *
F4	a940.32	671.38	144.17 *
F5	188.26 de	96.94 d	87.51 *
F6	97.60 e	93.60 cd	8.74 *
F7	554.94 b	300.60 d	.71 *
F8	217.60 de	117.60 d	82.66 *
L. S. D.	175.59 *	116.87 *	---
(P≤0.05) *			

F1, F2, F3, F4: Ethyl alcohol at concentrations of 60, 80, 90 and 100%. F5, F6: Methyl alcohol at concentrations of 60 and 80%. F7: Petroleum ether 100%. F8: Distilled water

Quantification of flavonoid components in defatted foxtail millet bran extracts

Table (7) shows the flavonoids concentration in ethyl alcohol (99%) extract and on the basis of rutin equivalents at 60 °C for the experimental bran extracts samples (whole foxtail millet bran (TF), defatted by the cold method (CF), and defatted by Soxhlet (SF)). The results indicated that the (TF) recorded the highest concentration of flavonoid compounds, followed by the (Cf), and the lowest value recorded by (Sf), which were (940.32, 646.936, 155.2) µg/g, respectively. Al-Mhyawi (8) assured the importance of proso millet fats in the bran and its high content of phenolic compounds and flavonoids, and the defatting process led to reduction in phenolic compounds and flavonoids content, as the concentrations of flavonoid for whole millet and whole bran powder were (281.59, 502.83) µg/g, respectively. This was attributed as mentioned previously to the role of its fat content from the phenolic compounds and the temperature role in enhancing release of these compounds to the extraction solution. These results agreed with results of this study, as the concentrations of flavonoid compounds TF was higher than that in (CF) and (SF).

Table 7. Concentrations of flavonoids of whole and defatted foxtail millet bran

No	Treatment	Concentrations of flavonoid compounds (µg/g)
1	TF	940.32
2	CF	646.936
3	SF	155.2
	L.S.D.	* 71.267
	.(P≤0.05) *	

TP: Whole millet bran extract. SP: Defatted millet bran extract (Soxhlet method). CP: Defatted millet bran extract (cold method).

Radical-Scavenging Activity (RSA) of different concentration of flavonoids extracted from whole foxtail millet using DPPH: Table (8) shows radical scavenging activity (using DPPH) for different concentration ethanolic extract from foxtail millet bran compared to the ascorbic acid at same concentrations. It is clear from the results that the antioxidant activity was proportional to the concentration of the alcoholic extract, as is the case with ascorbic acid, it reached (24.68, 40.18, 67.56, 81.98, 89.55%), respectively, compared to ascorbic acid, which was (40.14, 57.34, 73.46, 89.93, 99.55%). Yang *et al* .,(46) indicated that there is a direct relationship between the high content of phenolic compounds and antioxidant activity, where ethyl alcohol was used as a solvent at different concentrations (30, 50, and 70%). The concentrations of phenolic compounds reached (22.36, 29.39, 21.49) mg/ml, respectively. The oxidation power of these concentrations was (33.49, 35.79, 25.79) %, respectively, and this is consistent with what was reported in this study, as the high concentration of phenolic compounds led to a high antioxidant power.

Table 8. Radical scavenging activity (DPPH) of flavonoids extracted from foxtail millet bran by ethanol (99%).

Flavonoids concentration (mg/ml)	Radical scavenging activity (%)	Ascorbic acid concentration (mg/ml)	Radical scavenging activity (%)
0.125	24.68	0.125	40.14 [e]
0.250	40.18	0.250	57.34 [d]
0.500	67.56	0.500	73.46 [c]
0.750	81.98	0.750	89.93 [b]
0.800	89.55	0.800	99.55 [a]
L.S.D.	7.334*	L.S.D.	8.523 *

(P≤0.05) *

Radical scavenging activity of flavonoids extracted from whole and defatted foxtail millet bran using DPPH: Table (9) shows the radical scavenging activity of flavonoids extracted from whole and defatted foxtail millet bran by ethanol (99%) (using DPPH) compared to the same concentration of ascorbic acid (0.800 mg/ml). It is noted from the table above that there were significant differences in the antioxidant activity in treatment D1, compared to treatments D2 and D3 (defatted), and this proves the importance of the presence of fat in plant products. These results agreed with what was indicated by (11,17), who noticed a decrease in all phenolic compounds percentage and their antioxidant capacity compared to the non-defatted fractions. The radical suppression values of phenols indicated by (17) in the alcoholic extract (methanol 80%) of dehulled and whole foxtail millet were (67.3% and 90.8%), respectively. The same researcher also found a significant decrease in the values of free radical suppression using DPPH for free and bound phenols after peeling the foxtail variety compared to whole millet grains. These values also differed depending on the group of grains chosen and on the amount of removal of the outer layers (bran) rich in phenolic acids and fibers, (insoluble and soluble) as well as the amount of oil associated with these parts. While (11) found that when extracting phenolic compounds from wheat bran using distilled water, chloroform, ethanol, methanol, propanol, and ethyl acetate, their concentrations were (3700, 3110, 2750, 2860, 2850, 1050) mg/kg, respectively, and the antioxidant activity of these concentrations was (23, 22, 16, 22, 21, 17) %, respectively. A strong relationship was found between the concentration of extracted phenolic compounds and antioxidant activity, which indicates that these compounds, which are concentrated in the bran and oil, are responsible for antioxidant activity, they are either polar or non-polar in nature depending on the extraction conditions of these compounds.

Table 9. Radical scavenging activity (using DPPH) of flavonoids extracted from whole and defatted foxtail millet bran by ethanol (99%)

Treatment	Radical scavenging activity of treatments (%)	Radical scavenging activity of Ascorbic acid (%)	L.S.D.
D1	89.55^{a}	99.55	6.071 *
D2	71.22^{b}	99.55	8.552 *
D3	72.87^{b}	99.55	8.167 *
L.S.D.	7.695 *	---	---

.(P≤0.05) *

D1: Whole millet bran extract. D2: Defatted millet bran extract (Soxhlet method). D3: Defatted millet bran extract (cold method).

Inhibitory activity of millet bran extracts against test bacteria: Table (10) shows the inhibitory activity of millet bran extract (99% ethyl alcohol) against some microorganisms that cause food spoilage and foodborne illness. Two concentrations (50 and 100 mg/ml) of millet bran ethyl extract were used to study the effect of flavonoids against the growth of two types of Gram-negative bacteria. (*Escherichia coli, Salmonella typhimurium*) and gram positive (*Staphylococcus aureus, Bacillus cereus*). The microorganisms used partly cause food spoilage, and partly cause diseases that can be transmitted through food. The results indicated that the concentration (100 mg/ml) of millet bran extract had a higher inhibitory activity than the concentration (50 mg/ml) against the growth of the studied bacteria (*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus*). According to the results shown in Table 10, the diameter of the clear zone at a concentration of 100 mg/ml was (15.5, 18.5, 19, 19.5 mm), respectively. While the inhibition of growth zone at concentration of (50 mg/ml) was (14, 16, 16.5, 17 mm), respectively. It was noted that *Bacillus cereus* was more sensitive to the extract than the rest strains at both concentrations. These results were identical to the results of the researcher (36)when he used the aqueous extract of different types of millet (finger millet, foxtail millet, pearl millet, small millet, and kudu millet), where foxtail millet showed the largest zone of inhibition against *Staph. aureus, Salmonella tayphimurium*, and *Bacillus*, with diameters of 30, 29, and 35 mm, respectively. The zone of inhibition expanded with increasing concentration of the extract, which is identical to what was reported in this study. From the results it is clear that the inhibitory activity of alcoholic extracts is more effective against Gram-positive bacteria than Gram-negative bacteria. This can be attributed to the cell wall structure and outer membrane organization of Gram-negative and Gram-positive bacteria. Some studies indicated that the plant extract affects Gram-positive bacteria more than Gram-negative bacteria. Due to the differences in the outer layers of the walls of Gram-positive and Gram-negative bacteria, as negative bacteria contain special outer membranes that are not found in positive **bacteria. Antibacterial substances easily** damage the cell wall and cytoplasmic membrane of cells, causing the cytoplasm to spread outward and coagulate, as a result of which the cells die (39). Studies have shown that the alcoholic extract of millet bran is highly effective as an antibacterial. (40) found that methanol and ethyl acetate alcohol extracts were highly effective against Gram-positive and Gram-negative bacteria. The results showed high efficiency in the inhibition zone for Gram-negative and Gram-positive bacteria (*E. coli, Staph. aureus, B. subtills*). The inhibitory activity of the extract against bacteria generally depends on the amount of flavonoids, phenolic compounds and high antioxidant properties present in the alcoholic extract of the plant. Methanol extract of finger millet seed coat showed higher antimicrobial activity against *Bacillus cereus* and *Aspergillus flavus* compared to whole meal flour extract. From these observations, it can be concluded that polyphenols are responsible for the microbial activity of millet. The results indicate that there is a possibility of using finger millet seed layer as a natural antioxidant and (44) food preservative.

Table 10. Inhibitory activity of millet bran extract (ethanol extract) against the growth of *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus.*

Bacterial isolates	The rate of growth inhibition diameter (mm) Extract concentration (100 mg/ml)	Extract concentration (50 mg/ml)	L.S. D
Escherichia coli	15.5	14	* 1.48
Salmonella typhimurium	18.5	16	* 1.55
Staphylococcus aureus	19	16.5	* 1.59
Bacillus cereus	19.5	17	* 1.82
L.S.D.	* 2.285	* 2.056	---

.(P≤0.05)*

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