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Scientific Research

Evaluation of the efficiency of osmotic methods and the use of pretreatment in the extraction of active and antioxidant compounds from hibiscus tea and comparison with other methods

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ABSTRACT

This research examines the efficiency of various methods for extracting active compounds from hibiscus tea (Hibiscus sabdariffa), focusing on osmotic techniques and the application of specific pretreatments including ultrasound and ethanol. Hibiscus tea, known for its high content of anthocyanins, flavonoids, and phenolic compounds, possesses antioxidant, antibacterial, and blood pressure-lowering properties, making it a rich source of beneficial medicinal and nutritional compounds. In this study, fresh calyces of hibiscus tea were used for extraction, and the impact of different concentrations of osmotic solutions and ultrasound pretreatment in the presence of ethanol on the extraction yield of compounds was investigated. The results indicate that the combination of osmotic methods with ultrasound pretreatment and ethanol solvent significantly increases the yield of phenolic and flavonoid compounds. The highest amount of these compounds was obtained from the EO50S treatment, which included a 50% sucrose osmotic solution combined with ultrasound and ethanol. This method demonstrated better performance due to the preservation of the antioxidant properties of the extracted materials, especially compared to conventional extraction methods. Additionally, the results of the principal component analysis showed that the methods EO50S, EO40S, and O40S exhibited the highest efficiency in extracting plant active compounds. Overall, the findings of this research suggest that osmotic methods combined with ultrasound and ethanol provide an efficient and environmentally friendly approach for extracting active compounds from hibiscus tea, and could be applied in the pharmaceutical and food industries for extracting beneficial compounds from medicinal plants.

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1- Introduction

Medicinal plants are a rich source of bioactive compounds such as phenolic and flavonoid compounds [1]. Many medicinal plants contain specific compounds whose extracts serve as effective alternatives to chemical substances in the food and pharmaceutical industries [2,3]. Plant extraction is a well-established method for accessing bioactive compounds [4], and with advancements in technology, modern extraction methods have replaced conventional techniques, offering shorter extraction times, lower solvent consumption, and higher efficiency [2].

Osmotic extraction, developed based on ion exchange principles, is considered a green and sustainable method. Due to its simplicity, cost-effectiveness, and environmental compatibility, it has gained significant importance in various industries [5,6]. In this method, high-osmotic-pressure solutions are used to remove part of the water from the plant material, thereby transferring bioactive compounds into the solution [7]. Since this method does not involve thermal processing, it enables the preservation and extraction of heat-sensitive compounds without thermal degradation. The osmosis technique is recognized as an effective technology for enriching food products with functional compounds such as phenolic compounds, flavonoids, and antioxidants [8].

This technique has been used for extracting active components from biological sources, including flavonoids [9,10], alkaloids [11], phenolic compounds [12], polysaccharides [13], lignans [14], phenylethanoid glycosides [15], ginsenosides [16,17], and succinic acid [18]. Moreover, osmotic technology has promising applications in the separation and purification of active proteins such as lipase [19], phage [20], and serine protease [21].

In general, osmotic extraction is mainly used for liquid materials, and limited research has been conducted on the extraction of solid compounds. However, the extraction solvent faces challenges in penetrating plant tissues, making it difficult for bioactive components to diffuse out of plant cells.

During the osmotic process, the use of mineral salts and sugars has been shown to enhance extraction efficiency [9]. Additionally, pre-treatments such as ultrasound have been applied to improve the extraction yield of plant bioactives. Ultrasound generates microbubbles and cavitation effects, disrupting plant cell walls and facilitating the release of bioactive compounds [22]. This method, by maintaining low temperatures, enables the extraction of heat-sensitive compounds [23]. Ultrasound waves create and collapse bubbles, generating strong internal currents that ultimately promote the extraction of active compounds [24].

Hibiscus sabdariffa, commonly known as roselle, belongs to the Malvaceae family and is an annual or perennial plant with numerous medicinal properties. In traditional medicine, it has been used for the prevention and treatment of kidney and bladder stones, as an antibacterial and antifungal agent, as well as for its hypocholesterolemic, antispasmodic, and blood pressure-lowering effects [25]. Additionally, roselle is used for body cooling, blood purification, cholesterol control, and as a rich source of iron. The high anthocyanin content in its calyces plays a significant role in its antihypertensive and anticancer properties [26]. The calyces contain flavonoids such as gossypetin, hibiscetin, and sabdaretin, along with alkaloids, beta-sitosterol, anthocyanins, citric acid, and other beneficial compounds [27].

In this study, the effect of sugar-based osmotic extraction on the extraction of bioactive compounds from *Hibiscus sabdariffa* was investigated. Additionally, ultrasound pre-treatment was employed to enhance extraction efficiency, and this

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method was compared with other conventional extraction techniques.

2- Materials and Methods

The experiment was conducted during the fall and winter of 2023 (1402 in the Persian calendar) in the Department of Horticultural Science at Khuzestan Agricultural Sciences and Natural Resources University (Mollasani, 35 km northeast of Ahvaz).

2.1. Plant Material Preparation

The medicinal plant *Hibiscus sabdariffa* (roselle) was freshly collected in November 2023 from a farm located in Bavi County, Mollasani. The harvested plant material was stored at 4°C until the experiment was conducted.

2.2. Extraction Method

For plant extraction, 10 grams of fresh roselle calyces were mixed with a solvent in a 1:3 ratio (Table 1) and incubated in a shaker incubator under dark conditions for 12 hours. The solvent containing the extracted bioactive compounds was then separated, centrifuged, and stored at 4°C in a refrigerator.

For the ultrasound pre-treatment, treatment solutions containing 40% and 50% sugar solutions, both with and without ethanol, were subjected to ultrasound waves for 15 minutes using an H 200UP ultrasonic device (Euronda, Italy) with a power of 350 watts and a frequency of 34 kHz.

Table 1 presents the different extraction treatments applied to *Hibiscus sabdariffa* calyces, including various water and sucrose concentrations (30% to 60%), 10% ethanol-containing solutions, and ultrasound-assisted treatments. Each treatment was assigned a specific code, which is used in subsequent explanations.

Table 1. Solvents used to extract the active ingredients of fresh roselle sepals

No.	Solvents	Abbreviation
1	Water	W
2	Water/ 30% Sugar (Osmosis30)	O30
3	Water/ 40% Sugar (Osmosis40)	O40
4	Water/ 50% Sugar (Osmosis50)	O50
5	Water/ 60% Sugar (Osmosis60)	O60
6	10% Ethanol /Osmosis 30	EO30
7	10% Ethanol /Osmosis 40	EO40
8	10% Ethanol /Osmosis 50	EO50
9	10% Ethanol /Osmosis 40/Sonication	EO40S
10	10% Ethanol /Osmosis 50/Sonication	EO50S
11	Osmosis 40/Sonication	O40S
12	Osmosis 50/Sonication	O50S

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2.3. Measurement of pH and Electrical Conductivity

The samples underwent the osmotic process at room temperature (23°C) with varying sucrose concentrations. The pH and electrical conductivity (EC) values were measured at different time intervals: 0, 1, 6, and 12 hours.

pH Measurement: The pH of the osmotic solution samples was determined throughout the osmotic process using a pH meter.

Electrical Conductivity (EC) Measurement: The electrical conductivity of the osmotic solution samples was measured during the process using an EC meter, with results expressed in µS/cm.

2.4. Determination of Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent [28]. Initially, a range test was conducted using different sample volumes (5 to 200 µL) to determine the appropriate sample volume for further analysis.

For the final measurement, 200 µL of diluted extract was selected and diluted to 500 µL with distilled water. Then, 2.5 mL of Folin-Ciocalteu reagent was added, and the mixture was kept in the dark for 6 minutes. Afterward, 2 mL of 7.5% sodium carbonate was added, and the samples were incubated at room temperature in the dark for 90 minutes.

The absorbance was measured at 765 nm using a spectrophotometer. A standard calibration curve was prepared using different concentrations (5 to 100 mg/mL) of gallic acid, and the curve was plotted using Excel software, yielding the equation: $y = 0.002x + 0.2331$ ($R^2 = 0.9924$).

The absorbance values of the samples were substituted into the equation to determine x, representing the phenolic concentration. The total phenolic content was expressed as mg of gallic acid per 100 g of dry weight.

2.5. Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method [29]. For each plant methanolic extract, 200 µL was mixed separately with 1.5 mL methanol, 100 µL of 10% aluminum chloride (in methanol), 100 µL of 1M potassium acetate, and 2.2 mL distilled water. The mixture was then incubated at room temperature for 30 minutes.

The absorbance of each reaction mixture was measured at 415 nm using a spectrophotometer. A standard calibration curve was prepared using methanolic rutin solution (Sigma) at concentrations ranging from 10 to 100 µg/mL. The curve was plotted using Excel software, yielding the equation:

$y = 0.0743x + 0.0413$. The absorbance values of the samples were substituted into the equation to determine x, representing the flavonoid concentration.

2.6. Antioxidant Activity Based on DPPH Free Radical Scavenging

The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [30].

First, plant extracts were prepared in various concentrations (5-500 µL) in pure methanol to determine the optimal concentration. Based on the preliminary tests, 40 µL was selected as the appropriate concentration. The sample was then diluted to 100 µL with 100% methanol, and 350 µL of 1 mM DPPH solution was added. The total volume was adjusted to 2 mL, and the

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samples were incubated at room temperature for 30 minutes.

The absorbance was measured at 517 nm using a spectrophotometer, and the DPPH free radical scavenging percentage was calculated using the following formula:

Formula (1) $R\% = AD - AS/AD \times 100$

Where:

R%= Scavenging percentage

AD= Absorbance of the control (DPPH solution without sample)

AS = Absorbance of the sample containing the extract

2.7. Antioxidant Activity Based on ABTS Free Radical Scavenging

The antioxidant activity based on ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging was measured following the method described by [31].

First, equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate ($K_2S_2O_8$) solution were mixed and incubated in the dark at 25°C for 16 hours to generate the ABTS cationic radical solution. This solution was then diluted with methanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm.

Next, 100 µL of the extract was mixed with 3.9 mL of the ABTS radical solution, and the mixture was kept at room temperature for 6 minutes. The absorbance of the sample (As) was measured at 734 nm against a control (Ac, methanol instead of extract).

The ABTS radical scavenging activity was calculated using the following equation:

Formula (2) $I\% = ((Ac - As)/Ac) \times 100$

The antioxidant activity was expressed as IC_{50}, which represents the extract concentration required to scavenge 50% of the ABTS radicals.

2.8. Determination of Total Anthocyanin Content

The total anthocyanin content (TAC) was determined using the pH differential method with slight modifications [32].

Two 1 mL aliquots of the methanolic extract were taken. One was mixed with potassium chloride buffer (pH 1.0), and the other with sodium acetate buffer (pH 4.5), bringing each solution to a final volume of 10 mL. After 15 minutes of equilibration, the absorbance was measured at 520 nm and 700 nm using a spectrophotometer.

The total anthocyanin content was calculated in mg/L of cyanidin-3-glucoside using the following formula:

Formula (3) $Anthocyanin\ content\ (mg/g) = \frac{Abs}{eL} \times MW \times D \times \frac{V}{G}$

Where:

A = Difference in absorbance between pH 1.0 and pH 4.5

MW = Molecular weight of cyanidin-3-glucoside (449.2 g/mol)

DF = Dilution factor

ε = Molar extinction coefficient of cyanidin-3-glucoside (26,900 L/mol·cm)

V = Final solution volume (mL)

G = Dry weight of the sample (mg)

2.9. Statistical Analysis

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Initially, the effects of different extraction treatments on pH and EC (electrical conductivity) were analyzed using a factorial experiment at four time points (0, 1, 6, and 12 hours). The effects of different extraction methods on total phenol, total flavonoid, total anthocyanin, and antioxidant activity were evaluated using a completely randomized design (CRD).

For data analysis, SAS 9.4 software was used. Mean comparisons for pH and EC were performed using the LSMeans test at a 1% significance level ($p \leq 0.01$), while mean comparisons for qualitative data (phenol, flavonoid, anthocyanin, and antioxidant activity) were conducted using Duncan's multiple range test at a 5% significance level ($p \leq 0.05$).

Additionally, Pearson's correlation coefficient was calculated using SAS, and Principal Component Analysis (PCA) was performed using MATLAB.

3. Results and Discussion

The analysis of variance (ANOVA) results for the effect of different extraction methods on pH and EC at 0, 1, 6, and 12 hours after treatment (Table 2) showed that changes in acidity (pH) and electrical conductivity (µS/cm) over time were statistically significant at the 1% level.

Table 2. Analysis of variance (ANOVA) results for the effect of time and different extraction methods on the pH and EC levels of fresh roselle calyces extracts.

Sources	DF	Mean Squares	
		pH	EC
Treatment	11	0.11**	1213383.09**
Time	3	125.9**	26788483.25**
Treatment × Time	33	0.02**	239402.69**
Error	96	0.001	14.11
CV (%)	-	1.24	10.91

Table 3 presents a comparison of the effects of various extraction treatments on the pH changes of fresh *Hibiscus sabdariffa* calyx extract over four time intervals (0, 1, 6, and 12 hours) based on the LSMeans test (at a 1% significance level). The results indicate that in all treatments, the pH gradually decreases from time zero. The highest initial pH values were observed in treatments O40S, O40, O30, and W. Treatments containing higher concentrations of sucrose and ethanol exhibited lower pH values compared to the other treatments. For example, treatments O60, EO30, and EO40 showed lower pH at the start of the experiment (time zero). Furthermore, treatments that incorporated ultrasound processing, such as EO40S and EO50S, exhibited smaller pH changes over time, with the reductions at 6 and 12 hours being negligible compared to those without ultrasound. These pH variations over time may be attributed to the characteristics of the extracted compounds (acidic, basic, ionic, or non-ionic) during the osmotic process and other related phenomena. Moreover, the high concentration of the osmotic solution may create a buffering effect, reducing the mobility of ions and electrons. Additionally, ultrasound waves can facilitate the release of compounds and may affect the initial pH reduction, although their impact becomes less pronounced in later hours [2].

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Table 4 examines the effect of different extraction treatments on the changes in electrical conductivity (EC) of the fresh *Hibiscus sabdariffa* calyx extract over time. The results show that the electrical conductivity increases over time in all treatments, with the lowest EC recorded at time zero and the highest at 12 hours. This indicates that, as time progresses, more ions are transferred into the solution, thereby increasing its electrical conductivity. Extracts containing ethanol and higher sucrose concentrations exhibited lower EC at time zero. This initial reduction in electrical conductivity is attributed to ethanol's lower ability compared to water to solvate ions, and the high sucrose concentration, which may slow down ion mobility. Treatments processed with ultrasound (e.g., EO40S and EO50S) demonstrated smaller changes in EC over time. In these treatments, the increase in electrical conductivity over time was lower compared to other methods, whereas in the remaining treatments, significant differences were observed between the 0, 1, 6, and 12-hour intervals. It appears that the ultrasound pre-treatment results in a higher release of electrolytes from the plant tissues. A high concentration of ions is associated with high electrical conductivity, which is related to the migration of food compounds into the water [35]. Electrical conductivity is influenced by various factors, including increased temperature, solution concentration, particle size of food materials, and the type of pre-treatment applied, all of which contribute to its increase [36]. In one study, the effect of electrical conductivity resulting from different compounds (sugar solution versus sugar-salt solution) on the osmotic drying process of pineapple pieces was examined. The results indicated that, since high electrical conductivity reflects a greater presence of ions, the osmotic solution composed of a sugar-salt combination facilitated mass transfer and improved the efficiency of the osmotic process. Furthermore, in such a solution, the amount of released electrolytes (ions) was found to be lower [37].

Table 3. The effect of different extraction treatments on changes in pH of the fresh calyx of roselle per time

Treat		Time (h)			
		0	1	6	12
1	W	6.31(ab)	3.08(e)	2.78(gf)	2.58(ji)
2	O30	6.35(a)	2.77(gf)	2.67(ih)	2.45(onml)
3	O40	6.37(a)	2.72(gh)	2.55(jk)	2.37(orpq)
4	O50	6.25(bc)	2.71(gh)	2.52(jkl)	2.35(rqq)
5	O60	6.15(d)	2.75(gfh)	2.44(onmpl)	2.3(r)
6	EO30	6.24(bcd)	2.76(gf)	2.55(jk)	2.4(onmpq)
7	EO40	6.18(cd)	2.82(f)	2.49(nmkl)	2.4(onpq)
8	EO50	6.25(bc)	2.78(gf)	2.46(onmkl)	2.43(onmp)
9	EO40S	6.18(cd)	2.48(nmkl)	2.40(onmpq)	2.32(rq)
10	EO50S	6.25(bc)	2.43(onmp)	2.43(onmpl)	2.36(rpq)
11	O50S	6.25(bc)	2.38(orpq)	2.36(rpq)	2.32(rq)
12	O40S	6.38(a)	2.49(jmkl)	2.48(nmkl)	2.39(orpq)

Table 4. The effect of different extraction treatments on changes in EC of the fresh calyx of roselle per time

Treat		Time (h)			
		0	1	6	12
1	W	104.3(r)	1667.3(hij)	2383.3(cb)	2993.3(a)

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2	O30	104.3(r)	1251.3(lm)	1777.6(hfglj)	2543.3(b)
3	O40	86.03(r)	1096.3(nm)	1645.0(ij)	2206.6(cd)
4	O50	63.40(r)	656.0(oqp)	1165.6(nm)	1658.0(ij)
5	O60	41.63(r)	421.6(q)	950.3(on)	1274.3(lm)
6	EO30	88.90(r)	1280.0(lm)	2166.6(cd)	2630.0(b)
7	EO40	70.86(r)	883.3(onp)	1626.0(ij)	2064.6(fde)
8	EO50	49.80(r)	646.6(qp)	1313.6(klm)	1616.6(ij)
9	EO40S	70.80(r)	1848.6(hfgie)	1990.6(fdge)	2070.0(fde)
10	EO50S	49.46(r)	1542.3(klj)	1608.3(kij)	1714.6(hgij)
11	O50S	63.80(r)	1657.0(ij)	1660.6(ij)	1825.0(hfgij)
12	O40S	86.40(r)	1777.0(hfgij)	1957.3(hfgde)	2128.3(cde)

The ANOVA results (Table 5) examining the effects of different treatments on the total phenol content, total flavonoid content, anthocyanin content, and antioxidant activity of fresh *Hibiscus sabdariffa* calyces demonstrated that there are statistically significant differences at the 1% level among the treatments for all these parameters.

Table 5. The analysis of variance of the effect of different extraction methods on total phenol, total flavonoid, anthocyanin, and antioxidant activity of fresh roselle calyces.

Source	DF	Mean Square				
		Total Phenol	Flavonoid	Anthocyanin	IC50 (DPPH)	IC50 (ABST)
Treat	11	912.19**	46.29**	87.53**	9456.03**	13882.47**
Error	24	215.16	14.11	5.9	22.37	1112.46
CV. (%)	-	9.57	10.8	24.1	13.09	11.01

Comparison of the mean values between the osmotic extraction method and other methods on the total phenolic content, based on Duncan's multiple range test at the 5% level, revealed that the ultrasound pre-treatment had a significant effect on the extraction of total phenols. In this study, the highest total phenolic content was obtained in the EO50S treatment (192.69 mg/100 g of fresh material), which did not differ significantly from the EO40S treatment, while the lowest total phenolic content was found in the O30 treatment. No significant differences in total phenolic content were observed among the W, O30, O40, O50, and O60 treatments, nor among the EO40, EO50, and O50S treatments. The high sucrose concentration combined with the application of ultrasound pre-treatment and ethanol resulted in a better extraction of the phenolic compounds present in *Hibiscus sabdariffa*.

A study was also conducted on the penetration of grape phenolic compounds into fruits and vegetables by osmosis. The osmotic process was examined using a solution containing grape phenolic extracts at three different concentrations. The total amount of phenolic compounds absorbed at the end of the osmotic process was found to be between 5.0 and 5.1 times higher than that measured in the target fruits at the beginning of the experiment [38].

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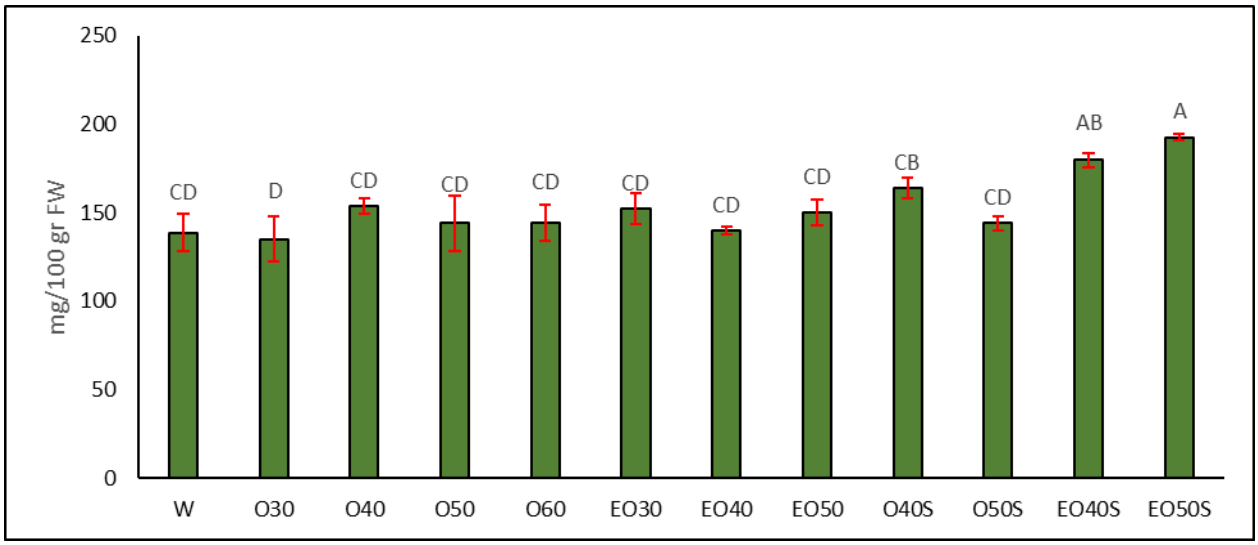

Fig1. The mean Comparison of osmosis extraction method on total phenol content based on Duncan's test ($p<0.05$)

Comparison of the mean values of the osmotic extraction method with other methods for total flavonoid content, based on Duncan's multiple range test at a 5% significance level, showed that the highest total flavonoid content was obtained in the O40S treatment, which did not differ significantly from the O30, O40, EO50, EO40S, and EO50S treatments. The lowest total flavonoid content was observed in the O50 and O60 treatments, which did not differ significantly from the O30, O40, O50S, and EO40, EO50 treatments.

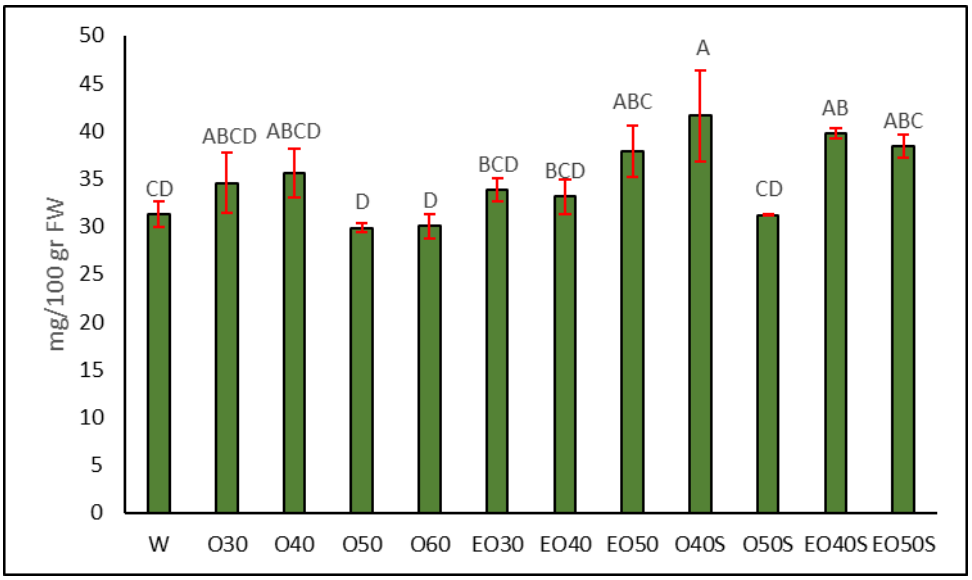

Fig2. The mean Comparison of the osmosis extraction method on total Flavonoid content based on Duncan's test ($p<0.05$)

Comparison of the mean values between the osmotic extraction method and other methods on the total anthocyanin content, based on Duncan's multiple range test at a 5% significance level, indicated that the highest anthocyanin content was obtained in the EO50S and EO40S treatments. No statistically significant

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differences were observed among the treatments O30, O40, O50, O60, EO40, EO50, and O50S in terms of the lowest anthocyanin content.

Basiri and Gheibi (1401) investigated the effects of osmosis and its pre-treatments (enzymatic and ultrasound) on the release of bioactive compounds from the medicinal plant thyme. Their results showed that the use of ultrasound and enzymatic pre-treatment, combined with the osmotic water absorption process, had significant effects on the release of active compounds from thyme [2].

Furthermore, Shahidi et al. (2020) evaluated the effect of osmotic and ultrasound pre-treatment on some quality characteristics of hot-air dried bananas. Various sugar osmotic solutions at different concentrations were used along with ultrasound pre-treatment with variable sonication times. The results indicated that the highest water removal, the greatest uptake of solids, and the lowest shrinkage were achieved by using a 50% glucose solution combined with ultrasound pre-treatment [39].

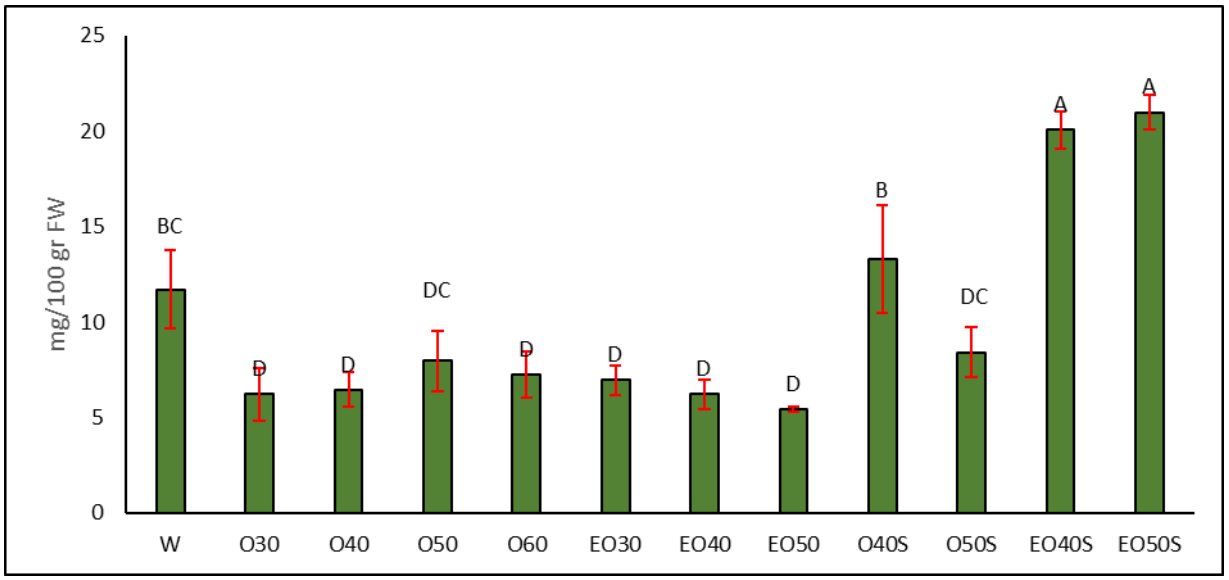

Fig3. The mean Comparison of osmosis extraction method on total Anthocyanin content based on Duncan's test ($p<0.05$)

The antioxidant activity based on the effective concentration required to inhibit 50% of the DPPH free radicals (IC50) was determined. Comparison of the mean values of the osmotic extraction method with other methods for IC50, based on Duncan's multiple range test at a 5% significance level, showed that the lowest effective concentration required for 50% free radical inhibition (in mg/mL) was obtained in the EO50S treatment, which did not differ significantly from the O40S and EO40S treatments. The lowest antioxidant activity was observed in the O30 treatment, with no significant differences compared to the other treatments.

Similarly, the antioxidant activity based on the effective concentration required to inhibit 50% of the ABTS free radicals (IC50) was determined. Comparison of the mean IC50 values, again based on Duncan's multiple range test at a 5% significance level, indicated that the lowest effective concentration (in mg/mL) was obtained in the EO50S and EO40S treatments, which did not differ significantly from the O40S treatment. The lowest antioxidant activity was observed in the O30 treatment, with no

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significant differences compared to the other treatments.

In a study by Feng et al. (2020), a novel green extraction technique for isolating flavonoids from blue lotus by modulating osmotic pressure was investigated. In this method, the extraction yield of flavonoids with high antioxidant activity was higher than that obtained using the conventional method. The interface effect contributed to enhanced extraction during the osmotic process, with osmosis serving as the primary factor in improving extraction efficiency [40].

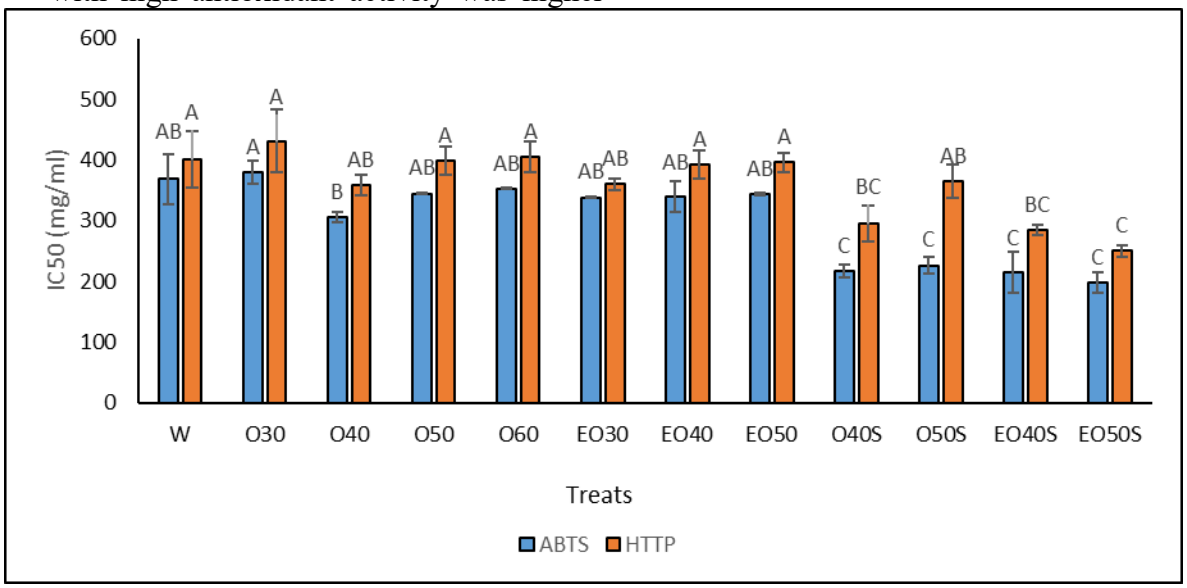

Fig4. The mean Comparison of osmosis extraction method on IC50 values of antioxidant activities, ABTS and DPPH radical scavenging based on Duncan's test ($p<0.05$)

Pearson correlation analysis was conducted to examine the relationship between total flavonoid, phenol, and anthocyanin content with their corresponding antioxidant activities. The results in Table 6 indicated a significant negative correlation between total phenolic, flavonoid, and anthocyanin content with the IC50 values of DPPH and ABTS radical scavenging activities.

A negative correlation means that higher levels of total flavonoids, total phenolics, and anthocyanins result in lower IC50 values for ABTS and DPPH inhibition. Since a lower IC50 indicates stronger antioxidant activity, this suggests that these bioactive compounds significantly contribute to the extract's antioxidant potential. Phenolic compounds are well known for their electron-donating ability, making them effective radical scavengers and strong antioxidants [41].

Table 6. Correlation of total flavonoid and phenolic and Anthocyanin content with IC50 DPPH, ABST scavenging activity.

Antioxidant Parameter	Pearson's Correlation Coefficient (r)		
	Total Phenol	Total Flavonoid	Anthocyanin
IC50 DPPH	-0.89**	-0.58**	-0.80**
IC50 ABST	-0.60**	-0.47**	-0.66**

**: significant at $p<0.01$

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The principal component analysis (PCA) of extraction methods, based on the results of phenol, flavonoid, anthocyanin content, and antioxidant activity, indicated that the first principal component (PC1), with an impact coefficient of 72.35%, demonstrated the highest differentiation among the various extraction methods. The second (PC2) and third (PC3) components contributed to 14.01% and 7.9% of the variations, respectively, showing a lower degree of distinction among the extraction methods.

PC1 revealed that the extraction methods EO50S, EO40S, and O40S exhibited the most significant differences compared to other methods. These methods were grouped separately on the right side of the diagram, indicating that they yielded higher amounts of bioactive compounds such as phenols, flavonoids, anthocyanins, or antioxidant activity. While PC2 and PC3 also effectively distinguished between extraction methods, their differentiation was less pronounced than that of PC1.

Certain extraction methods, such as O50, EO30, and O60, were positioned in the center of the diagram, indicating their moderate influence on bioactive compound extraction. These findings highlight that selecting an appropriate extraction method plays a crucial role in maximizing the yield of bioactive compounds, particularly osmosis and ultrasound-assisted methods, which were distinctly grouped in the analysis.

To achieve the highest extraction of bioactive compounds (phenols, flavonoids, anthocyanins, and antioxidant properties), the use of EO50S, EO40S, and O40S methods appears to be the most suitable approach.

The principal component analysis (PCA) based on the results of phenol, flavonoid, anthocyanin content, and antioxidant activity shows that the first principal component (PC1), accounting for 72.35% of the variance, provides the highest differentiation among the various extraction methods. As observed in the diagram, the extraction methods EO50S, EO40S, and O40S are distinctly separated from the other methods and positioned on the right side of the chart. This separation indicates the superior performance of these methods in extracting bioactive compounds such as phenols, flavonoids, and anthocyanins or enhancing antioxidant properties.

The second principal component (PC2), contributing 14.01% of the variance, shows less variation compared to PC1. While it helps differentiate some extraction methods, its impact is lower than that of PC1. The third principal component (PC3), with 7.9% of the variance, plays a minor role compared to the previous components but still contributes to distinguishing certain extraction methods.

Methods such as O50, EO30, and O60, which are located in the center of the diagram, exhibit moderate effects on the extraction of bioactive compounds. Their distinction from other methods is neither as pronounced as that of EO50S and EO40S nor completely insignificant.

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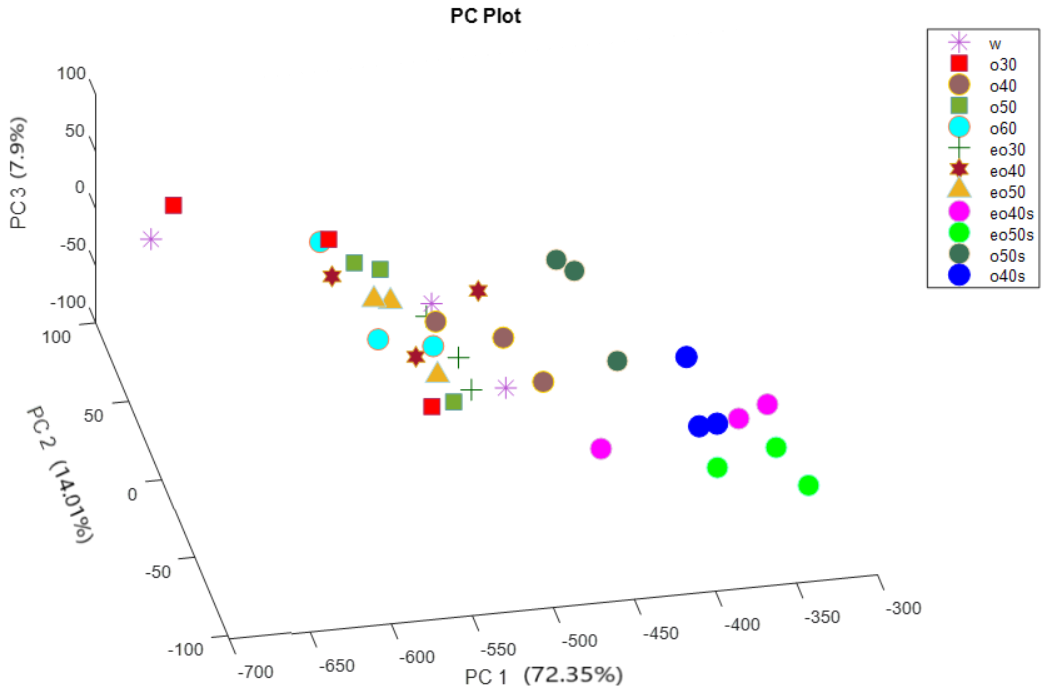

Fig 5. Principal Component Analysis (PCA) of Different Extraction Methods Based on Phenol, Flavonoid, Anthocyanin Content, and Antioxidant

4- Conclusion

In this study, sucrose solution with different concentrations, the application of ultrasonic pretreatment, and the use of alcohol as a solvent were examined for their effects on the extraction of compounds from fresh calyces of roselle tea. The results showed that pH decreased with increasing time and concentration, while EC increased over time but did not show significant changes with different concentrations. The application of ultrasonic pretreatment and the addition of ethanol had no significant effect on pH and EC. However, although the effect of sugar solutions on total phenols, total flavonoids, antioxidant activity, and total anthocyanins was not significantly different from the control (water) in many parameters, the combination of ultrasonic pretreatment with the addition of 10% alcohol had a notable impact on the extraction of bioactive compounds, making the osmosis method more effective. Therefore, the use of osmosis at a 40% concentration along with ultrasonic pretreatment and alcohol can be considered a suitable method for extracting bioactive compounds from fresh roselle.

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چکیده

این پژوهش به بررسی کارایی روش‌های مختلف استخراج مواد مؤثره از گیاه چای ترش (*Hibiscus sabdariffa*)، با تمرکز بر تکنیک اسمز و به‌کارگیری پیش‌تیمار فراصوت پرداخته است. چای ترش که به دلیل محتوای بالای آنتوسیانین، فلاونوئیدها و ترکیبات فنلی دارای خواص آنتی‌اکسیدانی، ضدباکتری و کاهنده فشار خون است، به‌عنوان منبعی غنی از ترکیبات مفید دارویی و غذایی شناخته می‌شود. در این مطالعه، از کاسبرگ‌های تازه چای ترش برای استخراج استفاده شد و تأثیر غلظت‌های مختلف محلول‌های اسمزی و پیش‌تیمار فراصوت بر میزان استخراج ترکیبات مورد بررسی قرار گرفت. نتایج حاکی از آن است که ترکیب روش اسمز با پیش‌تیمار فراصوت و حلال اتانول به طور معنی‌داری بازدهی استخراج ترکیبات فنلی و فلاونوئیدی را افزایش می‌دهد. بیشترین مقدار این ترکیبات در تیمارEO50S ، شامل محلول اسمزی ۵۰ درصد ساکارز و ۱۰ درصد اتانول به همراه فراصوت، به دست آمد. این روش به دلیل حفظ خواص آنتی‌اکسیدانی مواد استخراج‌شده، به‌ویژه در مقایسه با روش‌های استخراج مرسوم، عملکرد بهتری نشان داد. همچنین، نتایج تجزیه به مولفه‌های اصلی نشان داد که روش‌هایEO50S ، EO40Sو O40Sاز بیشترین بازدهی در استخراج مواد مؤثره گیاهی برخوردارند. در مجموع، یافته‌های این تحقیق نشان می‌دهد که روش اسمز با ترکیب فراصوت روشی کارآمد و دوستدار محیط‌زیست برای استخراج مواد مؤثره چای ترش است و می‌تواند در صنایع دارویی و غذایی برای استخراج ترکیبات مفید گیاهان دارویی به کار گرفته شود.

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