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Edible green solvent for optimized catechins extraction from green tea leaves: Anti-Hypercholesterolemia

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Abstract

Green tea's primary health advantages come from its catechin polyphenol content. It is still difficult to extract catechins from green tea (GTE) leaves under ideal circumstances. The industrial sector requires an extraction technology that is both efficient and cost-effective. We postulated that increasing the yield and biological activity of GTE catechin extraction by using certain extraction procedures in the presence of natural polymers and antioxidants would be possible. Separately and in tandem, the benefits of microwave (30-60 seconds irradiation in a standard household microwave) and ultrasonic aided extraction (UAE) were analyzed. Water, ascorbic acid, chitosan/ascorbic acid, carboxymethylcellulose/ascorbic acid, methylcellulose/ascorbic acid, chitosan/methylcellulose/ascorbic acid, methylcellulose, chitosan/acetic acid, and ethanol were among the nine edible green solventcombinations studied. HPLC-UV was used to measure the quantity of catechins extracted from green tea leaves.Extraction yields for catechins were found to be highest when the MAE & UAE approach was used. The best solvent for extracting catechins was found to be chitosan/ascorbic acid. After 3 weeks of once-daily oral treatment in studies with animals given a high-fat diet, GTE significantly decreased total cholesterol and LDL-C. Finally, green tea catechins that were effectively extracted and stabilized were shown to reduce the high fat diet-induced increase in blood cholesterol and LDL-C.

Keywords: hypercholesterolemia, dyslipidemia, green tea catechins, chitosan, edible solvent, and extraction

1. Introduction

Green tea, an aqueous infusion of dried "Camellia Sinensis" leaves, is one of the world's most popular drinks. Asia is where green tea was first developed. Even though the tea plant has a lot of leaves, only the top two leaves and the bud at the end of each young stem are used [1, 2]. You'll find polyphenols like flavonoids (like catechins and tannins) and alkaloids (like caffeine) in green tea, as well as free amino acids, alkaloids, ascorbic acid, saponins, and unsaturated fatty acids. Catechins such as epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) make up a significant portion of green tea's chemical makeup (30% or more) [3]. Green tea's primary catechin, epigallocatechin gallate (EGCG), is an antioxidant with various health advantages [4, 5]. EGCG makes up 9-13% of green tea by weight. Catechins have been linked to a variety of health benefits, including protection against cancer, inflammation, diabetes, infertility, atherosclerosis, ulcers, hypercholesterolemia, human platelet aggregation inhibition, skin wrinkle reduction, and cardiovascular disease prevention [5,6].

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The active compounds in green tea are extracted with varying degrees of success depending on a number of variables.
chemical make-up, length of storage, and presence of potentially disruptive chemicals [7]. Polarity of the solvent, degree of polymerization, interaction with other dietary ingredients, and formation of insoluble complexes all have a role in whether or not flavonoids are soluble [8-10]. Green tea flavonoids may be extracted in a variety of ways, but no one method has been shown to be universally effective [11,12]. Conventional solvent extraction with mechanical or thermal techniques, such as ultrasound assisted extraction (UAE) [13,14], microwave assisted extraction (MAE) [15,16], high hydrostatic pressure (HHPE) [17], and supercritical fluid extraction (SFE) [18], is the most frequently used extraction protocol. Conventional solvents for extraction typically consist of polar solvents like methanol, ethyl acetate, acetone, and water, or a mixture of these. In most cases, the time required for extraction is between 1 minute to 24 hours. Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) are said to degrade into gallocatechin gallate (GCG) and catechin gallate (CG) when subjected to high temperature (over 100 °C) and prolonged extraction time (over 2 hours). For this reason, green tea leaves must be processed at a low temperature in order to preserve the catechins. Because it is performed at a low temperature, ultrasonic aided extraction (UAE) is recommended for enhanced sensory qualities as it prevents the evaporation of volatile components and the heat destruction of active biomolecules. However, to increase polyphenol extractability with less time and energy expenditure, microwave aided extraction (MAE, at 60-80 0C for 5 min) is recommended. It is well accepted that catechins, being polyphenolic chemicals, are very susceptible to oxidation during the extraction process.

needed. The purpose of this study is to determine the optimal extraction method (UAE and/or MAE) and solvent composition for phytochemicals in green tea leaves, with the goal of achieving a high extraction yield in a short amount of time (Figure 1). Here, we provide a new method for extracting flavor from green vegetables.

Table 1 lists a variety of solvents, such as chitosan, ascorbic acid, carboxymethylcellulose (CMC), methylcellulose (MC), and combinations thereof, that meet the polarity and preservative storage network requirements of natural polymers.

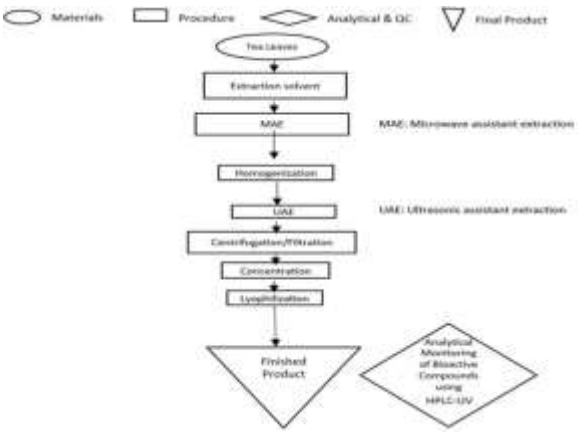

Figure 1: Schematic diagram for extraction protocol for catechins from green tea leaves.

Table 1: Extraction solvent compositions

	Extraction Protocol	**Solvent composition**
1	Water (control)	Deionized water
2	Ascorbic acid	1% Ascorbic acid in water
3	Chitosan/ascorbic acid	1% Ascorbic acid and 0.5% Chitosan in water
4	Carboxymethylcellulose (CMC)/ ascorbic acid	1% Ascorbic acid and 0.5% CMC in water
5	Methylcellulose (MC)/ascorbic acid	1% Ascorbic acid and 0.5% MC in water
6	Chitosan/Methylcellulose/ascorbic acid	1% Ascorbic acid, 0.25% MC and 0.25% chitosan in water
7	Methylcellulose (MC)	0.5% MC in water

8	Chitosan/acetic acid	1% Acetic acid and 0.5% chitosan in water
9	Alcohol extract	50% Ethanol in water

We hypothesized that the extracted catechins in the presence of ascorbic acid and chitosan will be simultaneously adsorbed inside the cavities of the polymer network leading to preservation of its stability and biological activities. In addition, the multivalent functional groups of the polymer offer

2. Materials and Methods

2.1 Chemicals

Green tea leaves (Organic certified, Gunpowder loose leaves, Davidson's, Reno, NV, USA), (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-gallocatechin gallate (GCG), (−)- epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG), gallic acid, caffeine, theobromine, theophylline, L-ascorbic acid, chitosan (50,000 - 150,000 Da), carboxymethylcellulose (CMC), methylcellulose (MC), ethanol, acetic acid and Amicon ultra-0.5 centrifugal filters, were purchased form Sigma-Aldrich (St. Louis, MO, USA).

2.2 Extraction procedure

Phytochemicals are extracted with nine extraction solvents (Table 1) using extraction techniques, i.e., UAE, MAE, and MAE/ UAE.

2.3 Effect of extraction technique

2.3.1Ultrasonic assisted extraction (UAE)

Two hours were spent steeping five grams of green tea leaves in 200 mL of deionized water while stirring at 37 °C. The ingredients were then homogenized at a speed of 20,000 rpm (PT3100D, Polytron, Kinematica, Bohemia, NY, USA) for 10 minutes, until a uniformly viscous dark green color was achieved throughout the mixture. Sonication occurred for 10 minutes using an ultrasonication probe with an 80 m amplitude (Q55, Qsonica LLC, Newtown, CT, USA) after homogenization. Double-filtering the mixture with 45

create a stable protective environment and many electrostatic attraction sites for the volatile active components [19]. The hypo-cholesterolemic effects of orally given GTE generated by the improved approach were then measured in blood total cholesterol and LDL-C in mice fed a high-fat diet. To get green tea extract powder [13,14], the solution was poured into freeze drying containers, frozen at -80 °C overnight, then lyophilized (MD85, Millerick technology, Kingston, NY, USA).

2.3.2 Microwave assisted extraction (MAE)

Microwave digestion at 60-80 0C for 5 minutes was performed on 5 grams of green tea leaves distributed in 200 mL of deionized water. After 5 minutes of homogenizing at 20,000 rpm (PT3100D, Polytron, Kinematica), the mixture had reached a uniform viscous dark green color. After passing it through 45 m and 22 m membrane filters twice, the resulting solution was as transparent as gold. Green tea extract powder [16] was made by pouring the solution into freeze drying containers, freezing them overnight at -80 °C, and then lyophilizing (MD85, Millerock technology).

2.3.3 Combined MAE and UAE (MAE/ UAE)

Microwave digestion at 60-80 0C for 5 minutes was performed on 5 grams of green tea leaves distributed in 200 mL of deionized water. After 5 minutes of homogenizing at a speed of 20,000 rpm (PT3100D, Polytron), the mixture was uniform in texture and color throughout. Once the mixture was homogenized, an ultrasonication probe was used to sonicate it for 10 minutes at 80 m amplitude (Q55, Qsonica). After passing it through 45 m and 22 m membrane filters twice, the resulting solution was as transparent as gold. After transferring the solution into freeze drying containers, it was frozen at -80 °C for a whole night before being lyophilized (MD85, Millrock technology) into a powder. Powders from each extract were weighed, and HPLC-UV spectrometry was used to quantitatively assess the extracts' catechin contents.

2.3.4 Effect of extraction solvent

Microwave aided extraction (MAE, 60-80 0C for 5 min) and ultrasound assisted extraction (UAE) were coupled to achieve a high extraction throughput (MAE/ UAE). There were nine different solvents tried for the

extraction process: water, ascorbic acid, chitosan/ascorbic acid, carboxymethylcellulose (CMC)/ascorbic acid, methylcellulose (MC)/ascorbic acid, chitosan/methylcellulose (MC)/ascorbic acid, methylcellulose (MC), chitosan/acetic acid, and 50% ethanol. The components of common extraction solvents are shown in Table 1. Microwave digestion at 60-80 0C for 5 minutes was performed on 5 grams of green tea leaves in 200 mL of extraction solvent. After 5 minutes of homogenizing at a speed of 20,000 rpm (PT3100D, Polytron), the mixture was uniform in texture and color throughout. After the mixture was homogenized, ten minutes were spent sonicating it at 80 m amplitude (Q55, Qsonica) using an ultrasonication probe. After passing it through 45 m and 22 m membrane filters twice, the resulting solution was as transparent as gold. Green tea extract powder was made by pouring the solution into freeze drying containers, freezing them overnight at -80 °C, and then lyophilizing (MD85, Millrock technology). The basic steps of the extraction process are shown in Figure 1. HPLC-UV (Waters, Milford, MA, USA) was used for quantitative analytical monitoring of bioactive chemicals.

USA). The nano formulations that will be utilized for the in vitro and in vivo biological assessments (the findings of which will be reported elsewhere) will be prepared using the extraction process that yielded the highest concentration of catechins.

2.4 HPLC sample preparation

Standard calibration solutions of EGCG, EGC, ECG, epicatechin, catechin, GCG, caffeine, theobromine,theophylline, and gallic acid solutions were prepared in 50% MeOH in water at concentrations of 500, 400,300, 200, 100, 10, and 1 ug/mL. Two grams of green tea extracts (GTEs) were dissolved in 1 mL of 50% MeOH in water. After vortex-mixing for 30 min, mixtures were centrifuged for 15 min; and aliquots of supernatant (100 uL) were used for HPLC-UV analysis in duplicate.

2.5 Quantitative analysis of catechins by HPLC- UV

The Waters 2695 Separations Module (Waters) and the Waters 2996 Photo Diode Array Detector (Waters) were used for the HPLC analysis. We used a Pursuit XRs 3 C18 column (150 mm x 4.6 mm, Agilent, Santa Clara, CA, USA) for the reversed-phase separation. The HPLC was operated and analyzed using Waters' Empower 3 software. For this experiment, we used mobile phases A) water and B) methanol, both of which contain formic acid. The volume was 1 mL per unit of time. The linear and constant gradient lasted for the whole 40 minutes, from 5% B at 0 minutes to 95% B at 40 minutes. The column temperature was maintained at room temperature, and the injection volume was 10 L. The analytes were identified using UV spectra collected from 210 to 400 nm. Phytochemical concentrations were determined using an external standard method and UV chromatogram peak areas at 275 nm [20]. A UV chromatogram is shown here as an example.See Figure 2 for evidence. Accuracy was estimated by analyzing three replicates of standard solutions at concentrations of 500, 100, and 10 ng/mL (n=3). The LOQ was determined by injecting a standard solution (10 g/mL) six times. Standard deviations (SD) of the analyte signals and the slope of the linear regression curve were used to compute the LOQ. The LOQ value is calculated as follows: LOQ = 10 x SD / inclineThe HPLC-UV method for standard solutions was linear from 10 to 500 g/mL ($r > 0.99$). The analytes were accurate to within a range of more than 90% and less than 110%. All analytes had LOQs below or equal to 10 g/mL under these conditions.

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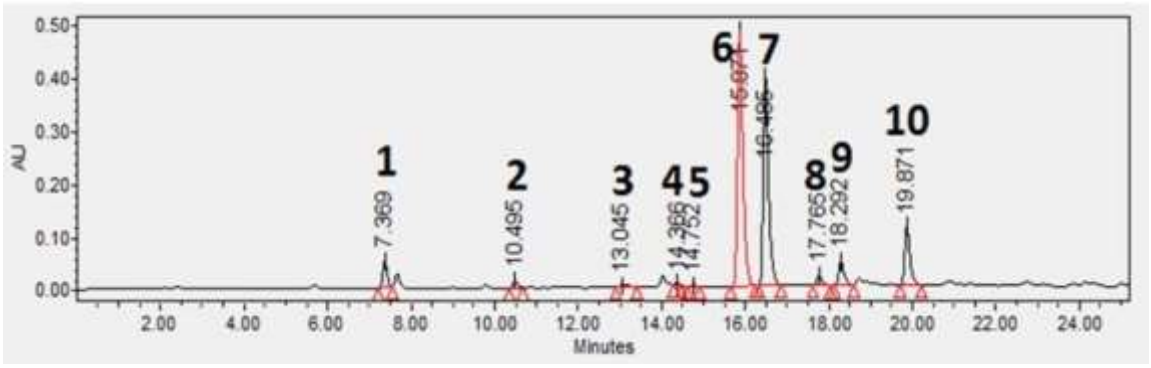

Figure 2: HPLC-UV chromatogram of phytochemicals in green tea extract, 1: Garlic acid, 2: Theobromine, 3: Theophylline, 4: EGC, 5: Catechin, 6: Caffeine, 7: EGCG, 8: GCG, 9: EC, 10: ECG

2.6 Animals

The in vivo studies were conducted at the animal facility of the Veterans Affairs Medical Center (VAMC), Albany, New York according to the guidelines of NIH and the institutional guidelines for humane animal treatment. The applied animal protocols were approved by the Institutional Animal Care & Use Committee (IACUC) at VAMC.

2.7 Pharmacodynamic study using nutritionally induced hypercholesterolemia model

High fat diet male C57BL/6 mice aged 4-5 weeks and weighing 20-25 g were purchased from Taconic Biosciences, Inc. (Germantown, NY, USA). Animals were maintained under specific pathogen-free conditions and housed under controlled conditions of temperature (20-24°C) and humidity (60-70%) and 12 h light/dark cycle with ad libitum access to water and high fat diet. Mice were fed a high-fat irradiated diet (TD.**D12492**, Research Diets, Inc., New Brunswick, NJ, USA) that provides 60% kcal from fat sources to increase total cholesterol. The composition of the diet is shown in Table 2.

Table 2: Diet Composition

Product #	**D12492**	
	gm%	**kcal%**
Protein	26.2	20
Carbohydrate	26.3	20
Fat	34.9	60
Total (kcal/gm)	5.24	100
Ingredient	**gm**	**kcal**
Casein, 80	200	800
Mesh L-Cystine	3	12
Corn Starch Maltodextrin	0	0
10 Sucrose	125	500
Cellulose, BW200	68.8	275.2

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Soybean Oil	50	0
Lard*	25	225
Mineral Mix,	245	2205
S10026 DiCalcium Phosphate	10	0
Calcium Carbonate Potassium	13	0
Citrate, 1 H2O	5.5	0
Vitamin Mix,	16.5	0
V10001 Choline Bitartrate	10.0	40
FD&C Blue Dye #1	2	0
	0.05	0
Total	**773.85**	**4057**

The mice were grouped into two arms (n=3) and administrated orally daily with water for vehicle control and GTE, (30 mg/kg) for 3 weeks. Plasma was collected after week 3 treatment to monitor the level of LDL-C as measured enzymatically. Blood samples (100 µl) were collected from the retro-orbital venous plexus via heparinized capillary tubes containing 2 USP units of ammonium heparin per tube (Carolina, Burlington, North Carolina, USA). Plasma was separated immediately using centrifugation (5,000 x g) for 5 min at room temperature and then kept at -80°C until assayed for lipid profile. Plasma total cholesterol and LDL-C levels were measured with the cholesterol and LDL assay kit according to the manufacturer's instructions(Abcam, Boston, MA, USA).

2.8 Statistical analysis

Statistical analysis was performed using GraphPad Prism (San Diego, CA, USA). All data are presented as mean ± standard error of the mean. The t-test t-was used to determine differences among the groups. ***P <0.001, **P <0.01, *P <0.05 wereconsidered as significant.

3. Result

3.1 Effect of extraction technique

The extraction technique is one of the most important factors that could affect the yield of biomolecules extracted from green tea leaves. Table 3 represents the results of quantitative HPLC-UV analysis of extracted bioactive ingredients using three extraction techniques, i.e., UAE, MAE, and combined MAE/ UAE, which were described in the methodology section. The results of quantification showed that extraction techniques have drastic impacts on the yield of catechins from green tea leaves. The yield of phytochemicals increased with the combined extraction technique (MAE/ UAE). For example, (−)-epigallocatechin gallate (EGCG), which is the dominant catechin, 80-90%, increased by four-fold compared to UAE technique and by two-fold compared to MAE technique. From the viewpoint of stability, ultrasonic assisted extraction (UAE) is the preferred method for catechins extraction due to the extraction process conducted at lower temperatures that avoid the degradation that would occur at high temperatures. The main disadvantage of UAE is that
it needs prolonged extraction time to accomplish high yields, which explain the low concentrations of the extracted active ingredients with UAE in this study.

3.2 Effect of extraction solvent

The extraction solvent is another key element affecting the extraction yield of catechins in addition to the extraction technique. We, therefore, investigated the effect of nine solvents on the extraction efficiency of catechins by the combined MAE/ UAE technique (Table 3).

Table 3: HPLC results of green tea extracted bioactive compounds (mg/g green tea extract) by UAE, MAE, andcombined MAE and UAE techniques

	UAE	MAE	MAE/ UAE
EGCG	34.84 ± 0.03	77.71 ± 0.07	142.80 ± 0.13
EGC	5.90 ± 0.03	7.94 ± 0.04	10.54 ± 0.06
ECG	2.22 ± 0.14	4.12 ± 0.27	7.39 ± 0.48
Epicatechin	0.32 ± 0.01	0.67 ± 0.01	0.78 ± 0.01
Catechin	0.16 ± 0.01	0.54 ± 0.05	0.74 ± 0.06
GCG	0.25 ± 0.02	0.70 ± 0.02	1.43 ± 0.02
Caffeine	6.35 ± 0.02	7.12 ± 0.03	8.81 ± 0.03
Theobromine	0.036 ± 0.002	0.096 ± 0.002	0.108 ± 0.002
Theophylline	0.064 ± 0.001	0.096 ± 0.005	0.378 ± 0.33
Gallic acid	0.198 ± 0.009	0.336 ± 0.019	0.636 ± 0.038

3.3 Pharmacodynamic study using nutritionally induced hypercholesterolemia model:

Animals were fed the high-fat diet and randomly assigned to one of the different groups such that the average of each biomarker level is comparable among the different groups. Plasma total cholesterol, free cholesterol [Note: no free cholesterol data shownin Fig. 3] and LDL-C levels were measured. Forplasma total cholesterol, mice treated with green tea extract (GTE) showed 25% total cholesterolreduction compared to vehicle group after three weeks oral treatment, Figure 3 A and B. Plasma LDL cholesterol (LDL-C) showed significant (*P<0.0.5) reduction in GTE was 15% compared to the control group after three-weeks treatment, as illustrated in Figure 3, C&D.

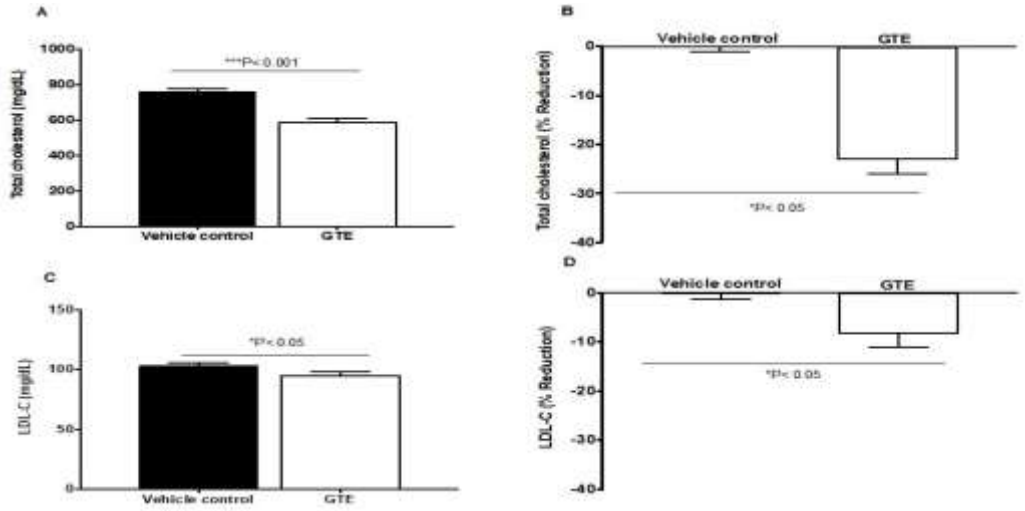

Figure 3: Levels of total cholesterol and LDL-C in plasma from a high-fat diet mice fed for 3 weeks and treated with A) GTE administered daily orally with water at 30 mg/kg versus vehicle control given water orally for 3 weeks. Blood was collected at week 3. B) Percentage reduction of plasma total cholesterol. C) LDL-C levels in vehicle control versus GTE treated daily for 3 weeks. D) Percentage reduction of plasma LDL-C was compared with control. Values are presented as mean ± S.D. Statistical analysis conducted using t-test to determine differences among the groups. GTE was statistically significant compared to vehicle control.Discussions

Our hypothesis was validated and accepted where indeed certain extraction techniques in the presence of natural polymers and antioxidants improved GTE catechin extraction yield, stability, and its biological activity. In MAE, the microwave oven generates electromagnetic radiation, which is absorbed by the water molecules in the extraction medium and by the green tea biomass, produces a drastic rise in temperature that disrupts the cell wall of the green tea leaves, and increases the solubility and diffusion coefficient of the bioactive ingredients [21-23]. The results in this study showed that the high temperature affected the yield of the extracted bioactive ingredients. The yield of catechins, alkaloids and gallic acid increased two-fold, however with MAE as compared to UAE, and this finding agrees with previous studies [23]. The main limitation of MAE is the high temperature, which can cause a drastic degradation of tea bioactive ingredients [24]. Therefore, it is necessary to optimize the extraction ata temperature as low as possible. The combination of MAE (60-80 °C) and UAE (MAE/ UAE) has dual benefits of the lower temperatures of ultrasonic waves by UAE and microwave heating by MAE, which maximize the extraction yield and minimizes the extraction time as shown in Table 3. The combined MAE/ UAE technique proved to be the best extraction technique method to maximize the efficiency of the extraction solvent. This combined protocol will impel us to overcome the limitations of high temperature, time consuming methods and low extraction efficacy. The results show that the chitosan/ascorbic acid protocol is the best extraction solvent composition for catechins from green tea leaves compared to the other solvents. It is interesting to note that this solvent composition had a strong impact on the yield of individual catechins and the yield of each catechin was affected differently by the extraction solvent compositions (Table 4). Similar observations were observed with caffeine, theobromine, theophylline, and gallic acid. It is known that the charge and the polarity of solvents influence the efficiency of the catechins extraction process. Catechins are electronegative polar molecules and attracted to the electropositive polar chitosan molecules; and such positive polarity of the chitosan network enhances the molecular interactions with catechins [25]. Ascorbic acid has a bifunctional role on extraction efficiency. It increases the polarity of the extraction medium due to the presence of four hydroxyl groups that will facilitate the dissolution of catechins in addition to its antioxidant activity that protects catechins from further oxidation and increases their stability [26].

Table 4: HPLC analysis of bioactive compounds in green tea extracts (mg/g green tea extract)

	Water	**Ascorbic acid**	**Chitosan /ascorbic acid**	**CMC / ascorbic acid**	**MC / ascorbic acid**	**Chitosan / MC /ascorbic acid**	**Chitosan / MC**	**Chitosan / acetic acid**	**50 % Alcohol**
EGCG	142.80± 0.1	99.64± 0.09	232.24± 0.21	13.248± 0.01	25.643± 0.02	44.884± 0.04	8.96± 0.01	65.28± 0.06	174.828± 0.16
EGC	10.54± 0.09	17.25± 0.09	39.36± 0.21	1.152± 0.00	15.31± 0.08	14.722± 0.08	1.456± 0.01	0.330± 0.00	0.634± 0.00
ECG	7.392± 0.29	4.508± 0.29	14.80± 0.95	0.576± 0.04	1.232± 0.08	2.055± 0.13	0.336± 0.02	4.295± 0.28	11.838± 0.76
Epicatechin	0.672± 0.01	0.874± 0.01	5.20± 0.07	0.576± 0.01	0.142± 0.02	0.239± 0.003	0.112± 0.002	1.746± 0.02	1.752± 0.02
Catechin	0.744± 0.07	0.368± 0.03	1.04± 0.09	0.192± 0.01	0.379± 0.03	0.335± 0.03	0.728± 0.06	0.802± 0.7	0.664± 0.06
GCG	0.696± 0.02	3.174± 0.02	9.52± 0.02	0.288± 0.02	2.512± 0.02	2.342± 0.02	0.112± 0.02	0.094± 0.02	0.242± 0.02
Caffeine	8.808± 0.03	12.512± 0.05	47.44± 0.19	21.50± 0.08	11.566± 0.04	11.090± 0.04	13.72± 0.05	14.77± 0.06	17.305± 0.07
Theobromine	0.096± 0.02	0.092± 0.002	0.72± 0.001	0.096± 0.02	0.095± 0.002	0.096± 0.002	0.728± 0.001	0.14± 0.001	0.393± 0.004
Theophylline	0.096±0.005	0.828± 0.077	0.40± 0.035	0.768± 0.071	0.569± 0.052	0	0.504± 0.045	0.09± 0.004	0.211± 0.16
Gallic acid	0.336± 0.02	0.782± 0.048	4.24± 0.28	3.84± 0.25	0.758± 0.046	0.765± 0.047	1.064± 0.067	1.038± 0.07	1.299± 0.082

Values are presented as mean value ± standard deviation (SD).

Epidemiological studies have shown an inverse association between coronary heart disease (CHD) risk and green tea consumption in humans [27]. Such lipid lowering action of green tea was attributed to its catechins content which inhibits the intestinal absorption of ingested lipids [28], and it inhibits PCSK-9 activity and up-regulates the LDL-R in liver tissues [29]. Hence, preservation of the polyphenol functional groups in GTE bioactive catechins protects the hypo-cholesterolemic effects of GTE bioactivepolyphenols.

4. Conclusion

We optimized the extraction technique and the solvent composition for phytochemicals in green tea leaves. The combined technique of microwave assisted extraction and ultrasonic assisted extraction (MAE/ UAE) was the optimized technique producing higher extraction yield compared to each technique separately. Also, the edible green solvent, chitosan/ascorbic acid in water was the optimized solvent with the highest extraction efficiency of catechins among the nine solvents investigated, which can be attributed to the high adsorbing capability of the chitosan network and the anti-oxidative protection of ascorbic acid for extracted catechins. Therefore, the combined MAE/ UAE technique with a chitosan/ascorbic acid solvents system is a rapid, scalable, and optimized approach for efficient extraction of catechins from green tea leaves that, preserves the hypo-cholesterolemic effects of GTE bioactive polyphenols.

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List of abbreviations: C: Catechin, **CG:** Catechin gallate, **CMC:** Carboxymethylcellulose, **EC:**Epicatechin, **ECG:** Epicatechin gallate, **EGC:** Epigallocatechin, **EGCG:** Epigallocatechin gallate, **GCG:** Gallocatechin gallate, **HHPE:** High hydrostatic pressure, **HPLC-UV:** High performance liquid chromatography-UV spectrometry, **MAE:** Microwave assisted extraction, **MC:** Methylcellulose, **SFE:** Supercritical fluid extraction, **UAE:** Ultrasonic assisted extraction, **LDL-C**: Low density lipoprotein-cholesterol

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Author Contributions: KF and TAS both contributed to the extraction and analysis of the bioactive compounds in green tea and writing the draft; KG contributed to the dyslipidemia studies, HVM contributed to data analysis and article review; and SAM contributed to the study design, data analysis, and write up.

Declaration: No conflict of interest to be declared by all authors.

Ethical approval: Animal protocol was approved by IACUC #545023, 2018.

Clinical trials (Human Subjects): N/A

Availability of Data and materials: All raw data are available at the Pharmaceutical Research Instituteupon request.

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