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ORIGINAL ARTICLE

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Functional and Novel Foods

Supplementation species effect on the phenolic content and biological bioactivities of the decocted green tea

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ABSTRACT

Background: Green tea, produced from the leaves of *Camellia sinensis*, is the most widely consumed beverage in the world after water, and it is widely sold and popular owing to its flavor, and its health benefits. Spices have been used by consumers worldwide to improve flavors of food including tea. Phenolics are the most common phytochemical found in herbal teas and are beneficial in promoting health or preventing diseases. **Aims:** This study aims to investigate the impact of the addition of mint (*Mentha piperita* L.), cinnamon (*Cinnamomum verum*), star anise (*Illicium verum*), ginger (*Zingiber officinale*), white mugwort (*Artemisia herba alba)* and clove (*Syzygium aromaticum*) on green tea biological properties, and on consumers' acceptance. **Subjects and Methods:** Different green tea blends were formulated with 10% of each proposed spice. Total phenolic and flavonoid contents of formulated blends were measured by Folin-Ciocalteu and aluminum chloride methods, respectively. The effect of these herbs supplementation on antioxidant activity of green tea was determined using three different methods, namely DPPH assay, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC). *In vitro* antibacterial activity was assessed by the disc-diffusion method. Consumers' acceptance of tea blends was evaluated by a sensory hedonic analysis performed by 127 panelists. **Results:** The results revealed significant variability among tea blends in terms of both total polyphenols (from 591.951 to 1027.387 mg GAE/L) and flavonoids (from 140.78 to 279.329 mg QE/L). As regards the antioxidant activity, control (no supplemented) green tea exhibited the highest ferric reducing antioxidant power ($RC_{0.5}$ = 27.25 mg AAE/g DE). Interestingly tea blends supplemented with white mugwort and ginger demonstrated the highest DPPH• scavenging effect with IC_{50} of about 3.37 and 3.38 mg AAE/g DE, respectively, while when supplemented with star anise, it showed the highest TAC (66.143 mg AAE/g DE). In the assessment of the antibacterial effect, control green tea showed the best efficacy, except for *Listeria innocua* which was particularly sensitive to samples flavored with star anise and clove. The sensory evaluation revealed that clove-flavored tea was the most preferred by the panelists (31.75%), closely followed by ginger green tea (30.95%), green tea and star anise flavored green tea (25.39%). **Conclusion:** The obtained results indicate that adding some species to green tea significantly enhance its phenolic content, boost its antioxidant capacity and improve its sensory properties.

Keywords: Flavored green tea; polyphenols; antioxidant activity; antibacterial activity; sensory characteristic.

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1 Introduction

Green tea, known as *Camelliia Sinensis* L. is most widely consumed beverage gaining in popularity in the western world. It has long been a staple food in Asian countries [1] and is considered as a nonalcoholic beverage in the world [2]. The global increase in tea consumption has been related to its *in vitro* and *in vivo* functionalities that have established a direct relationship between the regular tea consumption and the reduced risk of non-communicable degenerative diseases [3]. Green tea possesses high polyphenolic content and is considered as a good source of natural antioxidants [4]. It is characterized by its richness on flavan-3-ols, also known as catechins [5]. These compounds are widely used in the prevention and treatment of many diseases due to their demonstrated anti-inflammatory, antibacterial and antiviral effects. Moreover, they can prevent the development of cancer and diabetes [6]. In addition, catechins were found to be inhibitory against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Enterococcus faecalis* [7] and *Helicobacter pylori* involved in stomach and intestinal infections [8]. Furthermore, Izah et al. [9] reported that the ability of natural spices to inhibit pathogenic growth was found to be in this ascending order: nutmeg, cinnamon, kola nut, clove, garlic, ginger, ginger-garlic and lime.

Concerning its chemical composition, green tea contains proteins, which include predominant L- theanine amino acid, as well as trace elements such as copper, zinc, iron, selenium, sodium, and macro elements; it is a good source of fluorine and iodine. Green tea contains also carbohydrates such as glucose, lipids (linoleic and linolenic acids), vitamins B_2, B_3, and C [10]. Additionally, it contains organic compounds that readily produce vapors, contributing to its characteristic aroma [11].

Most of tisanes were prepared from a single ingredient like green tea, others were blended in polyherbal mixtures to improve their palatability [12] and maximize their effects [13]. Tea enriched with spices was popular in some countries like in Northern Indian, such as kahwa, a traditional mixture of green tea extract and other spices like saffron, cardamom, pepper or cinnamon and almond pieces [12]. In the northern African region, green tea is supplemented with mint, clove and cinnamon. Finimundy et al. [13] reported that these mixtures can increase the content of compounds of interest, improving the antioxidant status and the nutritional value of these beverages. Indeed, Zheng et al. [14] suggested the potential of Ganpu tea in modulating the gut microbiota to benefit human health because it increased the abundance of *Bifidobacterium*, *Lactobacillus*, and *Lactococcus*.

Herbs was an ancient source of medicine, flavoring, beverages, dyeing, fragrances, and cosmetics uses that have attracted biotechnology, cosmetics, pharmaceutical and food industries [15]. Mostly, the aromatic or spices plants contain compounds that possess potent anti-oxidative properties. Regularly consuming a wide variety of plants and spices is better for health than taking an antioxidant supplement [16]. Indeed, antioxidants are very important to human health [17]. Therefore, it is interesting to investigate whether the incorporation of spices has a positive effect on health.

The objective of our study was to evaluate the effect of the addition of selected spices: mint (*Mentha piperita* L.), cinnamon (*Cinnamomum verum*), star anise (*Illicium verum*), ginger (*Zingiber officinale*), white mugwort (*Artemisia lactiflora*) and clove (*Syzygiumaromaticum*) on the phytochemical composition, antioxidant, antibacterial activities and sensory characteristics of decocted green tea. The choice of these spices was made thanks to a survey carried out on tea consumption in Algeria, where people indicated the plants, they use to flavor the tea.

2 Material and Methods

2.1 Plant materials and chemicals

Green tea (*Camelia sinensis*) and the selected spices plants including mint (*Mentha piperita*), cinnamon (*Cinnamomum verum*), star anise (*Illicium verum*), ginger (*Zingiber officinale*), white mugwort (*Artemisia herba alba*) and clove (*Syzygium aromaticum*) were purchased from a local market (Bejaia, Algeria). The choice of these plants was made according to the results of a survey conducted on the consumption of tea in Algeria, where people gave the plants, they use to flavor tea.

All solvents and reagents were of analytical grade. Spectrophotometric measurements were obtained using a Perkin-Elmer Lambda 25 UV/Vis spectrophotometer.

2.2 Sample preparation

Decoctions of green teas were prepared at a concentration of 1%, which corresponds to the average volume of water and weight of tea recommended by manufacturers for a standard cup of tea. For flavored green tea (**GTC:** Green Tea flavored Cinnamon; **GTCl:** Green Tea flavored Clove; **GTG:** Green Tea flavored Ginger; **GTS:** Green Tea flavored Star anise; **GTM:** Green Tea flavored Mint; **GTW:** Green Tee flavored White mugwort), a mixture of 100 mg of each aromatic plant and 900 mg of green tea was extracted with 100 mL of distilled water for 5 min at 100 °C, subsequently, the obtained solutions were filtered. Non-flavored green tea was also prepared by adding 1g of green tea (**GT**) to 100 mL of distilled water and used as a control.

2.3 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectroscopy is a qualitative and quantitative analysis technique used to identify functional groups that appear in the form of absorption bands. This method involves exposing the molecule of interest to IR radiation. Tea IR spectra were recorded using a Fourier transform infrared spectrophotometer, the SHIMAZU FTIR-8400S model, controlled by a computer with processing software with a resolution of 4 cm^2, in the spectral range of 4000 cm^1 to 400 cm^3.

The analysis is carried out on mixture of grounded green tea extracts (20%) and potassium bromide (80%) (KBr). This powder mixture was then pressed in a mechanical die press to form a pellet under a pressure of 90 KN.

2.4 Determination of total polyphenol content (TPC)

TPC was determined by the Folin-Ciocalteau method [18], and the results were expressed in mg of gallic acid equivalent per liter of tea (mg GAE/L of Tea), calculated based on the linear regression value obtained from the gallic acid calibration curve. Briefly, a volume of 0.5 mL of each sample was mixed with 2.25 mL of distilled water and 0.25 mL of diluted Folin-Ciocalteu reagent (1/10, v/v). Then, the mixtures were agitated for 1 min. Eight minutes later, 2 mL of Na_2CO_3 (7.5% w/v) were added and the obtained solutions were left in the dark for 1 h for color development. At the end of the incubation time, the absorbance was measured at 765 nm using Thermo Scientific Evolution 220 Spectrophotometer against a blank solution. Each analysis was performed in triplicate.

2.5 Determination of total flavonoid content (TFC)

The aluminum trichloride method [19] was used to quantify flavonoids in different extracts. This method is based on the ability of flavonoid compounds to form chromogenic complexes with aluminum chloride ($AlCl_3$). One milliliter of each sample or of the quality standard (Quercetin), is added to 1 mL of the $AlCl_3$ solution (2%). After 10 min, the absorbance was read at 430 nm using a UV-Visible spectrophotometer. Flavonoid concentration of the different extracts was calculated based on the calibration curve, established with quercetin, and the results were expressed in milligrams of Quercetin Equivalents per Liter of Tea (mg QE/L).

2.6 DPPH free radical scavenging activity assessment

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a free radical compound widely used to test the free radical-scavenging ability of various samples. DPPH undergoes reduction reaction through capturing hydrogen or electron, resulting in a color change from purple to yellow [4]. To evaluate the radical scavenging capacity of different preparations, 1 mL of diluted extracts was added to 2 mL of DPPH solution (5.9 mg/100 mL ethanol). After 30 min of incubation in the dark, the absorbance (A) was measured at 517 nm. All measurements were made with distilled water as blank sample. The DPPH radical scavenging ability (%) of the samples was calculated as follow:

$$[(A_0 - A_{\text{Sample}})/A_0] \times 100$$

Where A_0 is the absorbance of the control solution (containing only DPPH) and A_{sample} is the absorbance of DPPH solution with the sample. The measurements were performed in triplicate for each sample. The kinetics of this activity was used to determine concentrations corresponding to a 50% inhibition (IC_{50}). The IC_{50} values were expressed as milligram Ascorbic Acid Equivalent per gram of Dry Extract (mg AAE/g DE) and the lowest IC_{50} corresponds to the highest activity.

2.7 Total antioxidant capacity (TAC) evaluation

The total antioxidant capacity (TAC) of extracts is assessed by the phosphomolybdenum method [20], which is based on the reduction of molybdenum Mo (VI), present in the form of molybdate MoO_4^{2-}, to molybdenum Mo (V) MoO^{2+} ions in the presence of the extract. This reaction takes place in an acidic medium and results in a formation of a green phosphate/Mo (V) complex.

An aliquot of 0.2 mL of the sample solution was combined with 2 mL of a reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The resulted mixtures were capped and incubated in a thermal block at 95°C for 90 min. After that, the samples were cooled to room temperature, and their absorbance was measured at 695 nm against a blank.

A typical blank solution, contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample, was incubated under the same conditions as the rest of the samples. The TAC was expressed as milligram Ascorbic Acid Equivalent per gram of Dry Extract (mg AAE/g DE).

2.8 Ferric reducing antioxidant power method (FRAP)

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant capacity. According to the method of Bhalodia, et al. [21], a series of aliquots containing various concentrations of the standard and test sample extracts (10 to 100 µg/mL) in 1.0 mL of deionized water were mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide solution (1%).

The mixture was incubated at 50 °C in water bath for 20 min before being allowed to cool. Subsequently, 2.5 mL aliquots of trichloroacetic acid (10%) were added to the mixture, followed by a centrifugation at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was combined with 2.5 mL distilled water and 0.5 mL of a freshly prepared ferric chloride solution (0.1%). The absorbance was measured at 700 nm using UV spectrometer, with ascorbic acid used as standard.

Results were expressed as mg Ascorbic Acid Equivalent per gram of Dry Extract (mg AAE/g DE).

2.9 Antibacterial activity assessment

Test microorganisms

The extracts were tested against four standard bacteria, encompassing three Gram-positive strains: *Listeria innocua* (Clip 74915), *Staphylococcus aureus* (SARM 43300), *Enterococcus faeccalis* (ATCC 25922), along with one Gram-negative strain*: Salmonella. sp.* (hospital strain), using the disc diffusion method. The bacterial cultures were maintained on nutrient agar and incubated at 37 °C for 18 h prior to their use.

Disc diffusion method

The antibacterial activity of green tea flavored with various species was performed using the disc diffusion method. This method has been chosen for its reliability and simplicity. It will provide preliminary results on the sensitivity of the bacterial strains and the antibacterial activities of the product, which can be observed through the measurement of inhibition zone diameters appearing around the discs.

Extracts were sterilized by filtration through a 0.45 µm Millipore Express filter. All bacteria were suspended in sterilized water, then diluted to 10^6 CFU/mL and negative controls were prepared using distilled water. As a positive control, Ciprofloxacin, Novobiocin and Doxycycline were used, at a concentration of 5 mg/disc, to determine the sensitivity of each microbial species to antibiotics.

The bacterial strains were spread onto Mueller Hinton Agar (MHA) plates. Subsequently, discs (6 mm Ø; Whatman No. 3) impregnated with different extracts were placed on the surface of these agar plates as described by Bachir et Benali [22]. After that, the inoculated plates were incubated at 37 °C for 24 h. The antibacterial activity was evaluated by measuring the diameter (mm) of the inhibition zone (DIZ) of the tested bacteria. All tests were performed in triplicate.

2.10 Sensory evaluation

Sensory evaluation was performed by the panelists, who were recruited from the staff and students of Abderrahmane Mira University of Bejaia (Algeria). Seven samples including green tea and 6 flavored green teas were prepared by decocting 1 g of tea in 100 mL of hot water (1g of tea/100 mL of water) and sweetened with sugar (2 g of sugar/100 mL of green tea decoction).

The hedonic analysis involved 127 untrained panelists of mixed gender aged between 18 to 66 years. Additionally, ten expert panelists aging from 27 to 50 years, who were able to identify specific characteristics of the sensory attributes namely, bitterness, aroma, color, odor, astringency, sweetness, and piquancy, were selected for a more detailed descriptive profiling test. This test aimed to detect the changes in the sensory quality between green tea and the flavored varieties using the following scale: 1. Absence, 2. Low, 3. Medium, 4. Strong, 5. Very strong.

The evaluation was conducted at room temperatures (20˚C - 22˚C) under natural light. The samples were coded with 3-digit numbers and randomly evaluated using a 9-point hedonic scale. This scale consisted of general preference accompanied by a scale of nine categories as: 1. Extremely unpleasant; 2. Very unpleasant; 3. Rather unpleasant; 4. Unpleasant; 5. Neither pleasant nor unpleasant; 6. Quite pleasant; 7. Pleasant; 8. Very pleasant; 9. Extremely enjoyable.

2.11 Statistical analysis

All data were reported as means ± standard deviation from three replicates. The analysis of the variance at $p<0.05$ was carried out using STATISTICA 5.5 to determine significant differences among the results.

3 Results and discussion

3.1 Fourier-transform infrared spectroscopy analysis

To identify the interactions between green tea and star anise, ginger, cinnamon, clove, and mint, the FTIR analysis based on the peak values within the region of IR radiation was used. During the FTIR analysis, the functional groups of these components absorb infrared radiation at characteristic wavelength [23]. The spectra of pure green tea and different varieties of flavored covering the range from 4000 to 400 cm^{-1} are displayed in Figure 1. Several distinct bands are observed for the spectra of pure green tea. The broad band appeared at~3387 cm^{-1} has been assigned to the OH stretching vibrations groups associated with polyphenol and/or catechin [24]. The peak observed at ~2937 cm^{-1} can be attributed to the aliphatic C–H stretching vibrations within methylene (CH_3) groups derived from carbohydrates and sugars can be seen [25].

It should be noted that the O–H stretching overlaps with the C–H stretching, but both absorptions are discernible. The strong absorption appeared at 1637 cm^{-1} suggests the presence of a carbonyl group (C=O) characteristic of catechin.

The presence of the band at 1450 cm^{-1} can be assigned to the symmetric bending of methylene groups. Additionally, the peak at approximately ~ 1039 cm^{-1} has been ascribed to the C–O stretching vibration of alcohol, phenol and/or C–H aromatic stretching [24]. It's worth noting that the region of the

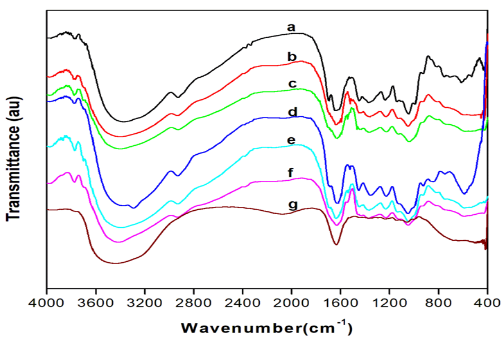


Figure 1. Representative FTIR spectrum of flavored decocted green tea

(a): Green Tea; (b): Green Tea flavored Star anise; (c): Green Tea flavored Ginger; (d): Green Tea flavored Cinnamon; (e): Green Tee flavored White mugwort; (f): Green Tea flavored Clove; (g): Green Tea

Table1. Comparative Table of FT-IR peak values of different functional groups of aqueous green tea and its flavored products

Functional groups	OH	C-H	C=O	C-H	C-O
Samples	Peak values (cm^{-1})				
GT	3387	2937	1637	1450	1039
GTW	3392	2933	1639	1456	1031
GTS	3414	2929	1629	1458	1033
GTG	3404	2933	1631	1460	1043
GTC	3431	2937	1637	1473	1043
GTCl	3427	2926	1625	-	1043
GTM	3452	-	1641	-	-

GT : Green Tea; GTC: Green Tea flavored Cinnamon; GTCl: Green Tea flavored Clove; GTG: Green Tea flavored Ginger; GTS: Green Tea flavored Star anise; GTM: Green Tea flavored Mint; GTW: Green Tee flavored White mugwort

infrared spectrum between 1000–600 cm-1 is commonly referred to as the "fingerprint" region. This region is characterized by a complex set of bending vibrations which cannot always be correlated with the structure of the green tea decoction [25]. When analyzing the spectral profile of green tea and various flavored products, it can be seen that their spectral characteristics are quite similar, except for GTM. Indeed, it was observed from the comparison of the spectra of GT and GTM (Fig. 1, Table 1), the overlapping of C–H bond as well as the "fingerprint" region.

The absorption bands of various functional groups appear within specific regions, and their positions are varied within a narrow range (Table 1), The O-H bond shows a shift from 3387 cm^{-1} in GT to higher frequencies of 3452 cm^{-1} in GTM, which can be attributed to the presence of internal O-H···O hydrogen bonding. Similarly, the C=O stretching peak observed at 1637 cm^{-1} in GT shifts to higher frequencies in flavored tea and GTM, and to lower frequencies in GTS, GTG, and GTCl. This phenomenon can be attributed to the formation of the conjugation between carbonyl and the double bond of the aromatic ring [26]. FT-IR peak values of different functional groups of aqueous green tea and its flavored products were shown in Table1.

3.2 Effect of the addition of species on total phenolic and flavonoid contents

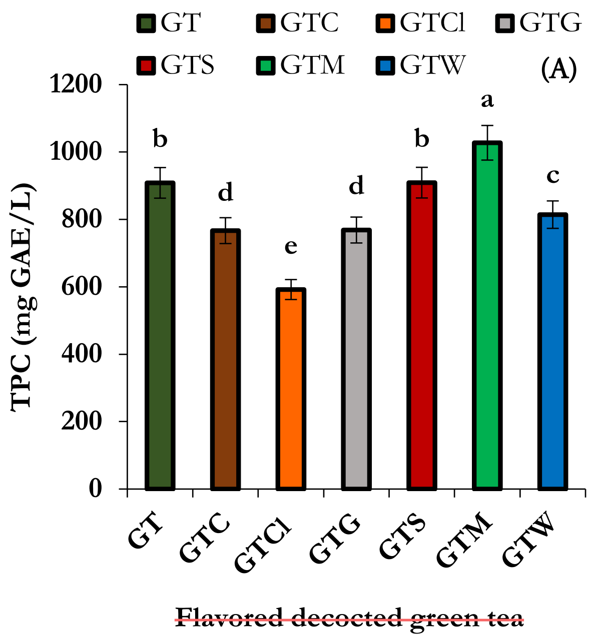
Results presented in Figure 2 showed significant differences at $p<0.05$ among tested samples. The beverage formulated with green tea flavored with mint (GTM) exhibited the highest polyphenol and flavonoid contents 1027.387 mg GAE/L and 279.329 mg QE/L, respectively.

In contrast, the lowest values were attributed to the green tea flavored with clove (GTCl) beverage with a polyphenol content of 591.95 mg GAE/L and flavonoid content of 140.787 mg QE/L.

The rank order in the TPC was: GTM >GTS - GT >GTW >GTG - GTC >GTCl, and that of TFC was: GTM> GTW > GTS> GTC>GTG-GT>GTCl. Green tea has a TPC of 908.46 mg GAE/L or 90.846 mg/g DW, lower than that obtained by Giménez et al. [27] which was about 12 g GAE/L of infused green tea, but higher than that reported by Oliveira [28] which was of 569 mg/L and by Tahir et al. [29] ranged from 146.97 to 148.94 mg/100 g for green tea.

Furthermore, we found a TFC of 18.73 mg QE/g in green tea which similar with that obtained by Hajimahmoodi et al. [30] ranged from 2.898 to 31.152 mg Eq catechin/g. Giménez et al. [27] reported that the main classes of polyphenols in green tea are flavanols and flavonols, and the most common flavonols described in green tea are quercetin, kaempferol and myricetin, commonly conjugated to a range of sugars.

The results of the present study showed that mixing some herbs with green tea such as mint, star anise, and white mugwort increased its TPC and TFC, while the addition of ginger, clove and cinnamon decreased its TPC, and clove decreased its TFC.



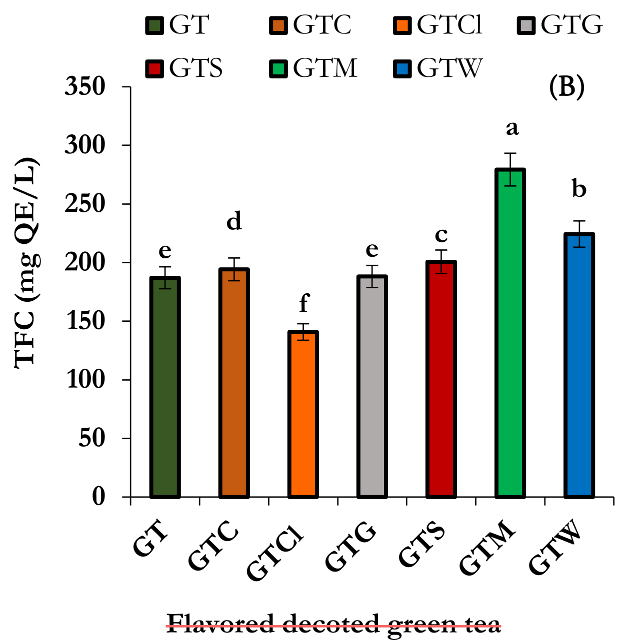


Figure 2. Total phenolic and flavonoid contents of flavored decocted green teas

(A): Total Phenolic Content; (B): Total Flavonoid Content. Values are averages ± standard deviation of triplicate analyses; different letters indicate significant difference at p<0.05. Results are ranked in ascending order; a>b>c>d>e>f. GT: Green Tea; GTC: Green Tea flavored Cinnamon; GTCl: Green Tea flavored Clove; GTG: Green Tea flavored Ginger; GTS: Green Tea flavored Star anise; GTM: Green Tea flavored Mint; GTW: Green Tee flavored White mugwort.

In their recent study Uduwana et al.[31] reported that the TPC was significantly higher ($p<0.05$) when both bee honey and *Citrus limonum* were added to plain green tea brew (3160.2 ± 117.1 µg GAE/mL) compared to the green tea alone (2276.0 ± 71.4 µg GAE/mL), but the total flavonoid content decreased unlike total phenolic content with the addition of bee honey and *Citrus limonum* extract from 6.6 ± 0.2 to 4.9 ± 0.4 µg QE/mL of infusion. Yap et al. [32] revealed that TC-16, a new polyherbal pormulation, comprising *Curcuma longa* L., *Zingiber officinale* var. Bentong, *Piper nigrum* L., *Citrofortunella microcarpa* (Bunge) Wijnands and *Apis dorsata* honey possessed the most abundant phenolic and flavonoid contents (46.14 ± 1.40 mg GAE/g and 132.69 ± 1.43 CE/g, respectively) followed by *Curcuma longa*.

In addition, Finimundy et al. [13] noted that blends containing 15% of *Laurus nobilis* L. and 15% of *Juglan regia* L. in their composition showed higher concentration of phenolic compounds (phenolic acids and flavonoids). The mechanisms of these interactions are difficult to explain because of the complex nature of mixtures, particularly plant extracts. Toydemir et al. [33] explained differences in the effect of pine honey for all the analyzed tea samples by differences in phenolic profiles of the herbal tea samples or the lack of the specificity of the Folin-Ciocalteau method for phenolic compounds. Indeed, this method is reported to be suffering from a number of interfering substances, including specifically sugars, aromatic amines, ascorbic acid, amino acids and proteins that can also react with this reagent.

3.3 Effect of the addition of species on antioxidant activity

Figure 3 showed the results of the three antioxidant assays. As it can be seen in Figure 3. A, the highest radical scavenging activity (IC_{50} value of 3.371 mg/g DE) was exhibited by the GTW, closely followed by GTG (3.377mg/g DE). The efficacy of tested samples was ranked depending in their radical scavenging ability as follow: GTW-GTG>GTS>GT>GTC>GTCl>GTM. In their recent study, Yap et al. [32], have reported the highest radical scavenging activity of *Zingiber officinal* plant.

Moreover, the ferric reduction antioxidant power and the total antioxidant activity were classified respectively as follows: GT> GTCl >GTC-GTG-GTM > GTW>GTS and

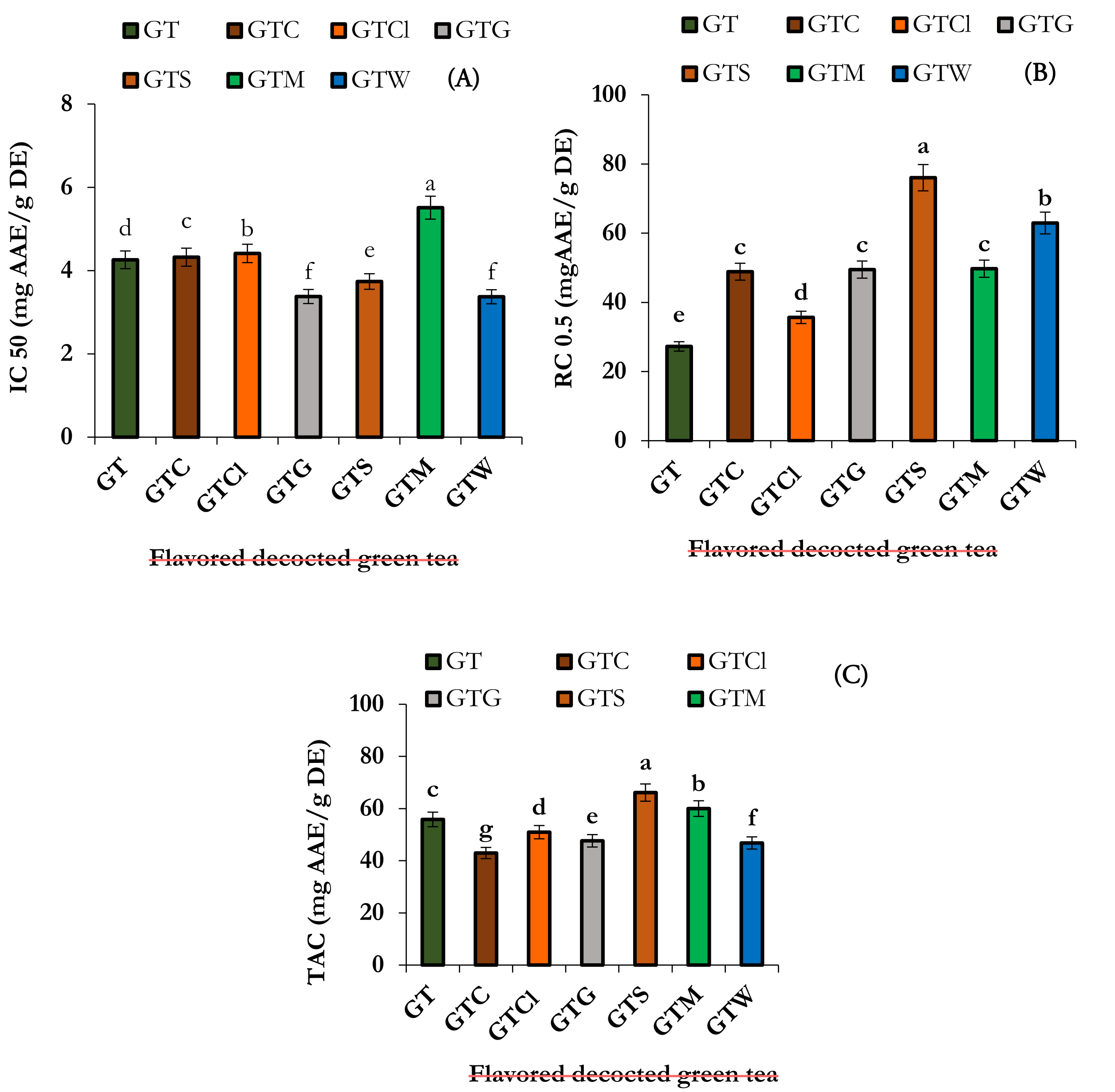


Figure 3. Antioxidant activity of flavored green teas

(A): Free Radical Scavenging Activity DPPH; (B): Ferric Antioxidant Reducing Power; (C): Total Antioxidant Capacity. Values are averages ± standard deviation of triplicate analysis; different letters indicate significant difference at $p<0.05$. Results are ranked in ascending order; a>b>c>d>e>f>g. GT: Green Tea; GTC: Green Tea flavored Cinnamon; GTCl: Green Tea flavored Clove; GTG: Green Tea flavored Ginger; GTS: Green Tea flavored Star anise; GTM: Green Tea flavored Mint; GTW: Green Tee flavored White mugwort.

GTS>GTM >GT> GTCl >GTG >GTW> GTC (Figure 3. B and C).

The obtained results show that the highest TAC was observed in green tea combined with star anise (GTS), which displayed a rate of 66.143 mg AAE/g DE, closely followed by green tea combined with mint (GTM) exhibiting an antioxidant activity of 60.006 mg AAE/g DE.

The pure green tea (GT) displayed an antioxidant capacity of 55.838 mg AAE/g DW, while green tea combined with clove (GTCl) showed a value of 50.958 mg AAE/g DE. In addition, green tea flavored with white mugwort (GTW) demonstrated an antioxidant capacity of 46.812 mg AAE/g DE. Finally, green tea combined with cinnamon (GTC) displayed a noteworthy antioxidant activity of 42.968 mg AAE/g DE.

Different results were observed for the blended teas in both FRAP assay (Figure 3.B) and the TAC assay (Figure 3.C). Unflavored green tea has obtained the lowest $RC_{0.5}$ value of 27.254 mg AAE/g DE, while the beverage GTS exhibited the highest value of 66.143 mg AAE/g DE in the total antioxidant assay. Yap et al. [32] argued that *in vitro* antioxidant capacity assays can be classified into two types, single electron transfer (SET)-based assays and hydrogen atom transfer (HAT)-based assays and the DPPH assay was found to use both HAT and SET. Difference in activity can be exhibited between different samples because DPPH assay only takes into account the hydrophobic antioxidants. The reducing properties are primarily due to the content of catechin and their gallic esters [34]. It has noticed that addition of some species to green tea improve its phenolic composition and its antioxidant power and the others conversely decreased it. Furthermore, Finimundy et al. [13] reported that the combination of several plants in a drink beverage can provide a synergistic effect in terms of bioactivities, as their combination improves the antioxidant status and reduces oxidative stress. However, Yap et al. [32] reported that antagonism can occur and the resultant antioxidant potential might not always be the additive value of the antioxidant properties of each component. Tipduangta et al. [35] showed that the summer tea containing *Aegle marmelos* L., *Steviarebaudiana.*, *Pandanus amaryllifolius* and *Morus alba* L. demonstrated the most promising antioxidant properties (*$p>0.05$*). Moreover, Zheng et al. [14] reported that Ganpu tea which is an emerging tea drink produced from Pu-erh tea and pericarp of Citrus enhance significantly (*$p<0.05$*) the superoxide dismutase (SOD) and glutathione peroxidase activities (GSH-Px activities) by 13.4% and 16.3%, respectively.

Combination of all these herbs with green tea can synergistically enhance antioxidant activity. Indeed, the results obtained by Jain, et al. [36] showed that combination of all extracts in ratio (5:3:3:3:3:3) of *Camellia sinensis*, *Vitis vinifera*, *Phyllanthus emblica* L., *Punica granatum*, *Cinnamomum cassia*, *Ginkgo biloba* L. exhibited the highest radical scavenging activity (IC_{50}= 33.5 mg/mL) among all other combinations as well as for individual extracts. Von Staszewski [37] proposed that many interactions take place between the compounds of tea infusions, leading to synergistic or antagonistic effects. In the other hand, Freeman et al. [38] reported that the interactions of antioxidants depend on the polarity of the interacting molecules, the reaction rates of the antioxidants, and their effective concentration at the site of oxidation. Uduwana et al. [31] explained the phenomenon of antagonism in antioxidants through a complex mechanism, including the regeneration of weaker antioxidants from the stronger ones, formation of complexes and adducts, polymerization reactions occurring between the antioxidants resulting in compounds with reduced antioxidant properties, reactions between free antioxidant radicals causing neutralization, and unpredictable mutual interactions among antioxidants. While, Olszowy-Tomczyk [39] explained the phenomenon of synergism by regeneration of the stronger antioxidant by the weaker one, formation of stable intermolecular complexes between the antioxidants which exhibits higher antioxidant activity than that of the parent compounds, differences in solubility and phase distributions of antioxidants near and at the interface, unpredictable interactions between compounds.

3.4 Effect of the addition of spices on the antibacterial activity

The results of the antibacterial activity of the tea samples are shown in Table 2, revealing that the extracts have a broad spectrum of activity against Gram-positive and Gram-negative bacteria.

In their study, Finimundy et al.[13] specified that polyherbal tisane composed of Bay laurel (*Laurus nobilis*) leaves and Walnut (*Juglans regia*) leaves at equal amounts possessed significantly high bioactivity compared to individual components. They noted that blends containing 15% *Laurus nobilis* L. and 15% *Juglan regia* L. displayed greater antioxidant, cytotoxic, and anti-inflammatory properties; as well as better antimicrobial effects against all the tested bacterial strains (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Micrococcus flavus*, *Enterobacter cloacae*, *Salmonella Typhymurium*) and fungal strains (*Aspergillus niger*, *Aspergillus versicolor, Aspergillus fumigatus*, *Penicillium funiculosum*, *and Penicillium aurantiogriseum*).

It was observed that both GT and GTC were significantly effectives against the three tested strains, surpassing the effectiveness of the tested anbtibiotics (Ciprofloxacin and Novobiocin). Particularly, GT was more potent against Salmonella sp with a DIZ value of 25.16 mm.

In addition, it has been reported by Almajano et al. [34] that tea compounds are characterized by their highest antioxidant and antimicrobial power. Indeed, the activity of bacterial agents depends on the type of microorganisms and is mainly related to their cell wall structure and the outer membrane arrangement [40]. Phenolic compounds can act at two different levels: the cell membrane and cell wall of the microorganisms

Table 2. Antibacterial activity of flavored decocted green teas

	Gram-positive			Gram-negative
	L. innocua	*S. aureus*	*E. Faecalis*	*Salmonella*
GT	20.00 ± 0.86^{b}	14.33 ±1.15^{f}	21.50 ± 0.50^{b}	25.17 ± 0.29^{c}
GTC	19.67 ± 1.00^{b}	15.00 ± 0.00^{e,f}	22.00 ± 0.57^{b}	17.67 ± 0.58^{b}
GTCl	14.00 ± 2.00^{cd}	14.33 ± 0.58^{f}	18.00 ± 0.29^{c}	14.33 ± 0.58^{d}
GTG	15.00 ± 1.04^{c}	19.00 ± 0.58^{cd}	18.00 ± 0.76^{c}	09.83± 0.28^{e}
GTS	16.00 ± 1.89^{c}	21.00 ± 1.41^{c}	16.5 0 ± 0.5^{c}	09.67 ± 0.76^{e}
GTM	16.33 ± 0.50^{c}	20.33 ± 0.58^{cd}	16.50 ± 0.86^{c}	15.00 ± 2.18^{c}
GTW	18.33 ± 0.28^{cd}	17.67±1.73d^{e}	17.00 ± 0.28^{c}	7.17 ± 0.28^{f}
CIP	26.00 ± 0.00^{a}	35.00 ± 0.00^{a}	35.00 ± 0.00^{a}	15.00 ± 0.00^{d}
DOXY	11.00 ± 0.00^{e}	27.00 ± 0.00^{b}	21.00 ± 0.00^{b}	0.00 ± 0.00^{e}
NOV	13.00 ± 0.00^{d}	27.00 ±0.00^{b}	35.00 ± 0.00^{a}	0.00 ± 0.00^{e}

Values are averages ± standard deviation of triplicate analysis. Different letter in the same column indicates a significant difference ($p<0.05$). Results are ranked in ascending order; a>b>c>d>e>f. GT: Green Tea; GTC: Green Tea flavored Cinnamon; GTCl: Green Tea flavored Clove; GTG: Green Tea flavored Ginger; GTS: Green Tea flavored Star anise; GTM: Green Tea flavored Mint; GTW: Green Tee flavored White mugwort, CIP: Ciprofloxacin; DOXY: Doxycyclin; NOV: Novobiocin.

[41]. They can interact with the membrane proteins of bacteria by means of hydrogen bonding through their hydroxyl groups which can induce changes in membrane permeability and cause cell destruction. They can also penetrate into Gram positive bacteria's cells and coagulate their cell content [42]. Moreover, antimicrobial mechanism of action of phytochemicals is related to the inhibition of ATP-ase activity, enzymatic reaction, toxins biosynthesis, biofilm formation, suppression of virulence factors, inhibiting nucleic acid synthesis, altering the lipid profile composition [43]. Phytoconstituents are responsible for protecting the plant against microbial infections [44]. For instance, Zhang et al. [45] reported that epigallocatechingallate (EGCG) gave rise to significant morphological alterations in *Shegella flexneri* cells, it might interfere with the production of proteins in bacterial cells.

In the current study, antibiotics have shown the best sensitivity against S. aureus, whereas, tea extracts exhibited the lower efficacy. Among antibiotics, ciprofloxacin displayed the most significant activity against S. aureus (DIZ: 35 mm), followed by novobiocin and doxycycline (DIZ: 27mm). while, GTS, GTM, GTG presented a DIZ of 21.00 mm, 20.33 mm and 19.00 mm, respectively. Indeed, Shan et al. [40] reported that *S. aureus* was the most sensitive bacteria (DIZ 15.7 mm) to the crude extract of cinnamon stick followed by *B. cereus* (15.4 mm), with *L. monocytogenes* being the most resistant one (11.5 mm). Generally, the Gram-positive bacteria were more sensitive to the crude extract than the Gram-negative bacteria.

The antibacterial activity of EGCG is associated to their interaction with surface proteins and/or producing hydrogen peroxide [46]. Cui et al. [47] have reported that EGCG binds directly to the peptidoglycan layer of *S. aureus*, causing damage to the cell walls. However, damage to Gram-negative bacteria, such as *E. coli*, cell walls has been induced primarily by the production of hydrogen peroxide, confirming that EGCG exerts different inhibitory mechanisms against Gram-negative and Gram-positive microorganisms. As we have already mentioned, the antibacterial activity of tea extracts can be due to their phenolic contents. Indeed, type-A procyanidinepolyphenols (TAPP) from cinnamon bark has been reported to exhibit activities against microorganisms [48]. In the other hand, the antibacterial and antifungal activity of methanolic leaf extract of *M. piperita* was probably due to the presence of tannins and flavonoids [49]. In the same context, Goudjil et al. [50] and Namita et al. [51] reported that the antibacterial activity of natural substances can be explained by their activity (lysis) on bacterial membranes, which leads to an extensive loss of critical molecules and ions and therefore to the cell death. Catechin of green tea has a broad spectrum of antimicrobial activities, including the effective prevention of the activity of food-borne bacteria [52,53].

3.5 Sensory analysis

Taste of green tea infusion is constituted of four taste-elements: bitterness, astringency, brothy taste and sweetness, and a good harmony of intensities of each element, within an intensity pattern specific to green tea, may give a good taste [54].

Our results showed that GTC is ranked in the sixth position with a high aroma, and a noticed odor and color intensity. In difference to our findings, Lutfiani et al. [55] reported that adding 5% cinnamon to instant green tea powder had the best effect on sensory properties and arises its acceptance.

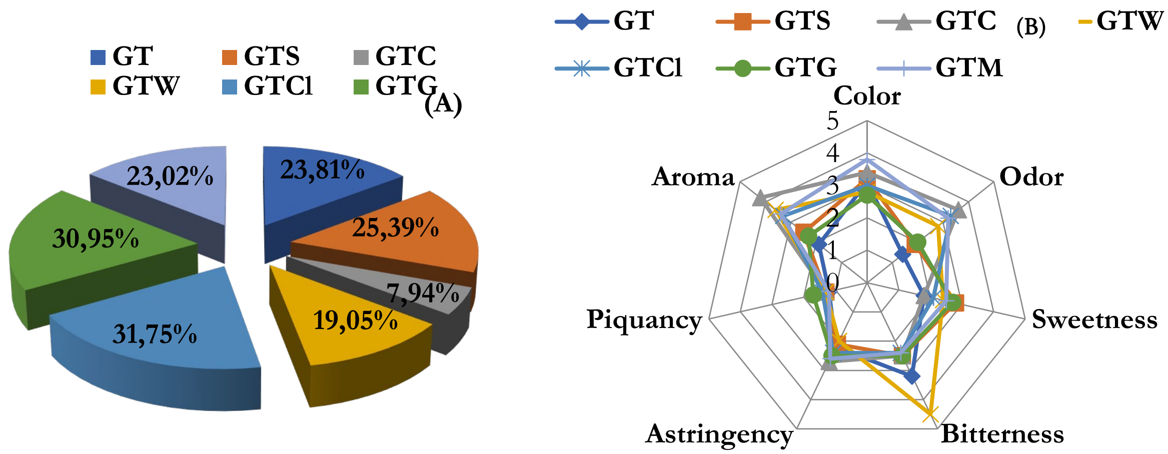


Figure 4. Sensory attributes of flavored decocted green teas

(A): Percentage of preferences; (B): Sensory attributes evaluation. Where GT: Green Tea; GTC: Green Tea flavored Cinnamon; GTCl: Green Tea flavored Clove; GTG: Green Tea flavored Ginger; GTS: Green Tea flavored Star anise; GTM: Green Tea flavored Mint; GTW: Green Tee flavored White mugwort.

In accordance with other findings, an improvement in the sensorial properties was mentioned in the majority of blends. Indeed, panelists detected that green tea with clove is the highest rank in taste (Figure 4). This preference might be related to its distinctive odor, aroma and color intensity. Indeed, Lutfiani et al. [55] reported that clove can give brownish color and a distinctive pungent. Additionally, eugenol, a compound that contained in clove, could processed into a synthetic vanillin compound which is an important flavor enhancer used in several food products including confectionary, chewing gum, cake, bread, and ice cream. Following the GTCl the preferred beverage was GTG, confirming the report of Supartono et al. [56] who postponed that green tea with ginger (GTG) gained almost highest consumer acceptance. However, only 23, 81% of the judges appreciated the GT which is ranked in the fourth position, mainly due to its bitterness. Hydrophobic amino acids contribute to the bitter taste, while some hydrophilic amino acids, due to their small molecular chains, contribute to the sweet taste sensation. Therefore, these amino acids are usually used as an evaluation index for assessing the tea quality as indicated by Zhang et al. [57].

Furthermore, the same researchers stated that cinnamon contains a viscous reddish liquid known as oleoresin, which gives a distinctive taste and aroma. This component can impart a reddish color to the tea, which is due to its constituents, anthocyanin and cinnam aldehyde. Regarding the GTM, the best consumed tea in the North Africa, was ranked in the fifth position but it has the best appreciated color and odor.

Finally, GTW ranked last in consumer preference, this can be explained by the strong and recognized bitterness which can be explained by the highest levels of polyphenols and flavonoids, as reported by Kraujalyte˙ et al. [58] and the monomeric flavan-3-ols, which contribute to this taste, while the presence of oligomers and polymers of flavan-3-ols contributes to astringency. Tang et al. [59] has also reported that catechin and tea polyphenols had significant effects on the bitterness and astringency of tea infusions but EGCG and tannins are involved in the establishment of color, taste and flavor of teas. Additionally, furfural and 5-HMF (5-Hydroxymethylfurfural) having a caramel flavor, are present in the Maillard reaction as an intermediate, likely contribute to the flavor quality of green tea [50,60].

4 Conclusion

The potential health benefits of green tea and herbal teas have led to an increased demand of specialty tea products in the food market. Nevertheless, a limited research studies on herbal mixtures have been conducted to show their benefits, polyphenol contents and their antioxidant activities. The results of this research have provided an insight into the differences in both antioxidant capacity and concentrations of polyphenols and flavonoids in flavored green tea decoctions, as well as their previously unstudied antibacterial activities.

The phenolic composition and the antioxidant activity of green tea has been improved through supplementation with some species like clove, ginger and white mugwort depending on the type of used test. The results showed a remarkable activity of both green tea (GT) and green tea flavored cinnamon (GTC) on the three tested strains *Salmonella*. sp, *E. faecalis* and *L.innocua*, when compared to that of antibiotics (ciprofloxacin and novobiocin). On the other hand, *S. aureus* exhibited sensitivity to all tested antibiotics, as well as to tea extracts, particularly in the case of pure green tea, green tea with cinnamon, and green tea with clove. Spices had a positive effect on sensory properties of green tea at various threshold ranges. The results showed that some spices mixed to green tea were preferred more than others. The four best rated spices included clove, ginger, star anise and mint. Consequently, it can be concluded that, apart from possible improvements in flavor and aroma, the mixed tea offers some advantages in terms of phenolic compounds, antioxidant and antibacterial activities, depending on the type of the strain.

The results of the sensory evaluation and antioxidant activity showed that green tea flavored with ginger is ranked in the second position, so we can advise people to consume ginger tea. Moreover, our results showed that interactions between individual herbs can positively affect the total antioxidant and antibacterial activities; it may also promote the consumption of GT blends that can act as a chemo preventive agent. So it would be interesting to carry out further studies on the ideal proportions to be used in these mixtures of herbs as well as on other blends. In addition, the identification of molecules responsible for the biological properties of plants, will allow a better understanding of the mechanisms involved in positive interactions.

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Authors' Contribution: Contributors should provide a description of contributions made by each of them towards the manuscript. Description should be divided into following categories, as applicable: concept, design, definition of intellectual content, literature search, experimental/clinical studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review. One author should take responsibility for the integrity of the work as a whole from inception to published article and should be designated as "corresponding author".

"e.g., **A.A.** conceived and designed the study, and undertook the literature research. All authors participated in the experiment and data acquisition. **B.B.** and **C.C.** performed the data analysis. **D.D.** carried out the statistical analysis, prepared, reviewed and drafted the manuscript. All authors approved the final version before submission. All authors have read and agreed to the published version of the manuscript."

Conflicts of Interest: A statement of financial or other relationships that might lead to a conflict of interest, if that information is not included in the manuscript itself or in an authors' form.

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