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Ultrasound-Assisted Green Extraction of Flavonoid-Rich Biocompounds from Camellia Sinensis Leaves and Evaluation of Antioxidant Activity

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Abstract

The present study investigated the ultrasound-assisted green extraction of Flavonoid-rich biocompounds from Camellia Sinensis leaves and evaluated their antioxidant activity. Ultrasound-Assisted Extraction (UAE) was employed as an environmentally friendly and efficient extraction technique to enhance the recovery of bioactive phytochemicals while reducing extraction time and solvent consumption. Different extraction times were examined to evaluate their effects on extraction yield, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity. The obtained results demonstrated that increasing ultrasonication time significantly improved extraction efficiency and recovery of phenolic and Flavonoid compounds. The highest extraction yield, TPC, and TFC were observed at 30 min of extraction. Antioxidant activity was evaluated using the 2,2−Diphenyl−1−Picrylhydrazyl (DPPH) radical scavenging assay, and the extracts exhibited considerable free radical scavenging activity that increased with increasing extraction time. The enhanced antioxidant activity was strongly associated with the elevated concentration of Flavonoids and polyphenolic compounds extracted from the green tea leaves. The findings of this study indicate that UAE is an effective green technology for the sustainable recovery of antioxidant-rich biocompounds from Camellia Sinensis. Furthermore, the obtained extracts demonstrate promising potential for applications in pharmaceutical, nutraceutical, food, and cosmetic industries due to their high antioxidant properties and natural bioactive composition.

Keywords: Camellia Sinensis, Flavonoids, Antioxidant activity, 2,2−Diphenyl−1−picrylhydrazyl assay, Biocompounds, Green tea.

1 | Introduction

Green tea (Camellia Sinensis) is one of the most widely consumed beverages worldwide and has attracted considerable scientific attention due to its rich composition of bioactive compounds and potential health-promoting properties [1–5]. Among the diverse phytochemicals present in Green tea, Flavonoids represent

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one of the most important groups of natural biocompounds owing to their strong antioxidant, anti-inflammatory, antimicrobial, anticancer, and cardioprotective activities. These compounds, particularly catechins such as Epigallocatechin Gallate (EGCG), Epicatechin (EC), Epicatechin Gallate (ECG), and Epigallocatechin (EGC), contribute significantly to the biological activity and therapeutic potential of Green tea extracts [6]. As a result, the extraction and recovery of Flavonoid-rich biocompounds from Green tea have become increasingly important in the pharmaceutical, nutraceutical, cosmetic, and food industries. Natural bioactive compounds derived from plants are receiving growing interest as sustainable alternatives to synthetic chemicals due to increasing consumer demand for natural products and environmentally friendly production technologies. Flavonoids are particularly valuable because of their ability to scavenge free radicals, inhibit lipid peroxidation, reduce oxidative stress, and modulate various cellular signaling pathways. Oxidative stress is strongly associated with the development of numerous chronic diseases, including cancer, cardiovascular disorders, diabetes, neurodegenerative diseases, and inflammatory conditions [7–11]. Therefore, plant-derived antioxidants have gained considerable attention as potential protective agents against oxidative damage. Green tea leaves are recognized as one of the richest natural sources of polyphenolic and Flavonoid compounds, making them an ideal candidate for the extraction of biologically active phytochemicals. Conventional extraction methods for recovering Flavonoids and other phenolic compounds from plant materials often involve prolonged extraction times, high solvent consumption, elevated temperatures, and relatively low extraction efficiencies. Traditional techniques such as maceration, Soxhlet extraction, and reflux extraction may also lead to degradation of thermolabile compounds and increased environmental impact due to excessive use of organic solvents. Consequently, there has been increasing interest in the development of green extraction technologies that are more efficient, sustainable, and environmentally friendly. In recent years, Ultrasound-Assisted Extraction (UAE) has emerged as one of the most promising green extraction techniques for the recovery of bioactive compounds from plant matrices [12].

UAE utilizes acoustic cavitation phenomena generated by ultrasonic waves in liquid media. The formation, growth, and collapse of microscopic cavitation bubbles create localized high pressure and temperature gradients that enhance mass transfer, disrupt plant cell walls, and facilitate the release of intracellular bioactive compounds into the extraction solvent [13]. Compared with conventional extraction methods, UAE offers several advantages, including shorter extraction time, reduced solvent consumption, improved extraction efficiency, lower energy requirements, and enhanced recovery of heat-sensitive compounds. Moreover, UAE is considered an environmentally sustainable technique that aligns with the principles of green chemistry and sustainable processing technologies. Several studies have demonstrated the effectiveness of UAE in improving the extraction of phenolic compounds, Flavonoids, alkaloids, and essential oils from various medicinal and aromatic plants [14–17]. The extraction efficiency of UAE is influenced by multiple parameters, including extraction time, solvent composition, temperature, ultrasonic power, and solid-to-solvent ratio. Optimization of these parameters is essential to maximize Flavonoid recovery while minimizing degradation of sensitive compounds. Ethanol-water mixtures are commonly used as extraction solvents due to their effectiveness in solubilizing phenolic compounds and their relatively low toxicity compared with synthetic organic solvents [18]. Furthermore, the use of food-grade and environmentally friendly solvents enhances the applicability of plant extracts in pharmaceutical, food, and cosmetic industries. In addition to extraction efficiency, evaluation of the biological activities of extracted biocompounds is an important aspect of natural product research. Antioxidant activity is one of the most widely studied biological properties of Flavonoid-rich plant extracts because of the critical role of oxidative stress in human health and disease. Various in vitro assays have been developed to evaluate antioxidant capacity, among which the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay is one of the most commonly used methods due to its simplicity, rapidity, reproducibility, and sensitivity [19–21]. The DPPH assay measures the ability of antioxidant compounds to donate hydrogen atoms or electrons and neutralize stable free radicals. The antioxidant activity observed in Green tea extracts is primarily attributed to their high concentration of Flavonoids and phenolic compounds capable of radical scavenging and metal-chelating activities. Despite extensive research on Green tea phytochemicals, continued efforts are needed to develop sustainable and efficient extraction strategies capable

of maximizing the recovery of biologically active compounds while preserving their functional properties. UAE represents a promising approach for enhancing the extraction of Flavonoid-rich biocompounds from Green tea leaves in an environmentally friendly manner. Moreover, understanding the relationship between extraction conditions and antioxidant activity is important for the development of high-value functional ingredients for industrial applications [21–23].

Therefore, the present study aimed to investigate the ultrasound-assisted green extraction of Flavonoid-rich biocompounds from Camellia Sinensis leaves and evaluate their antioxidant activity. The study focused on assessing extraction yield, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH radical scavenging activity under different extraction conditions. The findings of this work may contribute to the development of sustainable extraction technologies and support the utilization of Green tea-derived biocompounds in pharmaceutical, nutraceutical, cosmetic, and food applications.

2 | Materials and Methods

2.1 | Plant Material and Chemicals

Fresh Green tea leaves were obtained from a local commercial supplier and thoroughly washed with distilled water to remove dust and impurities. The plant material was air-dried at room temperature for several days under shaded conditions to prevent degradation of heat-sensitive phytochemicals. The dried leaves were subsequently ground into a fine powder using a laboratory grinder and stored in airtight containers until extraction. Ethanol, methanol, Aluminum Chloride ($AlCl_3$), Folin-Ciocalteu reagent, Sodium Carbonate (Na_2CO_3), quercetin standard, gallic acid standard, and DPPH were purchased from standard chemical suppliers and used without further purification. All chemicals and solvents used in the experiments were of analytical grade. Distilled water was used throughout the study [15].

2.2 | Ultrasound-Assisted Extraction of Flavonoid-Rich Biocompounds

UAE was performed to recover Flavonoid-rich biocompounds from Camellia Sinensis leaves. Briefly, 5 g of powdered plant material was mixed with 100 mL of 70% ethanol solution in a conical flask. The extraction process was carried out using an ultrasonic bath operating at a frequency of 40 kHz and an ultrasonic power of 250 W.

Different extraction times (10, 20, and 30 min) were investigated to evaluate their effects on extraction efficiency and recovery of bioactive compounds. During extraction, the temperature was maintained below 40°C to minimize degradation of thermolabile Flavonoids and phenolic compounds. Following ultrasonication, the extracts were filtered using Whatman No. 1 filter paper to remove plant residues. The filtrates were concentrated using a rotary evaporator under reduced pressure at 45°C to remove excess solvent. The obtained crude extracts were stored at 4°C in dark containers until further analysis [17]. The extraction yield (%) was calculated using the *Eq. (1)*:

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dry plant material}} \times 100\%. \quad \textbf{(1)}$$

2.3 | Determination of Total Phenolic Content

The TPC of the extracts was determined using the Folin-Ciocalteu colorimetric method with slight modifications. Briefly, 0.5 mL of plant extract was mixed with 2.5 mL of diluted Folin-Ciocalteu reagent and incubated for 5 min at room temperature. Subsequently, 2 mL of 7.5% sodium carbonate solution was added to the mixture, followed by incubation in the dark for 30 min. The absorbance was measured at 765 nm using a UV-Visible spectrophotometer. Gallic acid was used as the standard reference compound, and the results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) [18].

2.4 | Determination of Total Flavonoid Content

The TFC was evaluated using the aluminum chloride colorimetric assay. In this method, 1 mL of plant extract was mixed with 1 mL of 2% aluminum chloride solution and incubated at room temperature for 15 min. The absorbance of the resulting solution was measured at 415 nm using a UV-Visible (Ultraviolet–Visible) spectrophotometer. Quercetin was used as the calibration standard, and the Flavonoid content was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract) [17].

2.5 | 2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Assay

The antioxidant activity of the extracted biocompounds was evaluated using the DPPH free radical scavenging assay. Briefly, 1 mL of extract at different concentrations was mixed with 2 mL of freshly prepared DPPH methanolic solution (0.1 mM). The reaction mixtures were incubated in the dark at room temperature for 30 min. The decrease in absorbance was measured at 517 nm using a UV-Visible spectrophotometer. The radical scavenging activity was calculated using the *Eq. (2)*:

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{A0} - \text{A1}}{\text{A0}} \times 100, \quad \textbf{(2)}$$

where A_0 represents the absorbance of the control and A_1 represents the absorbance of the sample.

2.6 | Statistical Analysis

All experiments were carried out in triplicate, and the obtained results were expressed as mean ± Standard Deviation (SD). Statistical analyses were performed using standard analytical software. Differences between experimental groups were evaluated using one-way Analysis of Variance (ANOVA), and values of $P < 0.05$ were considered statistically significant.

3 | Results and Discussion

3.1 | Extraction Yield

The efficiency of UAE was evaluated by determining the extraction yield obtained under different extraction times. The extraction yield increased progressively with increasing ultrasonication time, indicating that prolonged exposure to ultrasonic waves enhanced the release of intracellular bioactive compounds from the plant matrix. The cavitation effect generated during ultrasound treatment likely promoted disruption of plant cell walls, improved solvent penetration, and facilitated mass transfer between the solvent and plant tissues. Among the investigated extraction conditions, the highest extraction yield was observed at 30 min of ultrasonication. The increased extraction efficiency may be attributed to enhanced diffusion of soluble compounds into the extraction medium as a result of prolonged acoustic cavitation. However, excessively long extraction times may potentially lead to degradation of sensitive phytochemicals due to localized heat generation and oxidative reactions.

The observed results demonstrate that UAE is an effective green extraction technique for improving the recovery of Flavonoid-rich biocompounds from Camellia Sinensis leaves while reducing extraction time and solvent consumption compared with conventional extraction methods. The extraction yields obtained under different extraction times are presented in *Fig. 1*.

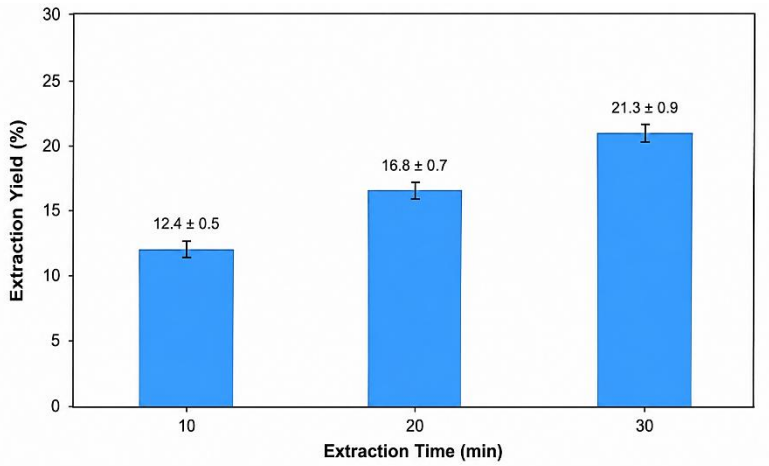

Fig. 1. Effect of UAE time on extraction yield of Flavonoid-rich biocompounds from Camellia Sinensis leaves.

3.2 | Total Phenolic Content

The TPC of the ultrasound-assisted extracts was determined using the Folin-Ciocalteu method in order to evaluate the recovery efficiency of phenolic biocompounds from Camellia Sinensis leaves. The results demonstrated that UAE significantly influenced the phenolic content of the obtained extracts. An increase in extraction time resulted in a gradual increase in TPC, suggesting enhanced release of phenolic compounds from plant tissues under prolonged ultrasonication conditions. The highest TPC value was obtained at 30 min of extraction, indicating that extended exposure to ultrasonic cavitation improved solvent penetration and facilitated the disruption of cellular structures, thereby increasing the diffusion of phenolic compounds into the extraction medium. The enhanced extraction efficiency may also be associated with improved mass transfer generated by acoustic cavitation effects. Phenolic compounds are considered important natural antioxidants due to their ability to donate hydrogen atoms or electrons and neutralize reactive oxygen species. Therefore, the relatively high phenolic content observed in the extracts may contribute significantly to their antioxidant potential. Green tea leaves are naturally rich in catechins and other polyphenolic compounds, which are recognized for their strong biological and pharmacological activities. The obtained results are consistent with previous studies reporting that UAE can improve the recovery of phenolic compounds from medicinal and aromatic plants while reducing extraction time and solvent usage. The TPCs obtained under different extraction conditions are presented in *Fig. 2*.

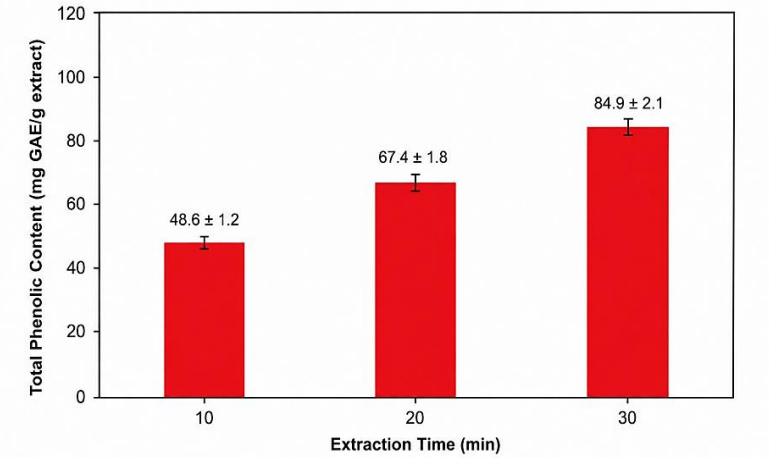

Fig. 2. Effect of UAE time on TPC of Camellia Sinensis leaf extracts.

3.3 | Total Flavonoid Content

The TFC of the ultrasound-assisted extracts was evaluated using the aluminum chloride colorimetric method to determine the efficiency of Flavonoid recovery from Camellia Sinensis leaves. The obtained results demonstrated that extraction time had a significant effect on Flavonoid extraction efficiency. A progressive increase in Flavonoid content was observed with increasing ultrasonication time, indicating enhanced release of Flavonoid compounds from the plant matrix under ultrasound treatment. The highest Flavonoid content was recorded at 30 min of extraction, which may be attributed to improved disruption of plant cell walls and enhanced penetration of the extraction solvent caused by ultrasonic cavitation. The mechanical effects generated during UAE likely facilitated the release of intracellular Flavonoids into the surrounding solvent medium. In addition, the relatively moderate extraction temperature used during the process may have contributed to the preservation of thermolabile Flavonoid compounds. Flavonoids are among the most important bioactive phytochemicals present in Green tea and are widely recognized for their antioxidant, anti-inflammatory, antimicrobial, and health-promoting properties. Major Flavonoids present in Camellia Sinensis include catechins and their derivatives, which contribute substantially to the biological activities of Green tea extracts. Therefore, efficient extraction of these compounds is essential for the development of functional ingredients and nutraceutical products. The observed increase in TFC with prolonged ultrasonication is consistent with previous studies demonstrating the effectiveness of UAE in improving the recovery of Flavonoids and other phenolic compounds from medicinal plants. The obtained TFC values under different extraction conditions are presented in *Fig. 3*.

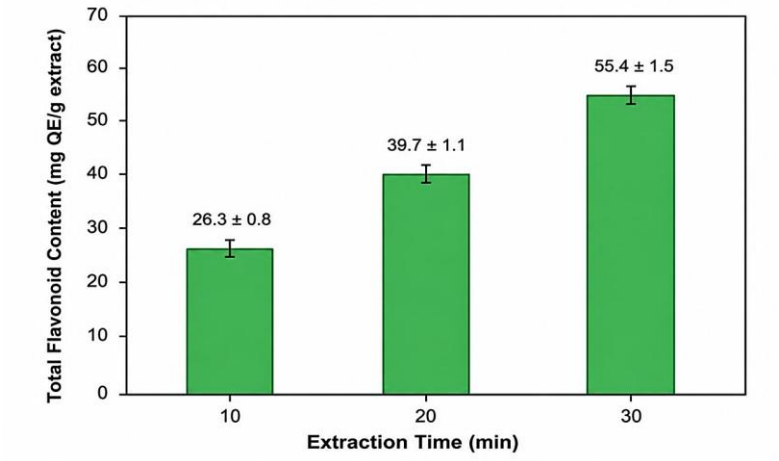

Fig. 3. Effect of UAE time on TFC of Camellia Sinensis leaf extracts.

3.4 | 2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Activity

The antioxidant activity of the Flavonoid-rich extracts obtained from Camellia Sinensis leaves was evaluated using the DPPH free radical scavenging assay. The results demonstrated that the extracts possessed considerable antioxidant activity, which increased progressively with increasing extraction time. This enhancement in radical scavenging activity may be associated with the higher recovery of phenolic and Flavonoid compounds during prolonged UAE. Among the investigated extraction conditions, the extract obtained after 30 min of ultrasonication exhibited the highest DPPH radical scavenging activity. The improved antioxidant capacity may be attributed to the increased concentration of catechins, Flavonoids, and other polyphenolic compounds extracted from the Green tea leaves. These phytochemicals are known to act as effective hydrogen or electron donors capable of neutralizing free radicals and reducing oxidative stress. The antioxidant mechanism of Flavonoids is mainly related to their hydroxyl functional groups, which can stabilize reactive oxygen species through electron transfer and radical scavenging processes. In addition, phenolic compounds may chelate metal ions and inhibit oxidation reactions, thereby contributing to the overall antioxidant potential of the extracts. The obtained results indicate a positive correlation between TPC,

TFC, and antioxidant activity of the extracts. The relatively high DPPH scavenging activity observed in the ultrasound-assisted extracts supports the effectiveness of UAE as a green extraction technology for recovering antioxidant-rich biocompounds from plant materials. The observed findings are in agreement with previous studies reporting strong antioxidant activity in Green tea extracts due to their abundant Flavonoid and catechin composition. The DPPH radical scavenging activities of the extracts obtained under different extraction conditions are presented in *Fig. 4*.

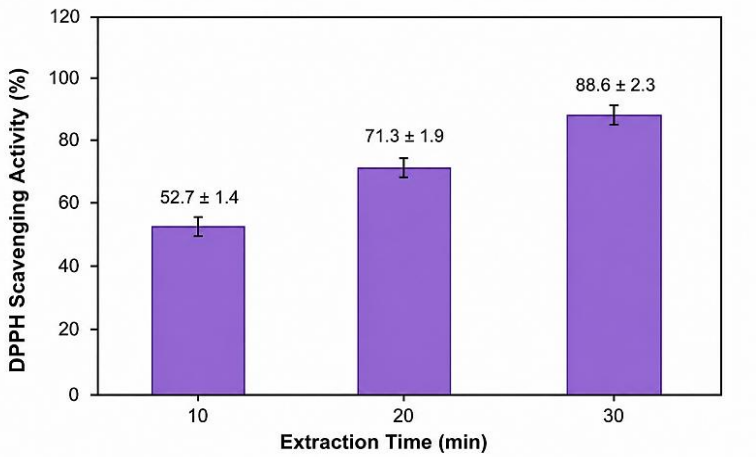

Fig. 4. DPPH radical scavenging activity of ultrasound-assisted extracts from Camellia Sinensis leaves under different extraction times.

3.5 | Correlation between Extraction Efficiency and Antioxidant Activity

The present study demonstrated a clear relationship between UAE efficiency and the antioxidant activity of the obtained Camellia Sinensis extracts. As extraction time increased, higher extraction yield, TPC, and TFC were observed, which corresponded to enhanced DPPH radical scavenging activity (*Fig. 5*). These findings suggest that the antioxidant potential of Green tea extracts is strongly influenced by the concentration of recovered phenolic and Flavonoid biocompounds. The positive correlation between phenolic content and antioxidant activity may be attributed to the chemical structure of phenolic compounds, particularly the presence of hydroxyl groups capable of donating electrons or hydrogen atoms to neutralize free radicals. Flavonoids and catechins present in Green tea are recognized as highly effective natural antioxidants due to their radical scavenging, metal-chelating, and oxidative stress-reducing properties. UAE appears to significantly improve the release of these antioxidant compounds from plant tissues through acoustic cavitation mechanisms. The collapse of cavitation bubbles generates localized pressure gradients and mechanical disruption of cell walls, thereby facilitating solvent penetration and diffusion of intracellular phytochemicals into the extraction medium. Consequently, increased extraction efficiency directly contributes to improved biological activity of the extracts. The results obtained in this study indicate that optimization of ultrasound extraction conditions can play a critical role in maximizing recovery of biologically active compounds from Green tea leaves. In particular, the 30 min extraction condition produced the highest extraction yield, phenolic content, Flavonoid content, and antioxidant activity, suggesting that this extraction duration may represent a favorable condition for efficient recovery of antioxidant-rich biocompounds. The observed correlation between phytochemical content and antioxidant capacity is consistent with previously published studies reporting strong associations between phenolic compounds and free radical scavenging activity in medicinal and aromatic plants. These findings further support the application of UAE as a sustainable and efficient green technology for the production of high-value natural antioxidants suitable for pharmaceutical, nutraceutical, cosmetic, and food applications.

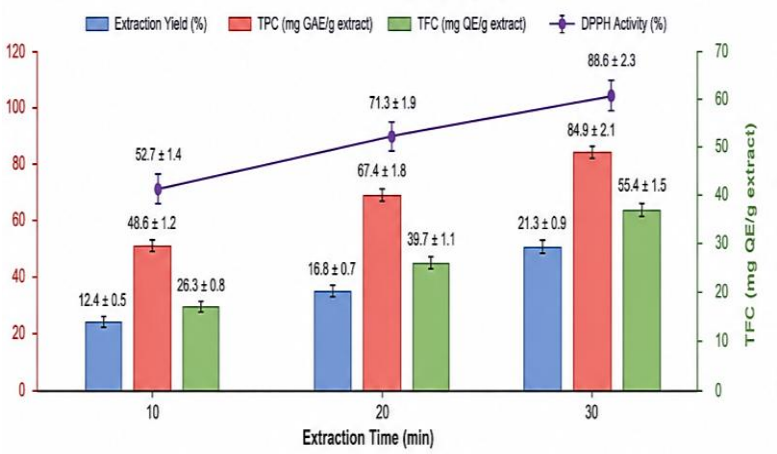

Fig. 5. Correlation between extraction yield, TPC, TFC, and DPPH radical scavenging activity of ultrasound-assisted Camellia Sinensis extracts.

3.6 | Potential Industrial and Biomedical Applications of Green Tea Biocompounds

The Flavonoid-rich biocompounds extracted from Camellia Sinensis leaves through UAE demonstrate considerable potential for industrial and biomedical applications due to their high antioxidant activity and rich phytochemical composition. Green tea-derived phenolic compounds and Flavonoids have attracted substantial attention in recent years because of their multifunctional biological properties, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, and neuroprotective activities. Consequently, these bioactive compounds are increasingly being explored as natural alternatives to synthetic additives and therapeutic agents in various industrial sectors. One of the most important applications of Green tea biocompounds is in the pharmaceutical and nutraceutical industries. The strong antioxidant activity observed in the present study suggests that the obtained extracts may serve as valuable sources of natural antioxidants for the prevention of oxidative stress-related disorders. Oxidative stress plays a critical role in the pathogenesis of numerous chronic diseases, including cardiovascular diseases, diabetes, cancer, and neurodegenerative disorders. Therefore, Flavonoid-rich Green tea extracts may contribute to the development of dietary supplements, functional ingredients, and natural therapeutic formulations aimed at reducing oxidative damage and improving human health. In the food industry, natural antioxidants derived from plant materials are increasingly utilized as alternatives to synthetic preservatives due to consumer preference for clean-label and naturally derived products. The antioxidant compounds extracted from Camellia Sinensis may help delay lipid oxidation, improve shelf life, and maintain the quality of food products during storage. In addition, Green tea biocompounds may be incorporated into functional foods and beverages to enhance their nutritional and health-promoting value. The cosmetic industry also represents an important area for the application of Green tea-derived biocompounds. Flavonoids and polyphenols possess anti-aging, anti-inflammatory, and photoprotective properties that may contribute to skin protection against oxidative stress and ultraviolet-induced damage. As a result, Green tea extracts are widely investigated for use in cosmetic formulations such as creams, lotions, serums, and skincare products designed to promote skin health and reduce signs of aging.

Furthermore, the use of UAE provides additional industrial advantages due to its environmentally friendly and sustainable characteristics. Compared with conventional extraction methods, UAE reduces solvent consumption, extraction time, and energy requirements while improving extraction efficiency. These characteristics make UAE highly attractive for large-scale industrial processing of natural biocompounds. The application of green extraction technologies also aligns with current global trends toward sustainable manufacturing, green chemistry, and environmentally responsible production systems.

The findings of the present study indicate that optimization of UAE conditions can significantly improve the recovery of biologically active Flavonoids and phenolic compounds from Green tea leaves. The obtained extracts may therefore serve as promising natural sources of antioxidant biocompounds for future pharmaceutical, food, nutraceutical, and cosmetic applications. Nevertheless, additional investigations including compound identification, toxicity evaluation, formulation development, and in vivo biological studies are required to further validate the industrial applicability and therapeutic potential of these extracts.

4 | Conclusion

The present study demonstrated the effectiveness of UAE as a sustainable and efficient green technology for the recovery of Flavonoid-rich biocompounds from Camellia Sinensis leaves. The results revealed that extraction time significantly influenced extraction yield, TPC, TFC, and antioxidant activity of the obtained extracts. Increased ultrasonication time enhanced the release of bioactive compounds from plant tissues, leading to improved recovery of phenolics and Flavonoids as well as increased DPPH radical scavenging activity.

Among the investigated extraction conditions, the 30 min UAE exhibited the highest extraction efficiency and antioxidant potential, suggesting that prolonged ultrasonication under controlled conditions may facilitate enhanced disruption of plant cell structures and improved mass transfer during extraction. The strong antioxidant activity observed in the extracts is likely associated with the high concentration of Flavonoids and polyphenolic compounds naturally present in Green tea leaves.

The findings of this study highlight the important relationship between phytochemical content and biological activity, demonstrating that UAE can effectively improve the recovery of natural antioxidant compounds while reducing extraction time and solvent consumption compared with conventional extraction techniques. In addition, the environmentally friendly nature of UAE makes it an attractive approach for sustainable processing of plant-derived biocompounds in accordance with green chemistry principles.

Overall, the obtained results support the potential application of Green tea-derived Flavonoid biocompounds in pharmaceutical, nutraceutical, food, and cosmetic industries due to their considerable antioxidant properties. The study also demonstrates the industrial relevance of UAE as a promising green technology for the large-scale production of biologically active natural compounds. Future studies focusing on optimization of extraction parameters, identification of individual phytochemicals, in vivo biological evaluations, and industrial-scale process development may further contribute to the practical utilization of Green tea biocompounds in various scientific and commercial applications.

Authors' Contributions

The author solely conducted the research and prepared the manuscript and has approved its final version.

Data Availability

The data are available from the corresponding author upon reasonable request.

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This work was carried out without financial support from any public, commercial, or non-profit organizations.

Conflict of Interest

There are no competing interests to declare.

Consent for Publication

The author confirms consent for the publication of this work.

Ethics Approval and Consent to Participate

This article does not include experiments involving humans or animals.

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