Research article

Enhancing the antioxidant properties of functional herbal beverages using Ultrasonic-Assisted extraction: Optimized formulation and synergistic combinations of taurine and vit. C

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ARTICLE INFO

ABSTRACT

Functional herbal beverages are gaining popularity in the beverage industry due to their natural antioxidants. However, the high concentration of antioxidants in these beverages can lead to increased toxicity, limiting their use. Moreover, the composition of tap water, including mineral salts and hydrogen carbonate ions, hampers the extraction process of polyphenolic compounds, thereby reducing the antioxidant properties. This study aims to address these challenges by enhancing antioxidant properties, reducing toxic effects, and improving the extraction process. Low-dose herbal extracts of green tea, rosemary, milk thistle, and sage were extracted using 100 ml of boiling water as a solvent, with ultrasonication employed for 20 min. Taurine, vit. C, and their combination were added to the extracts. The antioxidant properties, polyphenol, and flavonoid content were evaluated. The results demonstrated that the low-dose herbal tea combined with taurine and vit. C exhibited higher antioxidant activity compared to high-dose tea. Notably, the combination of taurine and vit. C showed the strongest synergistic effect. The addition of vit. C to these combinations eliminated any antagonism and resulted in a robust synergistic effect. The optimal conditions for enhancing antioxidant properties were determined as follows: an herbal type of 0.030 ≈ 0 (sage), vit. C concentration of 0.045 g/100 ml, and taurine concentration of 0.179 g/100 ml. The measured responses for reducing power, DPPH, and ABTS were 0.152 μg vit. C equivalent/ml, 67.778 %, and 87.630 %, respectively. This study provides valuable insights into optimizing the antioxidant properties of herbal beverages through the synergistic combinations of taurine and vit. C. By employing proper preparation techniques and including taurine and vit. C, the antioxidant capacity of these beverages can be significantly improved, potentially offering health benefits against degenerative diseases.

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1. Introduction

Functional beverages are a rapidly developing sector within the beverage industry, encompassing nutritional drinks, energy drinks, and sports drinks [1]. These beverages are enriched with bioactive compounds such as vitamins, minerals, polyphenols, and extracts from fruits, teas, and herbs. Energy drinks, specifically, contain ingredients like taurine, caffeine, herbal extracts, and vitamin B [1]. Popular functional ingredients found in many beverages include ascorbic acid (vit. C), taurine, and caffeine, which are known for their ability to support continuous physical activity, mental alertness, and provide antioxidant benefits [2]. Herbal drinks taken as natural antioxidants can help stop oxidative damage to cells [3]. However, their effectiveness is dependent on high concentrations, which can potentially increase toxicity and limit their use as beverages [4]. The composition of tap water and highly mineralized water contains significant amounts of dissolved mineral salts, such as calcium and magnesium salts, as well as hydrogen carbonate ions. These components can interfere with the extraction process and react with polyphenolic compounds and pectins found in herbal raw materials [5]. As a result, the extraction of both organic and inorganic compounds is reduced, and the antioxidant properties of the brewed beverage are also diminished [6]. Highly mineralized water leads to a substantial loss in antioxidant capabilities in brews, especially for green and black teas. The reduction in antioxidant qualities ranged from 70 % to 93 % [7].

In addition to the toxic effects of high doses, aqueous extraction of herbs reduces their value as antioxidants. Therefore, the study aims to reduce these effects by using a low-dose aqueous extract of herbs (rosemary (*Salvia rosmarinus*), green tea, milk thistle *(Silybum marianum)*, and sage herb (*Salvia officinalis*)) while enhancing it by adding the amino acid taurine and vit. C. When taurine and vit. C as antioxidants is used in single herbal extract formulations, enhancement of antioxidant effects is expected through either synergistic amplification or diminishment of possible adverse side effects.

Taurine, a non-proteinogenic sulfur-containing amino acid, holds a special place among the many antioxidants in the body and plays an important role as a natural modulator of the antioxidant defense system. The body's taurine requirement can be met through both synthesis within the body and dietary intake [8]. Taurine has been shown to inhibit oxidative stress-induced lung damage, and more recent studies have demonstrated that taurine supplementation can increase the activity of antioxidant enzymes in the lungs [9, 10]. The simultaneous administration of curcumin and taurine has demonstrated potential as a prophylactic intervention for individuals at high risk of developing liver cancer due to exposure to chemical substances with hepatocarcinogenic properties [11]. The synergistic effect resulting from the combination of curcumin and taurine surpasses the individual efficacy of both treatments. This synergistic interaction leads to increased cellular density and enhanced activation of immunological responses [12].

Taurine's action is demonstrated by its ability to detoxify hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and nitric oxide (NO) without acting as a typical scavenger of reactive oxygen species (ROS) [13–15]. Ascorbic acid, or vit. C, is commonly found in various foods and is known for its role in preventing scurvy [16]. The concomitant administration of taurine and ascorbic acid exhibited a noteworthy therapeutic effect in a rat model of spinal cord injury, as evidenced by the significant restoration of dysregulated antioxidant markers and a reduction in lipid peroxidation [16]. Interestingly, studies have shown that taurine levels in the brain decrease significantly with age, leading to investigations into the potential neuroprotective effects of supplemental taurine in various experimental models [17,18].

Taurine poses limitations and health risks for specific groups, including children, pregnant and breastfeeding women, individuals with cardiovascular issues, and those with liver or kidney problems [19]. Energy drinks containing taurine are not recommended for children due to high caffeine levels, which can negatively impact behavior and development taurine can also affect blood pressure and heart rate, potentially exacerbating cardiovascular conditions. Individuals with impaired metabolism or elimination of taurine, such as those with liver or kidney problems, may face additional risks [20].

Response surface methodology (RSM) is a statistical technique that is used to optimize the conditions for a process or an experiment. It involves the use of mathematical models to predict the response of a system to changes in the input variables and the use of experimental design to generate the data needed to build the models [21]. RSM has been widely used in the fields of chemistry, engineering, and biotechnology to optimize processes and formulations [22]. RSM can be used to optimize the formulation and processing conditions of a drug or nutrient to improve its vivo bioaccessibility and cardioprotective activity potential [23]. For example, RSM has been used to optimize the composition of lipid nanoparticles for poorly water-soluble drug delivery and to improve the conditions for the preparation of solid dispersions to enhance the dissolution and bioaccessibility of poorly water-soluble drugs [24].

The aim of this study is to optimize the antioxidant properties of functional herbal beverages by reducing toxic effects and enhancing the extraction process of polyphenolic compounds. The study specifically focuses on the use of low-dose herbal extracts of green tea, rosemary, milk thistle, and sage and investigates the effects of adding taurine and vit. C to enhance the antioxidant activity of these extracts. The purpose of the study is to identify synergistic combinations of these ingredients that would result in increased antioxidant activity. By optimizing the ratios of herbal extracts, taurine, and vit. C, the study aims to provide a better understanding of how to maximize the antioxidant properties of herbal beverages, potentially offering health benefits against degenerative diseases.

2. Material and methods

2.1. Material

Green tea, rosemary, milk thistle, and sage were obtained from a local market in Egypt. Folin Cio-calteu's phenol reagent, gallic acid, aluminum chloride, potassium persulfate, and Sodium nitrite were supplied from Merck KGaA (Darmstadt, Germany). DPPH

(2,2-diphenyl-1-picryl-hydrazyl-hydrate) reagent, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS$^{\bullet+}$), quercetin and all enzymes were supplied from Sigma-Aldrich Co. (Germany). Sodium chloride, ethanol, and methanol were purchased from Methanol J. T. Baker (Netherlands).

2.2. Methods

2.2.1. Ultrasound-Assisted extraction (UAE) of herbal extracts

Herbal aqueous extracts were prepared by adding boiling water (1:10, *w/v*) to each dried herb (rosemary (*Salvia rosmarinus*), green tea, milk thistle *(Silybum marianum)*, and sage herb (*Salvia officinalis*)) [25], with some modifications (Table 1). The samples were then subjected to probe sonication using a Sonics and Materials, Inc. Vibra-Cell instrument (USA). The sample was sonicated at a constant power of 160 W, a frequency of 20 kHz, and a pulse intensity of 50 %. The mixture was centrifuged (Labnet Spectrafuge 16M, Labnet International Inc., Woodbridge, NJ, US) at a speed of 5000 rpm for 20 min, followed by gathering the resulting supernatant. All the accumulated supernatants were combined and filtered. The remaining mixture was freeze-dried to obtain herbs phenolic extract powder and stored at −20 °C for further analysis. After cooling the herbal extract, taurine or vit. C alone or their combination was added (Table 1). After 30 min of infusion, the tea was filtered through 0.40 and 0.22 μm filters. These tea infusions were aliquoted and stored at −20 ^{O}C. The antioxidant activity, phenolic, and flavonoid content of the extracts were analyzed.

2.2.2. HPLC analysis

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse Plus C8 column (4.6 mm × 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.05 % trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82 % A); 0–1 min (82 % A); 1–11 min (75 % A); 11–18 min (60 % A); 18–22 min (82 % A); 22–24 min (82 % A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 μl for each of the sample solutions. The column temperature was maintained at 40 °C [26].

2.2.3. Gas chromatography-mass spectrometry (GC – MS) analysis

Solid-Phase Microextraction (SPME)/GC–MS Analysis.

Extraction of herbs (1 g), NaCl (1 g), distilled water (5 ml), and a magnetic bar were added into a 40 ml vial sealed with silicone septa (Sigma Chemical Co.), which was incubated at 90 °C. After 10 min, the SPME fiber was exposed in the headspace of the vial for 60 min and then the fiber was inserted directly into the GC-MS injector port at 250 °C for 5 min. Three parallel experiments were performed for each sample [27].

2.2.4. Total phenolic content (TPC)

The determination of the total phenolic content (TPC) in the tea beverage samples was carried out following a standardized methodology, as outlined in the prescribed protocol [28]. The extracted sample (200 μl) was then combined with 100 μl Folin-Ciocalteau reagent (1:10, *v/v*). After 8 min at 25 °C in the dark, 300 μl of sodium carbonate (20 %) was added, and the resulting mixture was incubated for 15 min. The TPC was measured using an automated UV-VIS spectrophotometer (Agilent Cary 60 UV–Vis, Spectrophotometer) at a wavelength of 765 nm; the quantification was performed using a gallic acid, the calibration curve ($y = 0.0032x − 0.0269$) with an R^2 value of > 0.99. The TPC values were reported in μg of gallic acid equivalents per ml extract.

2.2.5. Total flavonoid content (TFC)

The tea beverage samples were analyzed for their total flavonoid content (TFC) using the recommended methods [29]. The 0.25 ml of the extracted samples were taken and mixed with 1.25 ml pure water and added to 75 μl of $NaNO_2$ (5 %), then 150 μl of $AlCl_3.6H_2O$ (10 %) after 6 min 0.5 ml of NaOH (1 M) was added and completed to 2.5 ml with pure water after 11 min. Absorbance was read at 510 nm wavelength in the Synergy HT spectrophotometer immediately (Agilent Cary 60 UV–Vis, Spectrophotometer). The TFC was given as μg quercetin (Qu) per ml extract, the calibration curve (, with an R^2 value of >0.97.

2.2.6. Reducing power

The determination of reducing power in the tea beverage samples was conducted using the specified methods [30]. In 96-well plates, 10 μl of sample solution, 25 μl of 0.2 M phosphate buffer (pH 6.6), and 25 μl of K_3 [Fe(CN)$_6$] (1 % w/v) were added sequentially. The mixture was incubated for 20 min at room temperature, and the reaction was stopped by adding 25 μl of TCA solution (10 % *w/v*). Further, 85 μl of water and 8.5 μl of FeCl$_3$ (0.1 % w/v) were added to each well. The contents were mixed and incubated for another 15 min at room temperature, and absorbance was read at 750 nm wavelength in the Synergy HT spectrophotometer

Table 1
Nutritional components of herbal teas (HT) (g/100 ml H_2O).

Ingredient	Rosemary	Green tea	Sage	Milk thistle
Herbs	0.20	0.30	0.50	0.50
Taurine	0.40	0.40	0.40	0.40
vit. C	0.125	0.125	0.125	0.125
Taurine + vit. C	0.40 + 0.125	0.40 + 0.125	0.40 + 0.125	0.40 + 0.125

immediately (Agilent Cary 60 UV–Vis, Spectrophotometer). The Reducing power was given as µg vit. C equivalent/ml extract, the calibration curve ($y = 0.114x - 0.008$), with an R^2 value of >0.95

2.2.7. Antioxidant activity

2.2.7.1. DPPH radical scavenging assay. The antioxidant activity of the tea beverage samples was assessed using two methods. The first method employed was the DPPH radical scavenging assay, which measures the ability of the samples to neutralize free radicals [31]. In this method, 0.2 g of each sample were dissolved in 25 ml of methanol, and the resulting extract was filtered. One milliliter of the extract was mixed with a 0.1 mmol/l DPPH methanol solution and incubated for 30 min. The absorbance of the solution was then measured at 517 nm using a spectrophotometer (Agilent Cary 60 UV–Vis Spectrophotometer). The percentage of DPPH radical scavenging activity was calculated using Equation (1):

$$\% \text{ DPPH radical scavenging activity} = (Ac - As) / Ac \times 100, \tag{1}$$

where As represents the absorbance of the sample and Ac represents the absorbance of the control (without the sample).

2.2.7.2. ABTS radical scavenging assay. The second method used to evaluate the antioxidant activity was the ABTS method [32]. The ABTS·+ radical was generated by reacting ABTS (7 mM) with potassium persulfate (140 mM) and allowing the mixture to incubate in the absence of light for 16 h. A volume of 2 ml of the prepared ABTS radical solution was diluted in ethanol to a final volume of 150 ml, resulting in a solution with an absorbance of approximately 0.700. For the analysis, 300 µl of the ethanol extract was added to a test tube containing 2.7 ml of the ABTS radical solution. The tubes were covered and kept in the dark for 20 min. The absorbance was then measured at 734 nm using a UV–Vis spectrophotometer (Agilent Cary 60 UV–Vis Spectrophotometer). All assays were performed in triplicate. The percentage of inhibition, expressed as the absorbance at 734 nm, was calculated using Equation (2):

$$\% \text{ AA} = (Ac - As) / Ac \times 100, \tag{2}$$

where As represents the absorbance of the sample and Ac represents the absorbance of the control (without the sample).

2.2.8. Sensory evaluation

The National Research Center's Sensory Evaluation Lab was used to conduct sensory analyses of the four herbal beverages (HB1 (sage), 2(rosemary), 3(green tea), and 4(milk thistle)). Coded tea beverages and a questionnaire were given to each of the 40 panelists. They were asked to complete an acceptance test, rate the product on a 5-point hedonic scale (1 being "Extremely Dislike" to 3 being "Extremely Like"), and select their favorite product through a preference test. In addition, an expectorant cup and water for rinsing in between samples were provided to the panelists [33]. Until they were given to the panelists, the beverage samples were stored in the refrigerator.

2.2.9. Statistical analysis

Results are presented as means ± standard deviations using SAS system version 9.3. ANOVA was used to determine any significant differences among the treatment groups. The experiments were performed in at least three replicates, and the results were averaged. The significance was determined at $P \leq 0.01$ and $P \leq 0.05$.

Table 2
Concentration of the phenolic compounds (µg/g) present in Rosemary, green tea, Sage and milk thistle Extract by HPLC method.

	Rosemary	Green tea	Sage	Milk thistle
Gallic acid	29.78 ± 1.02	48.76 ± 2.01	7.80 ± 0.91	2.46 ± 0.09
Chlorogenic acid	–	–	–	6.93 ± 0.05
Catechin	21.16 ± 0.99	20.71 ± 1.02	2.80 ± 0.25	–
Methyl gallate	2.49 ± 0.01	–	0.91 ± 0.01	0.61 ± 0.01
Coffeic acid	12.84 ± 0.23	8.91 ± 0.04	13.51 ± 0.09	–
Syringic acid	4.77 ± 0.05	1.67 ± 0.01	2.02 ± 0.09	2.46 ± 0.02
Rutin	13.54 ± 0.54	4.65 ± 0.02	0.37 ± 0.01	–
Ellagic acid	9.61 ± 0.65	6.85 ± 0.03	4.03 ± 0.02	1.39 ± 0.03
Coumaric acid	0.26 ± 0.01	0.11 ± 0.01	0.79 ± 0.01	0.49 ± 0.03
Vanillin	15.46 ± 0.02	2.60 ± 0.01	14.47 ± 1.05	3.54 ± 0.03
Ferulic acid	5.82 ± 0.01	1.57 ± 0.01	0.60 ± 0.01	0.07 ± 0.01
Naringenin	3.35 ± 0.01	1.66 ± 0.01	1.31 ± 0.01	0.41 ± 0.00
Rosmarinic acid	527.43 ± 9.21	2.72 ± 0.01	101.62 ± 5.02	0.79 ± 0.00
Daidzein	12.25 ± 0.98	0.86 ± 0.02	0.24 ± 0.01	0.10 ± 0.00
Querectin	19.77 ± 1.05	2.06 ± 0.02	5.43 ± 0.05	–
Cinnamic acid	0.88 ± 0.05	0.26 ± 0.01	0.07 ± 0.00	0.06 ± 0.00
Kaempferol	2.66 ± 0.01	0.71 ± 0.01	13.46 ± 0.98	2.66 ± 0.01
Hesperetin	3.18 ± 0.03	6.62 ± 0.02	2.28 ± 0.05	3.08 ± 0.02

Values are the means of 3 replicates ± standard deviations.

3. Results

3.1. The chemical profiling of the herbs extract

3.1.1. HPLC

The major compounds present in rosemary, green tea, sage, and milk thistle were determined and quantified using HPLC analysis. Compounds were identified by comparing the retention durations of each peak with those of injected reference standards using the same chromatographic conditions. Table 2 lists each discovered compound's quantity (μg/ml) and retention duration (min). The predominant component in the extracts of rosemary and sage, with concentrations of 527.43 and 101.62 μg/ml, respectively, was rosmarinic acid. The two main compounds in the green tea and milk thistle extract were gallic acid and chlorogenic acid, which were detected at concentrations of 48.76 and 6.93 μg/ml, respectively. The other detected compounds were present with less.

3.1.2. The essential oils analysis (GC-MS)

The aromatic profile of the essential oils (EOs) liquid phase was carried out by gas chromatography-mass spectrometry (GC–MS), while the volatile composition of green tea and milk thistle was performed by headspace (HS)/SPME/GC–MS. The obtained results show that isoborneol (19.43 %) was the main compound, followed by α-Pinene and Eucalyptol (14.08 % and 14.97 %) in the liquid of Rosemary EO, respectively. The Eucalyptol was the main component of sage EO, reaching 39.23 % in the liquid phase. Regarding hydrolates (Hys), the obtained results show that turmerone and Hexanal (67.75 % and 12.38 %) were the main compounds of milk thistle and green tea (Table 3).

3.2. Response Surface Methodology (RSM) for experimental design optimization

The optimization of the experimental design using Response Surface Methodology (RSM) was undertaken to enhance the antioxidant properties of herbal beverages. Utilizing Design-Expert® software version 13, the Box-Behnken method was employed with three independent variables or experimental factors. The selected variables, along with their corresponding levels, were coded to generate experimental runs in the design matrix, as outlined in detail in Table 4.

Notably, different herbal tea types were numerically quantified, with sage represented as 0, milk thistle as 1, rosemary as 2, and green tea as 3. In contrast, the vit. C and taurine variables were assigned with three experimental levels - the highest, center point, and lowest values, as 0, 0.0625, and 0.125 g/100 ml H_2O for vit. C, and 0, 0.2, and 0.4 g/100 ml H_2O for taurine, respectively. This methodical approach facilitated a comprehensive exploration of the experimental space, yielding a nuanced understanding of the factors influencing the antioxidant properties, specifically reducing power, DPPH, and ABTS. The use of coded values established a standardized framework, ensuring precision in conducting experiments and analyzing outcomes during the optimization process.

3.3. Total phenolic and flavonoid content for low and high doses of herbal tea extract

The total phenolic and total flavonoid contents were also determined for the hot water extracts using colorimetric methods. The total amount of phenols and flavonoids present in the water extracts of the selected herbs is shown in Table 8. In high doses of herbal tea extract, rosemary had the highest total phenolic content (885 μg gallic acid equivalent/ml), while milk thistle had the lowest value (341 μg gallic acid equivalent/ml), using the standard curve of gallic acid ($R^2 = 0.9482$). The total phenolic content in decreasing order was rosemary > green tea > sage > milk thistle (p < 0.01, Table 3). Using the standard curve of quercetin ($R^2 = 0.9825$), the total flavonoid content of herbs varied from 0.04 μg to 0.01 QE/ml for green tea and sage, respectively. The total flavonoid content in decreasing order was green tea > rosemary > sage > milk thistle > (p < 0.01, Table 5). On the other hand, in low doses of herbal tea extract, the total amount of phenolics and flavonoids present in the water extracts of the selected herbs is shown in Table 3. Rosemary had the highest total phenolic content (59.95 μg gallic acid equivalent/ml), while green tea had the lowest value (50.29 μg gallic acid equivalent/ml), using the standard curve of gallic acid. The total phenolic content in decreasing order was rosemary > sage > milk thistle > green tea (p < 0.01). The total flavonoid content of herbs varied from 0.004 μg QE/ml to 0.001 μg QE/ml. The total flavonoid content in decreasing order was green tea > sage = milk thistle > rosemary (p < 0.01).

Antioxidant potential (DPPH, ABST radical scavenging activity, and reducing power) for low and high doses of herbal tea extract.

The antioxidant effects of herb (3 g herb/100 ml hot water) extracts were evaluated using a series of in vitro assays (DPPH•, ABTS•+ scavenging and reducing power, Table 5). Whereupon, non-toxic concentrations of the four most potent herb extracts (i.e., rosemary, green tea, sage, and milk thistle) were used as antioxidants (Table 5). The percentage of extract values in DPPH* and ABTS*+ scavenging assays was determined. Notable is that all the herb extracts that were tested exhibited DPPH radical scavenging activity: 54 % (Rosemary), 85.98 % (Green tea), 84.72 % (sage), and 88.18 % (milk thistle) (Table 5).

In the ABTS•+ assay, the extract of green tea and sage exerted the highest scavenging activity (95. 26 % and 96.56 %; Table 5). Similarly, all extracts (rosemary and milk thistle) exhibited potent scavenging activities against ABTS•+ with values of 89.19 % and 92.92 %, respectively (Table 8). The extract that exhibited the highest reducing power capacity was rosemary (0.424 μg Vit. C equivalent/ml), Table 2), followed by both milk thistle, sage, and green tea, 0.136, 0.219 and 0.220 μg vit. C equivalent/ml, respectively (Table 5). All low doses of herbal extract exhibited the lowest antioxidant values compared to high doses (Table 5).

Table 3

The relative percentage (%) of herbs essentialoils (EOs) using GC-MS.

No.	Identified compounds	Sage	Milk thistle	Rosemary	Green tea
1	Tricyclene			0.85±	
2	α-Pinene	4.81 ± 0.05		14.08±	
3	Camphene	4.78 ± 0.06		5.00±	
4	Sabinene			1.38±	
5	β-Pinene	7.11 ± 0.07		2.66±	
6	β-Myrcene	1.77 ± 0.01		0.59±	
7	α-Phellandrene			0.38±	
8	3-Carene			0.67±	
9	α-Terpinene			1.47±	
10	p-Cymene	0.70 ± 0.01		5.38±	
11	ᴅ-Limonene	2.32 ± 0.02		8.41±	
12	Eucalyptol	39.23 ± 2.05		14.97±	
13	γ-Terpinene			3.12±	
14	Terpinolene			0.78±	
15	Linalool			2.32±	
16	Thujone	1.60 ± 0.05			
17	β-Thujone	0.87 ± 0.01			
18	(+)-2-Bornanone	12.48 ± 1.05		2.92±	
19	Isoborneol	1.81 ± 0.05		19.43±	
20	Terpinen-4-ol	0.63 ± 0.02		0.93±	
21	α-Terpineol	2.01 ± 0.01		7.08±	
22	Linalyl acetate	0.79 ± 0.02			
23	(−)-Bornyl acetate	1.48 ± 0.06			
24	α-Terpinyl acetate	3.47 ± 0.09			
25	β-Caryophyllene	9.39 ± 0.25			
26	Aromandendrene	0.53 ± 0.06			
27	α-Humulene	3.17 ± 0.08			
28	Caryophyllene oxide	0.49 ± 0.01			
29	Viridiflorol	0.56 ± 0.01			
30	γ-Terpineol			1.17 ± 0.01	
31	Isobornyl acetate			4.08 ± 0.02	
32	β-Caryophyllene			2.31 ± 0.02	
33	Hexanoic acid		4.45 ± 0.29		
34	ᴅ-Limonene		3.54 ± 0.12		
35	Malonaldehyde, bis(dimethyl acetal)		2.99 ± 0.09		
36	Dimethylphenylamine		6.52 ± 0.09		
37	Nonanal		3.01 ± 0.08		
38	2,6-Dimethylphenol		3.04 ± 0.09		
39	aR-Turmerone		67.75 ± 1.05		
40	Curlone		3.79 ± 0.09		
41	n-Hexadecanoic acid		4.91 ± 0.05		
42	α-Methylbutanal				4.07 ± 0.09
43	Cyclopentanol				2.76 ± 0.06
44	4-Methylcyclohexanol				10.12 ± 0.92
45	.(2-Methyloctyl)benzene				1.75 ± 0.09
46	Hexanal				12.38 ± 0.07
47	2-Hexenal				2.45 ± 0.01
48	(E)-2-Hexenal				1.99 ± 0.01
49	(Z)-Hex-3-en-1-ol				5.88 ± 0.02
50	(E)-2-Hexen-1-ol				3.51 ± 0.01
51	Heptanal				1.04 ± 0.01
52	Methyl N-hydroxybenzenecarboximidoate				2.62 ± 0.01
53	Benzaldehyde				7.12 ± 0.03
54	1-Octen-3-ol				0.64 ± 0.01
55	6-Methyl-5-heptene-2-one				1.58 ± 0.01
56	trans-β-Terpineol				1.40 ± 0.01
57	2-Cyclopentene-1-undecanoic acid				1.08 ± 0.01
58	2,6-Dihydroxyacetophenone, 2TMS derivative				2.75 ± 0.02
59	2,4-Heptadien-1-al				1.18 ± 0.01
60	2-Ethyl-1-hexanol				1.11 ± 0.01
61	Linalool oxide				0.88 ± 0.01
62	Benzeneacetaldehyde				3.33 ± 0.02
63	1-Ethyl-1H-pyrrole-2-carboxaldehyde				0.60 ± 0.01
64	.(E)-2-Octenal				1.24 ± 0.03
65	Linalool oxide				2.85 ± 0.02
66	.(E)-Furan linalool oxide				3.09 ± 0.03
67	Linalool				9.44 ± 0.05
68	2,6-Dimethyl-1,7-octadien-3,6-diol				1.13 ± 0.06

Table 3 (*continued*)

No.	Identified compounds	Sage	Milk thistle	Rosemary	Green tea
69	2,5-Dihydroxybenzaldehyde, 2TMS derivative				0.52 ± 0.01
70	3-Oxo-betacycloclitral				1.44 ± 0.01
71	Methyl salicylate				5.25 ± 0.05
72	Estragole				0.38 ± 0.04
73	Decanal				0.38 ± 0.02
74	β-Cyclocitral				1.02 ± 0.01
75	Eugenol				0.77 ± 0.01
76	*trans*-β-Ionone				0.54 ± 0.01
77	Benzophenone				0.38 ± 0.01

Values are the means of 3 replicates $\pm$ standard deviations.

3.4. Synergistic antioxidant activity (DPPH, ABTS and reducing power assay) of herbal tea individual, with taurine and vit. C, at low dose of herb

In the present study, the effects of taurine and/or vit. C on the antioxidant activity of each herb were demonstrated (Table 6). The results showed that herbal tea individually attenuates free radicals, while taurine + vit. C combined with herbal tea has a greater potential to inhibit free radicals than each herbal tea alone. In the ABTS (radical scavenging activity %) assay in herbs combined with taurine and vit. C, milk thistle (98 %) exhibited the highest ABTS inhibition, while green tea (92 %), rosemary (97 %), and sage (94 %) showed the least effective ABTS inhibition (Table 6). In DPPH (radical scavenging activity %) assay in herbs combined with taurine and vit. C, the extracts of milk thistle, green tea, and rosemary were able to inhibit the DPPH radical ranging (98 %, 95 %, and 95 %, respectively), while sage showed the least effective DPPH inhibition (79 %) (Table 6). The order of antioxidant activity (DPPH assay) of herbal tea individually is green tea = rosemary > sage > milk thistle, while the order of antioxidant activity (ABTS assay) is green tea = rosemary > sage > milk thistle. The reducing property of herbal extracts is generally associated with the presence of reductants. The data presented here indicated that the marked reducing activity of the herbal extracts may be due to the presence of polyphenol, taurine vit. C, or their combination. The results showed that the reducing power of herbal extracts increased with the addition of taurine or vit. C or their combination. A strong reducing power was noted for water extracts of rosemary with vit. C and taurine (0.38 μg vit. C equivalent/ml). Much lower reducing power was noted for water extracts of sage with taurine and vit. C (0.314 (μg vit. C equivalent/ml)) and milk thistle (0.32(μg Vit. C equivalent/ml)). All herbal teas significantly increased the presence of vit. C and taurine (P ≤ 0.05) except the sage and milk thistle extracts, which showed no significant activity in the reducing power and ABTS test with added taurine. Likewise, there was no significant increase between green tea and vit. C in the reducing power test. In general, the antioxidant activity of each herb combined with taurine or vit. C or their combination is higher compared to the individual herbs, as such herbs could not effectively inhibit DPPH radical, ABTS radical, or reducing power. The data in Table 6 were subjected to Duncan's multiple range test to determine any significant differences among the herbs for each parameter. Superscript letters indicate significant differences at p ≤ 0.05.

3.5. Synergistic (+) and antagonistic (−) herbal tea water extract, at low dose, with taurine and vit. C as radical scavenging activity and their comparison to high dose herbal tea water extract

Through the study of antioxidants, it was found that the antioxidant effect of individual herbs at low doses of taurine and vit. C is the same as that of individual herbs at high doses. It should be noted, although, that low doses of green tea, rosemary, and sage herbal tea with taurine and vit. C are better when compared to high doses of individual herbs, as in radical scavenging activity, except milk thistle with reducing power activity, the presence of taurine and vit. C together with herbs remarkably enhanced antioxidative efficacy. On the other hand, it was found that the antioxidant effect of high doses (3g) of individual herbs is better than that of a low dose of individual herbs (0.2g–0.5g) without adding taurine and vit. C (Figs. 1–3). The synergistic potential of herbal tea with taurine and vit. C remarkably enhanced antioxidative efficacy. Antagonistic interactions were seen in several combinations, such as taurine/sage or taurine/milk thistle, in the reducing power test. The addition of vit. C to the antagonistic combination of taurine/sage or taurine/milk thistle removed the antagonism in the combination, resulting in strong synergism (Figs. 1–3).

Fig. 4 provides a detailed three-dimensional graphical representation specifically focusing on the antioxidant capacity analysis. This graph provides a nuanced/detailed characterization of both individual and collective effects of variables, offering insights into interrelationships among the effects within each treatment of different herbal tea beverage types fortified with vit. C and taurine.

The X-axis of the graph corresponds to the values of vit. C, ranging from 0 (control) to 0.125 g/100 ml H_2O, capturing the variable's impact on antioxidant capacity. Simultaneously, the Y-axis illustrates taurine addition, ranging from 0 (control) to 0.4 g/100 ml H_2O, showcasing its contribution to the overall antioxidant capacity. The interactions between the X-axis (vit. C) and the Y-axis (taurine) represent various mixture ratios between them, revealing the dynamic interplay within the experimental design.

To assess the antioxidant capacity, three different responses—Reducing power, DPPH, and ABTS—were assigned. These responses are intricately depicted within the three-dimensional space, offering a visual representation of how each variable influences the antioxidant properties.

In summary, this graphical representation goes beyond a simple portrayal of variables; it provides a visual insight into the specific impact of each variable and the interactive relationships between them. This approach enhances the understanding of the complexity

Table 4
Utilization of Box-Behnken design in response surface methodology (RSM) for optimizing antioxidant properties.

Run	Coded Factors			Experimental Factors				Results						
	1	2	3	Factor 1		Factor 2	Factor 3	Result 1		Result 2		Result 3	
				A: Herbal type		B: vit. C, g/100 ml H$_2$O	C: Taurine, g/100 ml H$_2$O	Reducing Power, µg vit. C equivalent/ml	Exp. Pred. Residual	DPPH, %	Exp. Pred. Residual	ABTS, %	Exp. Pred. Residual
				Coded	Actual name								
1	−1	−1	0	0	Sage	0.00	0.20	0.094	0.0012	61.585	−1.235	86.315	−0.015
2	1	−1	0	3	Green tea	0.00	0.20	0.154	−0.0018	75.15	0.43	76.23	0.09
3	−1	1	0	0	Sage	0.125	0.20	0.268	0.0032	85.795	1.155	93.37	0.33
4	1	1	0	3	Green tea	0.125	0.20	0.2325	−0.0025	91.925	−0.575	94.105	−0.405
5	−1	0	−1	0	Sage	0.0625	0.00	0.158	0.0022	73.425	0.825	88.6	−0.1
6	1	0	−1	3	Green tea	0.0625	0.00	0.0915	−0.0035	76	−0.4	85.99	0.39
7	−1	0	1	0	Sage	0.0625	0.40	0.204	−0.0012	73.955	0.355	91.085	−0.585
8	1	0	1	3	Green tea	0.0625	0.40	0.295	0.0018	91.075	0.475	84.345	0.055
9	0	−1	−1	1	Milk thistle	0	0.00	0.054	−0.0008	32.07	−0.53	75.29	−0.21
10	0	1	−1	1	Milk thistle	0.125	0.00	0.307	0.0022	96.74	0.14	93.7	0.2
11	0	−1	1	1	Milk thistle	0	0.4.00	0.057	−0.0005	78.42	0.02	77.22	−0.022
12	0	1	1	1	Milk thistle	0.125	0.4.00	0.323	−0.0013	98.23	−0.17	98.46	−0.036
13	0	0	0	1	Milk thistle	0.0625	0.2.00	0.18525	0.00025	76.365	0.135	86.1675	0.0025
14	0	0	0	2	Rosemary	0.0625	0.2.00	0.23625	−0.00075	83.645	−0.355	87.1875	−0.0625

Table 5

Total phenolic, flavonoids content and antioxidant potential (DPPH radical scavenging, ABTS radical scavenging) activity and reducing power for high and low doses of herbs (g/100 ml water).

	Rosemary		Green tea		Sage		Milk thistle	
	Level of dose		Level of dose		Level of dose		Level of dose	
	High 3.00 %	Low 0.20 %	High 3.00 %	Low 0.30 %	High 3.00 %	Low 0.50 %	High 3.00 %	Low 0.50 %
Phenolic (µg gallic acid equivalent/ml)	885.00 ± 10.34	59.95 ±2.48	502.00 ±5.22	50.29 ±5.74	353.00 ±8.34	58.85 ±5.14	341.00 ±10.34	54.40 3 ± 4.47
Flavonoid's content (µg Quercetin equivalent/ml)	0.033 ±0.001	0.001 ±0.0001	0.041 ±0.001	0.004 ±0.01	0.011 ±0.001	0.002 ±0.0001	0.017 ±0.002	0.002 ±0.0001
Reducing power (µg vit. C equivalent/ml	0.424 ±0.01	0.056 ±0.00	0.220 ±0.01	0.089 ±0.01	0.219 ±0.03	0.094 ±0.01	0.136 ±0.02	0.054 ±0.00
DPPH radical scavenging activity (%)	63.55 ±6.36	63.95 ±5.31	85.98 ±4.55	63.46 ±6.67	84.72 ±5.32	54.31 ±3.81	88.18 ±5.94	32.07 ±2.22
ABTS radical scavenging activity (%)	89.14 ±5.6	74.89 ±3.45	95.26 ±6.14	76.45 ±5.38	96.58 ±7.34	85.17 ±5.53	92.92 ±9.35	75.29 ±3.11

Values are the means of 3 replicates ± standard deviations.

and dynamics involved in optimizing the antioxidant properties of herbal beverages through synergistic combinations of vit. C and taurine.

3.6. Response surface model

The response surface model employed in this study involves three distinct equations: equations (3)–(5). These equations serve to encapsulate the relationship between the responses, namely reducing power, DPPH, and ABTS, and three experimental factors of herbal tea type, addition of vit. C, and addition of taurine acting as independent variables. The functional forms of these equations are linear models, offering a comprehensive representation of the intricate interplay between the responses and the experimental factors, contributing to a nuanced understanding of the experimental outcomes.

3.6.1. Top of form

In the analyses of reducing power, DPPH, and ABTS, strong positive correlations are observed between the independent variables and their respective dependent variables, as indicated by high multiple correlation coefficients (Multiple R) of 0.9343, 0.927, and 0.932, respectively. The coefficient of determination (R Square) values of 0.838, 0.887, and 0.943 for reducing power, DPPH, and ABTS, respectively, reveal that approximately 83.8 %, 88.7 %, and 94.3 % of the variability in the dependent variables can be explained by the independent variables (Equations (3)–(5)). These findings collectively demonstrate well-fitted models with a substantial proportion of variability explained. The multiple correlation coefficients highlight strong overall relationships, and the standard errors offer a measure of the precision of the model predictions.

$$Red.Power = 0.072589 - 0.00625A + 1.873B - 0.0453C - 0.255AB + 0.131AC + 0.26BC \ R^2 = 0.838, \tag{3}$$

$$DPPH = 40.195 + 2.098A + 460.11B + 77.55C - 19.827AB + 12.121AC - 897.2BC \ R^2 = 0.887, \tag{4}$$

$$ABTS = 82.71 - 2.673A + 74.56B + 6.33C + 28.853AB - 3.442AC + 56.6BC \ R^2 = 0.943, \tag{5}$$

where variable A represents different herbal tea types, each quantified with numerical values: sage is denoted as 0, milk thistle as 1, rosemary as 2, and green tea as 3. On the other hand, variable B corresponds to the addition of vit. C in grams per 100 mL of water, while variable C represents the addition of taurine in grams per 100 mL of water.

The ANOVA results for the analyses of reducing power, DPPH, and ABTS are summarized in Table 7, providing crucial insights into the overall significance of the regression models. For reducing power, the regression model is highly significant (F = 6.03, p-value = 0.0162), indicating that the model effectively explains the variation in reducing power. In the case of DPPH, the regression model is also highly significant (F = 9.11, p-value = 0.0051), suggesting its effectiveness in explaining the variability in DPPH levels. Similarly, for ABTS the regression model is highly significant (F = 19.21, p-value = 0.0005), indicating the model's efficacy in explaining the variability in ABTS levels. These results collectively affirm that the independent variables in each model significantly contribute to explaining the variation in the respective dependent variables (reducing power, DPPH, and ABTS), reinforcing the robustness of the regression analyses.

The optimal conditions are achieved with a herbal type of 0.030 ≈ 0 (sage), vit. C of 0.045, and taurine of 0.179. The responses for reducing power, DPPH, and ABTS are measured at 0.152 µg vit. C equivalent/ml, 67.778 %, and 87.630 %, respectively. The Desirability index is 1.000, indicating that this combination of factors is considered highly desirable based on the optimization criteria. In practical terms, this suggests that the herbal beverage formulated with the specified levels of herbal type, vit. C, and taurine resulted in optimal antioxidant properties, as indicated by the measured responses and the assigned desirability value.

Table 6

Synergistic antioxidant activity (DPPH, ABTS and reducing power assay) of herbal tea water extract, at low dose, with taurine and vit. C

	Rosemary(0.2)	Rosemary(0.2) + Taurine(0.4)	Rosemary(0.2) + vit.C(0.125)	Rosemary(0.2) + Taurine(0.4) +vitamineC(0.125)	Greentea(0.3)	Greentea(0.3) + Taurine(0.4)	Greentea(0.3) + vit.C(0.125)	Greentea(0.3) + Taurine(0.4) +vit.C(0.125)	Sage(0.5)	Sage(0.5) + Taurine(0.4)	Sage(0.5) + vit.C(0.125)	Sage(0.5) + Taurine(0.4)andvit.C(0.125)	Milkthistle(0.5)	Milkthistle(0.5) + Taurine(0.4)	Milkthistle(0.5) + vit.C(0.125)	Milkthistle(0.5) + Taurine(0.4) +vit.C(0.125)C(0.125)
Reducing power (μg VitC equivalent/ml)	0.06 ± 0.01[d]	0.16 ± 0.02[c]	0.35 ± 0.01[b]	0.31 ± 0.03[a]	0.09 ±0.01[c]	0.22 ±0.01[b]	0.09 ±0.02[c]	0.37 ±0.04[a]	0.09 ±0.01[c]	0.09 ±0.02[c]	0.22 ±0.04[b]	0.31 ±0.03[a]	0.05 ±0.00[d]	0.06 ±0.00[d]	0.31 ±0.02[a]	0.32 ±0.03[a]
DPPH radical scavenging activity (%)	63.95 ± 4.34[e]	88.06 ± 5.04[c]	87.07 ± 7.65[cd]	95.50 ± 4.30[a]	63.46 ±3.04[e]	86.84 ±5.30[c]	88.54 ±4.32[c]	95.31 ±4.36[a]	54.31 ±3.30[f]	68.86 ±5.03[d]	92.54 ±3.36[b]	79.05 ±2.32[d]	32.07 ±1.32[g]	78.42 ±2.09[d]	96.74 ±5.05[a]	98.23 ± 3.30[a]
ABTS radical scavenging activity (%)	74.89 ± 5.32[d]	87.37 ± 3.03[c]	88.97 ± 6.11[c]	97.52 ± 6.21[a]	76.45 ±3.35[cd]	76.01 ±7.04[d]	95.53 ±5.35[b]	92.68 ±4.14[b]	85.17 ±4.64[c]	87.46 ±3.95[c]	92.03 ±7.11[b]	94.71 ±3.33[ab]	75.29 ±7.37[d]	77.22 ±4.30[cd]	93.70 ±5.32[ab]	98.46 ±4.04[a]

Values are the means of 3 replicates ± standard deviations. Values with different superscript letters within the same row indicate significant differences among herbs according to Duncan's multiple range test (p ≤ 0.05).
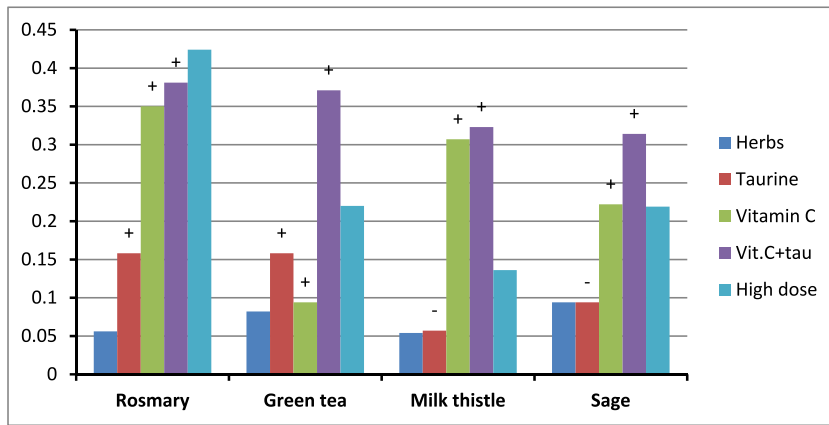
Fig. 1. Synergistic (+) and antagonistic (−) herbal tea water extract, at low dose, with taurine and vit. C as reducing power activity (µg Vic equivalent/ml) and their comparison to high dose herbal tea water extract from Table 5.
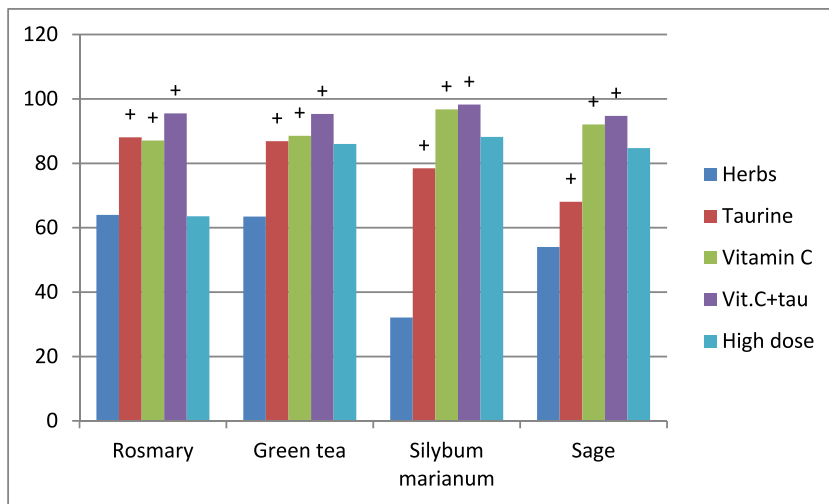

Fig. 2. Synergistic (+) herbal tea water extract, at low dose, with taurine and vit. C as DPPH radical scavenging activity (%) and their comparison them to high dose herbal tea water extract from Table 5.
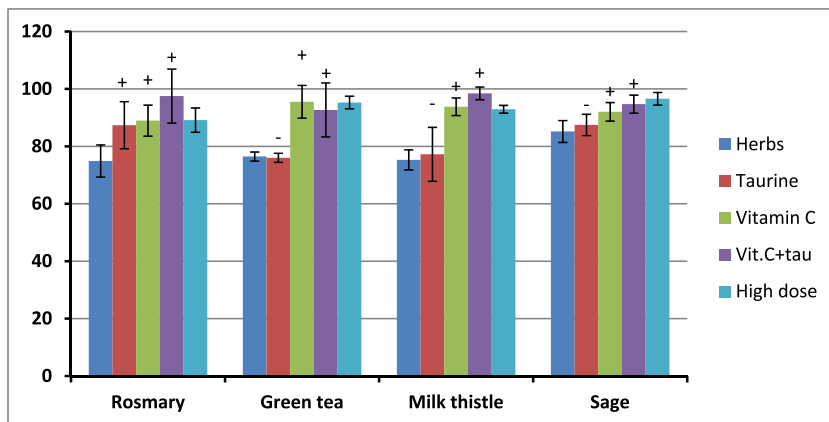

Fig. 3. Synergistic (+) and antagonistic (−) herbal tea water extract, at low dose, with taurine and vit. C as ABTS radical scavenging activity (%) and their comparison to high dose herbal tea water extract Table 5.

3.7. Sensory evaluation of four herbal beverage

Four herbal beverages (Codes HB1, HB2, HB3, and HB4) with taurine and vit. C additions were created for this study, and their color, flavor, and taste were assessed. The herbal tea beverage milk thistle (HB4) was shown to have the least liked herb tea in most of the sensory qualities, while Codes HB1, 2, and 3 (sage, rosemary, and green tea, respectively) had the greatest sensory scores. These findings indicated the possibility of bringing a polyherbal beverage with codes HB1, HB2, and HB3 that consumers would find acceptable to the market. This study has clearly demonstrated that the many purported therapeutic properties of polyherbal beverages are what make them so popular, notwithstanding their actual health advantages.
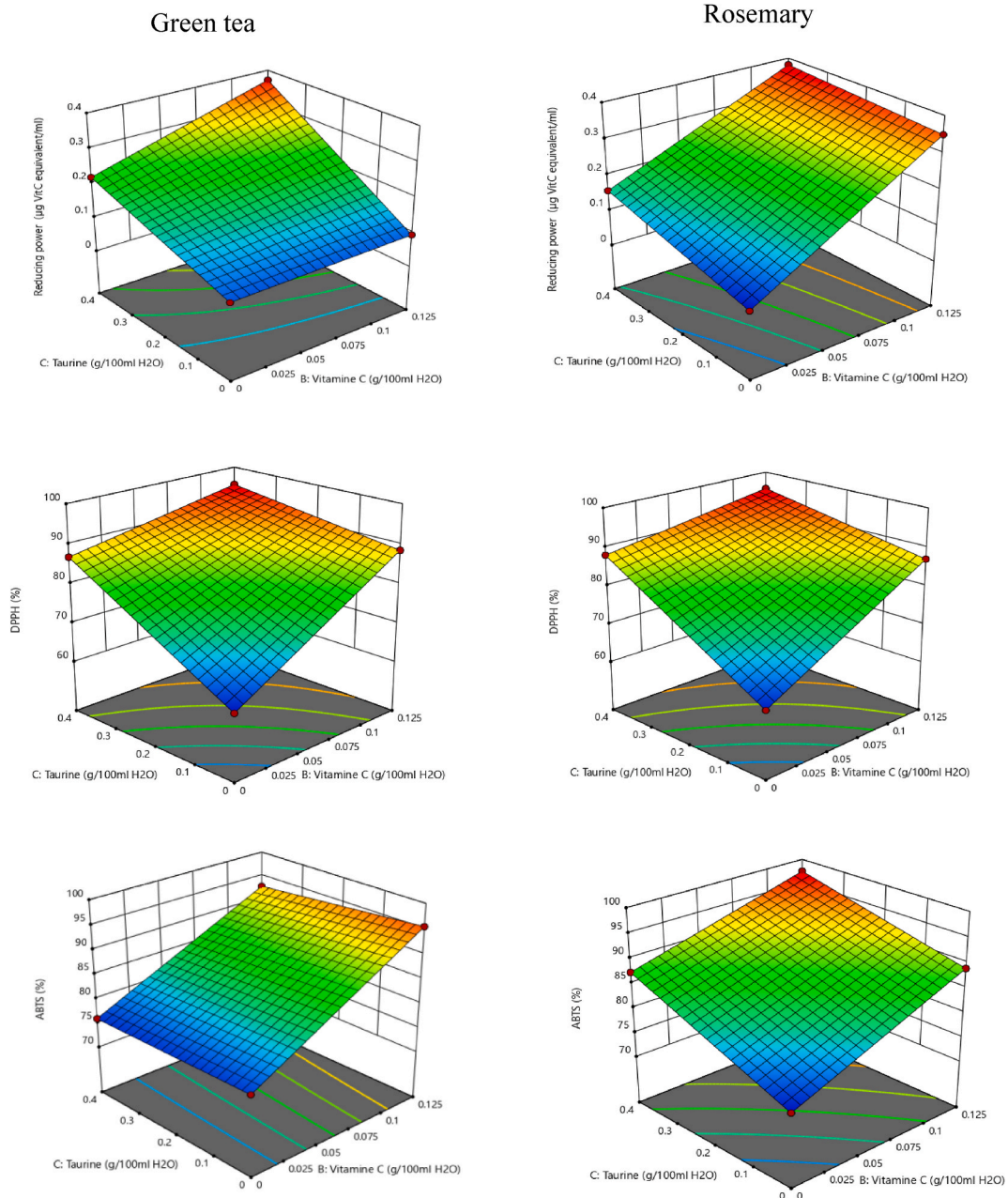

Fig. 4. Surface Response Plots and Contour Maps illustrating (a) Reducing Power, (b) DPPH, and (c) ABTS in relation to the dual factors of vit. C and taurine across four variants of herbal tea—green tea, rosemary, milk thistle, and sage.
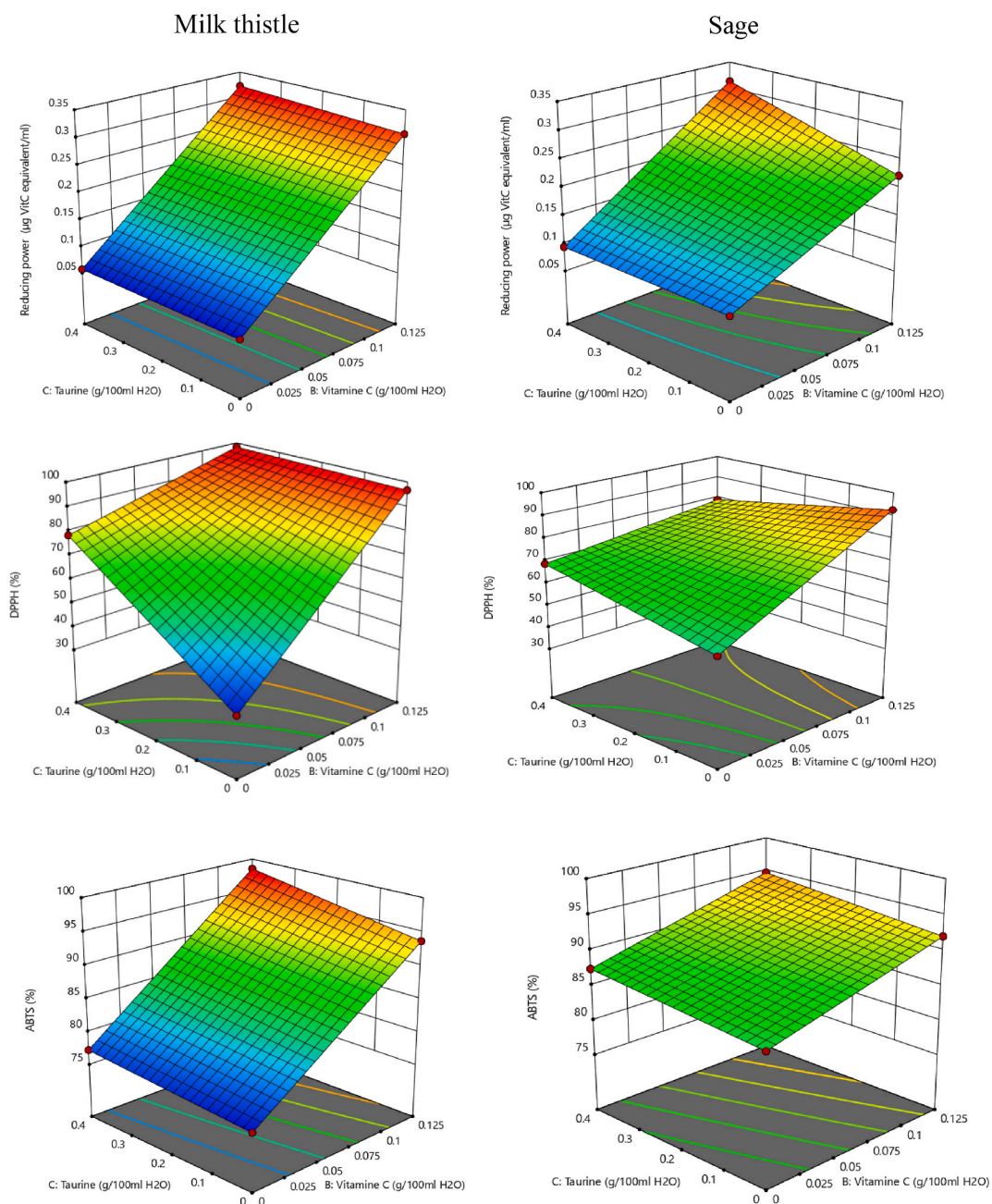
Fig. 4. (*continued*).

4. Discussion

The results of this study underscore the significance of antioxidant compounds found in medicinal plants in preventing oxidative reactions caused by free radicals. These natural compounds, including phenolic compounds and flavonoids, act as antioxidants and offer protection against oxidative damage in the body [34]. While synthetic antioxidants exist, their excessive consumption has been linked to toxicity and adverse health effects [35], prompting researchers to explore alternative sources of antioxidants from plant-based ingredients like vegetables, fruits, and traditional medicinal plants [36]. These natural sources hold promise for the development of safe and effective antioxidants that can promote health and well-being [37]. By harnessing the antioxidant properties of natural compounds, researchers aim to provide healthier alternatives for preventing oxidative damage and maintaining overall health.

Taurine (Tau), a β-amino acid with sulfur, plays a significant role as a natural modulator of the body's antioxidant defense systems.

Table 7
ANOVA analysis of reducing power, DPPH, and ABTS assays.

Reducing Power						
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0922	6	0.0154	6.03	0.0162	Significant
A-Herbal type	0.0003	1	0.0003	0.1178	0.7415	
B-vit. C	0.0744	1	0.0744	29.20	0.0010	
C-Taurine	0.0090	1	0.0090	3.54	0.1021	
AB	0.0023	1	0.0023	0.8948	0.3757	
AC	0.0062	1	0.0062	2.43	0.1627	
BC	0.0000	1	0.0000	0.0166	0.9012	
Residual	0.0178	7	0.0025			
Lack of Fit	0.0165	6	0.0028	2.12	0.4822	not significant
Pure Error	0.0013	1	0.0013			
Cor Total	0.1101	13				
DPPH						
Model	3234.60	6	539.10	9.11	0.0051	Significant
A-Herbal type	193.95	1	193.95	3.28	0.1131	
B-vit. C	1967.68	1	1967.68	33.25	0.0007	
C-Taurine	503.16	1	503.16	8.50	0.0225	
AB	13.82	1	13.82	0.2336	0.6437	
AC	52.89	1	52.89	0.8938	0.3759	
BC	503.10	1	503.10	8.50	0.0225	
Residual	414.20	7	59.17			
Lack of Fit	387.71	6	64.62	2.44	0.4544	not significant
Pure Error	26.50	1	26.50			
Cor Total	3648.81	13				
ABTS						
Model	607.66	6	101.28	19.21	0.0005	Significant
A-Herbal type	43.71	1	43.71	8.29	0.0237	
B-vit. C	521.32	1	521.32	98.89	<0.0001	
C-Taurine	7.09	1	7.09	1.34	0.2843	
AB	29.27	1	29.27	5.55	0.0506	
AC	4.26	1	4.26	0.8089	0.3983	
BC	2.00	1	2.00	0.3798	0.5572	
Residual	36.90	7	5.27			
Lack of Fit	36.38	6	6.06	11.66	0.2205	not significant
Pure Error	0.5202	1	0.5202			
Cor Total	644.56	13				

Table 8
Sensory Evaluation.

	Taste			Color			Flavor		
	Extremely like	Like	Extremely Dislike	Extremely like	Like	Extremely Dislike	Extremely like	Like	Extremely Dislike
Hb1	++++			++++			++++		
HB2	++++			++++			++++		
HB3	++++				+++			++	
HB4		+++			++			++	

The supplementation with both vit. C and taurine contribute to a reduction in lipid peroxidation, highlighting the potential of taurine in mitigating oxidative damage [38]. Additionally, the taurine has the ability to prevent oxidative stress [9]. However, it is important to note that taurine does not directly scavenge free radicals but plays a crucial role in maintaining mitochondrial health.

In contrast, vit. C acts as an exogenous antioxidant that can directly scavenge free radicals. This finding suggests that vit. C possesses direct radical scavenging properties [39].

The results of this study provide valuable insights into the antioxidant properties of various plant extracts and highlight the role of phenolic and flavonoid compounds in their antioxidant activities. Among the plants examined, rosemary exhibited the highest total phenolic content, indicating its potential as a rich source of phenolic compounds [40]. On the other hand, milk thistle had the lowest total phenolic content. The descending order of total phenolic content among the plants was rosemary > green tea > sage > milk thistle. A similar trend was observed for total flavonoid content, with green tea having the highest content, followed by rosemary, sage, and milk thistle. These results demonstrate that different plant species possess varying levels of phenolic and flavonoid compounds, which contribute to their antioxidant properties [41,42].

The protective effect against free radical oxidation observed in the water extracts of certain aromatic herbs can be attributed to their high phenol content [43]. Phenolic compounds, similar to taurine, vit. C, and phenols found in herbs, are believed to play a role in

neutralizing and suppressing the effects of reactive oxygen species. The antioxidant properties of phenolic acids and flavonoids are associated with their redox properties, metal-chelating abilities, and their ability to quench singlet oxygen [44].

It is important to consider that this study focused on the use of water as a solvent for extracting bioactive compounds. Water extraction, being a polar solvent, may have selectively extracted certain types of compounds while neglecting others [45]. Different solvents with varying polarities, such as hexane, diethyl ether, ethyl acetate, acetone, ethanol, and methanol, may yield different results [46]. Therefore, future studies should explore the use of these solvents to obtain a more comprehensive understanding of the bioactive ingredients present in water extracts of various herbs. Additionally, the literature does not provide extensive information on the presence of valuable bioactive ingredients in water extracts, highlighting the need for further research in this area [47].

In terms of antioxidant activity, the individual herbs alone were not as effective as taurine or vit. C, either individually or when combined, in inhibiting DPPH radical, ABTS radical, or reducing power. However, when taurine or vit. C was combined with the herbal extracts, a remarkable increase in antioxidant capacity was observed. This enhanced capacity can be attributed to the content and nature of phenolic compounds present in the herbs. The DPPH radical scavenging ability of various hot water extracts is as follows: clove (84.22 %) > thyme (70.79 %) > rosemary (56.98 %) > savory (53.51 %) > oregano (45.43 %) > basil (39.63 %) > cumin (35.02 %) > caraway (30.67 %), coriander (30.40 %), marjoram (30.22 %) > turmeric (24.43 %) > mace (20.94 %) > fennel (10.48 %) [48]. The differences in scavenging activities can be attributed to the presence of different compounds in each extract.

Although the DPPH radical scavenging activities of the spices were lower than that of ascorbic acid, the study indicated that most spices possess free radical scavengers or inhibitors, potentially acting as primary antioxidants [49]. Specifically, taurine and vit. C were found to exhibit enhanced antioxidant potential in the DPPH and ABTS assays, as well as in reducing power.

Although the individual herbs alone were not as effective as taurine or vit. C, either individually or in combination, in inhibiting DPPH radical, ABTS radical, or reducing power, the addition of taurine or vit. C to the herbal extracts significantly increased their antioxidant capacity. This enhancement can be attributed to the content and nature of phenolic compounds present in the herbs. However, it is important to note that certain combinations, such as taurine with sage or milk thistle, exhibited antagonistic interactions in the reducing assay. The inclusion of vit. C in these antagonistic combinations eliminated the antagonism and instead resulted in strong synergistic effects, as observed in previous studies [50].

Furthermore, the observed antagonistic effects in the antioxidant activity of the herbs can be attributed to various factors, including the regeneration of weaker antioxidants by stronger ones, the formation of complexes and adducts, and polymerization reactions that lead to compounds with reduced antioxidant properties. Additionally, interactions between different antioxidants in the system can result in neutralization. The weight ratio of extracts to vit. C was found to significantly influence the antioxidant activity and the type of interaction between these components [51]. The findings of this study demonstrated that the inclusion of 0.4 % taurine and 0.125 % vit. C increased the antioxidant activity of individual herbs, even at low doses of herbs.

5. Conclusion

The synergistic effect of taurine and vit. C demonstrated the highest antioxidative efficacy among the tested combinations. Notably, when taurine was combined with sage or milk thistle, antagonistic interactions were observed. However, the addition of vit. C effectively counteracted this antagonism, resulting in robust synergism. The combination of taurine and ascorbic acid exhibited significant increases in antioxidant power, ranging from 50 % to 200 % in the DPPH assay and 10 %–30 % in the ABTS assay, compared to individual herbs alone. By optimizing the weight ratio of herbal extract, vit. C, and taurine, the antioxidant activity of low-dose herbal tea was significantly enhanced. This study highlights the intricate dynamics involved in optimizing the antioxidant properties of herbal beverages through the synergistic combinations of taurine and vit. C. These findings contribute to the development of safe and effective antioxidants derived from natural sources. Further research is warranted to explore the optimal combinations and dosages of these natural compounds, maximizing their synergistic effects and potential health benefits.

Data availability statement

The data that support the findings of this study are available within the article.

Ethics statement

The experiment was conducted in accordance with established ethical guidelines, and all participants provided their full and informed consent. The study adheres to all regulatory requirements and provides confirmation of obtaining informed consent from all participants involved in this experiment.

CRediT authorship contribution statement

Kadry Z. Ghanem: Writing – original draft, Validation, Supervision, Resources, Investigation, Conceptualization. **Manal M. Ramadan:** Supervision, Investigation, Conceptualization. **Amira Taha Mohammed:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Abeer E. Mahmoud:** Investigation, Conceptualization. **Kirill Babintsev:** Methodology, Investigation, Conceptualization. **Wael M. Elmessery:** Writing – review & editing, Investigation, Conceptualization. **Tamer M. El-Messery:** Writing – review & editing, Visualization, Conceptualization.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Acknowledgments

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