Antioxidant, Anti-Inflammatory, and Antibacterial Potential of Different Drinks Based on Matcha Tea

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Abstract. The aim of this research was to present in vitro and ex vivo biological activities for three different Matcha teas and a juice based on Matcha tea, lingonberry and bacterial probiotic strain (as a control) and one commercial green tea. The results demonstrated a strong correlation between major bioactive compounds and three biological activities. Antioxidant potential was correlated with epicatechin levels and with a significant amount of caffeine. The level of these compounds was influenced by tea samples. Matcha tea (M1) was presented in the finest particles size and corresponded with obtaining the highest value of antioxidant potential. Chromatographic (qualitative) analysis has demonstrated a characteristic distribution, and epicatechin and rutin have been identified as major bioactive compounds. Correlation of the anti-inflammatory potential with the antibiofilm-inducing inhibition is a significant feature in the biological synthesis of new innovative products (eg, nanobiosystems) with antibiofilm effects.

1. Introduction
Oxidative dysfunction is associated with numerous health problems, and it is associated with many clinical implications. The presence of free radicals was associated with the onset of different pathologies that affect a large number of young individuals [1]. Foods cause free radical generation in large quantities; as such, one of the main concerns is to find functional foods and drinks that promote well-being. In this regard, herbal tea represents a potent product full of antioxidants and that exhibits many healthy effects on humans. These beneficial activities are based on the antioxidant and anti-inflammatory activities of the bioactive compounds found in tea drinks [2]. Major compounds, like phenolic compounds, are responsible for these in vivo/in vitro [3] activities, and all are correlated with strong antimicrobial potential, especially against pathogenic bacteria [4].

Matcha tea represents one of the many products to express such complex potential; it is a relatively new health product for Romanian consumers. Based on the recent findings on this Japanese tea, it was found Matcha tea consumption has numerous health benefits. Matcha tea has strong biological activities, which result from tea leaves that are dried and finely ground without any other chemical supplements. Tea preparation assures good release of some bioactive compounds in the water, and these compounds can be easily assimilated after consumption. The bioactive fraction has a positive action on a lepidic profile, anti-inflammatory activities, and energizing effects based on the caffeine content; it also helps lower blood pressure and improves memory [5]. For these reasons, the aims of the article are to demonstrate the correlation between major bioactive compounds, five biologic
activities (antioxidant, anti-inflammatory and antibacterial activities) from three different Matcha teas, a Matcha-based juice (as a control) and a green tea.

2. Experimental procedures

2.1. Biological material and sample preparation.

Three types of Matcha teas were bought from a specialized market in Romania (Bio Japan Matcha, Demmer GmbH, Wien, Austria (M1), Spring Markt (M3) and Thés George Cannon (M4)), one instant drink with Matcha, lingonberry, and cultures (Ikea PS Instant drink, commercialized by Ikea Romania (M2)) and green tea (The National Health Products Co., LTD., China; M5).

The samples (aqueous extracts) were prepared in accordance with previously described research [6]. For the dried powder Matcha tea, 2 g were mixed with 20 mL of boiling water and vertexing to obtain a homogeneous mixture; after that, boiling water was added to 200 mL. The instant drink was prepared in accordance with the manufacturer’s instructions by dissolving all contents of the sachet in 200 mL of boiling water.

2.2. Quantification of bioactive compounds

The total phenolic and flavonoidic contents were determined by spectrophotometric methods at 765 nm and 410 nm, respectively. The results were expressed as gallic acid equivalents in mg/100 g of extract and microgram quercetin equivalents/100 g of extract [3].

2.3. Determination of antioxidant activity

In vitro antioxidant activity was demonstrated by several spectrophotometrical methods: DPPH scavenging activity, chelating activity and reducing power. Scavenging activity was calculated using the following equation: % activity = (O.D. control – O.D. sample)/O.D. control × 100. Ascorbic acid was used as a standard for all methods [3, 4].

2.4. Determination of anti-inflammatory activity

The ex vivo anti-inflammatory activity was proven by human red blood cell (HBRC) membrane stabilization using a slightly modified method [7]. Fresh human blood mixed with sodium heparin, from the first author, was collected and transferred to centrifuge tubes [3]. After that, an equal volume of freshly collected blood was mixed with sterile Alsever’s solution (in g/L, NaCl 4.2; citric acid×3Na×2H2O 8.0; citric acid×H2O 0.55; D-glucose 20.5) and centrifuged at 3,000×g, for 10 minutes (Hettich EBA20). In a new Falcon tube (15 mL) 2 mL of HRBC suspension was mixed with an equal volume of drinks and kept at 37°C for 30 minutes (Memmert Incubator). The hemoglobin content was determined by spectrophotometric quantitative analysis at 560 nm. The % of protection was calculated with the following equation:

% protection = 100 – O.D. sample/O.D. control× 100. (1)

The second anti-inflammatory activity evaluated in this research was inhibition of lipid peroxidation. Inhibition was proven by using egg yolk homogenates, based on the previous protocol [8].

2.5. Determination of antibacterial activity

To evaluate the samples’ antimicrobial activity, a microdilution method was chosen, according to recommendations of the Clinical Laboratory and Standards Institute (CLSI) M07-A10. For this study, two strains of Gram-positive bacteria were selected (Bacillus cereus ATCC 11778; Staphylococcus aureus ATCC 6538), as were two Gram-negative strains (Escherichia coli ACTCC 25922; Pseudomonas aeruginosa ATCC 15442) and one yeast strain (Candida albicans ATCC 10231).

The microbial strains were seeded on boards with a specific BHI (bacteria) medium and YPG (yeast), and they were then incubated for 24 hours at 37°C. Following microorganism development,
suspensions were made in ADS with a density of 0.5 McFarland. In 96-well plates 100 µL of MH (bacteria) medium and RPMI 1640 medium were added with 0.165M MOPS, 0.03% L-glutamine, and no bicarbonate (yeast); over the medium, 100 µL of the sample were added and serial binary dilutions were made. Finally, 10 µL of microbial culture were added with a density of 5×10⁶ cells/mL. The plates were incubated for 20 hours at 37°C, performed in triplicate.

After CMI determination, the plates were cleaned with distilled water twice, fixed with 95% methanol for 5 minutes, and then stained with 1% purple crystal for 15 minutes. After staining, the plates were cleaned well to remove excess stain, and in every plate 130 µL of acetic acid (33%) were added. The optical density was determined at 490 nm to stabilize the minimum biofilm eradication concentration (MBEC) [9, 10].

2.6. Statistical analysis
All parameters for antioxidant activity were assessed in triplicate, and the results were expressed as the mean ± standard deviation (SD) values of three observations. The mean and SD were calculated with Excel (Microsoft Office 2016; Microsoft Corporation, Redmond, WA, USA).

![Figure 1. In vitro antioxidant capacity of tea samples.](image)

3. Results and discussions
3.1. Evaluation of antioxidant capacity
Matcha tea infusion M5 presented the highest antiradicalic potential compared with the other samples (Figure 1). This value was approximately 70%±0.25% higher than M1. The fact that M3, M4, and M5 determined DDPH scavenging activity values of a minimum of 70%±0.06% was noted. M1 and M5 were able to determine an inversely proportional relationship between DDPH scavenging activity and chelating ability. The results can exhibit large differences due to the water temperature, which could vary in association with the sample-processing time. This behavior was similar to that found in other previous studies regarding the influence of time, temperature, and size of white- and green-tea particles on antioxidant characteristics [11].

Instead, the reduction power was obtained for sample M2, where the lowest antiradicalic and chelating activity were observed. This result occurred because of the different compositions of the samples. For M2, the remainder of the instant product components and the presence of probiotic strains (Lactobacillus sporogenes) demonstrated their antioxidant potential [12] however, lingonberry
is also known to have a pronounced antioxidant effect due to its anthocyanin and phenolic acid content [13]. Furthermore, a high variation in chelating activity (approximately ±4.6%) and reduction power (approximately 0.25%) was significantly correlated ($P\leq0.005$) with the total phenol content variation.

![Figure 2. Anti-inflammatory activity of tea samples.](image)

### 3.2. Evaluation of anti-inflammatory activity
A significant number of studies proved the direct link between antioxidant activity and anti-inflammatory activity. The antioxidant potential, previously proven in vitro, showed a direct relationship with the composition of the tea samples. The anti-inflammatory potential proven in vivo and in vitro exhibited the same tendency (Figure 2). M4 and M5 presented maximum values. The correlation between methods and Matcha tea samples was significant ($P<0.05$). But, the M2 sample presented minimum values, which proved weak anti-inflammatory and antioxidant protection.

Similar to previous studies [14], non-polar solutions (M3, M4, and M5) had the highest anti-inflammatory activity, and these types of drinks represent functional alternatives to M2. It is worth mentioning the high anti-inflammatory value (84.2%) of the M1 sample, which showed the lowest scavenging effect on DDPH radicals (Figure 1). This behavior proved that the Matcha samples selectively expressed a biological response. The effect was directly correlated with the level of the main bioactive compounds (Figure 3).

### 3.3. Antimicrobial and antibiofilm activity
The M2 sample presented the most reduced activity against bacteria, while M1 did not have an antimicrobial effect. The M5 sample showed the highest antimicrobial activity, especially against *S. aureus* (ATCC 6538). The M2 and M4 CMI against *B. cereus* (ATCC 11778) and *P. aeruginosa* (ATCC 15442) was 50% higher (Figure 4). Strains of *E. coli* (ATCC 25922) and *C. albicans* (ATCC 10231) showed resistance to the presence of bioactive compounds in the five samples.

The main result was that biofilm inhibition directly resulted from the antimicrobial activity (e.g., samples M1 and M3). Conversely, M2, M4, and M5 did not inhibit biofilm formation in Gram-negative strains; the CMI value was the same as the CMEB value for *P. aeruginosa* (ATCC 15442) strains, while for *E. coli* (ATCC 25922) strains, the values were higher than in the control sample, which indicates a stimulation of biofilm formation.
In the case of *C. albicans* (ATCC 10231) strains, the values were very low; thus, it was not possible to determine biofilm formation. The results of the antibiofilm effect were opposite to those of the antioxidant effect for the M1 sample. These results were different for M2, M4, and M5, and they corresponded to other studies that proved the polyphenolic component effect against selected bacterial pathogens [15].

3.4. Evaluation of the bioactive compounds

It is worth noting the large difference between the level of flavonoids in samples M4 and M5 compared to the remaining samples. M5 had a minimum level of phenolic compounds of 0.4±0.01 mg of gallic acid/mL (Figure 3). High levels of flavonoids were also found in other studies when compared with other types of tea [16]. Thus, the high presence of flavonoids directly influenced the
antioxidant/anti-inflammatory activity (Figures 1 and 2). When compared to other previous studies, tea samples M4 and M5 had inversely proportional chelation activity, which had an opposite effect to the methanol extracts obtained from *Polyalthia longifolia* and *Cassia spectabilis* [2].

The M2 sample had a high phenolic acid content (1.85 mg gallic acid/mL). This was 10% greater than that for M1, on average, and it was due to the heterogeneous composition. The remaining samples showed significant variations of approximately 70%. A direct comparison with other studies [14] is not relevant, as the results depend on how the infusion is prepared, as well as on the analyzed tea sample and the expression of the results.

The epicatechin and the rutin were identified like major phenolic compounds (Figure 5, peak 30 and peak 31) in all samples except M4. Following the chromatographic analysis (qualitative), the existence of a significant number of bioactive compounds was also proven. Their quantitative distribution depended on each sample, but the substantial presence of flavonoids was characteristic. Depending on how the samples were prepared and presented, the qualitative analysis determined the identification of principal metabolites as a primary way of expressing the biological response. The spectrum of peaks from Figure 5 proved that epicatechin was a major bioactive compound, and it represented 40% of all these molecules.

4. Comparative discussions

According to previous studies, the biological action of bioactive compounds (e.g., polyphenol–carboxylic acids or flavonoids) is directly dependent on their chemical structure, which influences the assimilation capacity in vivo [17]. This result affected the anti-radical response *in vitro* (Figure 1). The values were poorly correlated with the chelation capacity ($R^2<0.01; P>0.05$), as they proved different mechanisms of action that depended on the bioavailability of the different categories of bioactive compounds. Instead, the strength of the correlation increased significantly with the anti-inflammatory activity *ex vivo* ($R^2>0.1; P<0.02$), but it tended to vary with the inhibition of lipid peroxidase *in vitro* ($R^2>0.36; P<0.04$).

The antimicrobial effect manifested in the Gram-positive strains was opposite to that which manifested against strains of *E. coli* (ATCC 25922) and *C. albicans* (ATCC 10231). These strains
were not inhibited, which indicated a selectivity toward the bioactive component. These samples, extracted with hot water, did not determine the presence of a specific concentration or of some phenolic compounds capable of inhibiting potential pathogenic strains. In addition, for the strain *S. aureus*, the M2 extract inhibits biofilm formation at high concentrations and stimulates its formation at concentrations less than 3% (the data did not demonstrate this). The extracts (the types of tea) analyzed stimulated the formation of biofilm in the case of *E. coli* strains (ATCC 25922). The biofilm formed by strains of *C. albicans* (ATCC 10231), *B. cerus* (ACTCC 11778), and *P. aeruginosa* (ATCC 15442) was very weak, and so the MBEC could not be precisely established. This behavior was interpreted as a direct bond with the antioxidant response, corresponding to the findings of some previous studies [18].

The antioxidant potential proven by *in vivo* and *in vitro* methods determined a biologic response similar to that found in previous studies, which showed a link between catechin level and high caffeine content [19]. The level of these compounds is influenced by how the tea samples are presented. M1 was presented with the finest particle size, and this corresponded to obtaining the highest antioxidant potential (Figure 1). Complete dissolution of the M2 sample determined an average antioxidant response, with preponderance influenced by the microbial component. Depending on the type of tea and the way in which the substrate was presented, there was a certain potentiation of the biological response; this was correlated with both antioxidant antimicrobial effects.

5. Conclusions

The consumption of beverages with different biological activities (antioxidant or antimicrobial) represents a broadly sought consumer tendency. Matcha tea has functional characteristics that can respond to this trend. Although the types of Matcha tea present different functional activities, they depend on the specificity of the active biologic compounds, particularly in terms of their ability to scavenge free radicals or inhibit the activity of microbial strains. The correlation of the two activities was a significant feature of M1 compared to the other types of evaluated tea types. This preliminary study showed the high biological potential of Matcha tea both *in vitro* and *ex vivo*. Protection against oxidative stress has been proven and was correlated with increasing antioxidant/antimicrobial protection capacity *in vivo* [20]. To validate these conclusions, studies on the assessment of gastrointestinal transit stability should be carried out, as the antibiotic effect plays a key role in the control of pathogenic strains.

6. References

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Acknowledgements
This work was partially supported by the Executive Agency for Higher Education, Research, Development and Innovation Funding – Human Resources, by the project PNCDI III UEFISCDI — Experimental demonstration project (PED), Project 171PED/2017 (http://www.robiomush.ro/biochaga/). English-language editing of this manuscript was provided by Journal Prep.