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Ultrasonic-assisted extraction of bioactive compounds from eggplant (*Solanum melongena* L.) calyx and evaluation of extract properties

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Abstract

Background: Eggplant (*Solanum melongena* L.) is widely consumed worldwide and generates considerable waste, including peel and calyx, during processing and consumption. Although often considered as by-products, these wastes are rich sources of bioactive compounds, which vary depending on the plant variety and cultivation location. Given that no studies have been reported on the bioactive compounds of the Iranian eggplant calyx, this study examines the extraction of bioactive compounds from the calyx of Iranian purple eggplant using a combined solvent and ultrasonic-assisted extraction method, including the determination of total phenolic and flavonoid contents and antioxidant capacity.

Results: The total phenolic content of hydroalcoholic and aqueous extracts was 8.72 ± 0.12 and 7.44 ± 0.041 mg gallic acid equivalents/g dry extract, respectively. Total flavonoid contents were 52.60 ± 1.15 and 49.44 ± 1.90 mg rutin equivalents/g dry extract. Both extracts showed strong antioxidant activity with 2,2-diphenyl-1-picrylhydrazyl radical scavenging percentages of 82.77% and 82.51%, and IC50 values of 1.47 and 4.16 mg/mL, respectively. Liquid chromatography–mass spectrometry profiling of the hydroalcoholic extract identified major bioactive compounds, including *p*-coumaric acid, caffeic acid, catechin, myricetin, rutin, luteolin, and kaempferol.

Conclusion: These results underscore the efficacy of ultrasonic-assisted solvent extraction in isolating phenolic and flavonoid compounds from eggplant calyx. Considering the abundance and accessibility of this plant material, coupled with its significant bioactive compound content, the eggplant calyx emerges as a promising, cost-effective source for the development of functional ingredients in the food, pharmaceutical, and cosmetic sectors, particularly in developing countries.

KEYWORDS
bioactive compounds, eggplant calyx, natural antioxidant, solvent extraction, ultrasonic-assisted extraction

INTRODUCTION

A significant portion of the food produced globally is wasted or discarded annually, amounting to approximately one-third of global food production, equivalent to about one billion tons of food, according to statistics provided by the Food and Agriculture Organization of the United Nations (FAO).[1,2] This wastage not only leads to the loss of food but also results in the squandering of underlying resources such

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as land, water, energy, and operational capital. Given the critical importance of this issue, reducing food waste by 50% has been identified as one of the main challenges for the FAO to address by 2050.[3] This goal underscores the urgent need for global action to combat food waste and mitigate its far-reaching consequences on resource management and sustainability. Among food products, fruits, vegetables, and summer crops contribute substantially to food waste. The by-products of these items—such as husks, seeds, roots, stems, peels, and calyxes—are typically discarded. However, many of these by-products contain beneficial bioactive components, including antioxidants, dietary fibers, and phytochemicals. When properly extracted and processed, these compounds can be repurposed for use in the food industry, pharmaceuticals, and cosmetics.[4] Consequently, extensive studies have focused on these waste materials and their extraction methods. For instance, a study in 2005 demonstrated that watermelon rind, an agricultural waste, is a natural source of citrulline, a nonessential amino acid.[5] Similarly, research conducted in 2017 revealed that the peel and seeds of cantaloupe melon are rich in phenols and possess strong antioxidant properties.[6] A recent study in 2025 showed that potato peel extract contains important bioactive compounds, including phenolics and glycoalkaloids.[7] Considerable research has been conducted on various members of the Solanaceae family, such as potato and tomato; however, one member of this family—eggplant (*Solanum melongena* L.)—has been relatively overlooked in scientific investigations.[8] Known globally as aubergine, brinjal, berenjena, or baigan,[9] eggplant has emerged as a notable functional food, distinguished by its remarkable antioxidant capacity due to its high phenolic content.[10] This widely consumed vegetable is valued not only for its high nutritional content but also for its versatility in culinary applications, including use in various dishes and pickles, as a meat substitute in vegetarian diets, and for its favorable cultivation, storage, and transport characteristics. Eggplant exists in various cultivars with diverse colors and shapes, such as white, reddish-purple, and dark purple varieties, as well as slender, egg-shaped, and globular forms. The plant consists of different parts, including flowers, leaves, fruit, peel, and calyx. While the fruit is edible, the peel and calyx are typically regarded as by-products and discarded. Research on eggplant has primarily focused on several key areas: the phytochemical properties of the fruit flesh,[10–12] comparisons among different cultivars regarding these properties,[13,14] investigations of the peel's constituents such as anthocyanins and phenolic compounds,[15–17] and the optimization of extraction processes to determine the most effective methods.[18,19] Extraction techniques vary widely, including boiling, maceration, solvent extraction, Soxhlet, and supercritical fluid extraction. A critical consideration in these methods is the preservation of phenolic compounds and the maintenance of antioxidant capacity. Recently, advanced extraction technologies such as ultrasonic-assisted, microwave-assisted, pulsed electric field, high-voltage electrical discharge, and pressurized liquid extraction have been developed to enhance efficiency while minimizing degradation of bioactive compounds.[19] These innovative methods offer advantages such as reduced processing time, lower solvent consumption, and improved retention of thermolabile compounds compared to conventional techniques. Numerous studies have demonstrated that ultrasonic-assisted extraction (UAE) is an effective technique for obtaining plant extracts with high efficiency, especially when combined with solvent extraction. This synergistic approach enhances extraction yield and preserves biological activity better than either method alone.[20,21] Considering the importance of extraction methods, the present study employs a combination of solvent extraction and UAE to investigate the phenolic content, flavonoid content, and antioxidant capacity of extracts obtained from eggplant calyx.

MATERIALS AND METHODS

Materials

Fresh purple eggplants were purchased from the Tehran fruit market. Subsequently, their calyxes were separated. After being washed and dried at room temperature, the calyxes were completely and uniformly powdered using the mill (Niko, MJ-367N).

Methods

Extraction method

In this research, a combination of two methods, including solvent extraction and ultrasonic waves, was used to prepare an extract of eggplant calyxes. For this purpose, 20 g of the powder prepared from eggplant calyxes was mixed with solvent in a 1:10 ratio. Two solvents, water and ethanol–water (50%), were used for the extraction test. The mixtures were placed on the magnetic stirrer (Sci-Finetech FTHPM) for 30 min to ensure the calyxes' powder was well exposed to the solvents. Subsequently, the samples were placed in an ultrasonic water bath (Parsonic 2600S) with an ultrasonic power of 70 W and an operating voltage of 220 VAC, at a temperature of 40°C for 30 min. After applying ultrasonic waves, the samples were stirred for another 30 minutes on a stirrer, and the obtained liquid was filtered by passing through Whatman filter paper and placed in a rotary evaporator (Hahnvapor HS-2005S-N) to separate the solvent from the extract and concentrate the extract. Finally, the obtained extracts were placed in a freeze dryer (LTE Scientific LTD) at a temperature of −49°C for 8 h and lyophilized, then stored in closed containers in the refrigerator for further measurements.

Estimation of total phenolic content

To measure the total phenolic content in eggplant calyx extract, a spectrophotometric method with Folin–Ciocalteu reagent was utilized. This method is based on reducing the Folin–Ciocalteu reagent by the phenolic compounds present in the extract, forming a blue complex.[22,23] Initially, 1 mg of the freeze-dried extract powder was dissolved in 1 mL of each solvent. Subsequently, 1 mL of Folin–Ciocalteu reagent (Merck) was added. After 5 min, 10 mL of a

7% Na_2CO_3 (Merck) solution and 13 mL of deionized distilled water were introduced to the mixture. The mixture was then left in the dark for 90 minutes at a temperature of 23°C. Finally, the absorbance of the samples was measured using a spectrophotometer (OPTIZEN 2120 UV PLUS) at a wavelength of 750 nm.[24] The samples were analyzed in triplicate, and the results were expressed as mean ± standard deviation. Different concentrations of gallic acid (25, 50, 75, 100, 125, and 150 µg/mL) were used to prepare the standard curve. Finally, the results of total phenol content are expressed in terms of mg gallic acid equivalent per gram of dry extract powder.[25]

Estimation of Total flavonoid content

The aluminum chloride colorimetric method quantified the total flavonoid content in eggplant calyx extract.[26,27] This technique relies on forming acid complexes between aluminum chloride and the hydroxyl groups present in flavonoids.[28] In the procedure, 0.3 mg of eggplant calyx extract was mixed with 3.4 mL of each solvent, 0.15 mL of $NaNO_2$ (Merck) (0.5 M), and 0.15 mL $AlCl_3.6H_2O$ (Merck) (0.3 M) and after a 5-minute interval, 1 mL of NaOH(Merck) (1 M) was introduced to the mixture. The mixture was then thoroughly stirred, and the absorbance of the samples was read using a spectrophotometer (OPTIZEN 2120 UV PLUS) at a wavelength of 506 nm.[24] Various concentrations of Rutin (Sigma-Aldrich) (62.5, 125, 250, 500, and 1000 µg /ml) were used to establish the standard curve. The total flavonoid content was reported as mg of Rutin per g of the dry extract powder.[29]

Estimation of antioxidant activity

The spectrophotometric method with a 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent was used to measure the antioxidant activity of eggplant calyx extract. The basis of this method is based on measuring the antioxidant capacity in scavenging the DPPH free radical.[30] To prepare the DPPH stock solution, 24 mg of DPPH in 100 mL of 50% water–ethanol solvent was used.[31] Different concentrations of the extract (0.93, 1.87, 3.75, 7.5, 15) mg/mL were prepared in the desired solvents. After adding the required amount of DPPH stock solution to a value where the optical absorbance reading was close to 1, the samples were placed in the dark for 30 minutes, and finally, their absorbance was read by a spectrophotometer at a wavelength of 517 nm. After determining the absorbance value of the blank (DPPH solution), the percentage of free radical scavenging activity was calculated using the following formula (Equation 1).[25,32]

$$\frac{\text{absorption of control} - \text{absorption of sample}}{\text{absorption of control}} \times 100 = \text{DPPH scavenging activity} \quad (1)$$

The IC50 value of the extracts was calculated from the graph plotting scavenging percentage against extract concentrations (mg/mL).[33,34]

Estimation of hydroalcoholic extract compounds by liquid chromatography–mass spectrometry (LC–MS)

To identify phenolic and flavonoid compounds in the hydroalcoholic extract of eggplant calyx, physical separation was performed using liquid chromatography and mass spectrometry, following a methodology consistent with Petra et al. In the process, the extract sample is introduced into a carrier solvent and passed through an analytical column, where different compounds are separated. Subsequently, the isolated compounds undergo mass spectrometry analysis. This analysis was performed using an Agilent 1200 series LC system coupled with a 6410 triple quadrupole mass spectrometer operating in negative ionization mode. The source temperature, capillary voltage, cone voltage, and gas flows were set according to manufacturer recommendations. Separation was performed by reversed-phase liquid chromatography using a Kinetex C18 column (100 × 2.00 mm, 2.6 µm particle size). The mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile (solvent B). The gradient program for the mobile phase was as follows: 10% B from 0 to 2 min; increased from 10% to 60% B between 2 and 20 min; 60% to 80% B from 20 to 21 min; held at 80% B from 21 to 25 min; decreased from 80% to 10% B between 25 and 26 min; and maintained at 10% B from 26 to 30 min. The injection volume was 10 µL, the column temperature was set at 25°C, and the flow rate was 0.3 mL/min.[35] Identification of phenolic and flavonoid compounds was based on their retention times, mass spectra, and molecular ion characteristics, compared against reference standards and spectral libraries.

Statistical analysis

All experiments were conducted in triplicate, and all reported results are presented as mean ± standard deviation (SD). The SPSS statistical software (version 26) was used. An independent samples *t*-test was used to compare differences between solvent types, while one-way ANOVA followed by Scheffé's post hoc test was used to assess differences among various concentrations. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

Determination of total phenol content

The most widely used method for quantifying the total phenolic content in plant extracts is the Folin–Ciocalteu assay. In this method, gallic acid is commonly employed as the standard compound for determining the phenolic content. Based on the established methodology, a standard curve was constructed using different concentrations of gallic acid, which showed a coefficient of determination (R^2) of 0.99 (Figure 1a), indicating the accuracy and reliability of the method. The total phenolic content was determined using the standard equation and measuring the optical absorption of the samples at a wavelength of 750 nm. The results showed that the total phenol content in the hydroalcoholic extract was 8.72 ± 0.12 and in the

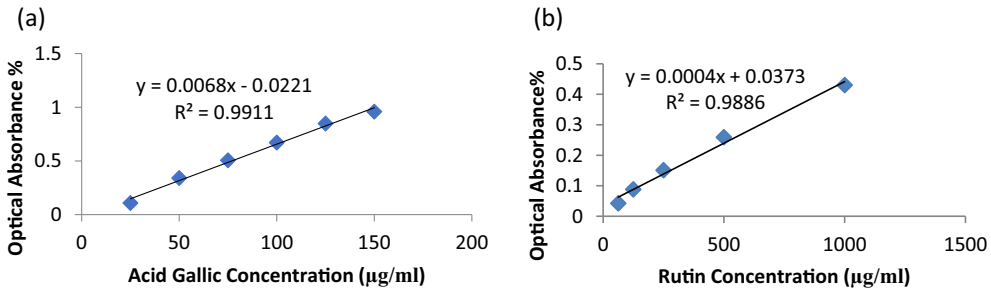

FIGURE 1 (a) Standard curve for different concentrations of gallic acid. (b) Standard curve for different concentrations of Rutin.

TABLE 1 Total phenol and flavonoid content of eggplant calyx extract.

Solvent	Total phenolic content (mg gallic acid equivalent/g dry extract)	Total flavonoid content (mg Rutin /g dry extract)
100% water	7.44 ± 0.041	49.44 ± 1.903
50% ethanol-50% water	8.72 ± 0.12*	52.60 ± 1.149*

Note: Results are presented as mean ± SD, based on three test repetitions. The values marked with an asterisk (*) are significantly different from the other values in the same column ($p < 0.05$).

aqueous extract was 7.44 ± 0.041 mg gallic acid equivalent per gram of dry extract ($p < 0.05$) (Table 1).

Determination of total flavonoid content

Colorimetric using iron chloride is a conventional method to measure the total amount of flavonoids in plant extracts. By plotting the linear graph of optical absorption at 506 nm wavelength for various Rutin concentrations as a reference for quantifying flavonoids, a standard line equation was derived with an R-squared value of 0.98 (Figure 1b), and then utilized to determine the flavonoid content in eggplant calyx extract samples. Based on this method, the total flavonoid amounts of aqueous and hydroalcoholic extracts were 49.44 ± 1.903 and 52.60 ± 1.149 mg Rutin per gram of dry extract, respectively ($p < 0.05$) (Table 1).

Determination of antioxidant capacity

To assess the antioxidant capacity of eggplant calyx extract, a spectrophotometric method using the DPPH reagent was employed. The inhibition percentage of DPPH free radicals was calculated by reading the optical absorption of the control sample (0.629) using Equation 1. The absorption values and inhibition percentage for different concentrations of extracts are shown in Figure 2.

The results demonstrate that as the concentration of the extracts increases, the scavenging percentage of free radicals also increases. Specifically, at concentrations of 15 mg/mL, the hydroalcoholic extract and aqueous extract exhibited inhibition percentages of 82.77% and 82.51%, respectively ($p > 0.05$). Accordingly, similar to the results of the previous sections, the hydroalcoholic extract has been more effective than the aqueous extract. To express the antioxidant activity in terms of IC50, a linear diagram was plotted based on the inhibition percentage of DPPH free radicals and the concentration of the extracts (Figure 3a,b). By substituting 50% inhibition in the obtained linear equation, the IC50 values were[33,36,37] 1.4714 mg/mL for the hydroalcoholic extract and 4.1566 mg/mL for the aqueous extract. The IC50 value denotes the concentration of an extract required to inhibit 50% of DPPH free radicals. Thus, extracts exhibiting lower IC50 values are considered to possess more potent antioxidant properties.[37,38] As observed here, a negative correlation exists between IC50 and % inhibition of DPPH.

Determination of phenol and flavonoids in hydroalcoholic extract by LC–MS

Figure 4 displays the chromatogram generated from the LC–MS technique. The vertical axis represents the concentration percentage of the identified compounds, while the horizontal axis indicates the retention time, which refers to the residence duration of each component within the column. Table 2 presents the types and quantities of the compounds detected in the hydroalcoholic extract of eggplant calyx. The identification of key phenolic and flavonoid compounds such as *p*-coumaric acid, caffeic acid, catechin, myricetin, rutin, luteolin, and kaempferol in the eggplant calyx extract demonstrates its considerable potential as a rich source of antioxidants and bioactive substances.

DISCUSSION

In this study, eggplant calyx extracts were successfully obtained through combined solvent-ultrasonic extraction, yielding substantial phenolic and flavonoid contents alongside significant antioxidant capacity. LC–MS analysis identified key bioactive compounds previously undocumented in this waste material, representing the first scientific investigation of Iranian purple eggplant calyx bioactivity. These findings establish eggplant calyx as a valuable resource with promising applications in food, pharmaceutical, and cosmetic industries.

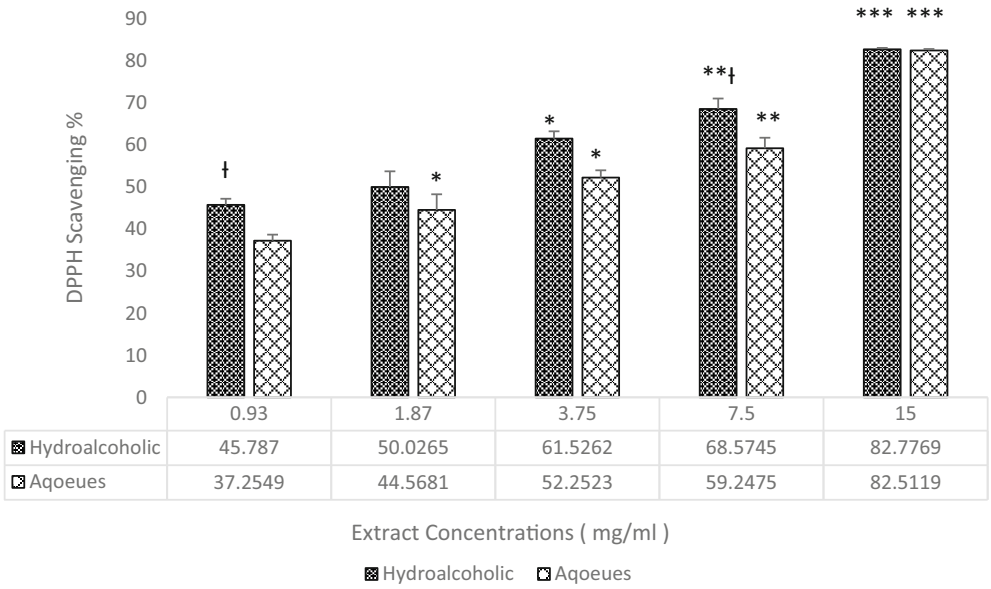

FIGURE 2 DPPH scavenging percentage of different concentrations of extracts. (The symbol ł indicates significant differences in the mean percentage of inhibition between different extracts at the same concentration ($p < 0.05$). In contrast, *, **, and *** represent significant differences within each extract type when compared to the lowest concentration, corresponding to $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.)

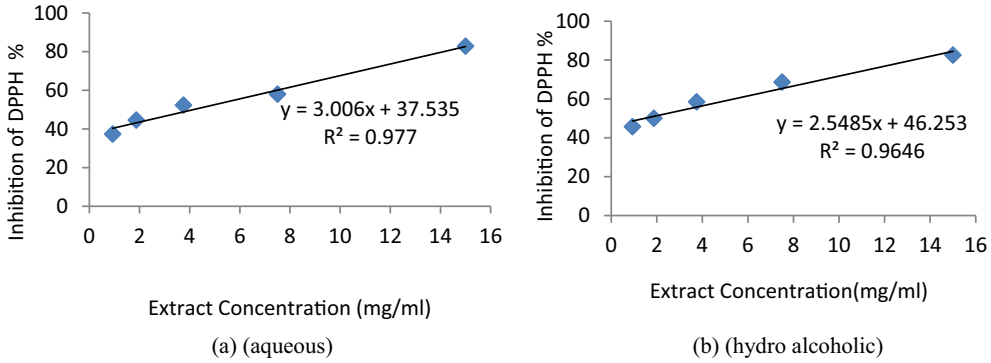

FIGURE 3 Effect of different concentrations of (a) (aqueous) and (b) (hydroalcoholic) extract of eggplant calyx on the inhibition of free radicals.

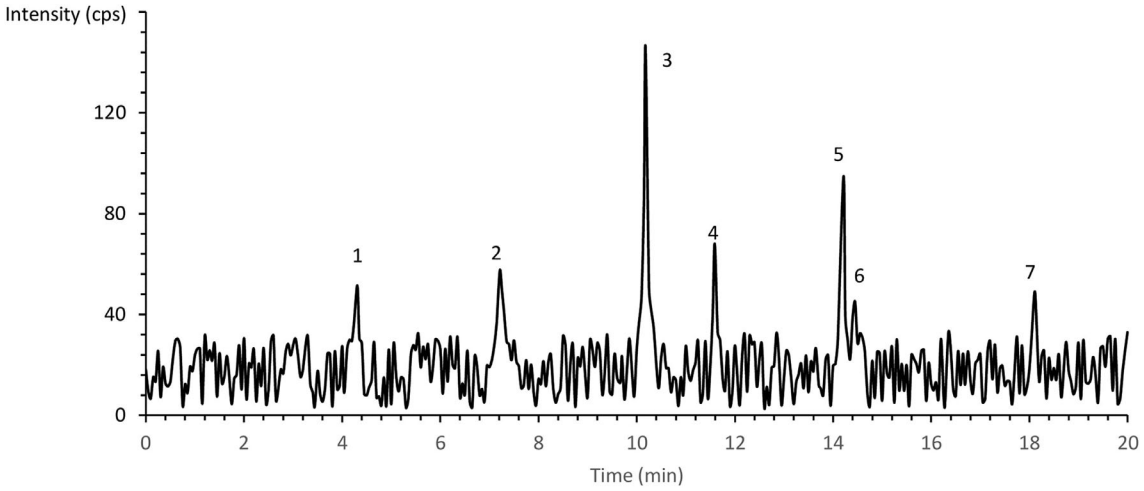

FIGURE 4 Liquid chromatography–mass spectrometry (LC–MS) chromatogram of the hydroalcoholic extract of eggplant calyx.

TABLE 2 Characteristics of the seven peaks of the major phenol and flavonoid compounds in the hydroalcoholic extract of eggplant calyx.

Peak No.	Compound	Retention time (min)	Peak area (%)	Recovery (%)	Concentration (μg/g)
3	ρ-Coumaric acid	10.18	28.5903	98	650.9
5	Caffeic acid	14.22	18.4662	93	488.2
1	Catechin	4.31	10.0487	95	305.4
4	Myricetin	11.59	13.3003	92	301.4
2	Rutin	7.21	11.1874	97	263.6
6	Luteolin	14.45	8.8087	89	261.8
7	Kaempferol	18.11	9.5980	94	257.2

Total phenolic content

The results obtained for the phenolic content showed that the hydroalcoholic extract was able to extract 17% more phenolic compounds than the aqueous extract. This difference can be attributed to the dual nature of ethanol. The hydroxyl group of ethanol interacts with polar compounds, and its ethyl group interacts with non-polar compounds present in the extract, so the water and ethanol mixture can extract a wider range of compounds than pure water. These findings are quite consistent with previous studies. For example, research conducted in 2024 demonstrated that a water–ethanol mixture with a 60:40 volume ratio (v/v) was more effective than pure water in extracting phenolic compounds from pomegranate peel and seeds.[39] Also, a study in 2020 showed that 70% ethanol was more effective than pure water in extracting phenols from eggplant calyx.[40] Various studies conducted on eggplant and its by-products have reported varying amounts of phenolic compounds.[18] For example, RO (2023) demonstrated that the phenolic content in eggplant flesh ranged from 0.1419 to 8.429 mg gallic acid per gram of dry extract.[41] Farwa (2023) also found that the extract of eggplant peel contained 7.03 mg gallic acid per gram of dry extract.[42] These findings are, to some extent, consistent with the phenolic values obtained in the present study. In another study, Al-Nachar (2020) investigated the extract of Syrian eggplant calyx and reported phenolic contents ranging from 9.95 to 12.2 mg gallic acid per gram of dry extract,[40] which is close to the findings of the current research. A common point in all these studies is that eggplant extracts, especially its calyx, have a relatively high concentration of phenolic compounds,[43] in line with the findings of the present study. The differences observed in the phenol values reported in various studies can be due to a number of factors, including the plant part used, variety and cultivation location of the plant, type of solvent, and extraction method used.[44–46]

Total flavonoid content

Flavonoids are characterized by the presence of multiple hydroxyl groups and aromatic rings, conferring them a moderate polarity. This structural feature allows flavonoids to interact favorably with solvents of similar polarity, such as hydroalcoholic mixtures. Hydroalcoholic solvents are particularly effective for the extraction of flavonoids because they can solubilize both the polar and nonpolar moieties of these compounds, thereby enhancing extraction efficiency compared to pure water. Moreover, the presence of alcohol in hydroalcoholic mixtures reduces the surface tension of water, which facilitates deeper penetration of the solvent into plant cellular matrices and promotes the release of intracellular flavonoid compounds. This improved cell wall permeability, combined with the moderate polarity of the solvent system, leads to higher extraction yields and more efficient recovery of flavonoids from plant materials.[47,48] Previous studies conducted on eggplant and its by-products have confirmed the presence of varying amounts of flavonoid compounds in different parts of this plant. For instance, Angulo (2022) reported that the flavonoid content in the root, leaves, and stem of eggplant was 46.66, 79.38, and 10.9 mg rutin per gram of dry extract, respectively.[49] Similarly, Horincar (2020) found that the peel extract of Romanian eggplant contained 35.82 mg rutin per gram of dry extract.[15] In another study, Jung (2011) reported the flavonoid content in the aqueous extract of eggplant calyx as 11.78 mg rutin per gram of dry extract.[50] The results of the present study are consistent with previous findings and demonstrate that the eggplant calyx contains a considerable number of flavonoids. The observed differences in flavonoid content reported in various studies may be attributed to differences in extraction procedures, cultivation conditions, and plant varieties. Overall, the findings of this research highlight the significance of eggplant calyx as a rich source of flavonoid compounds.

Antioxidant capacity

The results of the present study revealed that the eggplant calyx possesses strong antioxidant activity through effective inhibition of DPPH free radicals. This finding aligns closely with the work of Al-Nachar (2020), who reported antioxidant activity of approximately 82% and 90% for aqueous and alcoholic extracts of eggplant calyx, respectively, using the DPPH assay.[40] Although the majority of previous studies have primarily focused on the flesh and peel of eggplant, all consistently confirm that various parts of the plant—including the fruit, peel, and calyx—exhibit significant antioxidant properties.[15,51–53] Variations in antioxidant activity levels reported across studies may be attributed to differences in analytical methods (such as ABTS, FRAP, and DPPH assays), extraction procedures, solvent types, as well

as the specific eggplant varieties examined.[33,40,41] Given the crucial role of antioxidants in mitigating oxidative stress and related diseases, these findings highlight the potential of eggplant calyx as a valuable source of natural antioxidants.

LC–MS

The results of this technique are valuable in many research and industrial fields, including medicine, pharmaceuticals, and food, due to their high resolution, sensitivity, speed, and accuracy.[54] Notably, *p*-coumaric acid was the most abundant compound detected in the LC–MS chromatogram, recognized for its strong antioxidant activity and its ability to protect cells from oxidative stress by neutralizing free radicals.[55] A previous study had shown the presence of this substance in eggplant peel.[42] Caffeic acid, the second most prevalent compound, is also a potent antioxidant, playing an important role in disease prevention, including cancer, through the inhibition of free radical production and protection against oxidative cellular damage.[56,57] Catechin, another major constituent, is a powerful natural antioxidant that can improve lipid metabolism disorders and further reduce oxidative stress.[58] In addition, myricetin, rutin, luteolin, and kaempferol are flavonoids with notable antioxidant properties, contributing to the prevention of oxidative damage to cells. The research findings have shown that rutin, with its high antioxidant properties, possesses both analgesic and anti-inflammatory effects.[59] These findings highlight the potential of eggplant calyx extract as a valuable natural source of antioxidants for use in the pharmaceutical, nutraceutical, and food industries.

CONCLUSION

The results of this study demonstrated that the extract obtained from the calyx of the Iranian long purple eggplant, using a combined solvent and UAE method, contains high levels of phenolic and flavonoid compounds with notable antioxidant activity. These findings highlight the biological value of this often-discarded part of the plant, which has received little scientific attention to date. To the best of our knowledge, no previous studies have investigated the bioactive properties of the Iranian eggplant calyx, making this research one of the first to explore its potential. Given that the calyx is commonly treated as agricultural waste and is freely available, its utilization could contribute not only to reducing plant-based waste but also to the development of value-added products in the food, pharmaceutical, and cosmetic industries. In this study, LC–MS analysis successfully identified the key bioactive compounds present in the extract, further confirming its functional potential. Therefore, it is recommended that future research focus on evaluating the biological activity of these compounds in cell-based and in vivo models, as well as exploring their formulation into functional products. Additionally, due to the extract's rich phytochemical content, the green synthesis of metal nanoparticles using this material as a reducing and stabilizing agent could be investigated. This approach offers a sustainable route for developing eco-friendly nanomaterials with possible biomedical and antimicrobial applications.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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