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Research Article / Araştırma Makalesi

COMPARISION OF BLACK AND GREEN TEA INFUSIONS: CATECHIN CONTENTS, ENZYME INHIBITIONS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

YEŞİL ÇAY VE SİYAH ÇAY İNFÜZYONLARININ KARŞILAŞTIRILMASI: KATEŞİN MİKTARLARI, ENZİM İNHİBİSYONLARI, ANTİOKSİDAN VE ANTİBAKTERİYEL AKTİVİTELERİ

Burak TEMİZ[1]*, Gözde ÖZTÜRK[1], Serkan LEVENT[2], Hale Gamze AĞALAR[1]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskisehir, Türkiye
[2]Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, 26470, Eskisehir, Türkiye

ABSTRACT

Objective: *The operations involved in the process of tea production result in differences in the aroma and chemical profile of the final tea. In our work, the catechin contents in green and black tea were analyzed, and their application potential in skin care was compared. Under that framework, their tyrosinase (skin-lightening), elastase (anti-wrinkle), and antioxidative activities were studied, as well as the antimicrobial activities and synergistic interactions.*
Material and Method: *Black tea and green tea infusions were analyzed for the contents of catechin and total phenolic/flavonoid. Their antioxidant activities (DPPH$^{\bullet}$, ABTS$^{+\bullet}$, CUPRAC), tyrosinase, and elastase inhibitions were measured using the spectrophotometric assays. Infusions and catechin were combined with erythromycin and ciprofloxacin, and the synergistic activities were measured using the Checkerboard assay.*
Result and Discussion: *Green tea exhibited greater antioxidant and antielastase activity than black tea. Likewise, total phenol and total flavonoid contents and the amount of catechin in green tea were greater. The antityrosinase action of green tea was concluded to be lower than that in black tea. The samples displayed the greatest action against the Propionibacterium acnes strain. Although synergistic action wasn't found against the Staphylococcus aureus, it was seen that the infusions of black and green tea displayed the synergistic action with erythromycin against the Staphylococcus epidermidis.*
Keywords: *Antibacterial activity, antioxidant activity, catechin, inhibition of elastase, inhibition of tyrosinase, synergism*

ÖZ

Amaç: *Çay üretim sürecindeki işlemler, nihai çayın aromasında ve kimyasal profilinde farklılıklara neden olur. Çalışmamızda, yeşil ve siyah çaydaki kateşin içerikleri analiz edilmiş ve cilt bakımındaki uygulama potansiyelleri karşılaştırılmıştır. Bu çerçevede, tirozinaz (cilt aydınlatıcı), elastaz (kırışıklık karşıtı) ve antioksidatif aktivitelerinin yanı sıra antimikrobiyal aktiviteleri ve sinerjik etkileşimleri de incelenmiştir.*

* **Corresponding author:** Burak Temiz
E-mail: burak_temiz@anadolu.edu.tr
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Materyal ve Yöntem: *Siyah çay ve yeşil çay infüzyonları kateşin ve toplam fenolik/flavonoid içerikleri açısından analiz edildi. Antioksidan aktiviteleri (DPPH•, $ABTS^{+\bullet}$, CUPRAC), tirozinaz ve elastaz inhibisyonları spektrofotometrik testler kullanılarak ölçüldü. İnfüzyonlar ve kateşin, eritromisin ve siprofloksasin ile kombine edilmiş ve sinerjik aktiviteler Checkerboard testi kullanılarak ölçülmüştür.*

Sonuç ve Tartışma: *Yeşil çay, siyah çaya göre daha fazla antioksidan aktivite ve antielastaz etki sergilemiştir. Aynı şekilde polifenolik miktar olarak ölçüldüğünde toplam fenol ve toplam flavonoit içerikleri ile yeşil çaydaki kateşin miktarı daha fazla çıkmıştır. Tirozinaz enzim inhibisyonunun ölçüldüğü deneyde, kateşin bakımından zengin kateşin içeren yeşil çayın tirozinazı inhibe etmek yerine enzimi aktive ettiği görülmüştür. Böylece, yeşil çayın antitirozinaz etkisinin siyah çaydan daha düşük olduğu sonucuna varılmıştır. Örnekler en büyük etkiyi Propionibacterium acnes türüne karşı göstermiştir. Staphylococcus aureus'a karşı sinerjistik etki bulunmamasına rağmen, siyah ve yeşil çay infüzyonlarının Staphylococcus epidermidis'e karşı eritromisin ile sinerjistik etki gösterdiği görülmüştür.*

Anahtar Kelimeler: *Antibakteriyel etki, antioksidan etki, elastaz inhibisyonu, kateşin, sinerjizm, tirozinaz inhibisyonu*

INTRODUCTION

Tea, derived from the leaves of *Camellia sinensis* (L.) Kuntze, is one of the most widely consumed beverages globally, appreciated for its sensory diversity and health-promoting properties. Various processing steps such as fermentation, fixation, withering, and post-fermentation significantly influence the chemical profile and organoleptic characteristics of the final product. Among the different tea types -green, black, oolong, white, yellow, and dark- black tea is particularly predominant in Türkiye [1,2].

The bioactivity of tea is largely attributed to its rich content of flavan-3-ols, a subclass of phenolic compounds including catechin (C), epicatechin (EC), catechin gallate (CG), epicatechin gallate (ECG), gallocatechin gallate (GCG), and epigallocatechin gallate (EGCG). These compounds are especially abundant in green tea but undergo oxidation during fermentation to yield theaflavins (TFs), which not only contribute to black tea's distinct taste but also possess notable biological activities. Both TFs and catechins have been linked to various pharmacological effects, including antioxidant, anticancer, anti-diabetes, antiobesity, antihypertensive, and antimicrobial properties [3-13].

In recent years, interest has grown in the dermatological potential of natural products, particularly for conditions such as hyperpigmentation and premature skin aging. Tyrosinase and elastase are two key enzymes implicated in these processes (tyrosinase in melanin synthesis and elastase in the degradation of elastin fibers). Overactivation of these enzymes, often exacerbated by oxidative stress and bacterial colonization, can compromise skin health. Therefore, natural inhibitors of these enzymes, along with agents possessing antioxidant and antimicrobial properties, are being increasingly explored for therapeutic and cosmetic applications.

This study investigates and compares green and black tea infusions, both widely consumed in Türkiye, with a specific focus on their catechin content, total phenolic and flavonoid levels, antioxidant capacity, and inhibitory effects on tyrosinase and elastase enzymes. Additionally, the antimicrobial efficacy of the infusions was assessed, including their potential synergistic interactions with conventional antibiotics against clinical pathogens.

What distinguishes this study from prior research is the comprehensive scope of bioactivity analyses -spanning skin-relevant enzymes, oxidative stress parameters, and antimicrobial synergy. Notably, the observation that catechin and green tea extract may activate rather than inhibit tyrosinase provides a novel insight into the dual role of polyphenols and raises important considerations for their cosmetic application. Furthermore, the identification of synergistic antibacterial effects among skin infections, particularly against *Staphylococcus epidermidis*, adds value to ongoing efforts addressing antibiotic resistance through natural product combinations. Our findings revealed that green tea had superior antioxidant and anti-aging potential due to its rich polyphenolic profile. However, unexpected activation of tyrosinase by catechin-rich green tea limits its potential in skin-whitening applications. Conversely, black tea showed more promising tyrosinase inhibition. Antibacterial tests demonstrated notable activity, especially against *Propionibacterium acnes*, and synergistic effects with erythromycin

against *Staphylococcus epidermidis* highlight the relevance of tea infusions as adjuncts in dermatological antimicrobial therapy. These findings offer meaningful insights into the nuanced bioactivity of tea types and support their integration into cosmeceutical formulations.

MATERIAL AND METHOD

Chemicals

Catechin, dimethyl sulfoxide (DMSO), resazurin, ampicillin, cefuroxime, streptomycin, chloramphenicol, eritromycin and ciprofloxacin, gallic acid, quercetin, levodopa (L-DOPA), 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\bullet}$), kojic acid, trolox, lyophilized mushroom tyrosinase, and porcine pancreatic elastase were obtained from Sigma-Aldrich, Steinheim, Germany. Folin-Ciocalteu reagent, aluminium chloride hexahydrate was purchased from Merck, Darmstadt, Germany. All solvents that used was analytical grade.

Plant Materials and Infusion Preparation

Dried green and black tea which are very well-known in Turkish were purchased in local supermarket. 2% infusions were prepared by extraction of 2 g powdered tea samples with 100 ml boiled deionized water. After cooling to room temperature, infusions were filtered to remove the tea leaves. Aqueous aliquots were frozen at -20°C and lyophilized.

Phytochemical Analyzes of the Tea Infusions

Total Phenolic and Total Flavonoid Content

Infusions phenolic content was determined by using Folin-ciocalteu method [14]. Reaction was initiated with mixing the samples and Folin following by Na_2CO_3 addition. Abrosbances were measured at 760 nm after the 2h incubation. The calibration curve was generated from varying doses of gallic acid (1-0.8-0.6-0.4-0.2-0.1 mg/ml, y = 1.0703x + 0.0202, R^2 = 0.9996), and the results were given as mg gallic acid equivalent (GAE) / g extract.

The total flavonoid content was measured by using $AlCl_3$ [15]. The calibration curve was prepared from quercetin (1-0.8-0.6-0.4-0.2-0.1 mg/ml) and compared with the extracts (y = 1.7022x + 0.0312, R^2 = 0.9996). Results were given as mg quercetin equivalent (QE) / g extract.

Determination of Catechin Contents by LC-MS/MS

Catechin content was determined by Shimadzu LC-MS 8040 (Shimadzu, Kyoto, Japan) instrument using Electron Spray Ionization positive mode (ESI+). Shimpack FC-ODS C18 analytical column (150 mm x 2.0 mm, 3 μm) was used as stationary phase and water acetonitrile mixture (3:1, *v/v*) containing 0.1% formic acid as additive was used as mobile phase for chromatographic separation. Isocratic flow was performed for elution and flow rate was adjusted as 0.18 ml/min. For MS detection, Multiple Reaction Monitoring (MRM) was used and precursor ion was chosen as 291 and daughter ions were as 139 and 123. The method was optimized by instrument and the optimized MRM parameters were given at Table 1.

Table 1. Multiple reaction monitoring (MRM) parameters for the mass analysis of the catechin

Precursor *m/z*	Product *m/z*	Dwell Time (msec)	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
291.05	139.05	100	-11.0	-18.0	-25.0
291.05	122.95	100	-11.0	-15.0	-12.0

Evaluation of Antioxidant Activity of the Tea Infusions

DPPH Radical Scavenging Activity Assay

The experiment was performed in 96-well microplates. Briefly, 0.1 ml DPPH$^{\bullet}$ solution (0.2 mM in MeOH) was added into 0.1 ml of the 8-fold serial diluted samples, and incubated at 25°C for 30 min (A). Infusion control was prepared with addition of 0.1 ml MeOH instead of DPPH$^{\bullet}$ (B). 0.1 mM DPPH as a negative control (C), MeOH (D) as blank, and gallic acid as a positive control were used [16]. Absorbances were recorded at 515 nm, and inhibition percentages were given by following formula: [(C - D) - (A - B) / (C - D)] x 100.

Trolox Equivalent Antioxidant Capacity Assay

Stabile ABTS radical was obtained with mixing of aliquot solution of 7 mM $ABTS^{+}$ and 2.5 mM $K_2S_2O_8$ followed by incubation in the dark (16h, 25°C) [17]. Calibration curve was prepared from followed doses of Trolox; 2.5, 2, 1.5, 1, 0.5, 0.25, and 0.125 mM. 10 µl samples were added into 990 µl $ABTS^{+\bullet}$ and absorbances were recorded at 734 nm. Results were expressed as $mM_{TE}/g_{extract}$.

Cupric Reducing Antioxidant Capacity (CUPRAC) Assay

The method described by Apak et al. (2004) was used with minor modifications to evaluate the Cupric reducing properties [18]. $CuCl_2$, neocuproine, ammonium acetate buffer (pH 7.0), and infusions were mixed in 96-well plates. After 30 min incubation absorbances were recorded at 450 nm. The calibration curve was prepared from Trolox between to concentration range of 2 to 0.05 mM ($y = 1.4575x + 0.1506$, $R^2 = 0.9971$). The results were expressed as mM_{Te} / $g_{extract}$.

Evaluation of Enzyme Inhibitions of the Tea Infusions

Tyrosinase Inhibition Assay

Substrate (L-DOPA, 5 mM), enzyme (200 U/ml), and various doses of infusions were prepared in phosphate buffer (0.1 M, pH 6.8). 20 µl of sample and 20 µl of enzyme were mixed and incubated at 25°C for 10 min, and then the reaction was initiated by adding 160 µl of L-DOPA. After incubation at 25°C for 10 min, release amount of dopaquinone was measured at 475 nm (A). 20 µl buffer was added instead of enzyme for sample blank (B); 20 µl buffer was added instead of infusion for negative control (C); 20 µl buffer was used instead of enzyme for blank (D) [19]. The following equation was used to calculate the percentage inhibitions: [(A - B) - (C - D) / (A - B)] x 100.

Elastase Inhibition Assay

Elastase (0.5 U/ml), substrate (N-Suc-(Ala)-3-*p*-nitroanilide, 0.02%), and infusions were prepared in Tris-HCl buffer (0.1 M, pH 8.0). 10 µl sample, 45 µl buffer, and 130 µl substrate solution were mixed in a 96-well microplate and pre-incubated at 25°C for 5 min. Then, the reaction was initiated by adding 15 µl of enzyme and incubated at 25°C for 30 min (A). Sample control was prepared with the addition of 15 µl buffer instead of the enzyme (B). Negative control wells included 10 µl of buffer instead of the sample (C). Tris-HCl buffer was used as a blank (D). Oleanolic acid was used as the positive control. The amount of released p-nitroanilide was recorded at 410 nm [20]. The elastase inhibition of the tea infusions was calculated according to the following formula: [(C - D) - (A - B)] / (C - D) x 100

Broth Microdilution and Checkerboard Assay-Based Investigation of Antibacterial and Synergistic Activities of Tea Infusions

Test Microorganisms

Standard strains of *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC6538, *Streptococcus pyogenes* ATCC 13615, *Enterococcus faecalis* ATCC 51299, *Salmonella typhimurium* ATCC 13311, *Propionibacterium acnes* ATCC 11827, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* NRRL B-3008, *Bacillus subtilis* NRRL B-4378, *Serratia marcescens* NRRL B-2544 were used as test microorganisms and stored at -85°C in glycerol until inoculation and purity testing.

Antibacterial Activity by Broth Microdilution Assay

Standard strains of *B. subtilis* NRRL B-4378, *E. coli* NRRL B-3008, *E. faecalis* ATCC 51299, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC6538, *S. pyogenes* ATCC 13615, *S. typhimurium* ATCC

13311, *P. acnes* ATCC 11827, *S. epidermidis* ATCC 12228, *S. marcescens* NRRL B-2544 were used as test microorganisms and stored at -85°C until performing assay.

The activity of all samples was tested by broth microdilution assay according to a modified method as previously described [21]. The Minimum Inhibitory Concentrations (MIC) of samples were determined. The standard antibiotics ampicillin, cefuroxime, streptomycin, chloramphenicol (256-0.12 µg/ml) were used as positive controls. Antibacterial assays were repeated at least three times for all the test samples.

Synergistic Activity Determination Between Antibiotics and the Infusions

Synergistic activities of the test samples were determined by using the checkerboard microdilution assay according to a modified method as previously described [22]. The method was applied by calculating the fractional inhibitory concentration index (FICI). The activity types were defined as follows: FICI $\leq$ 0.5 = synergism; 0.5 < FICI $\leq$ 1 = additive; 1< FICI < 4 = indifferent; FICI $\geq$ 4 = antagonism.

Statistical Analysis

Experiments were performed in triplicate, and data were given as means ± standard deviation by using Sigmaplot 12.5 (Systat Software, Inc., San Jose, CA, USA). The differences between data were analyzed by one-way analysis of variance and Student's t-test, and $p < 0.05$ was considered significant. IC_{50} values were calculated by regression analysis.

RESULT AND DISCUSSION

Total Phenol and Total Flavonoid Content of the Tea Infusions

Phenolic compounds such as catechin and its derivatives are mainly found in green tea and black tea sapmles. The amounts of total phenols (TPC) and flavonoids (TFC) content determined in the infusions is reported in Table 2, and TPC and TFC values were found to be higher in green tea.

Catechin Contents (CC) of the Tea Infusions

A calibration set was created using 6 different concentrations of catechin standards dissolving in the appropriate amount of methanol between 0.115 mg/ml and 0.8625 mg/ml. A calibration plot was created from the signal Area vs. concentrations of the standard sets, and the equation was obtained from this plot as $y = 279276x + 970794$ and $R^2 = 0.9997$. By using that equation, catechin contents of samples were calculated and given in Table 2.

Table 2. Total phenolic, total flavonoid contents and catechin amounts of the tea infusions

Extract	TPC ($mg_{GAE}/g_{extract}$)	TFC ($mg_{QE}/g_{extract}$)	Catechin content (µg/mg)
Green tea	289.79 ± 1.83^{b}	1.12 ± 0.02^{b}	29.53^{b}
Black tea	152.89 ± 0.75^{a}	0.08 ± 0.001^{a}	0.32^{a}

[a,b] Same letter in the same column are not significantly different at $p < 0.05$

Antioxidant Activity Potentials of the Tea Infusions

Phenolic compounds are mainly known for their ability to reduce the oxidative stress with different mechanism such as free radical scavenging activity and metal chelating properties. Thus, infusions were comparatively tested for $DPPH^{\bullet}$, $ABTS^{+\bullet}$, and CUPRAC methods. Results of the $DPPH^{\bullet}$ scavenging activity (IC_{50}), Trolox equivalent antioxidant capacity (mM_{TE}/g_{sample}), and Cupric reducing antioxidant capacity (mM_{TE}/g_{sample}) is given in Table 3. Green tea presented the higher activity than the black tea in all methods. Even so, black tea infusions showed a remarkable radical scavenger activity. Gallic acid exhibited highest activity in DPPH with 1.13±0.03 µg/ml IC_{50} value.

A study evaluated the DPPH radical scavenging activity of polymeric phenolic and non-polymeric phenolic fractions prepared using the Sephadex LH-20 of commercial black and green tea infusions.

Polymeric fractions exhibited significantly higher activity than non-polymerics while green tea scavenged radical greater than black tea samples [23]. An on-line HPLC-DPPH study revealed that the EGCG was the most potent antioxidant followed by other catechin derivatives [24]. Another study compared the ABTS radical scavenging activity of green and black tea samples and green tea samples demonstrated the higher activity with approximately two-fold lower IC_{50} values [25]. Apak et al. (2006) determined the ABTS radical scavenging and CUPRAC properties of green and black tea samples from Türkiye and GT showed a better activity for both methods [26]. Furthermore, Zielinski et al. (2015) evaluated the comparative activity of green, black and white tea on DPPH, ABTS and CUPRAC. Green tea was found to be the highest antioxidant potential, while black tea was showed lowest activity [27].

In sum, fermentation step during the prosess of black tea results with a decrased amount of catechin derivatives due to oxidation of phenolics which cause the lower antioxidant activity.

Table 3. Antioxidant activitiy resutls of the tea infusions

Samples	**DPPH˙ scavenging activity**	**Trolox equivalent antioxidant capacity (TEAC)**	**Cupric reducing antioxidant capacity (CUPRAC)**
	(IC_{50}, µg/ml)	(mM Trolox equivalent/$g_{extract}$)	(mM Trolox equivalent/$g_{extract}$)
Green tea	9.56±0.16[b]	1409.05±20.92[b]	3896.17±56.61[b]
Black tea	16.89±0.5[c]	894.54±16.07[a]	1694.34±50.31[a]
Gallic acid*	1.13±0.03[a]	-	-

Data are given as mean±SD (n = 3). [a-c]Means in the same column followed by the same letter are not significantly different at $p < 0.05$. -, not performed, *, positive control

Tyrosinase Inhibition Potentials of the Tea Infusions

Tyrosinase plays a crucial role in the formation of skin color by taking part in the first two steps of melanin synthesis. Natural inhibitors have increased in importance due to medical treatments, the cosmetic industry, food storage and processing areas. L-DOPA was used as a substrate to evaluate the tyrosinase inhibitor properties of the tea infusions. Results revealed that black tea (IC_{50} 231.29±9.2 µg/ml) showed stronger inhibition properties than green tea (IC_{50} 460.83±8.9 µg/ml). Dose-dependent inhibition curve was given in Figure 1. Additionally, kojic acid (IC_{50} 14.28±0.6 µg/ml) inhibit the tyrosinase better than both tea samples.

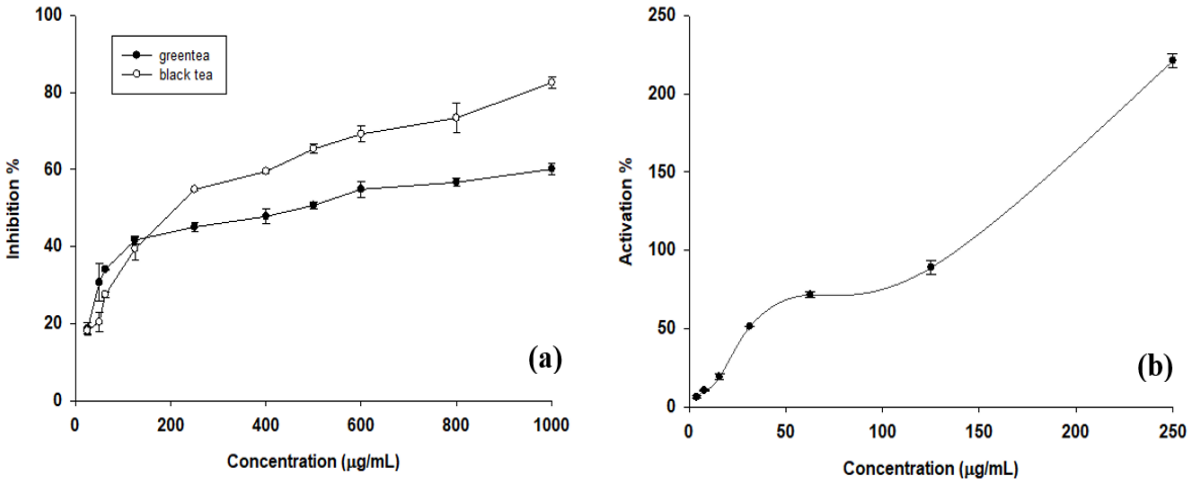


Figure 1. Tyrosinase inhibition properties of infusions (a) and catechin (b)

Mainly, polyphenolic compounds have increasingly extensive interest in term of natural tyrosinase inhibitors. In the literature, *Camellia sinensis* extracts were evaluated for their anti-tyrosinase properties. Different black tea cultivars and green tea leaves exhibited tyrosinase inhibition potentials with IC_{50} values between 208.82 ± 5.18 and > 1200 µg/ml [28]. On a comperative study on different tea extracts, green tea exhibited stronger inhibition than black tea on tyrosinase at a range of 0.25-0.5 mg/ml [29]. These effects are usually associated with tea polyphenols. Thitimuta et al. (2017) presented a comprehensive study on green tea polyphenols (EGCG, ECG, and EGC) and compared the activities with green tea. Results demonstrated that the effects on tyrosinase followed the order ECG > EGC > EGCG, and all polyphenolics were exhibited greater inhibition activity than green tea [30]. On the other hand, some polyphenols (eg. catechin) can act as a substrate or cofactors for tyrosinase. Therefore, effects of catechin on tyrosinase were examined, and as shown in Figure 1, activation of enzyme were observed instead of inhibition. Oliveira et al. reported that the catechin and epicatechin are better activators for tyrosinase rather than L-DOPA due to their higher interaction with Cu (II) ions [31]. Thus, the occurrence of catechin might affect the total activity due to its enzyme activator properties.

Elastase Inhibition Potentials of the Tea Infusions

Elastic fibers are responsible for skin elasticity and flexibility. Elastin is one of the main proteins found in this fiber, and it is degraded by the elastase enzyme. Therefore, the effects of tea infusions on elastase were studied to investigate the potential applications for skin health. Black and green tea samples were used at three different doses (Table 4). Green tea demonstrated a higher inhibitory effect on the enzyme compared to black tea. However, no effect observed at 0.05 mg/ml for both samples. Oleanolic acid, a member of pentacyclic triterpenoids, was used as a positive control and exhibited 37.11±5.31% inhibition at 0.05 mg/ml.

A recent study examined fifteen different tea cultivars from Sri Lanka related to elastase inhibitory activities, and fresh tea and black tea samples demonstrated notable activity with IC_{50} values between 427.25±0.97 to > 600 µg/ml and 36.7±6.38 to 444.63±2.11 µg/ml, respectively [28]. In another study, green tea extracts exhibited lower than 10% inhibition on elastase at 25 µg/ml [32]. Hong et al. (2014) examined the elastase inhibition of green tea extract treated with tannase to change the relative levels of tea polyphenols. According to the results, tannase treatment increased the activity, revealing that EGC, EC, and GA can act as better inhibitors than EGCG and ECG [33]. Liyanaarachchi et al. reported that the tea extract demonstrated 26.8% inhibition on elastase at 500 µg/ml. Studies are mainly focused on green tea instead of black tea samples [34]. Thus, considering the higher popularity of black tea compared to green tea in Türkiye, further studies are still needed to understand the effect of the fermentation process on elastase activity.

Table 4. Elastase enzyme inhibition results for tea infusions and oleanic acid

Samples	1 mg/ml	0.5 mg/ml	0.05 mg/ml
Green tea	78.9±2.99[b]	52.78±0.37[b]	-
Black tea	32.12±0.85[a]	27.01±0.85[a]	-
Oleanolic acid*	nt	nt	37.11±5.31

[a,b] same letter followed by same column are not significantly different at $p < 0.05$. -, no effect. nt, not tested. *, positive control

Antibacterial Activity Potentials of the Tea Infusions

The all extracts showed the highest activity against *P. acnes* strain (2.5, 10, 10 mg/ml). It was moderately effective against *P. aeruginosa, B. subtilis, S. pyogenes, S. typhimurium* and *S. epidermidis* strains (5 mg/ml). In contrast, the MIC value for green tea extract is quite high (> 20 mg/ml) against *E. faecalis* and *S. marcescens* strains. The antimicrobial activity of black tea was relatively low compared to green tea and antibiotics. Results were given in Table 5. In many studies, antibacterial activity of *C. sinensis* was tested against several pathogens; *Salmonella typhimurium, Acinetobacter baumannii, Escherichia coli* 0157, Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains (MIC 0.5-10

mg/ml) and *C. sinensis* has been reported to have effective antimicrobial activity against several Gram-positive and Gram-negative strains [35,36].

Table 5. Minimum inhibitory concentration (MIC) (mg/ml) values of the tea extracts and positive controls

Microorganisms	GT	BT	AMP	CEF	STREP	CHL
E. coli	10	20	0.004	0.064	0.002	0.004
P. aeruginosa	5	20	-	-	0.016	0.064
B. subtilis	5	> 20	-	-	0.004	0.004
S. aureus	10	> 20	0.002	-	0.032	0.032
S. pyogenes	5	> 20	0.008	-	0.002	0.016
E. faecalis	> 20	> 20	0.002	0.064	0.002	0.008
S. typhimurium	5	> 20	0.004	0.064	0.032	0.004
P. acnes	2.5	10	0.004	-	0.004	0.008
S. epidermidis	5	> 20	0.002	-	0.004	0.008
S. marcescens	> 20	> 20	0.064	-	0.008	0.032

GT: Green tea; BT: Black tea; Amp: Ampicillin; Cef: Cefuroxime; Strep: Streptomycin; Chl: Chloramphenicol

Synergistic Activity Potentials of the Tea Infusions and Antibiotic Combinations

Combinations of extracts and antimicrobial agents were investigated using the Checkerboard microdilution method, and the fractional inhibition concentration index (FIKI) was calculated. Values are given in Table 6 and Table 7. Synergistic activity was tested against *S. aureus* and *S. epidermidis* strains that cause serious skin diseases, by combining erythromycin and ciprofloxacin antibiotics with extracts and catechin. 4 independent and 2 antagonist activities were determined against *S. aureus* strain. 2 synergistic, 3 independent and 1 additive effect were determined against *S. epidermidis* strain.

Table 6. Synergistic activity results of tea infusions and antimicrobial agent combinations against *S. aureus*

Microorganisms	Ext. A	Ext. comb	FIC Ext.	Ant. A.	Ant. comb	FIC Ant.	FICI	Result
GT + Eri	1.56	0.78	0.5	0.0005	0.0005	1	1.5	Indifferent
BT + Eri	0.78	0.19	0.24	0.0005	0.0005	1	1.24	Indifferent
Catechin + Eri	0.78	0.19	0.24	0.0005	0.0005	1	1.24	Indifferent
GT + Cip	6.25	0.19	0.03	0.0005	0.002	4	4.03	Antagonism
BT + Cip	6.25	0.19	0.03	0.0005	0.002	4	4.03	Antagonism
Catechin + Cip	0.78	0.19	0.24	0.0005	0.0005	1	1.24	Indifferent

GT: Green tea; BT: Black tea; Amp: Ampicillin; Cef: Cefuroxime; Strep: Streptomycin; Chl: Chloramphenicol

Many studies have reported that green tea catechins have antimicrobial effects and have evaluated the synergistic activity of green tea catechins with known antimicrobial agents. Examples of these studies found that catechins worked strong synergistic activity with tetracycline against *S. aureus and S. epidermidis*; with penicillin, ampicillin/sulbactam, oxacillin, and imipenem against MRSA *S. aureus*, and with ciprofloxacin, chloramphenicol, and cefotaxime against *E. coli* [37-39]. Also, Farooqui et al. combined a 5% infusion prepared with *C. sinensis* purchased from the market with nalidixic acid and tested it against *S. typi* strain and this combination showed strong synergy (FICI 0.37) [40].

Green tea demonstrated greater antioxidant and antielastase activities than black tea. Similarly, higher values were recorded for total phenolic content, total flavonoid content, and catechin concentration in green tea, supporting its stronger bioactivity profile. These findings align with previous research emphasizing the enhanced pharmacological properties of minimally processed teas due to their preserved polyphenol structures.

Table 7. Synergistic activity results of tea infusions and antimicrobial agent combinations against *S. epidermidis*

Microorganisms	Ext. A	Ext. comb	FIC Ext.	Ant. A.	Ant. comb	FIC Ant.	FICI	Result
GT + Eri	3.12	0.19	0.06	0.0005	0.0002	0.4	0.46	Synergistic
BT + Eri	12.5	0.19	0.01	0.0005	0.0002	0.4	0.41	Synergistic
Catechin + Eri	0.78	0.19	0.24	0.0005	0.0005	1	1.24	Indifferent
GT + Cip	12.5	0.19	0.01	0.0002	0.0002	1	1.01	Additive
BT+ Cip	0.78	0.19	0.24	0.0002	0.0002	1	1.24	Indifferent
Catechin + Cip	0.78	0.19	0.24	0.0005	0.0005	1	1.24	Indifferent

GT: Green tea; BT: Black tea; Amp: Ampicillin; Cef: Cefuroxime; Strep: Streptomycin; Chl: Chloramphenicol

Interestingly, in the tyrosinase inhibition assay, catechin and catechin-rich green tea extract were found to activate the enzyme rather than inhibit it, suggesting a substrate-like behavior for certain polyphenols under specific conditions. This paradoxical effect highlights the complexity of tea polyphenol interactions with enzymatic systems and warrants caution in the cosmetic use of catechin-rich extracts for hyperpigmentation control. In contrast, black tea, despite its lower catechin content, exhibited stronger tyrosinase inhibition, suggesting that fermentation-derived compounds such as theaflavins may play a more pivotal role in this specific bioactivity.

From a dermatological perspective, the antibacterial findings of this study are particularly relevant. Both tea infusions displayed moderate to strong activity against several skin-associated bacterial strains, with the highest efficacy observed against *Propionibacterium acnes*, a key contributor to acne pathogenesis. Notably, green tea outperformed black tea in overall antimicrobial potency, potentially due to its higher catechin content, which has been extensively documented for its membrane-disruptive and bacteriostatic properties.

Beyond standalone antimicrobial activity, the synergistic effects observed between the tea infusions and erythromycin against *Staphylococcus epidermidis* are of high clinical interest. These results suggest that combining natural polyphenol-rich infusions with conventional antibiotics can enhance therapeutic efficacy, particularly against resistant or biofilm-forming skin flora. While no synergy was observed against *Staphylococcus aureus*, the lack of antagonism confirms that such combinations are safe and potentially beneficial in multi-agent dermatological formulations.

Overall, this study underscores the multifaceted dermal benefits of green and black tea infusions- not only as antioxidant and anti-aging agents but also as supportive antimicrobial components in topical therapeutics. Moreover, the demonstration of synergistic effects with antibiotics offers a promising strategy to combat microbial resistance, reinforcing the relevance of tea-derived phytochemicals in integrative skincare and pharmaceutical approaches.

Conclusion

As a conclusion, green tea showed stronger antioxidant and elastase inhibitory properties than black tea, due to its superior catechin and polyphenol content. These findings support its incorporation into anti-aging skincare products. However, its unexpected activation of tyrosinase presents a limitation for depigmenting applications, where black tea -with its fermentation-derived components- may serve as a better alternative. Both tea types displayed meaningful antimicrobial effects, particularly against *P. acnes*, a common acne pathogen. Moreover, the synergistic activity observed with erythromycin against *S. epidermidis* highlights the value of combining natural products with conventional antibiotics in topical treatments. These outcomes contribute to a deeper understanding of tea infusions' multifaceted roles in skin health and offer valuable insights for the design of targeted, evidence-based cosmeceutical formulations.

AUTHOR CONTRIBUTIONS

Concept: B.T., H.G.A.; Design: B.T., G.Ö., H.G.A.; Control: H.G.A.; Sources: B.T., G.Ö., S.L.; Materials: B.T., G.Ö., S.L.; Data Collection and/or Processing: B.T., G.Ö., S.L.; Analysis and/or

Interpretation: B.T., G.Ö., S.L.; Literature Review: B.T., G.Ö.; Manuscript Writing: B.T., G.Ö., H.G.A.; Critical Review: H.G.A.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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