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Comparison of Microwave, Ultrasonic Bath and Homogenizer Extraction Methods on the Bioactive Molecules Content of Green Tea (*Camellia Sinensis*) Plant.

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Abstract

In this study, the effects of three different extraction methods on the antioxidant capacity, phenolic component, volatile organic molecule and amino acid contents of green tea (Camellia sinensis) leaves were compared. Microwave digestion assisted extraction (MDAE), ultrasonic bath assisted extraction (UBAE) and homogenizer assisted extraction (HAE) methods were used as extraction methods. When the MDAE, UBAE and HAE extraction methods were compared according to the ferric reducing antioxidant power (FRAP) antioxidant activity results, 51.95, 50.9 and 13.8 TE/g dry weight (DW) results were obtained in green tea plant, respectively. DPPH activity in green tea was found to be 94.65, 69.7 and 36.35 TE/g dry weight in microwave extraction, ultrasonic bath and homogenizer extractions, respectively. Analysis in green tea leaves as a result of gas chromatography-mass spectrometry (GC-MS), caffeine, phytol, palmitic acid, beta.-monoglyceride, 13-docosenamide, (z)- and phytol were identified in all three extractions. Fourteen amino acid types were determined in green tea leaves by liquid chromatography-diode array detection (HPLC-DAD). Especially aspargine, glutamine, alanine, serine and L-theanine were determined in very high amounts. When the analysis data were compared, it was determined that the best results were in MDAE>UBAE>HAE, respectively.

Keywords: Extraction, green tea, HPLC-DAD, amino acid, phenolic compounds, LC-MS/MS.

1. Introduction

Extraction is defined as the whole process of separating bioactive components from plant or animal tissues from inert or inactive parts using selective solvents [1]. An ideal extraction method should be simple, inexpensive, fast and environmentally friendly, and should ensure that the desired component is obtained in high yield [2]. As a result of the extraction, the bioactive components should be obtained pure and should not be lost or decomposed. Efficiency in solid-liquid extraction based on the principle of obtaining one or a part of the components of a solid substance using a suitable solvent; it is affected by factors such as solvent type, pH, solid/liquid ratios, particle size, temperature and time [3]. Extraction of bioactive components from plant materials is of particular interest to the cosmetics, herbal medicine and food industries [4]. Modern extraction methods are encountered in classical extraction; efforts to eliminate the negativities such as long extraction time, high cost, high purity solvent requirement, the necessity of evaporation of large amounts of solvent, low extraction selectivity and thermal degradation of temperature-sensitive components have led to the development of new extraction techniques [3, 5]. The basic properties sought in extraction techniques developed today are use of more reliable chemicals, energy efficiency design, and use of renewable raw materials, prevention of pollution, shortened extraction time, low cost and prevention of accidents [5]. Methods such as, supercritical flow, pressurized liquid extraction, ultrasound, enzyme, microwave, accentuated electric field, homogenizer assisted extraction have been developed as modern extraction methods. These methods are referred to as 'green techniques' as they conform to standards set by the US Environmental Protection Agency [6]. Microwave Assisted Extraction is known as a new method that can be used for the extraction of components dissolved in the liquid from the material using microwave energy [3]. Microwaves are electromagnetic fields in the range of

300 MHz to 300 GHz. Pan et al. (2003) stated that in the microwave assisted extraction process they applied for the extraction of polyphenols and caffeine from green tea leaves, they obtained higher yields than other extraction methods applied in 20 hours at room temperature [7]. The Ultrasound Assisted Extraction method is a technique that uses ultrasonic waves, which are mechanical waves that propagate in an elastic medium, to degrade the plant cell wall and accelerate mass transfer, enabling the desired bioactive components to be obtained in a shorter time and with higher efficiency compared to classical techniques. In addition, it is an environmentally friendly technology with lower energy consumption and less solvent use [8,9]. Homogenizer Assisted Extraction is generally used to homogenize samples at high speed. At high cutting speed, it allows the plant to break in a few seconds and, as a result, to release the components. Since it has a high shear rate, it is faster to pass the bioactive components into the solution compared to other extraction methods. Once the mechanism is evaluated, the ultra-homogenizer can be used for the extraction of total phenolic compounds [6, 10, 11].

Tea is one of the most popular beverages worldwide. According to the production process, tea; It is classified as green and white tea (unfermented), oolong tea (semi-fermented), black tea (fully fermented) and puerh tea (post fermented) . It has been observed that tea has very important physiological and pharmacological activities due to characteristic components such as caffeine, polyphenol/flavonoids, amino acids (AA) and carbohydrates [12]. Green tea has various biological activities such as anti-tumor, anti-oxidation and anti-obesity. Therefore, it helps to reduce cancer and cardiovascular diseases [13,14]. According to the information in the literature, green tea is very rich in amino acids, polyphenols and purine alkaloids. The pharmacological effects of theanine and γ-aminobutyric acid (GABA) include nutritional role for essential amino acids. Theanine is an essential amino acid with important functions found in green tea. It is neuroprotective and strengthens cognition by lowering the levels of norepinephrine, serotonin and blood pressure in the brain. [15,16].

In this study, green tea leaves were extracted by three different methods. Three methods were used: microwave digestion, ultrasonic bath and homogenizer assisted. Methanol was used as solvent in all three extraction methods. In the obtained extracts, volatile organic compounds, phenolic and amino acids were determined by chromatographic methods. The antioxidant contents of the extracts were also analyzed. When all analysis data are compared, it was determined which of these three different extraction methods used was more efficient.

2. Materials and Methods

2.1. Chemicals and reagents

Green tea leaves were purchased from an herb market in Manisa city (38.749444°N 28.122778°E), Türkiye in August of 2020. The leaves were washed to remove impurities and then air-dried in the shade before extractions. All standards included chlorogenic acid (>99.0%), D-(+)-catechin (>99.8%), rutin (>99.7%), hyperoside (>99.8%), kaempferol-3-Orutinoside (>99.7%), astragalin (>99.8%), rosmarinic acid (>99.7%), polydatin (>99.8%), quercetin (>99.8%), apigenin (>99.8%), kaempferol (>99.8%) were purchased from Sigma-Aldrich Chemical Co., Ltd. (St. Louis, Missouri, USA). ABTS (2,2′ -Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), 1,1- diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich Chemical Co., Ltd. (St. Louis, Missouri, USA). Methanol, ethanol, acetonitrile (≥99.9%, (for HPLC) from Merck operates as Millipore Sigma, US. Amino acid standards 10X1 mL, Sigma/AAS18, Fmoc chloride (FMOC-Cl) (≥% 99.0) from Sigma-Aldrich Chemical Co., Ltd. (St. Louis, Missouri, USA), OPA (o-phthaldialdehyde) from Alfa Aesar, Thermo Fisher Scientific Chemicals, Germany.

2.2. Extraction methods of green tea leaves

2.2.1. Microwave digestion-assisted extraction (MDAE)

Dried leaves samples of 0.5 g were weighed, and 20 mL of pure methanol (Sigma-Aldrich) was added. Extraction was performed in a Microwave digestion device (Cem, Mars 6 version, NC, USA.), setting the temperature to 55 °C, 15 min ramp, 25 min hold and 20 min cooling program. The obtained extract solution was filtered. and kept at +4 °C in amber glass vials until the other analysis.

2.2.2. Homogeniser- assisted extraction (HAE)

Dried leaves samples of 0.5 g were weighed, and 20 mL of methanol was added. Extraction was performed by using an Ultra-turrax (IKA T25, Staufen, Germany) at 5000×g for 3 min at room temperature. The extracts were then centrifuged (Hettich- universal 320, Tuttlingen, Germany) at 10.000×g for 10 min at 4 °C. Finally, the resulting solutions were collected in amber glass containers until the other analysis.

2.2.3. Ultrasound bath- assisted extraction (UBAE)

Dried leaves samples of 0.5 g were weighed, and 20 mL of methanol was added. Extraction was performed by using an ultrasonic bath device (Wised, Wisd WiseClean,

Germany), for 30 min at 45 °C. The obtained extract solution was filtered and kept at +4 ºC in amber glass vials until the other analysis. For calculate the yield %, the obtained extract was filtered and the methanol was completely evaporated by rotary evaporator (IKA RV8, Germany). The yield % was calculated from the dry extract mass. The percentage yield % of extraction was calculated as:

Percentage yield = weight of dry extract/weight of dry plant material X 100%;

Percentage yield of microwave digestion - assisted extraction (MDAE): 22.96
Percentage yield of homogeniser- assisted extraction (HAE): 17.24
Percentage yield of ultrasound bath- assisted extraction (UBAE):15.48

2.3. Antioxidant activity assays

The FRAP analysis was performed according to the following procedure with some modifications [17]. Stock solutions in the experiment: 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$, 16mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$. The fresh FRAP solution was prepared as follows: 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5mL $FeCl_3.6H_2O$ solution and then warmed at 37 ^{0}C before use. Leaf extract (150 µL) and 2850 µL of FRAP were mixed in the dark for 30 minutes at room temperature conditions. Then, absorbance was taken at 593 nm using the spectrophotometer (TECAN, Männedorf, Switzerland). The standard curve was linear between 25 and 600 mM Trolox. Results were expressed in mM Trolox equivalents (TE)/g dry mass (DM).

The DPPH analysis was performed according to the following procedure with minor modifications [18]. Stock DPPH solution was prepared by weighing 24 mg of DPPH and dissolving it in 100 mL of methanol. The working solution was obtained by taking 10 mL of the stock solution and diluting it with 45 mL of methanol. Leaves extracts (150 µL) were allowed to react with 2850 µL of the DPPH solution for 2 h in a dark condition. Then, absorbance was taken at 515 nm using the spectrophotometer (TECAN, Männedorf, Switzerland). The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in mM Trolox equivalents (TE)/g dry mass. In all measurements, additional dilution was needed if the analysis value measured was over the linear range of the standard curve.

For ABTS assay of leaf extracts was performed according to the following method with some modifications [19]. A stock solution containing 7.4 mM ABTS and 2.6 mM potassium persulfate was prepared. The prepared stock solution was kept at room temperature for 12 h and then 1 mL was taken and diluted with 60 mL of methanol before the analysis. Leaves extracts (150 µL) were allowed to react with 2850 µL of the ABTS solution for 2 h in a dark condition. Then, absorbance was taken at 734 nm using the spectrophotometer (TECAN, Männedorf, Switzerland). The standard curve was linear between 25 and 600 mM Trolox. Results were expressed in mM Trolox equivalents (TE)/g dry mass.

2.4. Determination of phenolic compounds by LC-MS/MS

Phenolic profiles of leaf extracts were determined with Agilent 1260 Triple Quadrupole MS/MS. Separation was performed with a C18 ODS (25x4.6 mmx5 µm) analytical column. Analyzes were performed in three replications. Aqueous solution (0.1% formic acid) (A) and methanol (B) were used as mobile phase. The gradient method for chromatographic separation is as follows: 3 min 2% B, 6 min 25% B, 10 min 50% B, 14 min 95% B, 17.5 min 2% B. The method injection volume is 2 µL and flow rate 0.4 ml/min. The identification of compounds was achieved in positive and negative modes [20]. LC-MS/MS total ion chromatogram of phenolic compounds was given in figure 1.

2.5. Determination of volatile organic molecules by GC-MS

Methanol extracts were filtered with a 0.45 µm filter and analyzed in GC-MS. Volatile components in the extract were analyzed qualitatively by Agilent Technology 7890A GC-MS electron ionization method. Chromatographic separation was achieved with Agilent HP-5 MS, capillary column (30 mx0.25 mm, 0.25 mm film thickness). The oven temperature was started at 40°C and held at 40°C for 5 minutes, then increased to 280°C in increments of 5°C min-1 and held at this temperature for 5 minutes. Method flow rate is 1.5 mL min^{-1}, injector temperature is 250°C. Helium gas (99.999%) was used as carrier gas. Extract injection was performed in splitless mode with 1 µL. Interpretation of the mass spectrum was done according to the National Institute of Standards and Technology (NIST) and the Wiley Mass Spectral database.

2.6. Determination of amino acid contents by HPLC-DAD

2.6.1. Derivatisation of samples and amino acid standards

Before HPLC-DAD analysis, amino acid (AA) standards and samples were derivatized using o-phthaldehyde (OPA) for primary AA and 9-fluorenylmethyl chloroformate (FMOC) for secondary AA according to the method of Henderson et al al. (2000), modified to optimize the parameters for green tea plant leaves extraction analysis [21]. The derivatization solution was

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freshly prepared every day as follows: Borate Buffer: 0.4 M in water (pH 9.2), FMOC reagent, 0.2 mg/mL in acetonitrile, OPA reagent, 5 mg dissolved in 0.05 mL of methanol was added 0.45 mL of 0.4 M boric acid buffer (pH=9.5). Then 25 µL of β-mercapto ethanol was added. Derivatization of amino acids and samples was achieved by preparing a mixture of boric acid buffer/OPA/amino acid or sample/FMOC (5v/v/v/v). The mixture was vortexed for 2 min.

2.6.2. HPLC-DAD analysis

HPLC-DAD analysis was performed according to Wang et al. (2010) with some minor changes [22]. Agilent 1200 Infinity series HPLC system (Agilent Technologies, CA, USA) was used for the determination of amino acids. The separation was completed on a Zorbax Eclipse Inertsil ODS-3 column (250x4.6 mm, 5 µm, Agilent). The temperature of the column oven was set at 40 °C. The mobile phase consisted of methanol/acetonitrile/water (45/45/10, A) and phosphate buffer (pH 7.5, B). Elution was performed with following gradient as: 0–1.9 min, 100% A; 1.9-18.1 min, 0-58% B; 18.1–18.6 min, 58% B; 18.6–22.3 min, 58-70% B; 22.3–22.4 min, 70-100% B; 22.4–22.6 min, 100% B and 22.6–24 min, 100-0% B. The flow rate was 2.0 ml/min. The DAD was set at 338 nm to monitor the derivatized amino acids. The injection volume was 20 µL. Except for L theanine amino acid, 13 amino acids could be separated simultaneously with HPLC-DAD. Since the retention times of L- theanine and tyrosine are the same, a separate chromatogram was created for L theanine. Standard addition procedure was applied for each amino acid and validation was performed. Chromatograms of amino acids and samples were shown in figure 2.

Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) using the GraphPad Prism 8.4.2 program. Means were separated from each other using Bonferroni's multiple comparison test ($p<0.05$). All analyzes were achieved in triplicate.

3. Results and Discussion

3.1. Antioxidant activity results

Antioxidant activities measured in methanol extract of green tea leaves obtained using FRAP, DPPH and ABTS assays from a single extract were measured three times to test the reproducibility of the assays. Differences were observed according to the three different extraction methods and the results were shown in **Table 1** as TE/g dry mass (DM).

Table 1. Antioxidant activity results of three extraction methods of green tea leaves.

Extraction methods	FRAP mM TE/g DM	DPPH mM TE/g DM	ABTS mM TE/g DM
MDAE	51.95±0.7	94.65±11	47±2.4
UBAE	50.9±1.5	69.7±0.7	44.1±4.5
HAE	13.8±0.2	36.35±0.07	14.9±0.1

P<0.05, DM: dry mass

In green tea leaves ABTS antioxidant activity was obtained as 47.2, 44.1 and 14.9 TE/g dry weight, MDAE, UAE and HAE, respectively. When the three extraction methods were compared according to all analysis results, it was observed that the highest antioxidant activity was in microwave digestion extraction. Afterwards, it was determined that the ultrasonic bath and homogenizer extractions were respectively (Table 1). Some studies have listed the antioxidant properties of different teas as following order: green tea > oolong tea > black tea > puerh tea [23].

3.2. LC-MS/MS phenolic compound content results

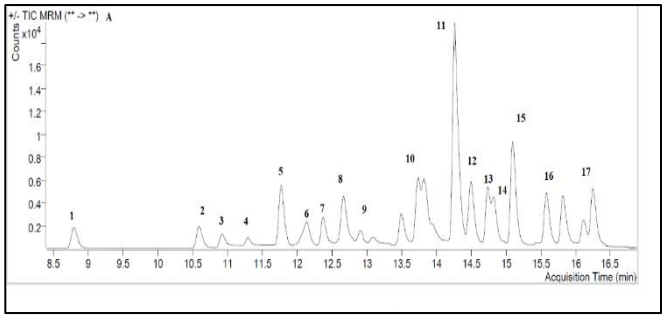

Figure 1. Total ion chromatograms of LC-MS/MS phenolic compounds. (1: Gallic acid, 2: Protocatechin acid, 3: 3,4-Dihydroxyphenylacetic acid, 4: (+)-Catechin, 5: Chlorogenic acid, 6: 4-Hydroxybenzoic acid, 7: (-)-Epicatechin, 8: Caffeic acid, 9: Vanillic acid, 10: p-Coumaric acid, 11: Hesperidin, 12: Rosmarinic acid, 13: Apigenin 7-glucoside, 14: Pinoresinol, 15: Eriodictyol, 16: Quercetin, 17: Kaempferol.

A total of 31 phenolic compounds were determined qualitatively by LC-MS/MS device. Gallic acid, catechin, epicatechin, hesperidin, hyperocyte were detected at a higher rate than other phenolic compounds.

When the total phenolic amounts were compared among the methods, it was seen that the most effective method was microwave decomposition (**Table 2**). Previous studies have found that catechins, chlorogenic acid, kaempferol-3-o-glucoside are the main antioxidants in tea leaves [24]. Catechins are natural antioxidants due to their free radical scavenging effects [25, 26]. The antioxidant activity of catechins differs from each other according to the plant species. Salman et al. (2022) when green tea was compared with other types of tea, they determined that the total catechin content was at the highest level. Accordingly, DPPH and ABTS antioxidant activities were determined more than other tea types [27].

Table 2. LC-MS/MS phenolic contents of three extraction methods of green tea leaves.

Phenolic content (µg/g Dry weight)	**MDAE**	**UBAE**	**HAE**
Gallic acid	366.21±1.16	250.64±0.16	200.39±1.69
Protocatechuic acid	75.44±0.66	45.92±0.26	31.11±0.02
Pyrocatechol	25.09±17.54	27.34±21.89	5.65±2.51
3.4-Dihydroxyphenylacetic acid	0.49±0.01	0.37±0.02	0.33±0.04
(±) -Catechin	320.30±2.47	229.24±4.33	202.94±7.67
chlorogenic acid	32.09±0.87	27.41±1.49	23.08±7.09
2.5-Dihydroxybenzoic acid	19.69±1.20	13.87±0.29	11.05±0.02
4-Hydroxybenzoic acid	5.99±1.00	5.62±0.50	4.07±0.26
(-) - epicatechin	3182.75±33.47	2265.61±231.6	2044.06±7.77
caffeic acid	0.32±0.05	ND	0.37±0.07
vanillic acid	2.88±2.62	3.17±4.46	7.87±4.57
syringic acid	1.40±0.31	1.26±0.15	1.12±0.32
3-Hydroxybenzoic acid	ND	1.06±0.66	0.49±0.56
Vanillin	1.24±0.10	1.33±0.32	0.81±0.19
verbascoside	4.17±0.38	0.69±0.06	1.31±0.21
Taxifolin	0.41±0.04	ND	0.11±0.01
sinapic acid	ND	ND	ND
p-kummeric acid	10.23±0.03	8.22±0.02	6.05±0.17
ferulic acid	1.94±0.27	1.21±0.08	1.02±0.04
Luteolin 7-glucoside	3.34±0.04	2.38±0.10	3.01±0.07
hesperidin	641.90±28.36	413.43±8.39	361.99±0.94
hyperocyte	357.12±15.52	224.87±4.45	195.99±0.35
Rosmarinic acid	16.75±0.42	13.64±0.36	89.71±1.60
Apigenin 7-glucoside	2.32±0.11	1.44±0.66	1.47±0.08
2-Hydroxycinnamic acid	0.15±0.01	0.18±0.02	0.17±0.04
pinoresinol	2.89±0.58	2.38±2.07	2.12±0.71
Eriodictyol	0.49±0.03	ND	0.11±0.07
Quercetin	34.68±3.73	18.03±0.83	11.72±0.36
Luteolin	ND	0.11±0.07	0.62±0.10
kaempferol	12.93±3.38	7.16±0.30	6.75±1.20
apigenin	ND	ND	ND
Total Phenolic	5123.21±114.36	3566.58±283.54	3215.49±38.73

P<0.05, ND: not defined.

3.3. GC-MS/MS Volatile organic compounds content

Three different extraction samples were introduced into the instrument and the results were compared according to their percentage similarity from the library in the database. The cas numbers, names, formulas and retention times (RT) of the determined volatile organic molecules with more than 80% similarity are shown in the tables (**Tables 3, 4, 5**). According to the GC-MS results obtained, similarities were obtained in the organic volatile molecules within the plants in three different extractions. The same molecules were identified as a

result of all three extractions. The most notable among these molecules is the squalene molecule. It has been reported that sharks are a rich source of squalene and that more than 40% of shark liver contains squalene, and therefore the absence of cancer in sharks is related to these high squalene levels [28, 29]. Squalene is a powerful antioxidant thanks to its wide electron exchange capability without undergoing molecular degradation. Experimental studies have shown that the use of squalene in the diet improves the performance of the immune system and increases macrophage function. Studies have shown that squalene keeps immune cell bio-membranes against oxidative stress during phagocytosis. [30].

Table 3. GC-MS volatile organic molecules of microwave-digestion extraction of green tea leaves.

CAS No	Name	Formula	Score	RT
64-19-7	Acetic acid	C2H4O2	97.54	3.719
87-66-1	1.2.3-Benzenetriol	C6H6O3	92.39	21.62
58-08-2	Caffeine	C8H10N4O2	96.1	32.80
23470-00-0	Hexadecanoic acid. 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	89.83	44.11
123-94-4	Octadecanoic acid. 2.3-dihydroxypropyl ester	C21H42O4	92.56	47.19
112-84-5	13-Docosenamide. (Z)-	C22H43NO	82.53	48.09
7683-64-9	Squalene	C30H50	86.36	48.78

+: Determined compound, Score: Similarity%, RT: Retention time

Table 4. GC-MS volatile organic molecules of ultrasound-bath extractions of green tea leaves

CAS No	Name	Formula	Score	RT
64-19-7	Acetic acid	C2H4O2	97.53	3.70
87-66-1	1.2.3-Benzenetriol	C6H6O3	92.87	21.59
58-08-2	Caffeine	C8H10N4O2	89.7	32.73
150-86-7	Phytol	C20H40O	93.57	37.53
23470-00-0	Palmitin. 2-mono-; Palmitic acid .beta.-monoglyceride	C19H38O4	90.59	44.10
123-94-4	Octadecanoic acid. 2.3-dihydroxypropyl ester	C21H42O4	92.93	47.21
112-84-5	13-Docosenamide. (Z)-	C22H43NO	82.61	48.11
7683-64-9	Squalene	C30H50	85.75	48.78

+: Determined compound, Score: Similarity%, RT: Retention time

Table 5. GC-MS volatile organic molecules of homogeniser extractions of green tea leaves

CAS No	Name	Formula	Score	RT
64-19-7	Acetic acid	C2H4O2	97.51	3.705
87-66-1	1.2.3-Benzenetriol	C6H6O3	93.53	21.58
58-08-2	Caffeine	C8H10N4O2	96.19	32.69
150-86-7	Phytol	C20H40O	93.23	37.53
23470-00-0	Hexadecanoic acid. 2-hydroxy-1-(hydroxymethyl)ethyl ester;	C19H38O4	90.66	44.11
123-94-4	Octadecanoic acid. 2.3-dihydroxypropyl ester	C21H42O4	93.21	47.20
112-84-5	13-Docosenamide. (Z)-	C22H43NO	82.77	48.11
7683-64-9	Squalene	C30H50	86.66	48.78

: Determined compound, Score: Similarity%, RT: Retention time.

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3.4. HPLC-DAD amino acid content results

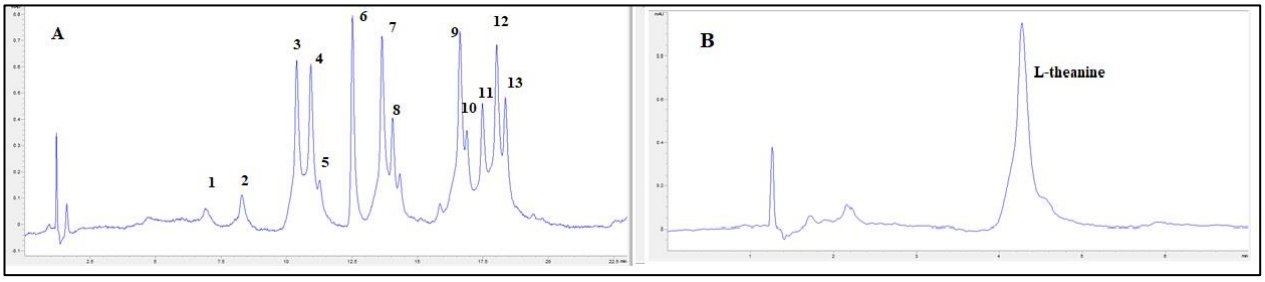

Figure 2. A: HPLC-DAD chromatogram of amino acid standards, B: HPLC-DAD chromatogram of L-theanine standard.

(1: Aspartic acid, 2: glutamic acid, 3: asparagine, 4: serine, 5: glutamine, 6: arginine, 7: alanine, 8: tyrosine, 9: valine, 10: tryptophan, 11: phenylalanine, 12: isoleucine, 13: leucine)

Table 6. Amino acid contents of three extraction methods of green tea leaves.

Amino Acid Content (µg/g kuru ağırlık)	MDAE	UBAE	HAE
Aspartic Acid	12.60±0.22	4.20±0.02	7.00±0.13
Glutamic acid	12.20±0.04	1.00±0.04	0.80±0.03
Aspargine	113.80±0.07	66.40±0.54	46.00±0.44
Serine	33.80±0.32	7.20±0.01	13.60±0.16
Glutamine	69.80±0.42	49.80±0.50	45.00±0.35
Arginine	10.00±0.14	24.80±0.07	16.20±0.32
Alanine	50.80±0.14	56.00±0.41	33.60±0.06
tyrosine	10.00±0.11	23.60±0.28	13.60±0.17
Valine	0.80±0.03	ND	ND
tryptophan	ND	ND	ND
Phenyl alanine	ND	ND	ND
isolaucine	ND	ND	ND
leucine	4.80±0.01	4.40±0.00	4.40±0.00
L-Theanine	57.80±0.16	49.80±0.01	52.40±0.08

P<0.05, ND: not defined

Amino acid analysis was performed with high performance liquid chromatography (HPLC) and diode array detector (DAD) detector. In MDAE, UBAE and HOME extractions, the amount of glutamine in green tea leaves was determined as 69.80, 49.80 and 45.00 µg/g dry weight, respectively. Aspargine, glutamine, alanine and L-theanine were determined at high levels in the green tea plant (**Table 6**). Theanine is one of the essential amino acids found in green tea due to its positive effects on the brain. Some of these effects include increasing neuroprotective and cognitive power by lowering levels of norepinephrine, serotonin and blood pressure. [16,17]. When three different extraction methods were compared from the results obtained, it was observed that the most effective extraction method was microwave decomposition extraction. Pan et al. (2003) stated that they obtained higher yields from the microwave assisted extraction process for the extraction of polyphenols and caffeine from green tea leaves compared to other extraction methods.

4. Conclusion

Extraction is the pre-treatment that must be done before bioactive component analysis. With an efficient extraction process, product loss, amount of solvent and wasted time must be minimized. Phytochemical analyzes were carried out on the extracts obtained with various chromatographic modern devices. There is no similar study in the literature yet. The detailed determination of antioxidant capacities and phytochemical contents of green tea leaves after extractions allowed comparison of extraction efficiency. The importance of natural products rich in bioactive components is increasing day by day. Therefore, this study may guide similar studies in the future.

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Author's Contributions

Hafize Dilek Tepe: Drafted and wrote the manuscript, performed the experiment and result analysis.
Fatma Doyuk: Assisted in analytical analysis on the structure, supervised the experiment's progress, result interpretation and helped in manuscript preparation.

Ethics

There are no ethical issues after the publication of this manuscript.

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