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Extraction of Bioactive Compounds of Broccoli with Deep Eutectic Solvents using Ultrasound Technique

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This research investigated the extraction of bioactive compounds from broccoli utilizing three choline chloride deep eutectic solvents (DESs)—DES1 (sorbitol), DES2 (glycerol), and DES3 (thiourea)—with ultrasound-assisted extraction. DES3 provided the greatest extract amount (7.8 g/100 g). HPLC showed higher levels of vitamins A and C, while vitamins E and K were present at lower levels. Successful identification and quantification of sulforaphane and several phenolic compounds, including catechin, gallic acid, and rutin, was accomplished. The existence of essential trace elements was confirmed by XRF spectrometry, including K, Mg, Ca, and Fe. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, terpenes, and glycosides. Quantum chemical calculations revealed that DES1 had the most stable structure. The findings illustrate that DESs are effective, green solvents for the extraction of a variety of bioactive compounds from broccoli, thus demonstrating the value of broccoli as a source of antioxidants and nutraceuticals.

Keywords: Broccoli, vitamins, DESs, amino acid, ultrasonic, phytochemical

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Broccoli, a versatile vegetable from the Brassicaceae family of cabbages, has a soft texture when cooked and a wonderful crunch when eaten raw [1]. Broccoli, closely akin to cauliflower in its growth habits, and thrives in cooler climates and gives significant health benefits. It plays an essential role in regulating blood sugar, and reducing cholesterol, and lowering blood pressure. Notably, broccoli is rich in sulforaphane, a compound with potent anti-cancer properties. While recognized for its nutritional value, broccoli cultivation remains exceedingly uncommon in Iraq, often being grown alongside cauliflower [2, 3]. Broccoli is a nutritional powerhouse, brimming with vitamins C and K, folate (vitamin B9), and dietary fiber. It also includes an array of antioxidants, including beta-carotene, and essential minerals such as potassium and manganese [4-6]. Consuming broccoli (Brassica oleracea var. Italica) gives a large number of fitness benefits. It strengthens the immune system, improves heart fitness, improves digestion, and can even have anti-cancer properties due to its rich antioxidant content [7]. Brassica oleracea var. Italica exhibits various functional properties associated with its secondary metabolites, including phenolic compounds, carotenoids, chlorophyll, alkaloids, glycosylates, and others, especially during extraction [8]. The liquid extraction technique is cost-effective, easy, fast, and gives excessive extraction efficiency. However, its number one drawback lies in the use of extraordinarily poisonous and environmentally unsafe solvents, along with chloroform, carbon tetrachloride, and chlorobenzene. As a result, there has been growing attention to greener, more environmentally friendly solvents as alternatives for liquid extraction [9-13]. Deep eutectic solvents (DESs), a brand-new era of green solvents, have attracted considerable interest from both medical and technological groups. Their enchantment lies in their precise characteristics, along with simple synthesis, low-value raw materials, and renewability [14-16]. DESs are normally composed of two or more environmentally friendly additives that interact through hydrogen bonding between a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) [17-19]. Hydrophilic DESs are broadly used in various fields, along with serving as solvents or catalysts in chemical reactions [20-22], applications in electrochemistry [23, 24], usage in pharmaceuticals [25], and involvement in separation techniques [26-28]. Liquid extraction- ultrasound with deep eutectic solvents (DESs) is a widely used extraction procedure that is cost-effective and straightforward. These extraction methods can be completed in less than one hour. Furthermore, this technique can be used to extract active substances and essential oils from plant sources. The extraction efficiency of active compounds from plant material depends on the type of deep eutectic solvents and the kind of plant material. In this study, various deep eutectic solvents were used to extract the bioactive compounds and evaluate the highest percentage of extraction of broccoli plants using the liquid extraction-ultrasound method.

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EXPERIMENTAL

Chemicals and Materials

The solvents and chemicals were used without purification. Three deep eutectic solvents were created: Choline Chloride and Sorbitol (DES1), Choline Chloride and glycerol (DES2), and Choline Chloride and thiourea (DES3). The Choline Chloride was acquired from ZhengZhou Mct Co. (China), Sorbitol from Zhucheng Dongxiao Co. (China), and glycerol and thiourea from Sigma-Aldrich Co. (Germany). The solvents prepared were characterized using FTIR spectra (Bruker, alpha 2 from Germany). This DES solvent was further characterized using 1H-NMR spectra (Bruker Bio Spin GmbH Spectrometer operating at 400 MHz, Germany), and this was executed in a deuterated DMSO solvent. For the extraction, an ultrasonic bath [Model XUB5, Formators (Type Solution), HPLC system (Sykam S3210, Germany), and X-ray fluorescence spectrometry (Rigaku Nex CG benchtop XRF, Texas, USA) was used to analyze samples.

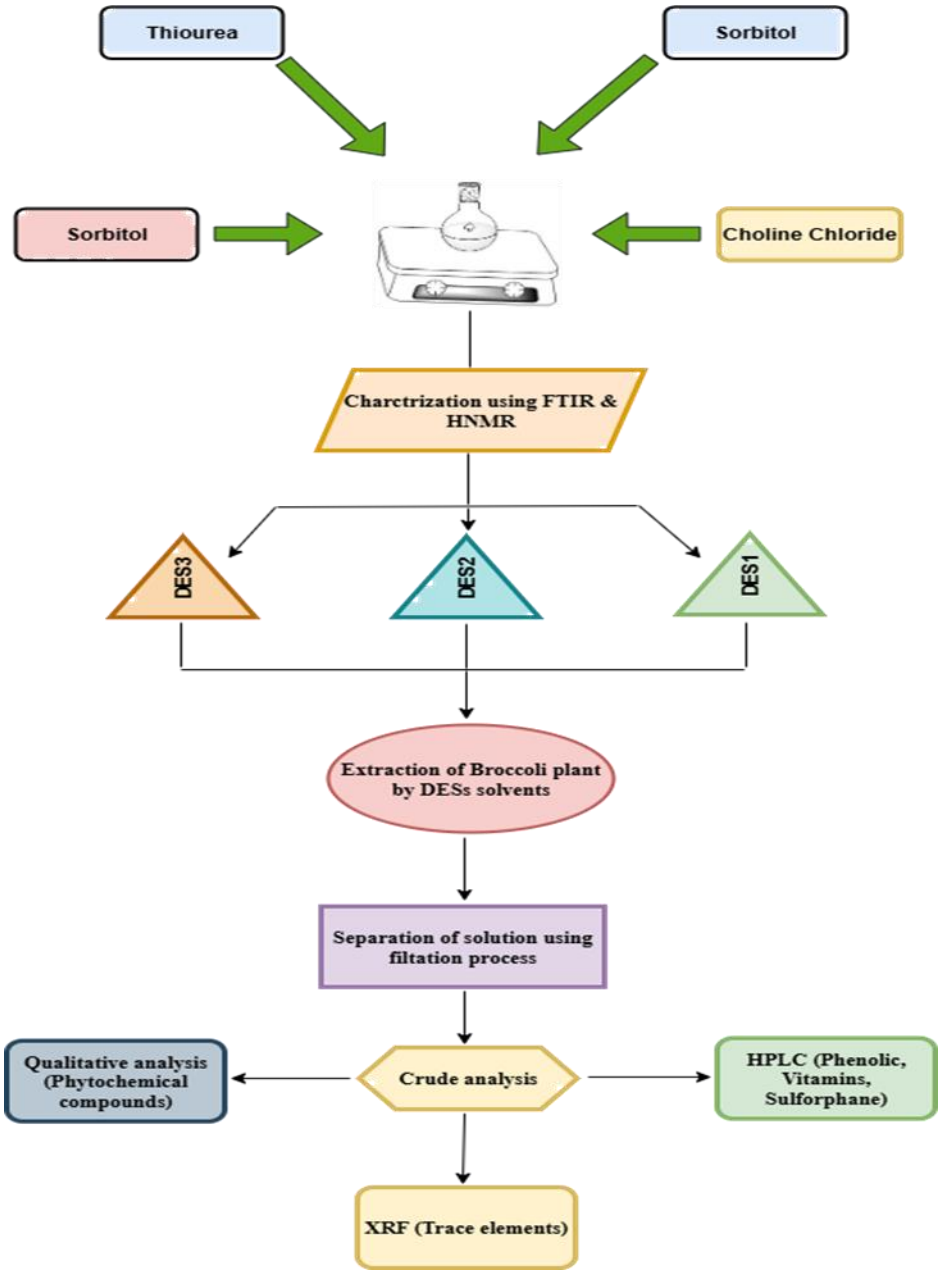

Figure 1. Schematic of Broccoli extraction.

Table. 1. Deep eutectic solvents used for extraction.

Abbreviation	Component 1	Component 2	Mole Ratio
DES1	Choline chloride	Sorbitol	1:1
DES2		Glycerol	
DES3		Thiourea	

Characterization Methods

Collecting Broccoli Plant

The Broccoli was acquired from a nearby market in Baghdad, Iraq, in September 2024. The entire plant material was rinsed with water to eliminate any soil particles. The substance is subjected to a process of air-drying at ambient temperature for many days, after which it is finely ground into a powder.

Preparation of Deep Eutectic Solvents (DESs)

The process of deep eutectic solvent preparation depends on the heating of the two individual components at 85 ^{0}C for DES1 and DES2, while DES3 was heated at 120 ^{0}C and continuously stirred on a magnetic stirring apparatus for 60–180min until the mixture was melted and the homogeneous solvents were formed [29]. The following DESs were prepared: Choline chloride + sorbitol (DES1), Choline chloride + glycerol (DES2), and Choline chloride + thiourea (DES3) (Table 1).

Extraction

The extraction was achieved with three deep eutectic solvents (DES1, DES2, and DES3) in a sonication water bath, providing dried powdered plant material: solvent ratio of 1:20. The conditions of the ultrasound device were set to 30 min at 50 ^{0}C. After the extraction, all samples were filtered, and the solution was collected for further analysis [38].

Determination of Vitamin Concentration

The HPLC system used in the experiment was a Sykam device from Germany. A C18 column with a diameter of 4.6 mm and a length of 25 cm was utilized. The UV detector was set to function at a wavelength of 280 nm, with a flow rate of 0.7 mL/min. The mobile phase consisted of an isocratic mixture of acetonitrile and distilled water in a 75:25 ratio. For sample preparation, (5 mL) of 0.1% butylhydroxytoluene in methanol was added to two mL of the sample, which was then saved in the dark for two hours in a tightly sealed container and stirred for 20 minutes at room temperature. Before injection into the column, the samples were centrifuged and analysed within 1 hour [30,31].

Determination of Sulforaphane

The HPLC equipment employed to determine the sulforaphane compound was the Sykam from Germany. The column used was a C18 column with a circumference of 4.6 mm and an overall length of 25 cm. The apparatus utilized for detecting UV at a wavelength of 235 nm. The liquid flow rate passing through the HPLC device is set at 1 mL /min. The mobile phase used consists of a combination of acetonitrile: Distilled Water: formic acid (75:25:5) [32].

Determination of Phenolic Compounds

The HPLC equipment utilized for the experiment was the Sykam from Germany. The column used was a C18 column with a circumference of 4.6 mm and an overall length of 25 cm. The apparatus is used for detecting UV at a wavelength of 280 nm. The flow rate of the liquid passing through the HPLC device is set at 1 mL per minute. The mobile phase used consists of a combination of methanol: Distilled Water: formic acid (75:25:5) [33,34].

Trace Elements Determination

The Iraqi Atomic Energy Commission/Central Laboratories Directorate in Baghdad used X-ray fluorescence spectrometry (XRF), Rigaku Nex CG benchtop XRF, Texas, USA, and Contemporary EDXRF spectrometers of the second generation, which provide fast and accurate identification and measurement of both main and trace atomic elements in a diverse range of samples. These samples can include oils, liquids, solids, metals, polymers, powders, pastes, coatings, and thin films. Extracts were administered intravenously into the device [35].

Phytochemical Compounds

The presence of active compounds in Broccoli extracts was determined through qualitative analysis using standard procedures. The following tests were conducted: alkaloids (Mayer's Test), steroids

(chloroform + concentrated H_2SO_4), terpenoids (Salkowski Test), carbohydrates (Benedict's reagent), glycosides (Keller Kilianin Test), proteins (NaOH + copper sulfate), saponins (Foam Test), phenols, tannins, and resins (lead acetate solution), coumarins (sodium hydroxide), and flavonoids (Alkaline reagent Test) [36].

RESULTS AND DISCUSSION

Three deep eutectic solvents (DES1, DES2, and DES3) were prepared using choline chloride as HBA and sorbitol, glycerol, and thiourea as HBD. The prepared compounds were characterized utilizing FTIR and H-NMR spectra.

DES1. FTIR: 3310 cm^{-1}(OH), 2810, 2930 cm^{-1} (C-H $_{al.}$); H-NMR (DMSO-d_6, 400 MHz): δ3.43 ($N(CH_3)$ of choline chloride), 3.53 (CH_2 of choline chloride), 3.65 (CH_2 attached with OH group), 4.10 (OH group in choline chloride), 2.71 (CH_2 attached with OH group in sorbitol), 4.31 (OH group of sorbitol).

DES2. FTIR: 3345 cm-1(OH), 2953, 2877 cm^{-1} ($CH_{al.}$); H-NMR (DMSO-d6, 400 MHz): δ3.30 ($N(CH_3)_3$ of choline chloride), 3.33(CH_2 attached with N(CH3)3), 3.36 (CH2 attached with OH group), 4.49 (OH- group in choline chloride), 3.2 (CH_2 attached with OH group).

DES3. FTIR: 3434 cm^{-1} (OH), 3296, 3210 (NH_2 in thiourea), 2975 cm^{-1}(C-H $_{al.}$); H-NMR (DMSO-d_6, 400 MHz): δ2.70 ($N(CH_3)_3$), 2.48 (CH_2 attached N(CH3)3), 3.38 (OH group of choline chloride), 7.13(NH group of thiourea).

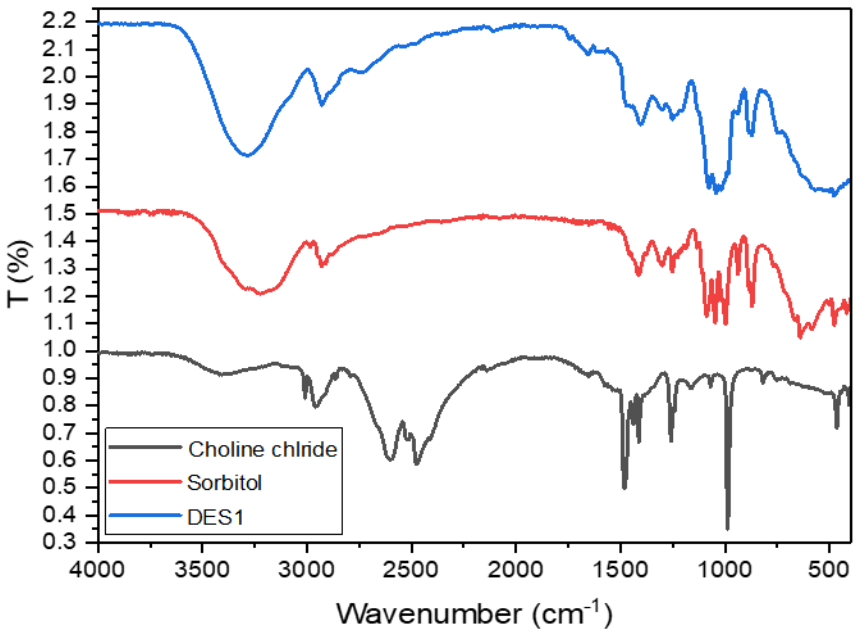

Figure 2. FTIR spectrum of DES1.

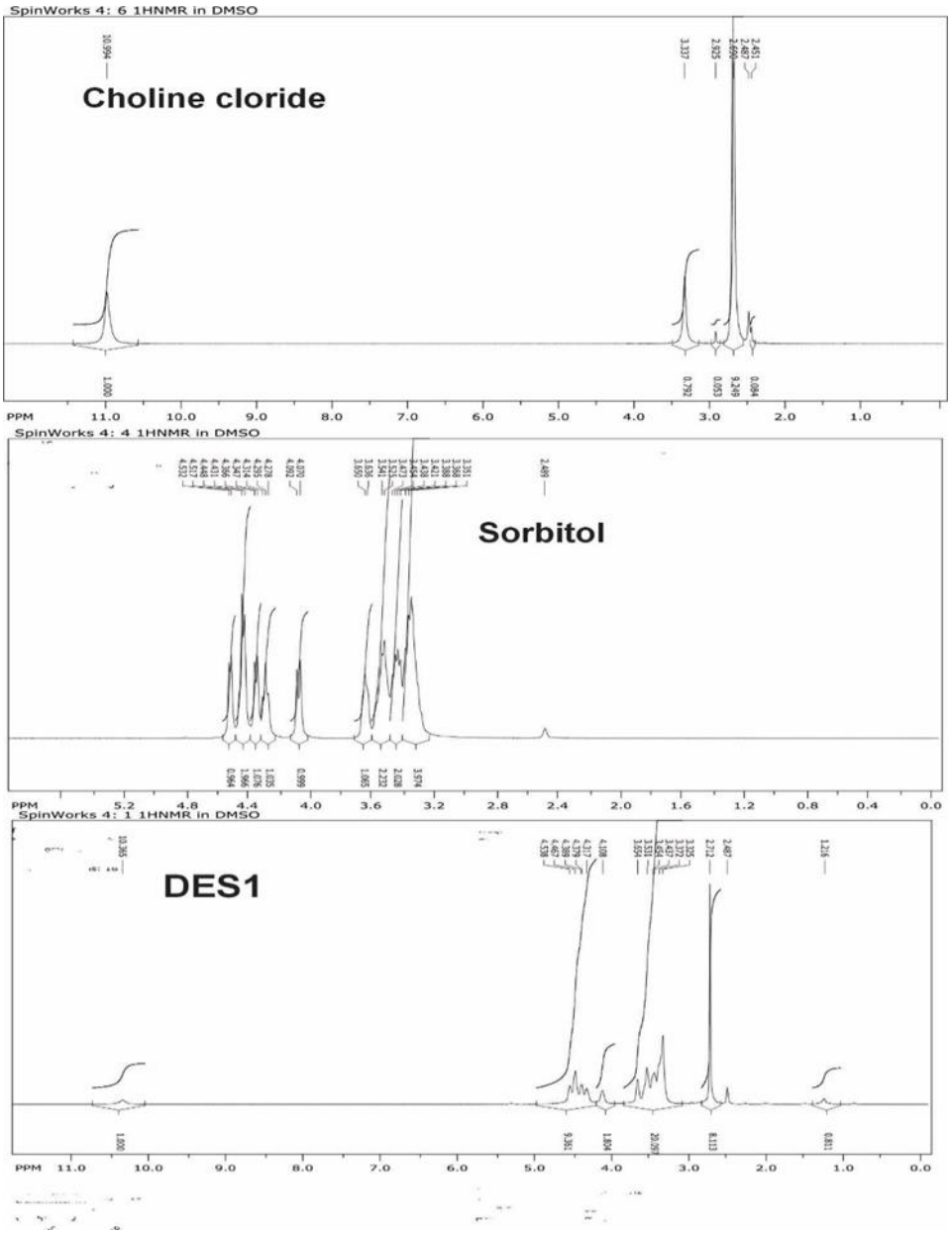

Figure 3. H-NMR spectrum of DES1.

Quantum Chemical Calculations

Figure 4 shows the structures of the DESs. Choline chloride interacted similarly with sorbitol, glycerol, and thiourea in a 1:1 ratio. In these combinations, the DESs were stabilized by hydrogen bonds between the chloride and the hydroxy group in sorbitol and glycerol, and also with the amino group in thiourea. Table 2 shows that DES1 was more stable than the others because of its better thermodynamic properties, such as the lowest total energy, strongest binding energy, most exothermic heat of formation, and most favorable electronic energy. These factors suggest that DES1 has a very stable and well-formed structure.

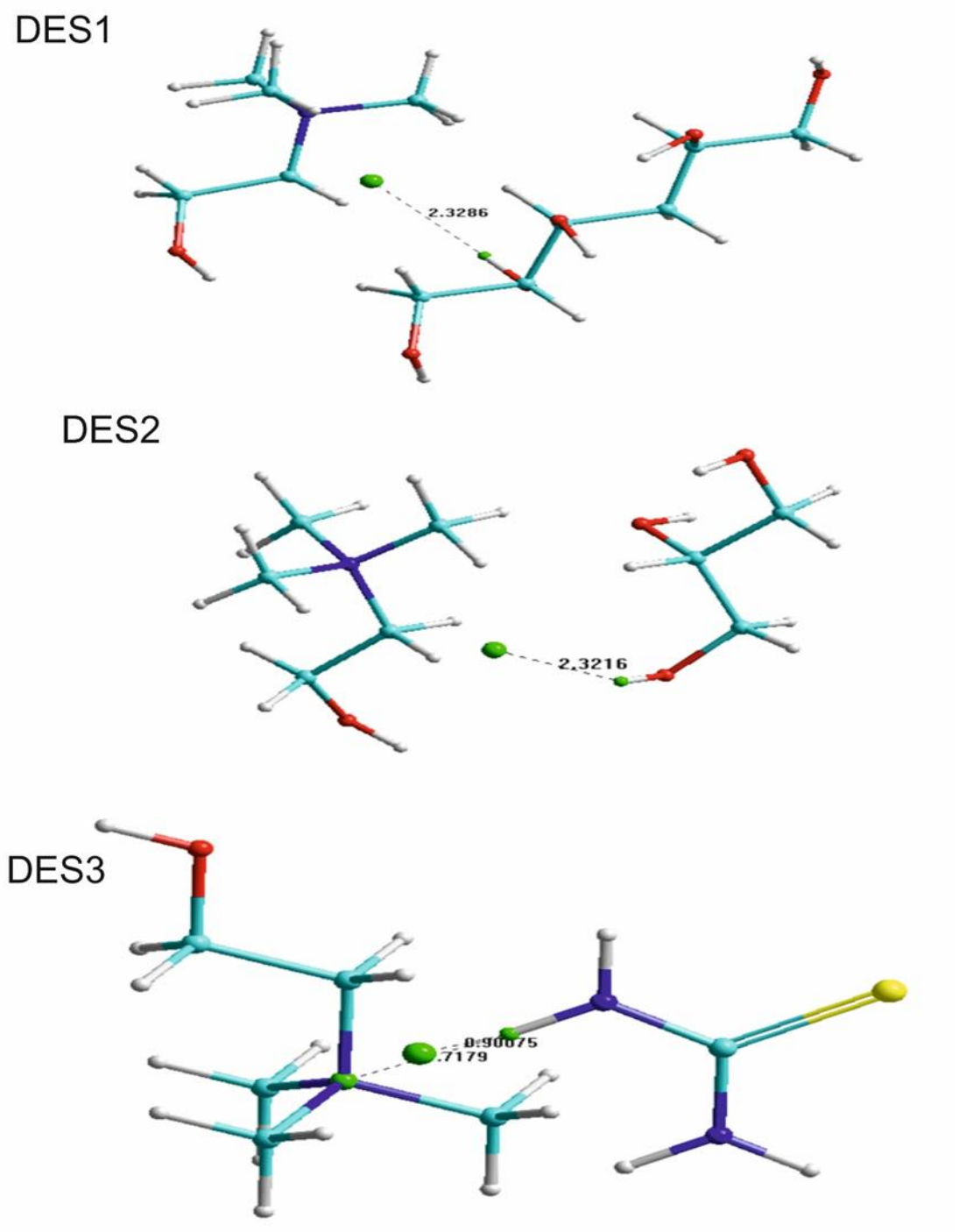

Figure 4. PM3-optimized structures of deep eutectic solvents.

After preparing the deep eutectic solvents, we use these solvents in the extraction of bioactive compounds from the broccoli plant utilizing an ultrasound device.

Vitamins Determination

Figure 5 (a, b, c) and Table 2 show the results of the HPLC system's measurements using Column C18 and wavelength (280 nm) with reference substances for each type of vitamin determined. Using the following equation, the area of the pick for the reference substance and the area of the pick for the required vitamin were compared to determine the concentration per vitamin:

$$C \text{ sample} = \frac{A\ sample}{A\ standard} \times C\ standard$$

Table 2. Thermodynamic properties of synthesized DESs.

Energy type	DES1	DES2	DES3
Total energy (Kcal/mol)	-91746.3	-64539.5	-52498.5
Binding energy (Kcal/mol)	-4150.6	-3297.4	-2605.4
Heat of formation (Kcal/mol)	-312.6	-188.3	-148.2
Electronic energy (Kcal/mol)	-669966.9	-391738.1	-315015.1
Nuclear energy (Kcal/mol)	578220.1	327198.6	262516.9
Hydrogen bonding (A^0)	2.328	2.3219	0.9007 1.7179

Table 3. Vitamin concentration extracted using DES1, DES2, and DES3.

Vitamins	DES1	DES2	DES3	Retention time (min)
Vit A (IU)	**415.6**	**400.5**	**441.2**	**3.62**
Vit C (ppm)	**288.7**	**260.4**	**321.4**	**5**
Vit K (ppm)	**19.8**	**12.1**	**22.8**	**7.6**
Vit E (ppm)	**78.9**	**70.1**	**83.9**	**8.14**

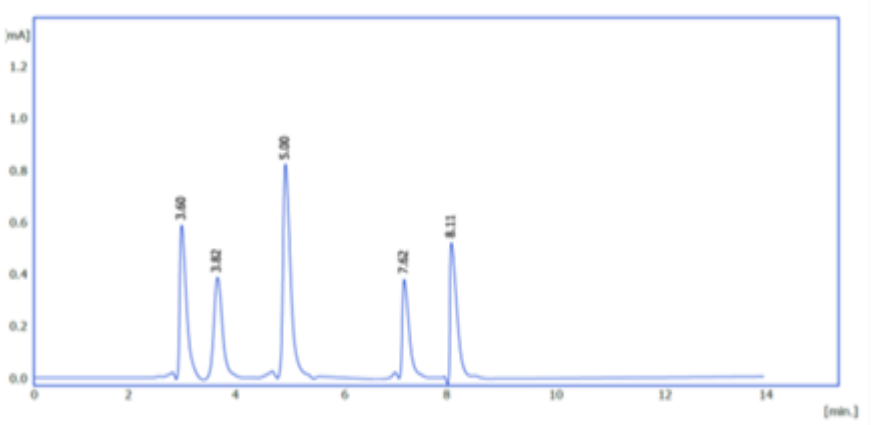

Figure 5(a). Chromatogram of DES1 extract for vitamins.

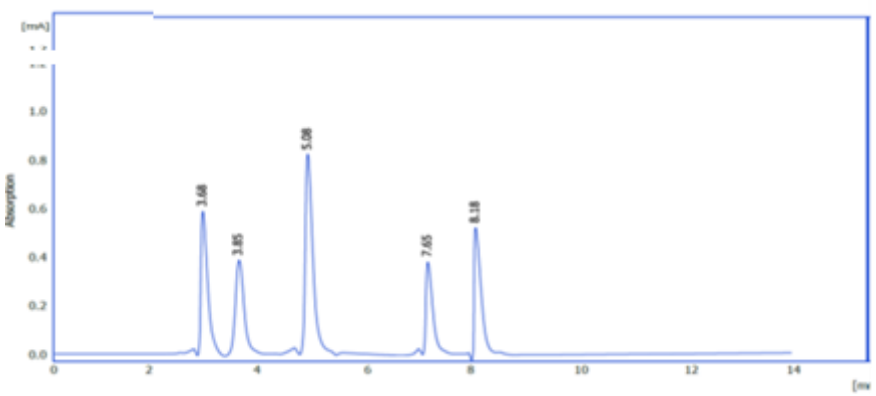

Figure 5(b). Chromatogram of DES2 extract for vitamins.

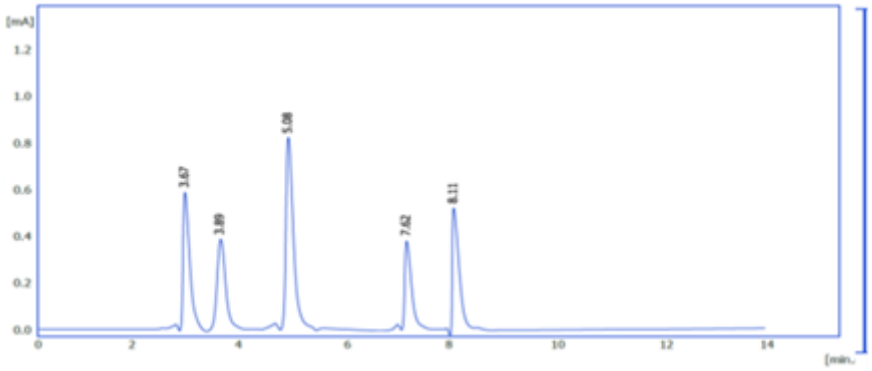

Figure 5(c). Chromatogram of DES3 extract for vitamins.

Table 4. Contents of trace elements in the analyzed samples of broccoli (ppm).

Element	DES1 extract (ppm)	DES2 extract (ppm)	DES3 extract (ppm)
Na	29413	21336	49477
Cl	22423	33732	55891
K	1858	2078	1841
S	858	986	73078
P	702	969	680
Mg	647	679	552
Al	507	618	880
Ca	181	153	189
Sn	93	89.2	104
Si	67.9	104	114
Te	53	39.5	59
Fe	22.1	24.7	38.5
Ni	19.2	20.5	26.1
Sb	13.5	7.03	15.9
Ag	7.84	7.26	9.52
Cu	3.58	3.42	4.92
Zn	2.96	3.68	3.98
Br	1.03	0.765	3.80
Cr	0.789	0.865	2.14
Rb	0.489	0.505	0.584
Mn	ND	4	6.88
Co	ND	0.977	2.25
V	ND	ND	3.28
Hf	ND	ND	2.29

Trace Elements Determination

The concentration of trace elements in the broccoli plant's three extracts (DES1, DES2, and DES3) has been measured by the XRF technique. The result is shown in the table 4.

Sulforaphane Determination

The results from the HPLC system measurements are shown in Table 4. These results were gathered using Column C18 at 235 nm for Sulforaphane, which was determined using reference substances. The concentration of Sulforaphane was found by comparing the peak area of the reference substance to the peak area of the Sulforaphane in question, using this equation:

$$sample = \frac{A\ sample}{A\ standard} \times C\ standard$$

Table 5. Quantification of sulforaphane concentration in the DES1, DES2, and DES3 extracts of the Broccoli plant.

Compound	Concentration of DES1 extract	Concentration of DES2 extract	Concentration of DES3 extract	Retention time(nm)
Sulforaphane (ppm)	221.3	203.9	208	6.12

Phytochemical Compounds

Qualitative phytochemical screening for active components in broccoli plant extracted in three solvents (DES1, DES2, and DES3) is shown in Table 6 below:

Table 6. Experiment Reagents and Chemical Detection broccoli extracts (DES1, DES2, and DES3) Active Compound.

No.	Type of Test	DES1 extract	DES2 extract	DES3 extract
1	**Tannins Test**	+	+	+
2	**Carbohydrate Test**	+	+	+
3	**Glycosides Test**	+	+	+
4	**Phenols Test**	-	+	+
5	**Resins Test**	-	-	-
6	**Flavonoid's Test**	+	+	+
7	**Saponin Test**	+	+	+
8	**Alkaloid Test**	+	+	-
9	**Protein Test**	-	-	-
10	**Coumarins Test**	+	+	-
11	**Terpenes Test**	+	-	+
12	**Steroids Test**	-	-	-

Table 7. Quantification of phenolic compounds concentration in the DES1, DES2, and DES3 extracts of the Broccoli plant.

Compound	Concentration of DES1 extract	Concentration of DES2 extract	Concentration of DES3 extract	Retention time (nm)
Catechine	22.6	33.6	28.7	5.78
Epicatechine	18.7	25.4	22.6	8.3
Gallic acid	41.6	53.6	50.9	10.22
Kaempferol	11.6	20.3	17.4	3.15
Qurcetine	30.1	40.6	45.6	2.00
Rutin	35.9	44	40.4	4.09

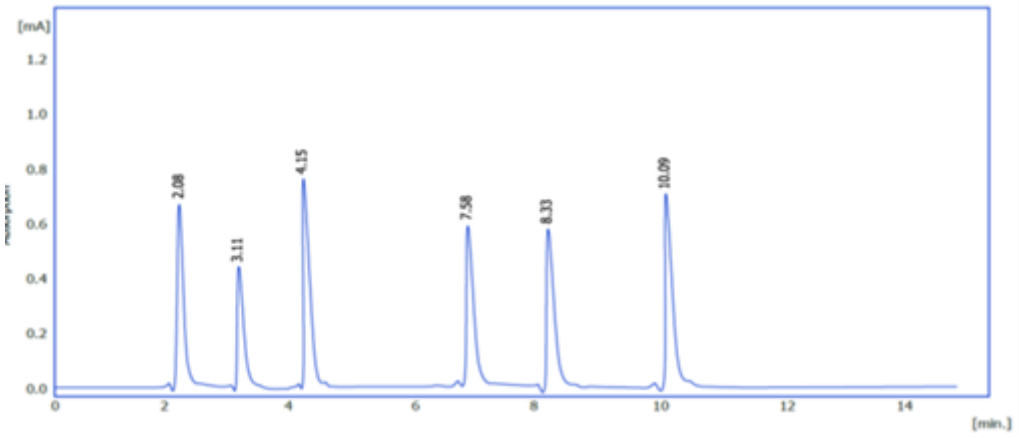

Figure 6(a). Chromatogram of DES1 extract for phenolic compounds.

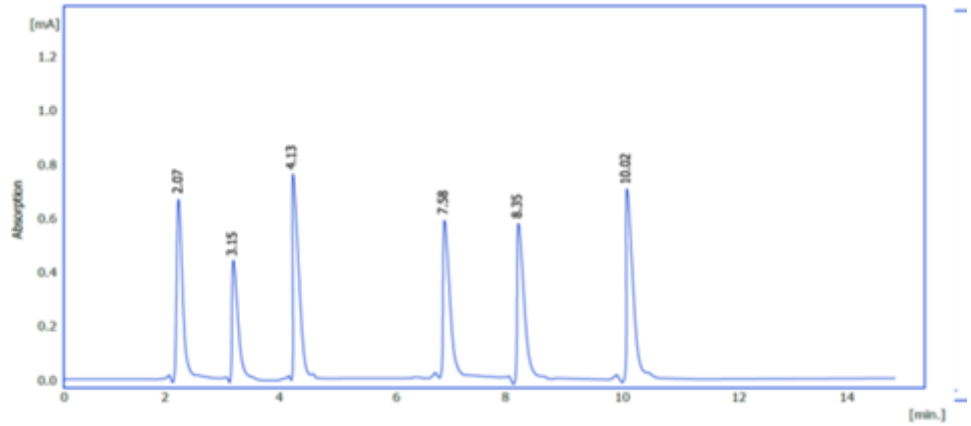

Figure 6(b). Chromatogram of DES2 extract for phenolic compounds.

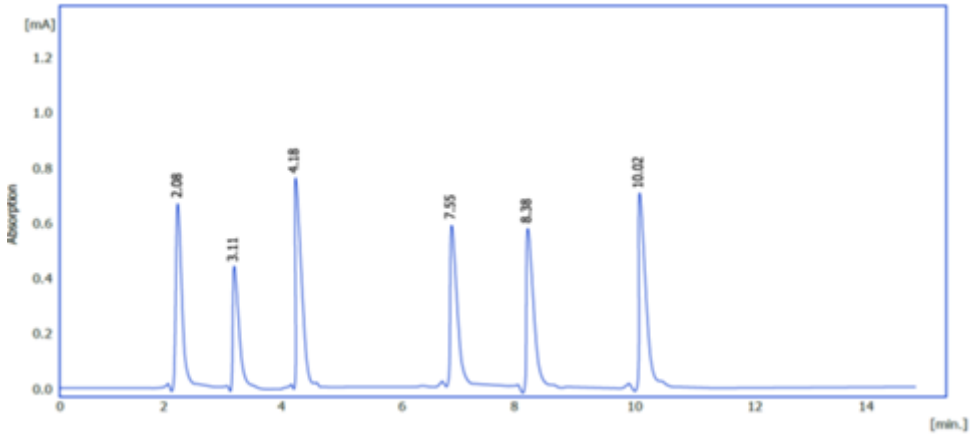

Figure 6(c). Chromatogram of DES3 extract for phenolic compounds.

Phenolic Compounds Determination

The phenolic compounds were extracted with three deep eutectic solvents (DES1, DES2, and DES3), and the concentration was determined using the HPLC approach. The results are shown in Table 7 and Figure 6 (a,b,c).

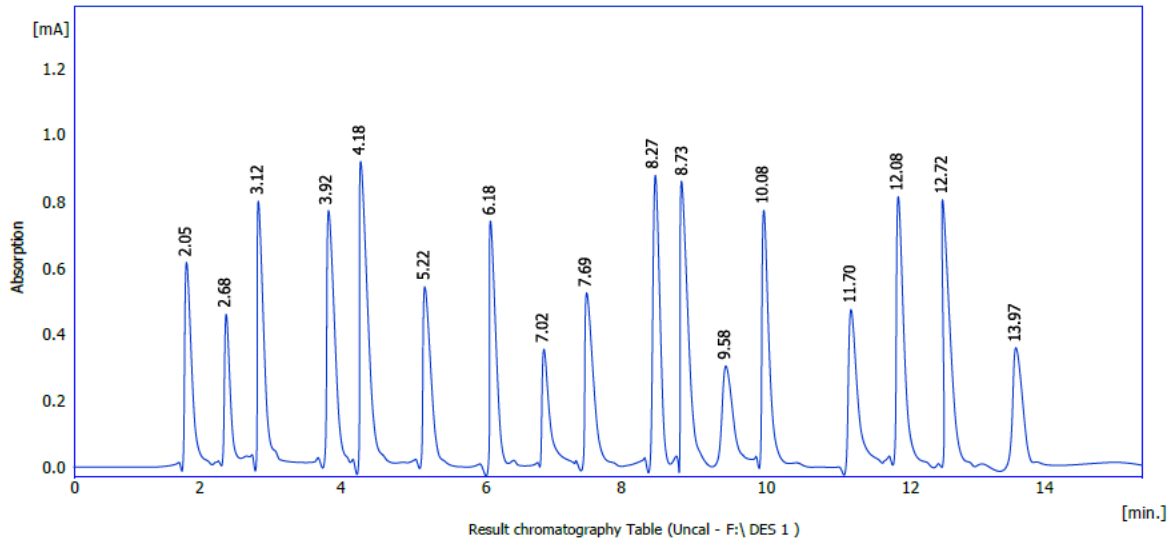

Figure 7(a). Chromatogram of DES1 extract for amino acids.

Table 8(a). DES1 extract for amino acid.

No	Retention.Time [min]	Area [mAU.s]	Height [mAU]	Amount (g/100gm)	Calculation	Peak type	Compound Name
1	2.05	2105.6	600.1	6.25	Calibration carve	Order	Aspartic acid
2	2.68	1452.6	435.6	4.11	Calibration carve	Order	Glycine
3	3.12	3587.7	789.8	9.58	Calibration carve	Order	Lysine
4	3.92	3698.8	774.9	10.22	Calibration carve	Order	Serine
5	4.18	4569.7	820.6	13.08	Calibration carve	Order	Threonine
6	5.22	1874.4	564.9	6.25	Calibration carve	Order	Isoleucine
7	6.18	3022.5	755.8	8.22	Calibration carve	Order	Alanine
8	7.02	1102.6	365.9	4.12	Calibration carve	Order	Valine
9	7.69	1578.9	524.9	6.25	Calibration carve	Order	Tyrosine
10	8.27	3554.1	811.2	12.33	Calibration carve	Order	Arginine
11	8.73	3412.0	807.9	15.65	Calibration carve	Order	Cysteine
12	9.58	856.9	264.7	3.25	Calibration carve	Order	Methionine
13	10.08	2564.1	741.1	10.12	Calibration carve	Order	Proline
14	11.7	1010.1	412.6	5.77	Calibration carve	Order	Histidine
15	12.08	3665.0	710.5	10.36	Calibration carve	Order	Lucien
16	12.72	3547.7	711.9	12.65	Calibration carve	Order	Glutamic acid
17	13.97	986.4	365.0	3.14	Calibration carve	Order	Phenylalanine

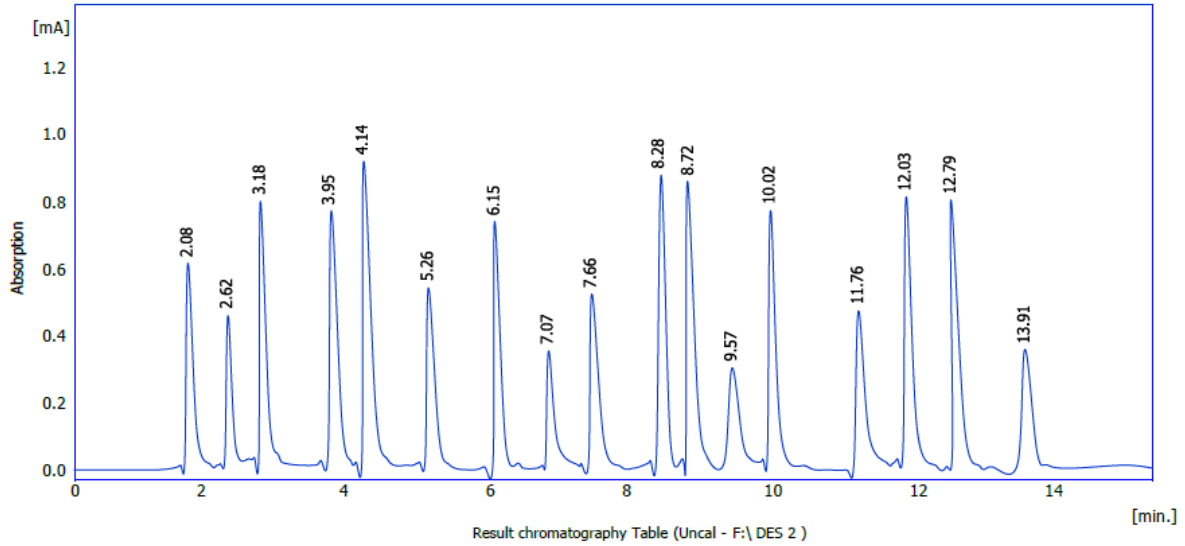

Figure 7(b). Chromatogram of DES2 extract for amino acids.

Table 8(b). DES2 extract for amino acid.

No	Retention.Time [min]	Area [mAU.s]	Height [mAU]	Amount (g/100gm)	Calculation	Peak type	Compound Name
1	2.08	2250	602.6	7.45	Calibration carve	Order	Aspartic acid
2	2.62	1512.6	434.9	6.08	Calibration carve	Order	Glycine
3	3.18	3611.4	781.5	11.62	Calibration carve	Order	Lysine
4	3.95	3795.1	776.5	13.65	Calibration carve	Order	Serine
5	4.14	4632.5	824.6	15.98	Calibration carve	Order	Threonine
6	5.26	1952.1	575.9	8.74	Calibration carve	Order	Isoleucine
7	6.15	3225.9	765.0	10.33	Calibration carve	Order	Alanine
8	7.07	1321.4	374.5	6.12	Calibration carve	Order	Valine
9	7.66	1698.5	532.6	8.97	Calibration carve	Order	Tyrosine
10	8.28	3789.0	812.5	14.15	Calibration carve	Order	Arginine
11	8.72	4125.9	81.1	18.07	Calibration carve	Order	Cysteine
12	9.57	1120.5	275.3	6.25	Calibration carve	Order	Methionine
13	10.02	6521.4	745.0	13.44	Calibration carve	Order	Proline
14	11.76	2650.1	415.0	8.70	Calibration carve	Order	Histidine
15	12.03	3985.8	715.7	14.66	Calibration carve	Order	Lucien
16	12.79	7845.9	713.5	15.98	Calibration carve	Order	Glutamic acid
17	13.91	1124.5	366.9	6.90	Calibration carve	Order	Phenylalanine

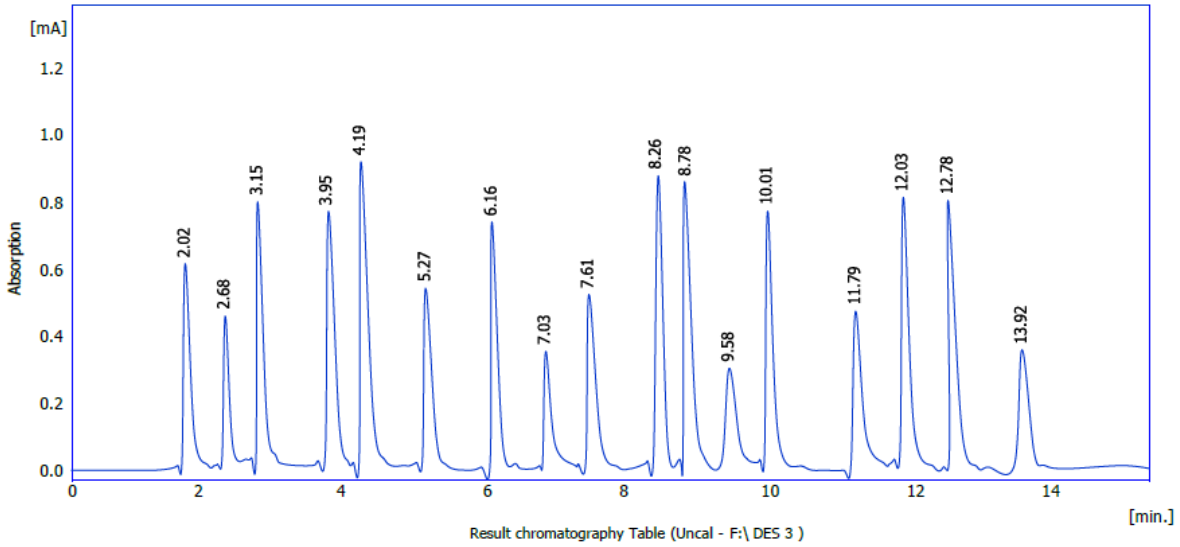

Figure 7(c). Chromatogram of DES3 extract for amino acids.

Table 8(c). DES3 extract for amino acid.

No	Retention.Time [min]	Area [mAU.s]	Height [mAU]	Amount (g/100gm)	Calculation	Peak type	Compound Name
1	2.02	2201.1	605.9	7.01	Calibration carve	Order	Aspartic acid
2	2.68	1488.7	431.4	5.85	Calibration carve	Order	Glycine
3	3.15	3555.8	785.9	11.14	Calibration carve	Order	Lysine
4	3.95	3621.3	771.4	13.32	Calibration carve	Order	Serine
5	4.19	4512.6	822.0	15.41	Calibration carve	Order	Threonine
6	5.27	1822.5	573.6	8.25	Calibration carve	Order	Isoleucine
7	6.16	3165.1	765.0	10.00	Calibration carve	Order	Alanine
8	7.03	1235.8	376.4	6.00	Calibration carve	Order	Valine
9	7.61	1574.3	532.8	8.52	Calibration carve	Order	Tyrosine
10	8.26	3521.4	817.7	13.89	Calibration carve	Order	Arginine
11	8.78	4085.7	813.6	17.84	Calibration carve	Order	Cysteine
12	9.58	1099.8	271.4	13.00	Calibration carve	Order	Methionine
13	10.01	6265.9	741.1	13.00	Calibration carve	Order	Proline
14	11.79	2521.0	410.9	8.52	Calibration carve	Order	Histidine
15	12.03	3845.1	713.5	14.32	Calibration carve	Order	Lucien
16	12.78	7774.2	711.4	15.41	Calibration carve	Order	Glutamic acid
17	13.92	1065	365.9	6.36	Calibration carve	Order	Phenylalanine

Amino Acid Determination

Amino acids provide numerous benefits for human health, as they play roles in antibacterial, antioxidant, anti-thrombotic, immuno-stimulating, and anti-inflammatory activities, among other positive effects on the body. These compounds are present in the broccoli plant, and their extraction and identification necessitate different analytical techniques, such as supercritical fluid extraction (SFE), solid-phase extraction (SPE), acid digestion, hydrolysis, or solvent extraction, due to the complexity of these compounds [37]. We used solvent extraction with deep eutectic solvents to extract the amino acids and determine their concentration using the HPLC technique, as shown in Table 8(a-c) and Figure 7 (a, b, c).

The results from this study demonstrate that broccoli extract includes numerous active compounds, highlighting its potential as a highly active plant with significant implications for pharmaceutical and biological functions. Phytochemical screening of medicinal plants indicates a correlation between active constituents and various biological and pharmacological properties. All the findings of this work, concerning the vitamin, mineral, amino acids, or chemical compound composition of the broccoli plant, aligned reasonably well with those from different previous studies. The notable differences in results compared to earlier research can be attributed to the diverse growth habitats of the plants studied, as well as environmental factors such as soil and irrigation water quality, temperature, climate, and other influences that significantly affect the chemical composition of plants.

CONCLUSION

In summary, this study has shown that three choline chloride-based deep eutectic solvents (DESs) can be effective "green" media for the ultrasound-assisted extraction of a wide range of bioactive compounds present in broccoli. Among the three solvents, DES3 (choline chloride-thiourea) was found to be the most effective in the extraction, yielding the highest extract. Following further analysis, no less than significant amounts of ER vitamins (especially A and C), sulforaphane, which is currently recognized as an important anti-cancer compound, a range of different phenolic compounds, numerous amino acids, important essential trace elements, and further a variety of phytochemicals such as flavonoids and alkaloids had been extracted. The presence of exciting phytochemicals during extraction, followed by the screening of isolated compounds, is supportive of the plant having potential health benefits and antioxidant properties. Overall, this study confirms that DESs are a viable alternative "green" option to organic solvents for valorizing broccoli and still maintaining its health benefits. These bioactive compounds extracted highlight the potential for broccoli to be an inexpensive and surplus source of nutraceuticals and functional food components. The green extraction process proposed in this study presents further options for the utilization of natural products in the pharmaceutical and food industries, while incorporating sustainable chemistry principles.

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REFERENCES

1. Baltacıoğlu, C. and Tartıcı, R. (2022) Bioactive component extraction from broccoli (Brassica oleracea L. var. italica) and optimization by Taguchi method. *Niğde Ömer Halisdemir Üniversitesi Mühendislik Bilim. Derg.*, **11(4)**, 945–951.

2. Gudiño, I., Casquete, R., Martín, A., Wu, Y. and Benito, M. J. (2024) Comprehensive Analysis of Bioactive Compounds, Functional Properties, and Applications of Broccoli By-Products. *Foods*, **13(23)**, 3918.

3. Zainab, N. M. H. A. -B. and Al-Sudani, A. J. (2021) Effect of Planting Date and Spraying with Sulfur on Growth and Yield of Broccoli in the Desert Lands of Southern Iraq. *Euphrates J. Agric. Sci.*, **13(1)**.

4. Uvaraj, D., Alharbi, N. S., Kadaikunnan, S., Thiruvengadam, M. and Venkidasamy, B. (2024) Comprehensive study on the differential extraction and comparison of bioactive health potential of the Broccoli (Brassica oleracea). *Int. J. Med. Sci.*, **21(4)**, 593,

5. Siomos A. S., Koularmanis, K. and Tsouvaltzis P. (2022) The impacts of the emerging climate change on broccoli (Brassica oleracea L. var. italica Plenck.) crop. *Horticulturae*, **8(11)**, 1032.

6. Syed, R. U., Moni, S. S., Bin Break, M. K., Khojali, W. M. A., Jafar, M., Alshammari, M. D., Abdel Salam, K., Taymour, S., Alreshdi, K. S. M. and Mohan, S. (2023) Broccoli: A multi-faceted vegetable for health: An in-depth review of its nutritional attributes, antimicrobial abilities, and anti-inflammatory properties. *Antibiotics*, **12(7)**, 1157.

7. Zhang, Y. (2010) Allyl isothiocyanate as a cancer chemopreventive phytochemical. *Mol. Nutr. Food Res.*, **54(1)**, 127.

8. Le, T. N., Chiu, C. -H. and Hsieh, P. -C. (2020) Bioactive compounds and bioactivities of Brassica

oleracea L. var. italica sprouts and microgreens: An updated overview from a nutraceutical perspective. *Plants*, **9(8)**, 946.

9. Kareem, N. K., Thani, M. Z. and Al-Rawi, K. F. (2022) New microextraction methods for the evaluation of bromhexine HCl in pure and pharmacological formulations. *Egypt. J. Chem.*, **65(4)**, 1–2.

10. Kareem, N. K., Thani, M. Z. and Al-Rawi, K. F. (2022) New approach for determination of Phenylephrine HCl in Pure and Pharmaceutical Formulation using a various Microextraction Methods. *Res. J. Pharm. Technol.*, **15(4)**, 1648–1652.

11. Dadoosh, S. A., Thani, M. Z., Abdullah, A. M., Fahad, A. S. and Fahad, Y. S. (2021) Development of an Ecological-friendly Method for Dexamethasone Determination and Cloud Point Extraction in pharmaceutical formulations using Schiff Base Reaction. *Egypt. J. Chem.*

12. Thani, M. Z., Dadoosh, S. A., Fahad, A. S., Abdullah, A. M. and Fahad, Y. S. (2022) Synthesis of New Azo Compound and its Application for Spectrophotometric Determination of Sulfamethoxazole and Extraction using Cloud Point Extraction. *International Journal of Drug Delivery Technology*, **12(03)**, 1311–1318.

13. Ge, D., Shan, Z., Pang, T., Lu, X. and Wang, B. (2021) Preparation of new hydrophobic deep eutectic solvents and their application in dispersive liquid–liquid microextraction of Sudan dyes from food samples. *Anal. Bioanal. Chem.*, **413(15)**, 3873–3880.

14. Qu, Q., Lv, Y., Liu, L., Row, K. H. and Zhu, T. (2019) Synthesis and characterization of deep eutectic solvents (five hydrophilic and three hydrophobic), and hydrophobic application for microextraction of environmental water samples. *Anal. Bioanal. Chem.*, **411**, 7489–7498.

15. Zhang, H., Qiao, X., Cai, T., Chen, J., Li, Z. and Qiu, H. (2017) Preparation and characterization of carbon dot-decorated silica stationary phase in deep eutectic solvents for hydrophilic interaction chromatography. *Anal. Bioanal. Chem.*, **409**, 2401–2410.

16. Tang, B., Park, H. E. and Row, K. H. (2014) Preparation of chlorocholine chloride/urea deep eutectic solvent-modified silica and an examination of the ion exchange properties of modified silica as a Lewis adduct. *Anal. Bioanal. Chem.*, **406**, 4309–4313.

17. Ozturk, B., Parkinson, C. and Gonzalez-Miquel, M. (2018) Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. *Sep. Purif. Technol.*, **206**, 1–13.

18. Trivedi, T. J., Lee, J. H., Lee, H. J., Jeong, Y. K. and Choi, J. W. (2016) Deep eutectic solvents as attractive media for CO 2 capture. *Green Chem.*, **18(9)**, 2834–2842.

19. Germani, R., Orlandini, M., Tiecco, M. and Del Giacco, T. (2017) Novel low viscous, green and amphiphilic N-oxides/phenylacetic acid based Deep Eutectic Solvents. *J. Mol. Liq.*, **240**, 233–239.

20. Lamei, N., Ezoddin, M., Ardestani, M. S. and Abdi, K. (2017) Dispersion of magnetic graphene oxide nanoparticles coated with a deep eutectic solvent using ultrasound assistance for preconcentration of methadone in biological and water samples followed by GC–FID and GC–MS. *Anal. Bioanal. Chem.*, **409**, 6113–6121.

21. Li, Z., Jia, J., Wang, M., Zhang, H., Yan, H. and Qiao, F. (2017) Bifunctionalized ordered mesoporous organosilica synthesized in deep eutectic solvent for extraction of triazine herbicides from watermelon. *J. Chromatogr. A*, **1529**, 50–56.

22. Massolo, E., Palmieri, S., Benaglia, M., Capriati, V. and Perna, F. M. (2016) Stereoselective organocatalysed reactions in deep eutectic solvents: Highly tunable and biorenewable reaction media for sustainable organic synthesis. *Green Chem.*, **18(3)**, 792–797.

23. Abbott, A. P., El Ttaib, K., Frisch, G., Ryder, K. S. and Weston, D. (2012) The electrodeposition of silver composites using deep eutectic solvents. *Phys. Chem. Chem. Phys.*, **14(7)**, 2443–2449.

24. Jhong, H. -R., Wong, D. S. -H., Wan, C. -C., Wang, Y. -Y. and Wei, T. -C. (2009) A novel deep eutectic solvent-based ionic liquid used as electrolyte for dye-sensitized solar cells. *Electrochem. Commun.*, **119(1)**, 209–211.

25. Morrison, H. G., Sun, C. C. and Neervannan, S. (2009) Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles. *Int. J. Pharm.*, **378(1-2)**, 136–139.

26. Shishov, A., Bulatov, A., Locatelli, M., Carradori, S. and Andruch, V. (2017) Application of deep eutectic solvents in analytical chemistry. A review. *Microchem. J.*, **135**, 33–38.

27. Fu, N., Lv, R., Guo, Z., Guo, Y., You, X., Tang, B., Han, D., Yan, H. and Ho Row, K. (2017) Environmentally friendly and non-polluting solvent pretreatment of palm samples for

polyphenol analysis using choline chloride deep eutectic solvents. *J. Chromatogr. A*, **1492**, 1–11.

28. Posada, E., Roldán-Ruiz, M. J., Riobóo, R. J. J., Gutiérrez, M. C., Ferrer, M. L. and Del Monte, F. (2019) Nanophase separation in aqueous dilutions of a ternary DES as revealed by Brillouin and NMR spectroscopy. *J. Mol. Liq.*, **276**, 196–203.

29. Martinović, M., Krgović, N., Nešić, I., Žugić, A. and Tadić, V. M. (2022) Conventional vs. green extraction using natural deep eutectic solvents—Differences in the composition of soluble unbound phenolic compounds and antioxidant activity. *Antioxidants*, **11(11)**, 2295.

30. Zhang, Y., Zhou, W., Yan, J., Liu, M., Zhou, Y., Shen, X., Ma, Y., Feng, X., Yang, J. and Li, G. (2018*)* A review of the extraction and determination methods of thirteen essential vitamins to the human body: An update from 2010. *Molecules*, **23**(**6)**, 1484.

31. Ibraheem, A. K., Thani, M. Z. and Mohammed, M. T. (2023) Determination of Vitamins, Trace Elements, and Phytochemical Compounds in Ginkgo Biloba Leaves Extracts. *Egypt. J. Chem.*, **66(4)**, 159–166.

32. Li, Z. (2012*)* Development and verification of sulforaphane extraction method in cabbage (Brassica oleracea L. var. capitata) and broccoli (Brassica oleracea L. var. italica Planch.). *J. Med. Plants Res.*, **6(33)**, 4796–4803.

33. AlJuhaimi, F., Mohamed Ahmed, I. A., Özcan, M. M., Uslu, N. and Albakry, Z. (2024) Quantitative Determination of Biogenic Element Contents and Phytochemicals of Broccoli (Brassica oleracea var. italica) Cooked Using Different Techniques. *Plants*, **13(10)**, 1283.

34. Hashim, H. A., Mohammed, M. T. and Thani, M. Z. (2023) Study of Some Boswellia Carterii Contents and Effect of their Extracts as Anti-oxidants and Antibacterial. *J. Surv. Fish. Sci.*, **10(3S)**, 5704–5718.

35. Chaplenko, A., Monogarova, O. and Oskolok, K. (2018) X-Ray Fluorescence Determination of Trace Elements in Vitamin-Mineral Complexes and Medicinal Herbs Using Chemically Modified Polyurethane Foam Sorbents. *Iraqi J. Pharm. Sci. (P-ISSN 1683-3597 E-ISSN 2521-3512)*, 1–6.

36. Harborne, A. J. (1998) Phytochemical methods a guide to modern techniques of plant analysis. *Springer Science & Business Media*.

37. Ares, A. M., Nozal, M. J. and Bernal, J. (2013) Extraction, chemical characterization and biological activity determination of broccoli health promoting compounds. *J. Chromatogr. A*, **1313**, 78–95.

38. Martinović, M., Krgović, N., Nešić, I., Žugić, A. and Tadić, V. M. (2022) Conventional vs. green extraction using natural deep eutectic solvents—Differences in the composition of soluble unbound phenolic compounds and antioxidant activity. *Antioxidants*, **11(11)**, 2295.