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Original Article

The interaction effect of ginger extract and ascorbic acid on antioxidant activity

Apakorn Singprecha, Liudmila Yarovaya, and Watcharee Khunkitti*

Department of Pharmaceutical Technology, Faculty of Pharmaceutical Science, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand

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Abstract

The antioxidant effects of individual natural compounds have been extensively investigated. However, studies on their interactions are still lacking. This study aimed to observe the interaction effect of ginger extract (GE) combined with ascorbic acid on antioxidant activity by applying response surface methodology. The results from the quadratic model showed that individually tested DPPH free radical scavenging capacities of GE and ascorbic acid manifested positive effects on the antioxidant index (AI%). However, interaction of their combination demonstrated an antagonistic effect that negatively influenced the AI%. An optimization process revealed that the highest AI% could be achieved by applying maximum effective concentrations of GE (0.49 mg/mL) and ascorbic acid (0.82 mg/mL). Although, the mechanisms behind these interactions need to be further explored, the results of this study suggested that antagonistic interaction between the combination of ginger or other herbal extracts with ascorbic acid should be taken into consideration to prevent any potential health complications.

Keywords: ginger extract, ascorbic acid, antioxidant, antagonism, interaction

1. Introduction

Free radicals are generated endogenously from the cellular metabolism or triggered by solar radiation, pollution, and other external sources (Pham-Huy, He, & Pham-Huy, 2008). When free radicals are produced in the amount exceeding the capacity of the human antioxidant defense system, accumulation of free radicals in the body leads to formation of oxidative stress. This process, in turn, plays a major role in the aging of skin (Liguori *et al.*, 2018). Natural antioxidants, including vitamins and phytochemicals, are the most efficient free radical scavengers, and even in combination, are assumed to provide superior protection against oxidative stress (Sonam, & Guleria, 2017). However, the mixtures of antioxidants in different concentrations may generate either synergistic, additive or antagonistic effects. According to Wang *et al.* (2011), synergism refers to the effect exceeding the sum of individual components. The additive effect occurs when the combination of components is equal to the sum of the effects of the individual components, and antagonism refers to the sum of the effects below the sum of the individual components.

The herbal extracts of chia seeds (*Salvia hispanica* L.), rosella flowers (*Hibiscus sabdariffa* L.), white crane flower leaves (*Rhinacanthus nasutus* L.), and ginger rhizomes (*Zingiber officinale* Roscoe) have been reported to have high contents of polyphenols related to strong antioxidant capacity. However, a comparison of the antioxidant capacities of these anti-aging herbal extracts has not been reported. Chia seeds were found to provide effective anti-aging properties by minimizing the appearance of wrinkles due to the improvement of the skin barrier function and enhancing overall skin hydration (Qu & Saito, 2013). Rosella flowers demonstrated the effective inhibition of collagenase, elastase, and hyaluronidase, thereby enhancing skin elasticity and preventing it from aging (Widowati *et al.*, 2017). White crane flowers have shown effectiveness in the reduction of acne scars and fine lines of the aging skin (Singprecha & Khunkitti, 2019). Owing to these properties, these herbal extracts have found wide applications in anti-aging cosmetic products. In

*Corresponding author
Email address: watkhu@kku.ac.th

this study, we focused mainly on ginger rhizome extract since it has potential anti-aging properties.

Ginger (*Zingiber officinale* Roscoe) is a herb that is widely distributed in Asia (Li, Tran, Duke, & Roufogalis, 2012). The rhizome of ginger is a major source of polyphenols accountable for numerous therapeutic properties. Gingerols and shogaols are the main ginger polyphenols that demonstrate potent antioxidant and anti-inflammatory activities that control the process of aging (Dugasani *et al.*, 2010). In addition, gingerols and shogaols have been reported to have antimelanogenic effects by inhibiting tyrosinase activity and reducing melanin content in B16F10 melanoma cells through their antioxidant properties (Chan *et al.*, 2008; Huang, Chang, Wu, Ke, & Chang, 2014; Huang, Chou, Wu, & Chang, 2013).

Ascorbic acid (vitamin C) is a potent antioxidant found in high concentrations in the normal skin. It plays an important role in the regulation of collagen biosynthesis and improvement in the fine wrinkle status, thereby averting the process of skin aging (Traikovich, 1999). Along with ginger rhizome, ascorbic acid acts as a depigmenting agent inhibiting tyrosinase activity and decreasing production of melanin. However, in light of the ascorbic acid instability, it is commonly combined with other antioxidants like alpha-tocopherol (vitamin E) to prevent its oxidation (Bissett, 2006).

The majority of the currently existing over-the-counter skin care products that aim to prevent skin from premature aging contain combinations of natural antioxidants rich in phenolic compounds. This tendency is supported by the fact that synergistic effects of combined antioxidants yield more effective formulations compared to single antioxidants (Campos, Gianeti, Mercurio, & Gaspar, 2014; Mao *et al.*, 2017). The synergism between ginger extract (GE) and green tea extract was proven by increased antioxidant activity of their mixture (Selvakumar, Kumar, Gandhi, & Geetha, 2015). Lin *et al.* (2003) reported superior photoprotective effects of a combination of topical L-ascorbic acid and alpha-tocopherol applied to the skin alone. Although GE and ascorbic acid were found to have interactions with other potent antioxidants, thereby presenting a good potential for anti-aging cosmetic applications, currently no study has reported the antioxidant activity of a combination of GE and ascorbic acid. To analyze the interaction between these two antioxidants, quadratic models of response surface methodology (RSM) can be used. RSM is applicable for evaluation of the effects of multiple variables and their interactions on the output variables with a reduced number of trials. Several RSMs have been applied to optimize the antioxidant activity of plant extracts (Wani *et al.*, 2017). Pereira *et al.* (2015) implemented RSM to analyze the potential interaction between six tropical fruits to obtain an optimized juice with high levels of ascorbic acid, polyphenols, and total antioxidant capacity. Therefore, the objective of this study was to observe the antioxidant activities of GE combined with ascorbic acid by applying RSM. In the cosmetic point of view, this study aimed to provide factors that affect the combination of ascorbic acid and ginger root extract in cosmetic products to improve the effectiveness of anti-aging products.

2. Materials and Methods

2.1 Materials

Gallic acid, Folin-Ciocalteu reagent and quercetin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Sodium carbonate was obtained from RFCL, Ltd. (New Delhi, India). Ethanol was purchased from Liquor Distillery Organization (Chachoengsao, Thailand). Trolox and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Steinheim, Germany), and butylated hydroxyltoluene (BHT) was purchased from Namsang Co., Ltd. (Bangkok, Thailand). L-ascorbic acid and vitamin B3 were purchased from S. Tong Chemicals Co., Ltd. (Bangkok, Thailand). All assays were performed using Milli-Q water system (Massachusetts, USA). All solvents and reagents were of analytical grade.

Chia seeds (*Salvia hispanica* L.) (CE) were macerated in 95% ethanol (1:3 ratio) for one week following by filtration. The obtained extract was evaporated by rotary vacuum evaporator (Buchi Co., Ltd., Thailand) at 40 °C. Rosella flower (*Hibiscus sabdariffa* L.) ethanolic extract (RE), ginger rhizome (*Zingiber officinale* Ros.) ethanolic extract (GE), and white crane flower leaf (*Rhinacanthus nasutus* L.) ethanolic extract (WE) were purchased from Thai-China Flavours and Fragrances Industry Co., Ltd. (Phra Nakhon Si Ayutthaya, Thailand).

2.2 Methods

2.2.1 Determination of total phenolic content

Determination of total phenolic content in the ethanolic extracts of CE, RE, GE, and WE was carried out using the Folin-Ciocalteu assay reported by Singleton *et al.* (1999). Samples of ethanolic herbal extracts at different concentrations (0.1–100 µL) were mixed with 1 mL of 10% Folin-Ciocalteu's reagent and 0.8 mL of 7.5% w/v sodium carbonate. The absorbance was measured after 2.5 h at 760 nm. The content of phenolics in the extracts was expressed in terms of gallic acid equivalent (GAE) in mg per 1 g of extract dry weight. The calibration equation for gallic acid was $y = 0.0025x + 0.0871$ ($R^2 = 0.9989$).

2.2.2 DPPH free radical scavenging capacity assay

The determination of antioxidant activity of CE, RE, GE, and WE extracts was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity adapted from Brand-Williams *et al.* (1995). The standard solutions of Trolox, BHT, vitamin C, vitamin B3 were used as positive controls and the samples of CE, RE, GE, and WE extracts were serially diluted with ethanol in a 96-well plate in the ranges of 1–20 mg/mL and 2–20 mg/mL, respectively. A quantity of 50 µL of 0.004 M DPPH was added to 50 µL of the standards and samples, except for the wells that contained extracts and ethanol (blank) and DI water (background). The reaction mixtures were then kept in the dark for 30 min. The absorbance (A) was measured at 517 nm using a microplate reader (EnSight™, PerkinElmer, MA, USA). The results were expressed as IC_{50} values. The antioxidant index (AI%) was

calculated for all plant extracts and compared to the standard solutions using the following formula.

$$\text{Antioxidant index (\%)} = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} \times 100$$

2.2.3 Combination antioxidant activity of ginger extract and ascorbic acid

The experimental design for investigation of interaction between GE and ascorbic acid affecting antioxidant activity was RSM using Design-Expert® 7.1 software (Stat-Ease Inc., Minneapolis, MN, USA). The three-level, two-factorial miscellaneous experimental design was used. The design was composed of low (–1), medium (0) and high (+1) levels corresponding to the concentrations of independent variables including GE (X_1) and ascorbic acid (X_2) (Table 1). Antioxidant activity reported as AI% did not fit the model, thus absorbance (Y) measured in DPPH free radical scavenging assay was used as a response variable (Table 2). A total of 13 runs were carried out in triplicate to optimize the level of independent variables.

Table 1. Coded and actual values for independent variables.

Independent variables	Parameters levels		
Concentration (mg/mL)	-1	0	+1
GE, X_1	0	0.4	0.8
Ascorbic acid, X_2	0	0.6	1.2

Table 2. Three-level, two-factorial miscellaneous design matrix and actual values of experimental parameters and their responses.

Run	Factors		Response 1	Antioxidant index %
	GE, X_1 (mg/mL)	Ascorbic acid, X_2 (mg/mL)	Absorbance, Y_1 (nm)	
1	0	0	0.817	0.000
2	0.4	0	0.384	53.007
3	0.8	0	0.247	69.710
4	0	0.6	0.324	60.362
5	0.4	0.6	0.114	86.002
6	0.8	0.6	0.123	84.884
7	0	1.2	0.245	69.940
8	0.4	1.2	0.118	85.537
9	0.8	1.2	0.125	84.659
10	0.4	0.6	0.117	85.669
11	0.4	0.6	0.120	85.351
12	0.4	0.6	0.126	84.530
13	0.4	0.6	0.105	87.158

The relationship between the predicted response and independent variables was estimated applying the general second order polynomial models given in Equation 1.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \,, \quad (1)$$

where Y is the predicted response, β_0, β_i, β_{ii}, and β_{ij} are the intercept, linear, quadratic, and interaction regression coefficients, respectively, and X_i and X_j are the independent variables that affect responses.

The analysis of variance (ANOVA) was applied to determine the significance of the model and the regression coefficients were then used to generate response surfaces. The fitness of the model was assessed by the lack of fit. A $P<0.05$ was considered to be statistically significant. The determination of a combination antioxidant activity between GE and ascorbic acid was carried out using DPPH free radical scavenging capacity assay as described earlier.

2.2.4 Optimization of the model

To achieve optimization of the model, two-dimensional overlay contour plots between absorbance and AI% as the response variables were derived. The optimum combination of the GE and ascorbic acid was then selected to obtain the most effective concentration of these two antioxidants.

The reliability of the model was confirmed by validation experiments based on the predicted by optimization process conditions. Six points were selected at the optimized area of the overlay contour plot between absorbance and AI% correlating to the concentrations of GE and ascorbic acid. The actual values of the responses were then evaluated. The percentage prediction error (PPE) was calculated by the following equation:

$$\text{\% prediction error} = \frac{|\text{measured value} - \text{predicted value}|}{\text{predicted value}} \times 100$$

The model was considered reliable, if the prediction error was ≤5%.

2.3 Statistical analysis

All data are presented as the mean±standard deviation (SD) of at least three determinations. Statistical analysis of the experimental data was performed by analysis of variance (ANOVA), followed by Tukey's multiple comparison test using the Statistical Package for Social Sciences (SPSS version 17.0, SPSS Inc., Chicago, IL, USA). All differences were statistically significant at $P<0.05$.

3. Results

3.1 Total phenolic content of herbal extracts

Total phenolic content of the CE, RE, GE, and WE extracts was determined (Table 3). The amounts of total phenolic compounds varied among the four herbal extracts. The highest polyphenolic content was found in GE (62.51 mg GAE/g dry weight) and the order of ranking was GE> RE>CE>WE.

3.2 *In vitro* antioxidant activity

The DPPH free radical scavenging potentials (IC_{50}) of the four plant extracts compared to Vitamin C, Vitamin B3,

Table 3. Total phenolic content of chia seeds, rosella flowers, ginger rhizomes, and white crane flower extracts.

Extracts	Total phenolic (mg GAE/g dry weight)
Chia seeds	29.34±0.19 [a]
Rosella flowers	37.67±0.44 [b]
Ginger rhizomes	62.51±0.97 [c]
White crane flower leaves	22.31±0.18 [d]

Data with different superscript letters in the same column differed significantly. (P<0.05)

BHT, and Trolox as the positive controls are shown in Table 4. The highest DPPH scavenging potential was demonstrated by GE (0.05±0.001 mg/mL) following by RE (1.12±0.01 mg/mL), CE (3.31±0.11 mg/mL), and WE (4.92±0.78 mg/mL). GE was higher but not significantly different from BHT (0.06±0.01 mg/mL), Vitamin C (0.07±0.01 mg/mL) or Trolox (0.08±0.002 mg/mL). Vitamin B3 showed the lowest capacity to inhibit DPPH (IC_{50}=30.95±3.91 mg/mL) and was significantly different from the other tested compounds. These results, therefore, indicated that GE appeared to be a potent antioxidant demonstrating comparable DPPH scavenging capacity with BHT, Vitamin C, and Trolox.

Table 4. Antioxidant activity of chia seeds, rosella flowers, ginger rhizomes, and white crane flower extracts.

Tested compound	DPPH
	IC_{50} (mg/mL)
Vitamin C	0.07±0.01
Vitamin B3	30.95±3.91 [a]
Trolox	0.08±0.002
BHT	0.06±0.01
Ginger rhizomes	0.05±0.001
Chia seeds	3.31±0.11 [b]
Rosella flowers	1.12±0.01 [c]
White crane flower leaves	4.92±0.78 [b]

* Mean of triplicate determinations±SE
Data with different superscript letters were differed significantly (P<0.05)

3.3 GE and ascorbic acid interaction effect on antioxidant activity

According to the results obtained from the previously described assays, GE demonstrated high total phenolic content as well as powerful antioxidant activity of all herbal extracts and was chosen as the candidate for further analysis of its antioxidant activity in combination with ascorbic acid.

The potential interaction between antioxidant activities of GE (X_1) and ascorbic acid (X_2) in combination was carried out by the RSM approach applying the two-level, three-factorial miscellaneous experimental design. The results of the absorbance responses were in the range of 0.384–0.105, and the AI% was in the range of 53.01–87.16%.

The relationship between the absorbance response and independent variables in terms of the coded factors was presented by the following polynomial equation:

$$Y = 0.35 - 0.13X_1 - 0.14X_2 + 0.06X_1X_2 + 0.10X_1^2 + 0.12X_2^2 \quad (2)$$

where Y is the absorbance measured at 517 nm, and X_1 and X_2 are the concentrations of GE and ascorbic acid.

The coefficient of determination (R^2) value for the quadratic model was 0.9924 demonstrating the quality of the model fitness. Adjacent R^2 (0.9871) was in close agreement with the predicted R^2 (0.9465). Adequate signal to noise ratio was indicated by the adequacy precision value of 44.478 which suggested that this model can be used to navigate the design spaces.

The results of the analysis of variance (ANOVA) for the regression equation are shown in Table 5. The higher significance of the applied model and contributing coefficient terms were demonstrated by the larger F-values and the smaller P-values. The P-values of less than 0.05 were considered to be statistically significant. Thus, P<0.0001 indicated apparent significance of the model. In addition, the lack of fit was not significant (P>0.05) which demonstrated that the model accurately predicted the response.

Table 5. Results of analysis of variance (ANOVA) for the regression equation.

Source	Sum of squares	df*	Mean square	F-value	P-value Prob>F	Significance
Model**	0.34	5	0.68	183.97	<0.0001	significant
X_1	0.098	1	0.098	263.76	<0.0001	significant
X_2	0.11	1	0.11	308.22	<0.0001	significant
X_1X_2	0.018	1	0.018	47.36	0.0002	significant
X_1^2	0.028	1	0.028	75.66	<0.0001	significant
X_2^2	0.041	1	0.041	111.30	<0.0001	significant
Residual	2.592E-003	7	3.703E-004			
Lack of fit	2.056E-003	3	6.852E-004	5.11	0.0745	not significant
Pure error	5.361E-004	4	1.340E-004			
Cor total	0.34	12				

* df is the degree of freedom.
** X_1 is the concentration of GE (mg/mL) and X_2 is the concentration of ascorbic acid. (mg/mL).

3.4 Analysis of RSM

According to the results of the second order equation (Equation 2), individually tested concentrations of GE and ascorbic acid presented negative effects ($P<0.0001$) on absorbance which indicated an increase in antioxidant activity identified by the DPPH assay. In contrast to these results, interaction between the GE and ascorbic acid combination presented a positive effect ($P=0.0002$) and indicated antagonistic interaction between these variables. According to the DPPH free radical scavenging activity assay, antioxidant activity increased as the AI% decreased suggesting that there is an antagonistic effect between ascorbic acid and GE. These effects can also be observed in two-dimensional and three-dimensional plots (Figures 1A and 1B).

3.5 Optimization of the model

The goals for optimization in this study were to minimize absorbance and maximize AI% to obtain the most effective concentrations of GE and ascorbic acid combination. Figure 2 demonstrates the central points of the optimum combination regions corresponding to the maximum effective concentrations of GE (0.49 mg/mL) and ascorbic acid (0.82 mg/mL). Thus, applying these conditions, the highest antioxidant index could be achieved.

Validation experiments were carried out at the predicted conditions derived from the RSM analysis to confirm reliability of the model. The predicted and calculated values based on the actual results of PPE are shown in Table 6. The PPE was found to be less than 5% for all tested combinations of GE and ascorbic acid demonstrating the adequate correlation between experimental values and predicted values. The results of validation experiments confirmed the reliability and fitness of the model.

4. Discussion

In this study, total phenolic content and antioxidant activity of four plant extracts were determined. The content of polyphenolic compounds acts as a reliable indicator of the antioxidant activity. The correlation between phenolic content and the scavenging ability of plant extracts was proved by several studies (Fitriansyah, Aulifa, Febriani, & Sapitri, 2018; Piluzza & Bullitta, 2011). The correlation analysis reported by Akinola *et al.* (2014) showed a close relationship between total phenolic content and DPPH radical scavenging activity of ginger rhizomes, including *Zingiber officinale*. Ginger contains a high content of compounds demonstrating antioxidant properties such as β-carotene, ascorbic acid, terpenoids, alkaloids, and polyphenols such as flavonoids and tannins (Shirin & Prakash, 2010). Shogaol, gingerol, and their related compounds are the major polyphenols of the ginger rhizome responsible for its antioxidant activity (Dugasani *et al.*, 2010; Kikuzaki & Nakatani, 1993). In our previous study, we found that total flavonoid content of ginger extract was about three times lower than the total phenolic content (Singprecha & Khunkitti, 2019). Teixeira *et al.* (2017) reported a lower contribution of flavonoid content to the antioxidant activity. However, the free radical scavenging activity of ginger can account for the synergistic effect of

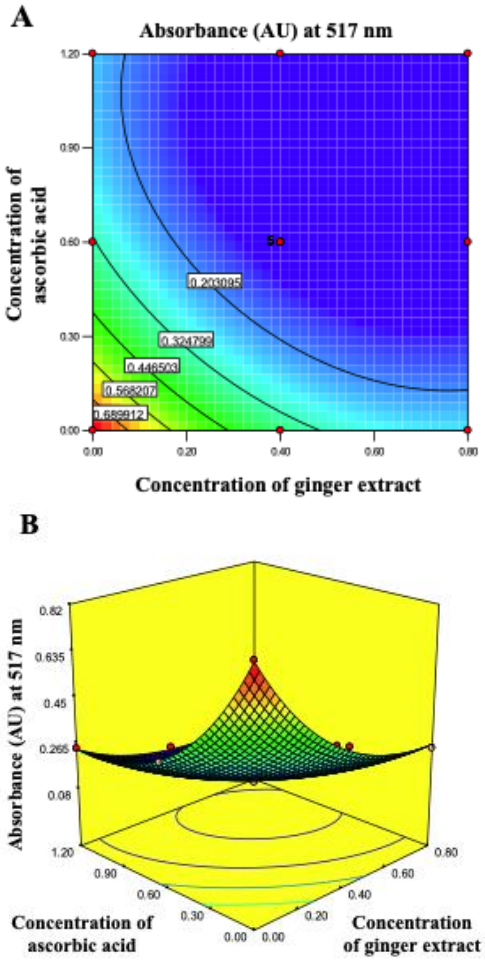

Figure 1. (A) Two-dimensional and (B) three-dimensional and contour plots of antioxidant activity (DPPH assay) in terms of measured absorbance (AU) of combinations between concentrations of ginger extract (X_1) and concentrations of ascorbic acid (X_2).

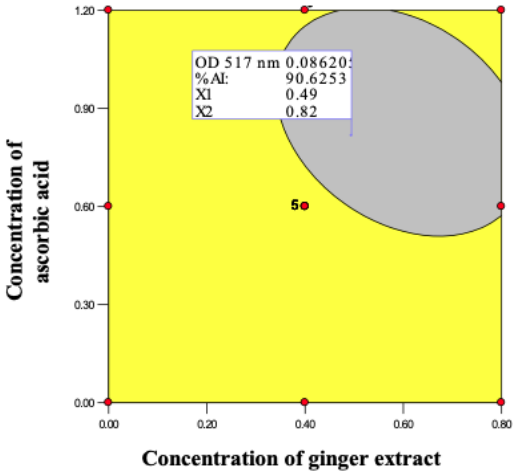

Figure 2. Overlay plot between the absorbance and AI%, and optimized region of GE and ascorbic acid concentrations (mg/mL).

Table 6. Predicted values and percentage prediction error (PPE) of interaction between ginger extract and ascorbic acid combinations.

Predicted values	F1	F2	F3	F4	F5	F6
GE (mg/mL)	0.5	0.6	0.7	0.7	0.6	0.8
Ascorbic acid (mg/mL)	0.8	0.6	0.6	1	0.9	0.6
Absorbance (AU)	0.13±0.00	0.15±0.00	0.15±0.00	0.155±0.00	0.154±0.00	0.16±0.01
Antioxidant index %	93.80±0.19	92.78±0.15	92.72±0.10	92.72±0.26	92.77±0.14	92.28±0.08
PPE	0.79±0.20	1.41±0.17	0.79±0.11	2.12±0.29	1.08±0.15	1.68±0.09

several compounds present in this plant. In addition, the total content of flavonoids and phenolics, and antioxidant activities of the plants are influenced by various biological and environmental factors as well as the extraction methods (Ghasemzadeh, Jaafar, & Rahmat, 2010). In this study, the highest phenolic content and antioxidant activity were observed in GE, and therefore it can evidently find its application as a powerful free radical scavenger.

The RSM analysis of interaction between GE and ascorbic acid was conducted by means of the DPPH assay. The mechanism based on the acceptance of hydrogen from antioxidant by DPPH• radical is visually confirmed by the colorimetric reaction. The color change from purple to yellow demonstrates the formation of DPPH, and associated with it reduction of UV absorbance which indicates increasing antioxidant activity. The effects of GE and ascorbic acid alone to absorbance were negative ($P<0.0001$). However, their combination resulted in antagonistic interaction explained by increased absorbance, which in turn was related to a reduction of antioxidant activity. The free radical scavenging capacity of single and combined antioxidants may depend on the effects of pH, light, polarity, and the presence of prooxidants in the reaction media that influences test systems. Considering the differences in chemical basis of methods, it is recommended to use at least two assays to obtain correlation analysis data.

Several studies have discussed mechanisms of synergistic and antagonistic interactions between combinations of natural antioxidants like ascorbic acid and plant extracts rich in polyphenolic compounds. Peyrat-Maillard *et al.* (2003) proposed a mechanism in which combinations of antioxidants produce a synergistic effect when a weaker antioxidant regenerates the stronger one and an antagonistic effect when stronger regenerates the weaker. Another study hypothesized that synergistic effects are associated with heterogeneity of polyphenols contained in individual extracts, while antagonistic effects are associated with their homogeneity. However, these assumptions require further investigation (Wang *et al.*, 2012).

The majority of studies reported positive outcomes from combinations of natural antioxidants demonstrating improved activity from either synergistic or additive effects. However, a few have discussed the negative effects of antioxidant combinations. The synergistic and antagonistic interactions between the mixtures of different polyphenolic compounds in green tea were associated with the chemical structures influencing total antioxidant capacity (DPPH assay) of each combination (Colon & Nerín, 2016). In a study conducted by Bolling *et al.* (2013), ascorbic acid antagonized the phenols of grape juice in a DPPH assay, while interaction with non-polyphenolic compounds was highly synergistic in a dose-dependent manner. This finding can be explained by an interaction effect that might not be attributed to its interaction with polyphenols alone but it might depend on polyphenols and non-polyphenols as well as the level of ascorbic acid. Therefore, this suggests that polyphenol and other chemical constituents in herbal extracts may play an important role on the interaction with ascorbic acid.

In addition, ascorbic acid is known as a highly unstable antioxidant that easily oxidizes in an aqueous environment. In the presence of oxygen, the breakdown products generated from the higher concentrations of ascorbic acid behave as prooxidants. Choueiri *et al.* (2012) has reported the elevated antagonistic effect of ascorbic acid acting as a prooxidant when its concentration was increased in the mixture with catechins (Choueiri, Chedea, Calokerinos, & Kefalas, 2012). The antagonism produced in a mixture can be also explained by degradation of ascorbic acid during measurements that may affect the results of the experiments. Thus, when conducting experiments with ascorbic acid, the relatively low stability should be considered (Sazhina, Plotnikov, Korotkova, Dorozhko, & Voronova, 2018).

Although, the mechanisms behind the interactions of potent antioxidants need to be further explored, the results of this study suggest that careful selection of antioxidant combinations is required for amplification of synergism and attenuation of antagonism in their free radical scavenging activity. In particular, when combining ascorbic acid with ginger or other herbal extracts, the antagonistic interaction should be taken into consideration to prevent any potential health complications.

5. Conclusions

The RSM approach facilitated the analysis of the interaction effects of variables on the target response. The quadratic model provided a good fit for the relationship between ginger extract and ascorbic acid that resulted in the antagonistic effect of this combination. The optimization was an important technique used to achieve the highest antioxidant index. However, further analysis implementing *in vivo* studies on relevant animal models followed by clinical trials need to be conducted in the future to evaluate the antagonistic effects between these compounds.

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