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RESEARCH ARTICLE

# Determination of the antioxidant activity in different widely consumed hot beverages

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## Abstract

**Aim:** Flavonoids are a natural compound widely spread in the plant kingdom. Flavonoids have been identified as having extraordinary clinical properties, such as anti-inflammatory, anti-allergic, antiviral, antibacterial, and antitumoral activities. Flavonoids are present abundantly in vegetables and fruits, including tea. It is proven that flavonoids, as antioxidants, scavenge free radicals. The balance between free radicals and antioxidants in the biological system is crucial to prevent the development of diseases. Any defect in this required homeostatic state leads to what is known as oxidative stress. The present study aimed to determine the antioxidant activity in different widely consumed hot beverages.
**Material and Methods:** Different types of tea were used in this study, including honey tea, herbal tea, black tea and matcha, to determine the antioxidant activity by changing water temperature for preparation using the DPPH methodology.
**Results:** This study revealed that tea prepared in Extra Boiling Water (E.B.W) showed the highest antioxidant activity compared with Boiling Water (B.W). In addition, herbal tea prepared in E.B.W was characterized by higher antioxidant potential compared with the same tea in B.W (≈ 5% compared to ≈ 30%, respectively) and among others.
**Discussion:** Our data suggest that the water temperature used to prepare different teas is an important parameter influencing the activity of antioxidants in different teas.

**Keywords:** Flavonoids, ROS, Oxidative stress, Antioxidants, Tea

## Introduction

Flavonoids are a natural compound widely spread in the plant kingdom. They are polyphenolic phytochemicals grouped into subclasses according to their molecular structure and the degree of oxidation of the core pyran ring. A recent study suggested that there are more than 4,000 different types of flavonoids present in nature, including flavonols, flavanones, flavones, catechins, isoflavones, dihydroflavonols, chalcones, and anthocyanidins (Meleleo et al., 2023 and Ozgen et al., 2016). The class flavonols contain quercetin, a (3,5,7,3',4'-pentahydroxyflavone), present abundantly in

vegetables and fruits like apples, onions, berries, red grapes, kales, broccoli, cherries and as well as in tea (Xu et al., 2019). Flavonoids have been identified as having extraordinary clinical properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoral activities (Azeem et al., 2023).

It has been well known that flavonoids have a protective effect against Reactive Oxygen Species (ROS), which are produced as by-products under normal metabolism or induced by extracellular stimuli. Free radicals, also referred to as ROS, are independent molecules containing unpaired electrons and can react as oxidants or reductants with other molecules. They share many properties as being unstable and highly reactive (Zhu et al., 2023). Within the body, they react with many molecules such as lipids, carbohydrates, proteins and, more importantly, DNA, causing damage to these molecules and harmful damage to the cells and body components (Giri et al., 2017). These species are formed normally in the body because of metabolic reactions or due to exposure to different environmental factors such as smoking, x-ray radiation, pollutants, and chemicals (Lobo et al., 2010). Although ROS have an important role in stimulating the signalling pathway and play an important role in homeostasis for normal functioning, over accumulation of these species over time upsets the balance between oxidants and antioxidants and may contribute to disease development (Reuter et al., 2010).

On the other hand, antioxidants are compounds that are stable enough to give an electron to an out of control free radical and neutralize it, thus lowering the radical's potential for harm. These antioxidants' ability to scavenge free radicals is primarily responsible for delaying or inhibiting cellular damage. These antioxidants with low molecular weight can effectively react with free radicals to stop the continuous cycle before important molecules are harmed (Lobo et al., 2010). The balance between free radicals and antioxidants in the biological system is crucial to prevent the development of diseases. Any defect in this required homeostatic state leads to what is known as oxidative stress (Wang et al., 2022). Oxidative stress can lead to undesirable transformations of lipids, proteins and nucleic acids in the cell and is linked with different pathological conditions, including diabetes, hepatocellular damage, atherosclerosis, cancers, inflammatory disorders, and aging, as a result of changes in macromolecules and DNA mutations (Reuter et al., 2010 and Phaniendra et al., 2015).

It is proven that flavonoids, as antioxidants, scavenge free radicals, form insoluble complexes with metal ions and deactivate both lipoxygenase and cyclogeneses, minimizing oxidative rancidity in food (Embuscado et al., 2015). Naturally occurring antioxidants are gaining high interest for their promising possibilities in the prevention of health defects. Plants are rich in well-known antioxidant compounds such as vitamins A, C and E, besides carotenoids and phenolic acids. These could be found in vegetables, fruits, herbs, and tea (Hęś et al., 2019).

Tea is formed of polyphenols, caffeine, minerals, and trace amounts of vitamins, amino acids, and carbohydrates (Wierzejska et al., 2014). Polyphenol compounds are considered potent antioxidants. They have a more robust antioxidant effect in comparison to vitamins such as vitamins C and E. In addition, they include carotene and tocopherol, which have the potential to ameliorate oxidative stress (Miazek et al., 2022). Interestingly, heat or steam leads to inactivation of the enzymes responsible for degradation, maintains a considerable amount of the original biologically active components of green tea and also produces some unique products (Komes et al., 2010).

Tea is considered one of the most widely consumed beverages and is available in different forms. Thus, the aim of the present study is to determine the antioxidant activity in different widely consumed hot beverages, including honey tea, herbal tea, black tea and matcha, using DPPH methodology for preparation through changing water temperature.

## Materials and Methods

### Tea samples

Al Rabea black tea and matcha were purchased from the local market in Saudi Arabia. Two types of Pukka herbal tea: the first one mix of lemon, ginger, and Manuka honey, and the second contain a clean fusion of aniseed, fennel, and cardamom from the pharmacy in Saudi Arabia and stored at room temperature in a dry place.

Two different forms of prepared tea were examined in the study: Extra Boiling Water (E.B.W) and Boiling Water (B.W).

## Preparation of tea extract

8 Different cups containing 75 ml of water were prepared and divided into 4 cups of boiling water and 4 cups of hot water. Another 8 cups containing 50 ml of water divided into 4 cups of boiling water and 4 cups of hot water. 0.5 grams of each type of tea was added. The extract of each tea type was prepared in two versions as E.B.W and B.W.

## DPPH radical scavenging assay

2,2-diphenyl-1-bicrylhydrazyl (DPPH) (D9132-1G, Sigma-Aldrich Chemical, Pool, UK) kit was used for this assay. The DPPH solution was prepared according to the procedure described by Maria Agostina (Frezzini et al., 2019). In brief, 0.1 mM solution of DPPH in ethanol (99.5%) was prepared. This solution 1 ml was added to 3 ml of each extract. The mixture was shaken vigorously and kept at room temperature in the dark for 30 min. then, absorbance was measured at 517 nm using spectrophotometer. The experiment was performed in duplicate.

## DPPH radical scavenging assay principal method

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is one of the inexpensive and easy techniques for the measurement of antioxidant potentials. This technique is based on the use of the free radicals used for assessing the samples to serve as hydrogen providers or free-radical scavengers (FRS). This technique is associated with the elimination of DPPH, which would be a settled free radical. The free-radical DPPH then interacts with an odd electron to yield a strong purple colour. In other words, an FRS antioxidant reacts to DPPH and forms DPPH-H, which has a lower absorbance than DPPH. It is radical in comparison to the DPPH-H form, which causes decolorization, or yellow colour, as the number of electrons absorbed increases. Thus, decolorization affects the lowering capacity significantly (Fig 1).

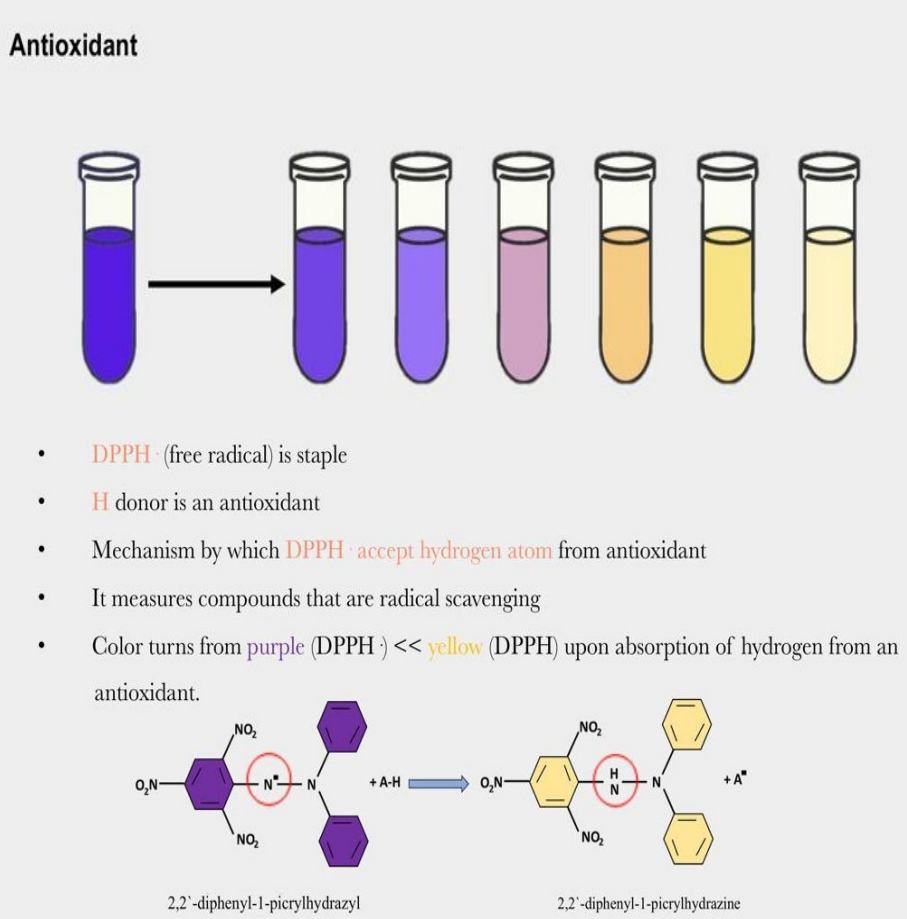


**Figure.1. Antioxidant activity is inversely proportional with absorbance.**

### Statistical analysis

Statistical analysis was performed using Graph Pad Prism 9. Mean ± standard error of the mean was used for descriptive data analysis of the study sample. The independent sample t-test was used to evaluate the difference in teas between the E.B.W and B.W.

## Results

The results reported in this article show how the temperature of water used in preparing tea would affect the tea's antioxidant activities. Teas prepared in E.B.W represent the highest antioxidant potential compared with teas prepared in B.W.

Interestingly, herbal tea prepared in E.B.W was characterized by higher antioxidant potential compared with the same tea in B.W (≈5% compared to ≈30%, respectively) and among other teas (Fig. 2-3). Generally, statistically significant increases were observed between all teas prepared in E.B.W compared with B.W (Fig. 2).

Additionally, matcha tea prepared in E.B.W has the highest antioxidant potential differences than matcha tea prepared in B.W (≈25% compared to ≈150%, respectively) and among all other teas.

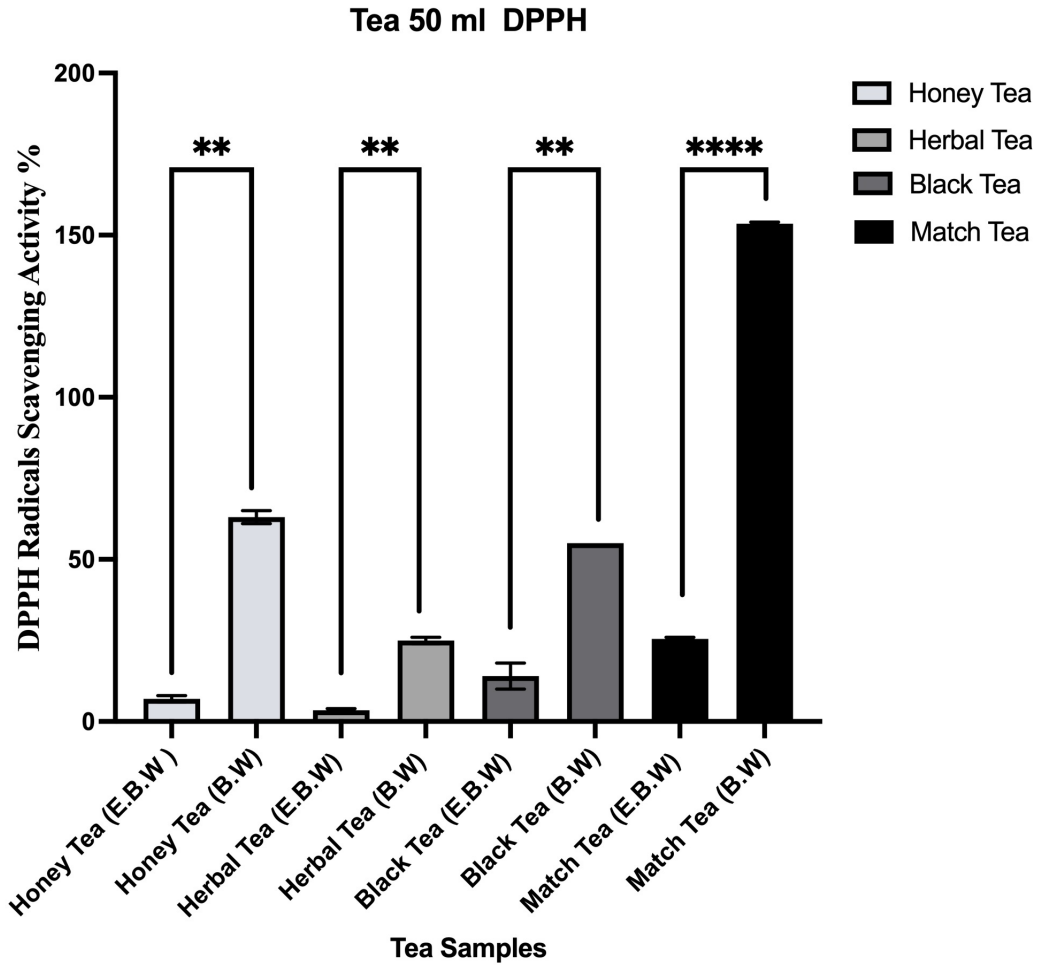


**Figure 2. Tea prepared in Extra Boiling Water (E.B.W) represents the highest antioxidant potential than boiling one (B.W).**

Densitometric analysis was pooled from two independent experiments. Error bars = SEM, **p<0.01 and ****p<0.0001.

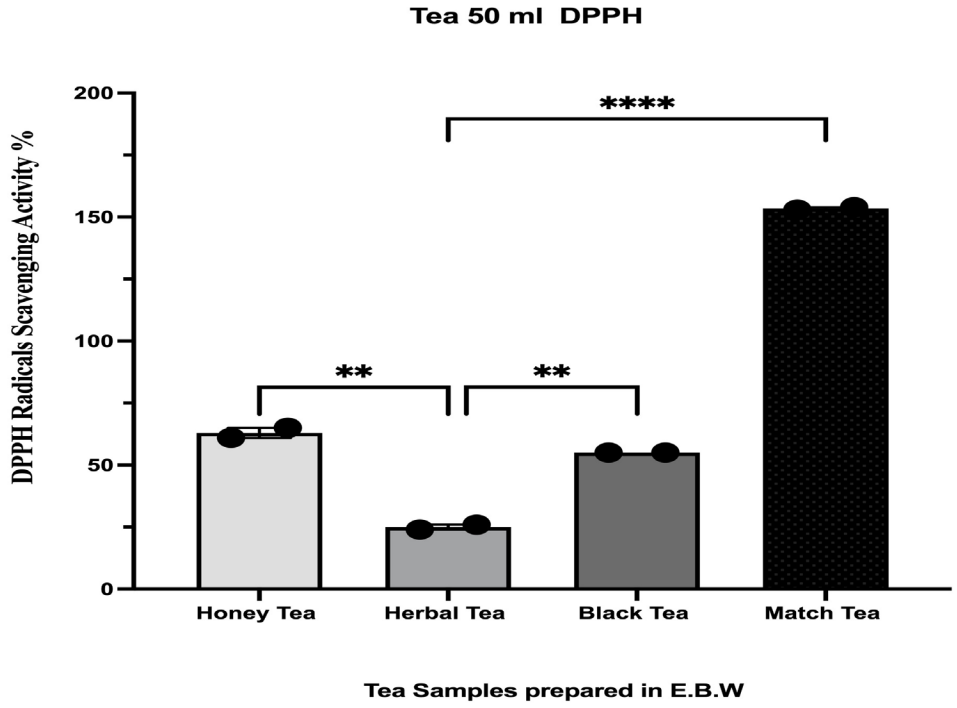


**Figure 3: Herbal tea prepared in Extra Boiling Water (E.B.W) represents the highest antioxidant.**

Densitometric analysis was pooled from two independent experiments. Error bars = SEM, **p<0.01 and ****p<0.0001.

## Discussion

Humans and other living organisms such as animals and plants are in continuous danger of free radicals. These molecules could be either developing from outside environmental factors (exogenous) such as pollution, smoking and radiation or developing from inside biological system factors (endogenous) because of normal metabolic processes or inflammatory responses in different disease conditions (Nimse et al., 2015). They are oxygen compounds such as superoxide, peroxyl and hydroxyl unstable due to a lack of unpaired electrons in their electron shell. They react with other molecules to trap their electrons or donate, causing DNA and tissue damage (Phaniendra et al., 2015).

On the other hand, biological systems develop a defensive mechanism as antioxidants, including enzymes and non-enzymes, are capable of scavenging these free radicals and minimizing their harmful effect. Enzymatic antioxidants as superoxide dismutase and peroxidase work by converting radicals to hydrogen peroxide $H_2O_2$ and then to water. Non-enzymatic molecules as vitamins (A, C, E), carotenoids and polyphenols are present in our diet, mainly fruits and vegetables. They minimize free radical activity by interrupting their chain reaction (Haida et al., 2019).

Consumption of tea has been linked with many health advantages, including cancer, neurodegenerative and metabolic disease as a result of the presence of many biologically active components (Lange et al., 2022). These include caffeine, inorganic salts, theobromine, and polyphenols; tea's important phenolic compounds are flavonoids linked to antioxidant, anti-inflammatory and antimicrobial effects (Dwyer et al., 2013). In this study, the aim was to examine the antioxidant activity of different types of tea, one of the most widely used beverages worldwide, using two different temperatures for the preparation.

The results confirmed that the water temperature used to prepare different teas is an important parameter influencing the acquired results. In the DPPH method, teas prepared in E.B.W represented the highest antioxidant activity compared with B.W in honey, herbal, black and matcha tea. Cheng et al., 2023 illustrated that the extraction efficiency was improved by raising the water temperature, and surprisingly, the highest product of antioxidants was performed at 100 ◦C in green, Oolong, black, and scented teas (Cheng et al., 2023). Additionally, Jakubczyk et al., 2020 showed that the antioxidant potential in green tea was higher at higher water temperatures at 90 ◦C and the lowest at 25◦C (Jakubczyk et al., 2020). The reason might be associated with the greater liberation of biologically active compounds and

the higher kinetic energy at higher temperatures, which was also indicated in similar studies (Komes et al., 2010, Fujioka et al., 2016).

Herbal tea infusions, such as honey, cardamom, lemon, aniseed, fennel, and ginger, are very popular. It is well known that these infusions, composed of valuable parts of herbs, are among the major contributors to phenolics in the diet. Interestingly, the results showed that herbal tea containing aniseed, fennel, and cardamom prepared in E.B.W, has the highest antioxidant activity among all types in both E.B.W and B.W. Many studies confirmed that antioxidant activity was enhanced by increasing water temperature in black tea (Chang et al., 2020) and herbal tea (Ueda et al., 2019). The reason behind this might be that the concentration of antioxidants depends on the time the plant material was harvested, besides the water temperature used to prepare it (Jakubczyk et al., 2020).

## Conclusions

Our DPPH method results proved that the water temperature used to prepare tea is an important parameter that helps increase the tea's antioxidant activity. Therefore, future studies should explore the beneficial effect of tea as a source of antioxidants by investigating how the water temperature used to prepare tea would affect this potential activity.

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## Conflict of Interest

The authors declare no conflict of interest.

## Animal and Human Rights Statement

No animal or human studies were carried out by the authors for this article.

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## Scientific Responsibility Statement

Authors declare that they are responsible for the article's scientific content. These responsibility areas include study design, data collection, analysis and interpretation, writing, preparation and scientific review of the contents, and approval of the final version of the article.

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