The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2024), 48(1), 163-177

ORIGINAL RESEARCH PAPER

PHYTOCHEMICAL SCREENING AND PRELIMINARY BIOLOGICAL *IN VITRO* ASSESSMENT OF *CAMELLIA SINENSIS* L. ETHANOLIC EXTRACT

ANDREEA GEAMANTAN-SÎRBU[1,2], ALEXANDRA SEMENESCU[1,2], ALINA MOACĂ[1,2], RAZVAN SUSAN[3*], ROBERT JIJIE[1], CRISTIAN VLAD[3]

[1]*Faculty of Pharmacy, "Victor Babes" University of Medicine and Pharmacy Timisoara, 2nd Eftimie Murgu Square, Timisoara 300041, Romania*

[2]*Research Centre for Pharmaco-Toxicological Evaluation, "Victor Babes" University of Medicine and Pharmacy, 2nd Eftimie Murgu Square, Timisoara 300041, Romania*

[3]*Faculty of Medicine, "Victor Babes" University of Medicine and Pharmacy Timisoara, 2nd Eftimie Murgu Square, Timisoara 300041, Romania*

Correspondence: razvansusan@umft.ro

Received on 16 May 2024
Revised on 16 July 2024

Abstract

Camellia sinensis L. (*Theaceae*), is the most frequently consumed beverage worldwide, due to its human health benefits. The antioxidant compounds are known to act as a protective barrier of cell membranes against oxidative injury caused by various free radicals as well as by reactive oxygen species. Additionally, even phenolic compounds have pharmaceutical effects on oxidative injury from diverse causes. Therefore, the present study was conceived to establish the phytochemical screening, as well as the physicochemical profile of an ethanolic extract of aerial parts of *Camelia sinensis* L. acquired from a local authentic herbal distributor. In this respect, the total phenolic and flavonoid contents were investigated, as well as the antioxidant capacity of the green tea ethanolic extract obtained. For the physicochemical profile, the organic functional groups present in the green tea extract, and their thermal behavior were identified. Moreover, a preliminary *in vitro* biological assay was performed, through the evaluation of the antitumor potential of the green tea ethanolic extract obtained on the colon adenocarcinoma cell line (HT-29) compared to a healthy cell line, HaCaT (human immortalized keratinocytes). The results after the biological assessment suggest that the extract decreases the viability of HT-29 colon tumor cells (67% at a concentration of 100 µg/mL), showing a lesser effect on HaCaT cells; this being a first step in exploring the anticancer properties of the obtained extract.

Keywords*:* green tea, DPPH, FT-IR, colon cancer

https://doi.org/10.35219/foodtechnology.2024.01.10

Introduction

Originating from the Chinese area, tea is one of the most consumed beverages among the global population (Zhao *et al*., 2022). The Tea tree (*Camellia sinensis* L. - Cs) is a member of the Theaceae family and is cultivated mainly in tropical and subtropical areas (Sirotkin and Kolesarova, 2021). The tea plant is known as a rich source of bioactive components from several types of metabolites, with polyphenols making up one third of its composition (Koch *et al*., 2019). Unfermented green tea retains its original components. Since the 19th century, it has been known that over 500 chemical components have been isolated from tea, 400 of which are organic components and about 40 are inorganic components. The chelated metal ion of copper was reported as a possible component responsible for the anti-peroxidative mechanisms of green tea (Zhao *et al*., 2022). The production of green tea is based on a hot steam treatment of fresh leaves to prevent fermentation, and then they undergo the drying process (Koch *et al*., 2019). The medical-pharmaceutical industry is facing major challenges due to the increasingly rapid spread of diseases of various etiologies, and current treatments are not always effective or have severe side effects on the body. Natural products, including green tea, are triggering incredible research attention to explore their therapeutic actions that are produced by the versatility of the components and are tools that have already proven their efficacy in various conditions. Herbal products such as *C. sinensis* L. are being investigated as complementary or sometimes alternative therapies in many pathologies, thanks to their limited adverse effects, which is an important aspect, but also because the population has been developing resistance to conventional therapies (Nag *et al*., 2023).

The diverse composition of polyphenols (such as theaflavins), flavonol glycosides, L-theanine, caffeine, and volatile organic compounds is responsible for the versatility of green tea's health promoting benefits. Among the most important therapeutic effects are antioxidant, anti-inflammatory, hepatoprotective, anticancer, antimutagenic, antimicrobial and antiparasitic properties (Aboulwafa *et al*., 2019). Moreover, green tea has proven its efectiveness in cardiovascular diseases, diabetes, obsesity and gastric disorders or dysfunctions (Samanta, 2022). Compared to other types of tea, it has the highest antioxidant content, but, the composition of the tea depends on the fermentation process used to obtain it (Prasanth *et al*., 2019). Green tea also contains other elements such as phenolic acids (e.g. gallic acid), flavonols (e.g. quercetin, myricetin), vitamins (B, C and E), minerals, trace elements such as calcium, magnesium or zinc, and proanthocyanidins. Polyphenols can be direct antioxidants by scavenging reactive oxygen species (ROS), but they can also be indirect antioxidants by increasing the level of stimulation of phase II oxidative enzymes (Spadiene *et al*., 2014). In general, health-promoting effects are correlated with the presence of polyphenols and flavonols (which constitute 30% of the dry weight of fresh leaves) (Chacko *et al*., 2010). Polyphenols can modulate inflammatory processes; they are known as secondary plant metabolites that provide protection against UV radiation and against pathogens. They can also activate the

transcription factor Nrf2, a critical key element in providing protection to cells against inflammation or oxidative stress (Hussain *et al*., 2016).

The antioxidant activity of green tea is attributed to its ability to neutralize free radicals in the body, which can cause serious cellular damage and contribute to cancer development. In addition to this, it has also been established that green tea extracts may have anticancer effects. The mechanisms of action involved in the biological activity of green tea are not complete and require further investigations (Brown, 1999). Also, for these future evaluations, it is imperative to know and characterize the compounds included and the possible targets of each compound.

The purpose of the present study was the establishment of phytochemical and physicochemical profiles of an ethanolic extract of *Camelia sinensis* L. (green tea) plant material, through the determination of its polyphenols and flavonoid contents, as well as through the evaluation of its antioxidant capacity. The physicochemical characterization was performed both to identify the organic functional groups of the potential biologic active compounds present in the green tea extract, as well as to assess the purity and thermic stability of the green tea extract, for future pharmaceutical formulations. Therefore, to establish the biological effect of the prepared green tea extract, a preliminary *in vitro* evaluation was conducted to determine the antiproliferative effect of ethanolic green tea extract on colorectal adenocarcinoma cells (HT-29), compared to its safety on human healthy cells (human immortalized keratinocyte cell line - HaCaT).

Materials and methods

Reagents

For the obtaining of the ethanolic extract, ethanol (≥ 99.8%, p.a.) was used, having been acquired from Carl Roth Company (Karlsruhe, Germany). For the total phenolic content assessment, the Folin-Ciocalteu reagent (purchased from Merck, Darmstadt, Germany), Na_2CO_3 99%, and gallic acid 98% (both purchased from Roth, Dautphetal, Germany) were used. For the total flavonoid content assessment, were used $AlCl_3$ 98% from Roth (Dautphetal, Germany), $NaNO_2$ from Merck (Darmstadt, Germany), and NaOH pellets, from ChimReactiv SRL (Bucharest, Romania) were utilised. The standard used was (+)-Cathechin hydrate 98%, acquired from Sigma-Aldrich.

For the antioxidant potential assessment, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used, purchased from Sigma Aldrich (Steinheim, Germany). The standard used as etalon was the ethanolic solution of ascorbic acid (Lach-Ner, Prague, Czech Republic).

In regard to *in vitro* studies, the culture medium used for the cell lines - McCoy's 5A Medium and Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS) and trypsin-EDTA were purchased from PAN Biotech (Aidenbach, Germany); while dimethyl sulfoxide (DMSO - solvent), phosphate-buffered saline, MTT [3-(4,5-dimethylthiazole-2-bromide)-yl)-2,5-diphenyltetrazolium)] kit, and

penicillin-streptomycin mixture, were provided by Sigma-Aldrich, Merck KgaA (Darmstadt, Germany).

Cell culture

For the *in vitro* study, the cell lines used were HT-29 (HTB-38™) (colorectal adenocarcinoma) procured from the American Type Culture Collection (ATCC, Manassas, VA, USA) and HaCaT (300493) (human immortalized keratinocytes) from CLS Cell Lines Service. HT-29 cells were grown in McCoy's 5A Medium, while HaCaT cells were grown in Dulbecco's Modified Eagle's medium (DMEM), both specific culture mediums being supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin mix. Cells were incubated at 37°C and 5% CO_2.

Extract preparation protocol

Camellia sinensis L. (batch no. 11/21) herbal material was purchased from a local authentic herbal distributor—Favisan SRL (Lugoj, Romania). According to the protocol proposed by Ameer and co-workers (Ameer *et al*., 2022), the green tea ethanolic extract was obtained. Briefly, five grams of green tea leaves were grounded and subjected to the sonication process, after mixing them with 300 mL of high purity ethanol. The sonication process was conducted using a Q 700 sonicator (Qsonica Sonicators, Newtown, CT, USA) at an amplitude of 50% for 10 minutes. After sonication, the ethanolic extract was filtered and concentrated at 35 °C under reduced pressure, using a rotary evaporator (Heidolph G3, Schwabach, Germany). Finally, the extract was lyophilized in a Christ Alpha 1–2 freeze dryer (Osterode, Germany) at −60 °C, then stored in a refrigerator until further use. For the biological analysis, the Cs lyophilized extract was mixed with 0.5% DMSO, until a stock concentration of 1 mg/mL was obtained.

Phytochemical investigations of green tea ethanolic extract

The total phenolic (TPC) and flavonoid (TFC) contents of green tea ethanolic extract were conducted according to the protocol detailed in the reference (Moaca *et al*., 2019). The results obtained were expressed as milligrams of gallic acid equivalent (GAE) per gram extract, for the total phenolic content, and as milligrams of catechin equivalent per gram extract (mg CE/g extract), for the total flavonoid content.

Physicochemical investigations of green tea ethanolic extract

Organic functional group identification by Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectrum of the lyophilized green tea-dried extract was performed using a Prestige-21 spectrometer (Shimadzu, Duisburg, Germany) at room temperature. The spectral region ranged from 4000-400 cm^{-1}, the resolution was of 4 cm^{-1}, using KBr pellets (Semenescu *et al*., 2023).

Thermal behavior of the green tea ethanolic extract

The thermal behavior of the lyophilized dried green tea extract was conducted using the Netzsch STA 449C instrument (Selb, Germany), in the temperature range of 25–900 °C. The instrument was equipped with alumina crucibles and the working parameters for the thermogravimetric (TG) and differential scanning calorimetry

(DSC) curves included a heating rate of 10 °C/min and a flow rate of 20 mL/min in an artificial air atmosphere.

Antioxidant capacity assay

The antioxidant capacity of the green tea ethanolic extract was determined by DPPH free-radical scavenging assay, the working protocol being detailed in reference (Moaca *et al*., 2019). One milligram of the lyophilized extract was weighed in order to prepare a stock solution with a concentration of 1 mg/mL of green tea in ethanol. Further, through the dilution of the stock concentration, another two samples were obtained, as follows: 0.5 mg/mL and 0.1 mg/mL. The results obtained were expressed in comparison to an ethanolic solution of ascorbic acid 0.2 mM (used as etalon).

***In vitro* biological assay**

Cellular viability evaluation

To observe the *in vitro* impact of *Camellia sinensis* L. and its action on colorectal adenocarcinoma cells as well as on healthy cells, the MTT assay was performed. The test was carried out on HT-29 cells (colorectal adenocarcinoma) and the healthy cell line (HaCaT cells - human immortalized keratinocytes). Cells were cultured in a 96-well plate and allowed to attach to the plate. When the desired confluence was reached, the cells were treated with green tea extract at concentrations of 25, 50, and 100 µg/ml for 24 hours. After the 24 hours of incubation, the medium was replaced with 100 µL/well of fresh medium and, subsequently, 10 µL of MTT Kit-1 was added to each well; then the 96-well plate was placed in the incubator for 3 hours. After this time, 100 µL of Kit 2-solubilization buffer solution was added using a multichannel pipette to each well, and the plate was kept at room temperature and protected from light for 30 minutes. The final step was to measure absorbance at 570 nm using Cytation 5 (BioTek Instruments Inc., Winooski, VT, USA.). Cell viability was calculated and compared to untreated control cells and expressed in percentages (%), as described in a previous study (Semenescu *et al*., 2024).

Results and discussion

Phytochemical analysis

Table 1 depicts the results obtained after investigating the total phenolic and flavonoid contents of the green tea ethanolic extract.

Phytoconstituents that are generally investigated in medicinal herbs are polyphenols and flavonoids due to their important biological activities, including their antioxidant capacity. Our ethanolic extract exhibited a high amount of TPC and TFC, agreeing with other research studies (Ydyrys *et al*., 2021; Rafique *et al*., 2023), considering the extraction method used, the amount of the plant material subjected to the extraction process, as well as the solvent concentration used. This high amount of polyphenols content in the ethanolic extract of green tea suggests the important role of this extract in the prophylactics of several diseases (Lorenz *et al*., 2013).

Table 1. Total phenolic and flavonoid contents of the ethanolic green tea extract

Sample	TPC, mg GAE/g Extract	TFC, mg CE/g Extract
Green tea ethanolic extract	476.15 ± 1.26	1904.6 ± 5.34

Physicochemical investigations

Organic functional group identification by FT-IR

To identify the functional groups that are present in the lyophilized green tea extract, the FT-IR spectroscopy was employed. This analysis is based on the match of a peak value, recorded in the region of IR radiation, with the absorption peak of an organic molecule from the library. The FT-IR spectrum of the lyophilized green tea extract is shown in Figure 1.

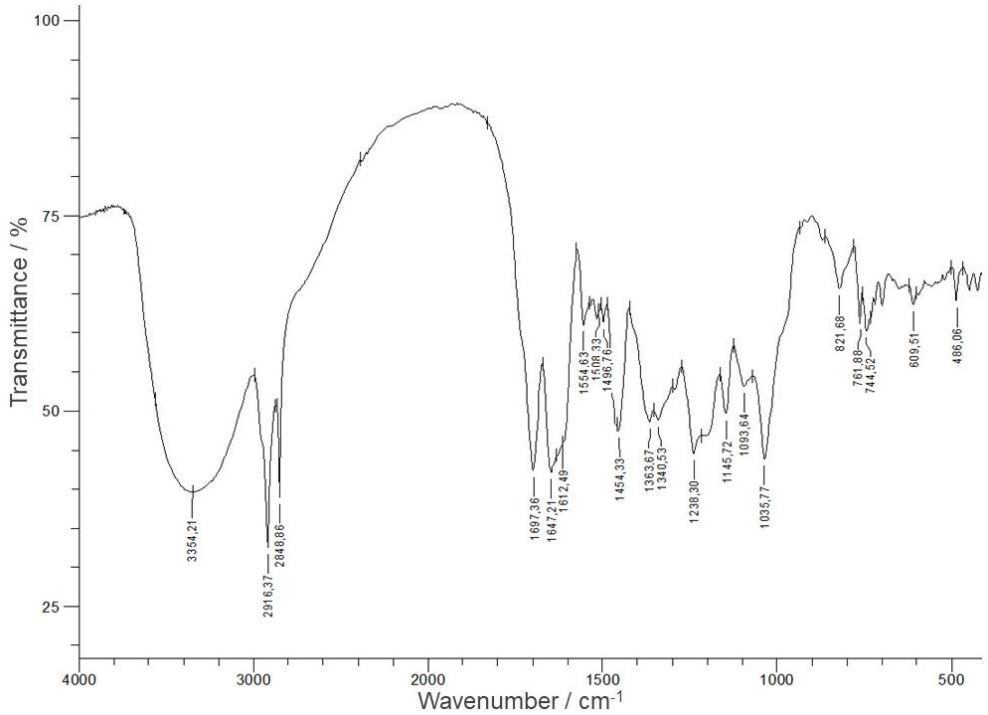

Figure 1. The FT-IR spectrum of the lyophilized green tea extract

The most important bands recorded in the IR region are at 3354.21 cm^{-1}, a strong and broad absorption band that corresponds to the O-H stretching of hydroxyl groups from alcohols, phenols, or carboxylic acids present in ethanolic green tea extracts (Szymczycha-Madeja *et al*., 2013). The intense absorption band recorded at 2916.37 cm^{-1}, attributed to the N-H stretching groups (Mohani *et al*., 2014), highlights the amine salts. The medium absorption band, recorded at 2848.86 cm^{-1}, can correspond

to the C-H stretching functional groups from alkanes. The intense absorption bands, which are around 1600 cm^{-1}, can be attributed to the C=O stretching functional groups present in primary amides, to the C=C stretching functional groups present in alkenes, as well as to the C=C stretching functional groups from α,β-unsaturated ketones. At the same time, the peak, recorded at 1612.49 cm^{-1}, could confirm the presence of N-H amine functional groups (Kumar 2012).

The medium multiple absorption bands, recorded between 1554.63 - 1340.53 cm^{-1}, can correspond to the C-H bending functional groups from alkanes (the binding of the aromatic ring –C-H) or aldehydes compounds. However, at the same time, the region between 1454.33 - 1340.53 cm^{-1} highlighted the O-H bending functional groups, either from carboxylic acids or from alcohols. This spectral region confirms the presence of organic compounds with an aromatic ring (Mohani *et al*., 2014). At 1238.30 cm^{-1}, the absorption band of the aromatic acid esters' C-O stretching vibration is recorded (Li *et al*., 2013). Between 1035.77 and 1145.72 cm^{-1}, the bands recorded correspond to the C-O stretching functional groups from primary, secondary, and tertiary alcohols (Li *et al*., 2013). The bands, recorded in the range 821.68 - 486.06 cm^{-1}, correspond to the out-of-plane bending vibration of =C-H functional groups from the aromatic alkenes (Heredia-Guerrero *et al*., 2014).

The FT-IR investigation gives us only structural information regarding the identification of functional organic molecules present in a plant extract, but for completing the phytochemical profile of ethanolic extract of green tea, a quantitative analysis of the extract, which provides the amount and confirms the identity of a bioactive compound, is required.

Thermal behavior of the green tea extract

The thermal behavior of the lyophilized green tea extract was performed in order to establish the purity of the extract, as well as its thermal stability, considering that after the complex physicochemical investigation and a preliminary *in vitro* evaluation, the lyophilized green tea extract would be further used as raw material in various pharmaceutical formulations for *in vivo* studies.

Figure 2 depicts the TG-DSC curves of the lyophilized extract of *Camelia sinensis* L. plant material. One can observe that the thermal analysis of the lyophilized green tea extract revealed a total mass loss of 98.97% in four stages. In the first stage, the green tea ethanolic extract loses 4.65%, without any thermal effects recorded. This slight mass loss can be attributed either to the water rest elimination or to the partial decomposition of green tea extract. The second stage is located between 200 °C and 400 °C; within this interval, the sample loses 52.29% of the total mass without any exothermic or endothermic effect. In this stage, an important mass loss was recorded without energy changes observed on DSC curves, which means that in this stage the degradation of the phenolic acids and flavonoids contained in the green tea extract occurs. The phenolic acids and flavonoids may be present in the green tea extract in a low content, since no thermic effect was recorded on the DSC curve. Their presence in the extract is confirmed by the mass loss recorded (52.29%), which is relatively reduced, in this stage of the analysis. In the third stage, that of extract degradation, an exothermic process occurred at 431.1 °C, and a total mass loss of

7.68% was recorded. In this stage, the exothermic process accompanied by the lowest mass loss can be related to the initial degradation of aromatic amino acids and/or carbohydrates present in the green tea extract. In the final stage of extract degradation, an important mass loss (34.44%) occurs, but with a strong exothermic effect recorded at 560.6 °C, which can be related to the complete degradation of aromatic compounds, carbohydrates, and aromatic amino acids present in the green tea extract.

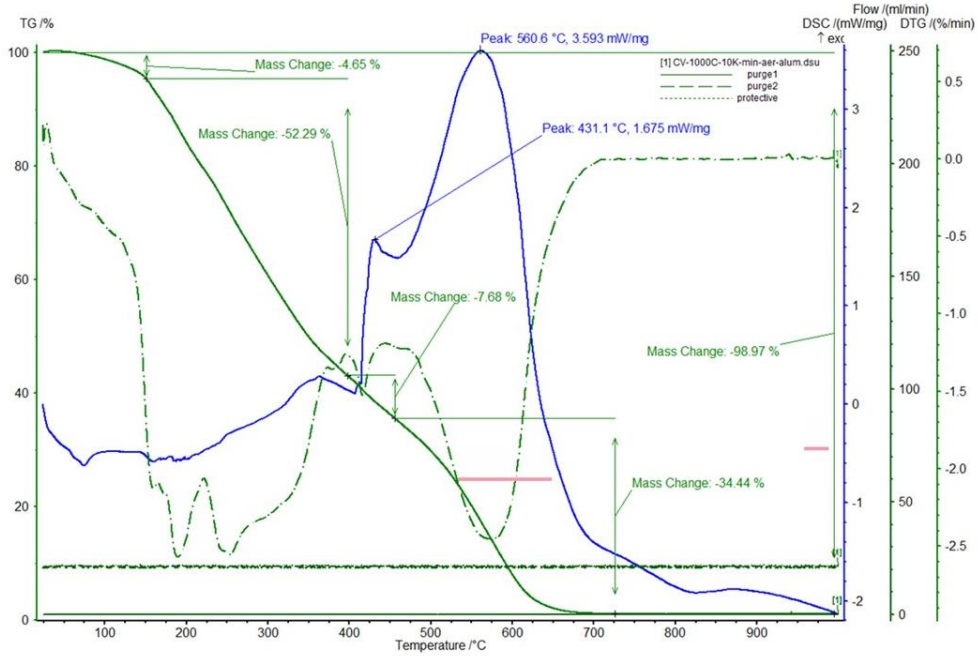

Figure 2. TG-DSC curves of the lyophilized green tea extract

Antioxidant capacity evaluation

To evaluate the antioxidant capacity (AC) of the as-prepared green tea ethanolic extract, the stock solution (1 mg/mL), as well as the samples obtained through the dilution of the stock concentration (0.5 mg/mL and 0.1 mg/mL), were investigated. The inhibition capacity of the green tea ethanolic extract samples were evaluated in time, continuously, for 20 minutes, and the values obtained were compared to the values of the ethanolic solution of ascorbic acid (used as etalon), and the results were depicted in Figure 3. One can notice that the green tea ethanolic extracts show antioxidant effects during the time frame of the evaluation. Moreover, the AC values of green tea ethanolic stock solution (1 mg/mL) are almost comparable with the AC values of the etalon (ascorbic acid solution of 0.2 mM concentration).

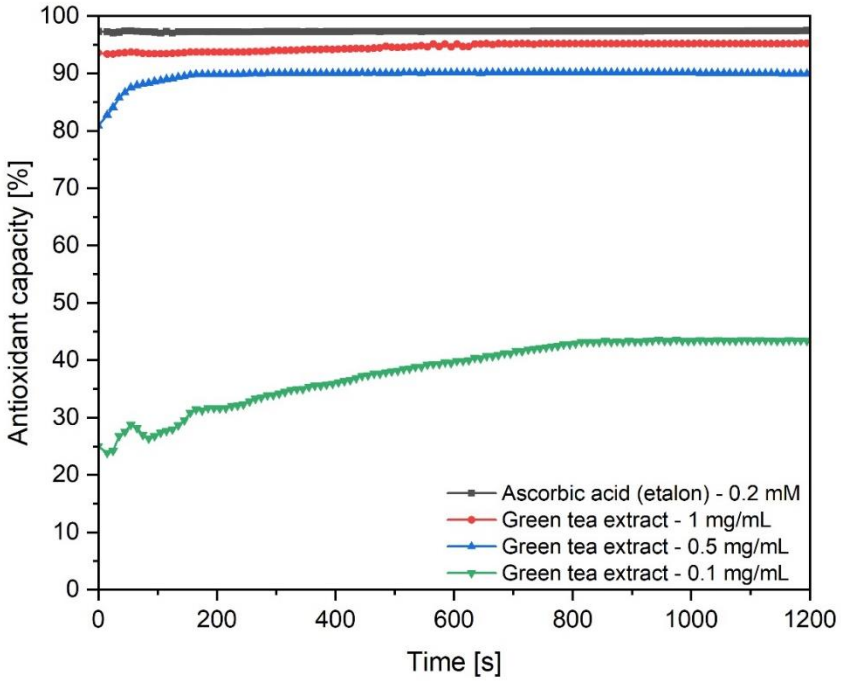

Figure 3. The time-dependent inhibition percentage of green tea ethanolic extracts vs. ascorbic acid ethanolic solution

The reduction rate of the DPPH free radical (an ethanolic solution of 1 mM concentration) is different for the three extracts. One can observe that the stock solution of green tea ethanolic extract (1 mg/mL) consumed the DPPH free radical very quickly, and the kinetics of the reaction were kept in equilibrium throughout the recording time of the analysis. The green tea ethanolic extract of 0.5 mg/mL concentration quenches the DPPH free radicals after 200 s; subsequently, the reaction reaches equilibrium. The final concentration of green tea extract tested (0.1 mg/mL), reacted slowly with the DPPH free radicals, scavenging the substrate after 800 s, reaching the equilibrium after that. Moreover, the 0.1 mg/mL green tea extract concentration displayed some fluctuation during the analysis, but this phenomenon could be due to the relatively low amount of antioxidant compounds that remained in the sample after successive dilution. The inhibition percentages were calculated with the equation described by Moaca and co-workers (Moaca *et al.*, 2019). Table 2 presents the inhibition percentage of the green tea ethanolic extracts, calculated as a mean ± standard deviation of three different measurements.

Regarding the antioxidant capacity of the green tea ethanolic extracts investigated in this study, it was demonstrated that the antioxidant capacity increases with the concentration increase, meaning, the antioxidant potency is concentration dependent. Our results are in agreement with those obtained by Rafique and co-workers (Rafique *et al*., 2023), who investigated the antioxidant activity of 80% green tea ethanolic extract and showed a DPPH free radical scavenging activity of

77.70%. Khalaf and co-workers (Khalaf *et al*., 2008) reported higher values of antioxidant activity of ethanolic and methanolic green tea extracts through DPPH assay.

Table 2. The inhibition percentages of green tea ethanolic extracts, as compared to the inhibition percentage of ascorbic acid ethanolic solution of 0.2 mM

Green tea ethanolic extracts		Ascorbic acid (etalon)	
Concentration, mg/mL	Inhibition percentage, %	Concentration, mM	Inhibition percentage, %
1	95.24±0.017	0.2	97.47±0.017
0.5	89.92±0.004		
0.1	43.41±0.025		

The polyphenolic compounds (flavonoids and phenolic acids) are usually responsible for the antioxidant effect of plant materials (Nooreen *et al*., 2017). Antioxidants are crucial substances that have the potential to prevent or delay some types of cell damage, being the most employed compounds worldwide. Antioxidants are natural products, enzymatic and nonenzymatic, which possess a protective mechanism in battle and are useful in eliminating the reactive oxygen species (ROS). Through ROS overproduction, due to the metabolic reaction that uses oxygen, oxidative stress is formed (Birben *et al*., 2012). The human body produces antioxidants known as endogenous antioxidants; while the exogenous antioxidants could be synthetic or natural such as vitamins A, C, D, and E; beta-carotene; lycopene; flavonoids; flavonols; polyphenols; phytoestrogenes; lutein; etc. Plants and food contain antioxidants that are the most radical scavengers that can be found, sensitive to oxidation and that could mitigate the damaging effects of ROS in the human body (Huang, 2018). The hydroxyl groups from organic molecules have the hydrogen-donating property, thus making plant polyphenols act as reducing agents, as well as antioxidants (Aberoumand and Deokule, 2008).

In vitro biological assay

Cellular viability test

As a preliminary study, due to the results observed in the physicochemical analyses and in order to observe the *in vitro* anticancer abilities of the compound of interest, the MTT assay was performed to evaluate the cell viability following the treatment with *Camellia sinensis* L. extract (Cs) on a colorectal adenocarcinoma cell line (HT-29) and on a healthy cell line (HaCaT). After the cells reached the desired confluence, they were treated with 25, 50 and 100 µg/ml Cs for 24 hours. Evaluation results showed a dose-dependent decrease in cell viability in HT-29 colorectal adenocarcinoma cells, with the most significant reduction, approximately 67%, being observed at the highest concentration (100 µg/ml), as shown in Figure 4A. For HaCaT cells (Figure 4B), however, Cs appears to have lower potency, with cell viability percentages dose-dependently reducing slightly to about 89% at treatment with the concentration of 100 µg/ml.

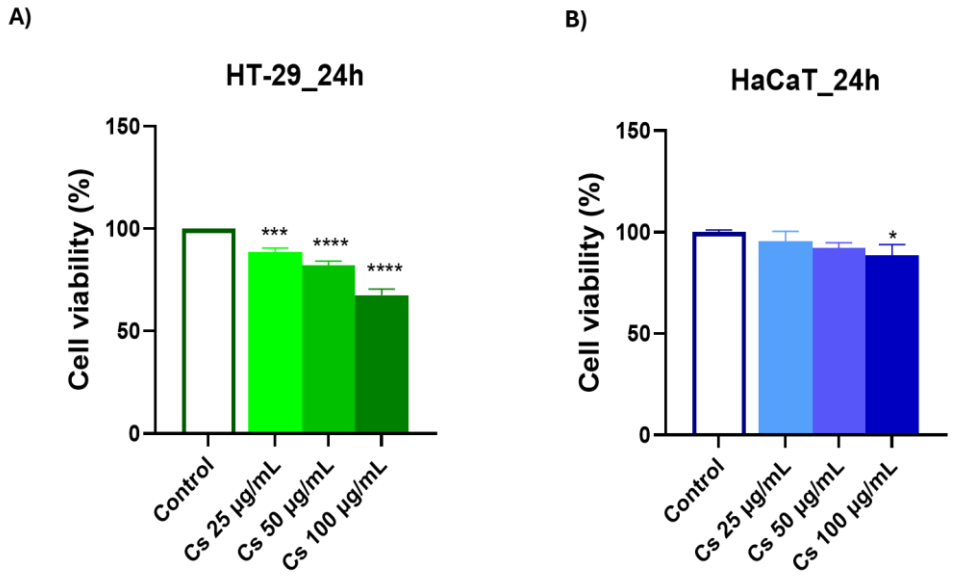

Figure 4. *In vitro* cell viability evaluation of the Cs extract in A) HT-29 cells and B) HaCaT cells after 24h of treatment. The statistical differences between the untreated group and the control group were confirmed by applying the one-way ANOVA test followed by Dunnett's multiple comparisons post-test (* $p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$).

Colorectal cancer (CRC) is a type of cancer with a poor prognosis, especially when diagnosed in its early stages. It can occur sporadically, but there are also inherited patterns. Adenocarcinoma accounts for about 95% of all forms of CRC, being a real global health problem (Thrumurthy *et al*., 2016).

Studies in the literature have also tested green tea extracts on various cancer lines, and the data obtained correlates with antiproliferative action on tumor cells. Furthermore, green tea extracts have been shown to act on several types of cancers. Zhang *et al.* (2022) used Aged Chinese Green Tea Extract (0-1.0 mg/ml) on the HT-29 cell line. Results obtained at 24 and 48 hours, respectively, showed a more prominent decrease in cell viability with increasing concentration, and, subsequently, cell viability decreased more after 48 hours, which also indicates a time-dependent manner of cytotoxicity. The findings of this evaluation reinforced that the cell proliferation was inhibited along with cell cycle progression and promoted apoptosis (programmed cell death) by inactivating PI3K/AKT (Zhang *et al*., 2022). Mehrabani (2022) evaluated aqueous green tea extract on the HT-29 cell line. Their results suggest, based on the MTT assay, that the extract produces a moderate decrease in cell viability after 48 hours of treatment, the effects also being dose and time dependent. The safety of the extract was addressed on the 3T3 line, which showed no significant changes after treatment. Trypan blue dye exclusion test revealed that the extract was toxic on HT-29 (at a concentration of 100 µg/mL) after 48 hours (Mehrabani *et al*., 2022).

Santos and co-workers (2021) evaluated *Camellia sinensis* L. extract on breast cancer *in vitro* (MDA-MB-231 cell line) at concentrations of 32.5-1000 µg/mL, with cell viability results after 24 and 48 hours also revealing a decrease in viable cells. Similarly to the HT-29 cell line, cell viability decreases in a dose-dependent manner, with the greatest reductions in viability indicated at the highest doses. No significant cytotoxic effects were reported on non-tumor MCF-10A cells, indicating that green tea extract was specifically cytotoxic on cancer cell lines. Furthermore, the extract reduced cell migration in both cancer cell lines, with more significant effects on MDA-MB-231 (Santos *et al*., 2021). Other studies have observed that green tea extract can induce autophagy in A549 (non-small lung cancer cell line) cells, characterized by vacuoles filled with organelle remnants and swollen mitochondria devoid of cristae. In addition, in the same study on A549, green tea extract caused no significant decrease in cell viability (Izdebska *et al*., 2015).

In principle, the selectivity of an agent is an important property, and the need for the compounds that act specifically on cancer cells, while not affecting normal cells, is increasing. This selectivity feature has become a challenge, but many preclinical studies are focused on investigating options to protect normal cells (Blagosklonny, 2023). In the current investigation, HaCaT cells revealed that they are not as sensitive to Cs action as HT-29 cells. Pooja Makhija and associates also evaluated an extract of black tea on the HaCaT cell line, and following treatment, with a concentration of 0.1 mg/ml, the percentage of cell viability was about 90% (Makhija *et al*., 2021). Additionally, green tea polyphenols have shown a promising effect in protecting HaCaT cells against UVB destruction (WU *et al*., 2009). Furthermore, an ethanolic extract of green tea was tested in another study conducted by Sarita Sangthong and co-workers on HaCaT and MRC-5 cells at concentrations between 62.5 - 1000 µg cm-3, and the results of their MTT assay indicated that in HaCaT cells the extract was slightly cytotoxic, while on MRC-5 cells it showed no cytotoxicity (Sangthong *et al*., 2024).

In the light of the above mentioned, the present study provides a first step in the visualization of green tea ethanolic extract as a potential candidate in the management strategy for colon cancer; therefore, future investigations on the antitumor potential of this plant material are necessary to observe the mechanism of action underlying the effects.

Conclusions

One of the objectives of the current study was the investigation of the phytochemical profile as well as the physicochemical screening of the lyophilized green tea extract. According to the results, the green tea ethanolic extract possesses a valuable amount of polyphenols and flavonoid compounds, whose functional groups were confirmed by the IR spectroscopy. As regards the antioxidant potency, the results obtained showed that all the three test samples have significant antioxidant potential in a concentration-dose-dependent manner. The lyophilized green tea ethanolic extract was shown to be of high purity, and stable up to 430 °C when the degradation of the aromatic amino acids and/or carbohydrates began. The second objective of the study

was the evaluation of the *in vitro* biological effects of the as-prepared green tea ethanolic extract on colon adenocarcinoma cells and a normal cell line. The MTT assay results indicate that the cellular viability decreased in a dose-dependent manner after the HT-29 cells were treated with the green tea extract, avoiding a marked cytotoxicity towards the HaCaT cells. However, even if a first satisfactory result was obtained in the preliminary *in vitro* biological evaluation, the correlation between green tea extract and the inhibition of adenocarcinoma cells still represents an important concern. Therefore, besides the results gained from the current study, further explorations remain necessary to clarify the biological potential of the green tea ethanolic extract.

References

Aberoumand, A., Deokule, S.S. 2008. Comparison of phenolic compounds of some edible plants of Iran and India. *Pakistan Journal of Nutrition*, **7**(4), 582–585.

Aboulwafa, M.M., Youssef, F.S., Gad, H.A., Altyar, A E., Al-Azizi, M.M., Ashour, M. L. 2019. A Comprehensive Insight on the Health Benefits and Phytoconstituents of Camellia sinensis and Recent Approaches for Its Quality Control. *Antioxidants (Basel, Switzerland)*, **8**(10), 455.

Ameer, K., Ameer, S., Kim, Y. M., Nadeem, M., Park, M. K., Murtaza, M. A., Khan, M. A., Nasir, M. A., Mueen-ud-Din, G., Mahmood, S., Kausar, T., Abubakar, M. 2022. A hybrid RSM-ANN-GA approach on optimization of ultrasound-assisted extraction conditions for bioactive component-rich Stevia rebaudiana (Bertoni) leaves extract. *Food*, **11**(6), 883.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O. 2012. Oxidative Stress and Antioxidant Defense. *World Allergy Organization Journal*, **5**, 9–19.

Blagosklonny, M. V. 2023. Selective protection of normal cells from chemotherapy, while killing drug-resistant cancer cells. *Oncotarget*, **14**, 193–206.

Brown M.D. 1999. Green tea (Camellia sinensis) extract and its possible role in the prevention of cancer. *Alternative medicine review : a journal of clinical therapeutic*, **4**(5), 360–370.

Chacko, S.M., Thambi, P.T., Kuttan, R., Nishigaki, I. 2010. Beneficial effects of green tea: a literature review. *Chinese medicine*, **5**, 13.

Condello, M., Meschini, S. 2021. Role of Natural Antioxidant Products in Colorectal Cancer Disease: A Focus on a Natural Compound Derived from Prunus spinosa, Trigno Ecotype. *Cells*, **10**(12), 3326.

Heredia-Guerrero, J.A., Benítez, J.J., Domínguez, E., Bayer, I.S., Cingolani, R., Athanassiou, A., Heredia, A. 2014. Infrared and Raman spectroscopic features of plant cuticles: A review. *Frontiers in Plant Science*, **5**, 305.

Huang, D. 2018. Dietary Antioxidants and Health Promotion. *Antioxidants (Basel)*, **7**, 9.

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., & Rahu, N. 2016. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us?. *Oxidative medicine and cellular longevity*, **2016**, 7432797.

Izdebska, M., Klimaszewska-Wiśniewska, A., Hałas, M., Gagat, M., Grzanka, A. 2015. Green tea extract induces protective autophagy in A549 non-small lung cancer cell line. *Advances in Hygiene and Experimental Medicine*, **69**, 1478-1484.

Khalaf, N. A., Shakya, A. K., Al-Othman, A., El-Agbar, Z., Farah, H. 2008. Antioxidant activity of some common plants. *Turkish Journal of Biology*, **32**(1), 51–55.

Koch, W., Zagórska, J., Marzec, Z., Kukula-Koch, W. 2019. Applications of Tea (Camellia sinensis) and its Active Constituents in Cosmetics. *Molecules (Basel, Switzerland)*, **24**(23), 4277.

Kumar, P., SenthamilSelvi, S., LakshmiPraba, A., PremKumar, K., Ganeshkumar, R.S., Govindaraju, M. 2012. Synthesis of silver nanoparticles from Sargassum tenerrimum and screening phytochemicals for its anti-bacterial activity. *Nano Biomedicine and Engineering*, **4**, 12–16.

Li, Y.Q., Kong, D.X., Wu, H. 2013.Analysis and evaluation of essential oil components of cinnamon barks using GC–MS and FTIR spectroscopy. *Industrial Crops and Products*, **41**, 269–278.

Lorenz, M. 2013. Cellular targets for the beneficial actions of tea polyphenols. *The American Journal of Clinical Nutrition*, **98**, 1642S–1650S.

Makhija, P., Kathuria, H., Sethi, G., Grobben, B. 2021. Polymeric Hydrogels for Controlled Release of Black Tea and Coffee Extracts for Topical Applications. *Gels* **7**, 174.

Mehrabani, M., Amirheidari, B., Ahmadinia, N., Forooghi-Nematollahi, M., Sabouri, S. 2022. Antioxidant and Cytotoxic Effects of Aqueous Extract of Iranian Green Tea (Camellia sinensis L.): Antioxidant and Cytotoxicity of Camellia sinensis. *Iranian Journal of Pharmaceutical Sciences*, **18**(1), 77–86.

Moacă, E.A., Pavel, I.Z., Danciu, C., Crăiniceanu, Z., Minda, D., Ardelean, F., Antal, D.S., Ghiulai, R., Cioca, A., Derban, M., Simu, S., Chioibaş, R., Szuhanek, C., Dehelean, C.A. 2019. Romanian Wormwood (Artemisia absinthium L.): Physicochemical and Nutraceutical Screening. *Molecules*, **24**(17), 3087.

Mohani, N., Ahmad, M., Mehjabeen Jahan, N. 2014. Evaluation of phytoconstituents of three plants Acorus calamus linn. Artemisia absinthium Linn and Bergenia himalaica boriss by FTIR spectroscopic analysis. *Pakistan Journal of Pharmaceutical Sciences*, **27**, 2251–2255.

Nooreen, Z., Singh, S., Singh, D.K., Tandon, S., Ahmad, A., Luqman, S. 2017. Characterization and evaluation of bioactive polyphenolic constituents from Zanthoxylum armatum DC., a traditionally used plant. *Biomedicine and Pharmacotherapy*, **89**, 366–375.

Rafique, S., Murtaza, M.A., Hafiz, I., Amee,r K., Qayyum, M.M.N., Yaqub, S., Mohamed Ahmed, I.A. 2023. Investigation of the antimicrobial, antioxidant, hemolytic, and thrombolytic activities of Camellia sinensis, Thymus vulgaris, and Zanthoxylum armatum ethanolic and methanolic extracts. *Food Science Nutrition*, **11**(10), 6303-6311.

Samanta S. 2022. Potential Bioactive Components and Health Promotional Benefits of Tea (Camellia sinensis). *Journal of the American Nutrition Association*, **41**(1), 65–93.

Sangthong, S., Phosri, S., Thitilertdecha, N., Yaowiwat, N., Chaiwut, P., Wilai, M., Sermkaew, N., Sarker, S.D., Nahar, L., Theansungnoen, T. 2024. Phytochemicals and antimicrobial properties of Thai edible plant extracts and their prebiotic-like effects. *J Agric Food Res* **16**.

Santos, R.A., Andrade, E. D. S., Monteiro, M., Fialho, E., Silva, J. L., Daleprane, J. B., & Ferraz da Costa, D. C. 2021. Green Tea (Camellia sinensis) Extract Induces p53-Mediated Cytotoxicity and Inhibits Migration of Breast Cancer Cells. *Foods (Basel, Switzerland)*, **10**(12), 3154.

Semenescu, A-D., Moacă, E-A., Chioibaş, R., Iftode, A., Tchiakpe-Antal, D-S., Vlase, L., Vlase, A-M., Muntean, D., Dehelean, C-A. 2023. Phytochemical and biological screening of aqueous Galium verum L. extract. *Annals of the University Dunarea de Jos of Galati. Fascicle VI – Food Technology*, **47**(2), 172-189.

Semenescu, A-D., Moacă, E-A., Iftode, A., Dehelean, C-A., Tchiakpe-Antal, D-S., Vlase, L., Rotunjanu, S., Muntean, D., Chiriac, S.D., Chioibaș, R. 2024. Recent Updates Regarding the Antiproliferative Activity of Galium verum Extracts on A375 Human Malignant Melanoma Cell Line. *Life*, **14**(1), 112.

Sirotkin, A.V., Kolesárová, A. 2021. The anti-obesity and health-promoting effects of tea and coffee. *Physiological research*, **70**(2), 161–168.

Spadiene, A., Savickiene, N., Ivanauskas, L., Jakstas, V., Skesters, A., Silova, A., Rodovicius, H. 2014. Antioxidant effects of Camellia sinensis L. extract in patients with type 2 diabetes. *Journal of Food and Drug Analysis*, **22**, 505-511.

Szymczycha-Madeja, A., Welna, M., Zyrnicki, W. 2013. Multi-element analysis, bioavailability and fractionation of herbal tea products. *Journal of the Brazilian Chemical Society*. **24**, 777–787.

Thrumurthy, S.G., Thrumurthy, S.S., Gilbert, C.E., Ross, P., Haji, A. 2016. Colorectal adenocarcinoma: risks, prevention and diagnosis. *BMJ (Clinical research ed.)*, **354**, i3590.

WU, L.-Y., ZHENG, X.-Q., LU, J.-L., LIANG, Y.-R. 2009. Protective effect of green tea polyphenols against ultraviolet B-induced damage to HaCaT cells. *Hum Cell* **22**, 18–24.

Ydyrys, A., Zhaparkulova, N., Aralbaeva, A., Mamataeva, A., Seilkhan, A., Syraiyl, S., Murzakhmetova, M. 2021. Systematic Analysis of Combined Antioxidant and Membrane-Stabilizing Properties of Several Lamiaceae Family Kazakhstani Plants for Potential Production of Tea Beverages. *Plants (Basel)*, **10**(4), 666.

Zhang, X., Huang, H., Sun, S., Li, D., Sun, L., Li, Q., Chen, R., Lai, X., Zhang, Z., Zheng, X., Wong, W. L., Wen, S. 2022. Induction of Apoptosis via Inactivating PI3K/AKT Pathway in Colorectal Cancer Cells Using Aged Chinese Hakka Stir-Fried Green Tea Extract. *Molecules (Basel, Switzerland)*, **27**(23), 8272.

Zhao, T., Li, C., Wang, S., Song, X. 2022. Green Tea (Camellia sinensis): A Review of Its Phytochemistry, Pharmacology, and Toxicology. *Molecules (Basel, Switzerland)*, **27**(12), 3909.