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Research Article
Effects of Extraction Variables on Pharmacological Activities of Vine Tea Extract (*Ampelopsis grossedentata*)

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Abstract

Background and Objective: Vine tea *(Ampelopsis grossedentata)* may potentially perform multiple pharmacological roles, including antibacterial, anti-cancer, antioxidant, hepatoprotective and anti-hypertension functions. But effects of extraction polarity, time and temperature on the recovery of bioactive compounds along their pharmacological activity of vine tea extract have not been reported yet. The present study reports on the importance of extraction variables for obtaining vine tea extract aiding in purification and isolation of functional and polyphenolic compound and their pharmacological activity (polyphenols) from vine tea. **Materials and Methods:** The effects of extraction variables (time, temperature and polarity) on Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Condensed Tannin Content (CTC) were evaluated using single factor experiment. Antiradical capacity assay and radical-scavenging capacity 2,2-diphenyl-1-picrylhydrazyl (DPPH) were tested for evaluating the antioxidant activities of vine tea crude extract. To analyze data, one-way analysis of variance (ANOVA) was established followed by the Tukey's test and to test the correlation, Pearson correlation coefficient was tested. **Results:** Data explained that extraction polarity, extraction time and the temperature had a significant effect ($p<0.05$) on yield and their anti-radical activities. The optimized extraction parameters for TFA and CTC were 40% aqueous ethanol at 45 while 50 for TPC for 180 min. Whereas, maximum yield were 40.01 μg Catechin Equivalent per gram (CE g^{-1}) of Dry Weight (DW) for TFA, 15.12 μg, Gallic Acid Equivalent per gram of DW (GAE g^{-1}) for TPC and 12.70 μg CE g^{-1} DW for CTC. Ethanol concentration showed a significant effect ($p<0.05$) on extraction of phenolic compounds and their pharmacological activity especially antioxidant capacity and DPPH assay. **Conclusion:** This study showed that extraction variables were greatly influence on the pharmacological activities of vine tea crude extract and this study can be used as preliminary and key information to design central composite rotatable design for Response Surface Methodology (RSM). Moreover, this approach can be used to determine significant factors that influence functional compounds and their other pharmacological activities.

Key words: Vine tea, extraction variables, single factor, gallic acid, 2,2-diphenyl-1-picrylhydrazyl, response surface methodology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ampelopsis grossedentata is a vine plant which grows prevalently in Southern China, especially in regions South of the Yangtze River and as a Chinese herbal medicine, it has been used for about more than 1500 years. During recent times, awareness in medicinal plants and natural phenolic extracts has been developed for their antioxidant potential and significance in curing of diseases[1,2]. However, some studies have been reported that use of synthetic antioxidants can cause some toxic side effects[3].

Recent reports have revealed that vine tea has health-promoting characteristics[4], antimicrobial[5], anti-diabetics[6], anti-inflammatory effect[7] cholesterol-lowering[8] and hepta-protective[8]. Many of the perceived pharmaceutical and general health-promoting properties of *Ampelopsis grossedentata* have been confirmed by modern scientific investigations, including clinical studies[9].

Extraction is the basic step of the polyphenolic analysis, which comprises of phenolic compounds extraction from plants. Therefore, the method used in this procedure becomes crucial for precise determination and quantification of antioxidant analysis[10]. Numerous extraction techniques are described in the previous studies, but there is no single extraction technique which may be recommended as a standard[11,12]. The chemical properties of phenolic compounds, in consort with various interfering substances, influence the effectiveness of the extraction methods[13,14].

Optimization of extraction process can be accomplished by two methods: (1) Single factor approach and (2) Response surface methodology. The prior approach also known as One-factor-one time approach, in this approach single element is changeable, whereas, other variables will be constant at one time[15,16]. One-factor-one time approach was used in this study to optimize parameters involved in vine tea phenolic extraction.

The present study was designed to investigate the impact of 3 variables on the extraction of bio-active and functional compounds (polyphenols) from vine tea and their pharmacological activities. This vine tea crude extract gained could be a part of purification and isolation of functional compounds. For further application in food, cosmetic or medicines industries as a natural resource.

MATERIALS AND METHODS

Plant material: Fresh and dried leaves of vine tea (*Ampelopsis grossedentata*) produced in Southern China, especially in regions South of the Yangtze River purchased from Chinese medical herbs/plants supplier (Guizhou Miaoyao Biotechnology Co. Ltd., 5 kg) and authenticated as ampelopsis grossentata (Hand.-Mazz) W.T. Wang. In March 2016, grinding and extraction procedure were performed in the College of Food Science and Technology, Nanjing Agriculture University, Jiangsu, China. Ground samples were stored at 4 prior to analysis.

Chemical reagents: Folin-Ciocalteu (FC) reagent, concentrated hydrochloric acid (37% purity), sodium carbonate ($\geq$99% purity), gallic acid (98% purity), methanol ($\geq$99.8% purity), 2, 2-diphenyl-1-picrylhydrazyl (DPPH, 95% purity), sodium nitrite, aluminium chloride-6-hydrate ($\geq$99% purity), sodium hydroxide and absolute ethanol ($\geq$99.4% v/v), were bought from Sinopharm Chemical reagents Co., Ltd (China). Standard of (+) catechin hydrate ($\geq$98% purity) was purchased from Solarbio (China). All other chemicals used were of analytical grade and Milli-Q water purifier system (Millipore cooperation, USA) was used to purify water required throughout the experiment.

Solvent extraction procedure: Three grams dry powder of vine tea along with 50 mL of ethanol was taken into 100 mL volumetric flask. Aluminum foil was used to wrap the flask to protect from light exposure. After that, vine tea and ethanol and water solution were shaken at 150 rpm. Temperature controlled water bath shaker was used for this purpose to achieve controlled temperature for certain time period. The levels of extraction polarity, time and temperature were established as defined in experimental design. Vine tea extract was filtered through filter paper after filtration, the extract was stored at a dark place to avoid spilling. The extractions were performed in replicates.

Experimental design: One-factor-one time approach was executed to optimize phenolic compounds extraction from vine tea and evaluating the impact of three independent variables named as extraction polarity, time and temperature. The levels of each independent factor was decided by results of 5 responses of each variable (time temperature and ethanol-water concentration).

Extractant polarity: To study the impact of solvent concentration, the extraction time and temperature were kept as a constant at 150 min and 50°C While, binary solvent (ethanol-water) of different concentration (20, 40, 60, 80 and 100% v/v) was tested. Best extraction polarity was chosen depending on the yields of phenolic compounds. Based upon these data further study was conducted to know the best time and temperature conditions for the extraction of functional compounds.

Extraction time: Time range required for extraction of functional compounds was the second step of the experiment. To evaluate the range of extraction time, 5 different levels (60, 120, 180, 240 and 300) were established and find the optimal extraction time condition. While during this step the temperature was kept constant at 50°C.

Extraction temperature: The third step of the experiment was to determine the optimal heating condition for the extraction of functional compounds from vine tea. To evaluate the best extraction temperature, extraction vine tea samples were extracted at 40, 45, 50, 55 and 60°C and were evaluated for the effect of extraction temperature on the concentration of bioactive and functional compounds.

Chemical analysis

Total Phenolic Content (TPC): Folin-Ciocalteu colorimetric method as described by Wong *et al.*[17] and Li *et al.*[18], was used with slight modification for determination of Total Phenolic Contents (TPC). The reading of extracted sample and control were taken at 765 nm by UV-VIS spectrophotometer (Model SHIMADZU UV-2600). A calibration curve was prepared by using gallic acid and results were represented as μg GAE g^{-1} DW, where, GAE is gallic acid equivalent per gram of dry weight sample and equation was given as:

$$y = 19.994\ x+0.0169\ (R^2 = 0.9987)$$

Determination of Total Flavonoid Content (TFC): For the determination of Total Flavonoid Contents (TFC) of vine tea extract, method explained by Ozsoy *et al.*[19] was used with slight modification. The reading of extracted sample and control were taken at 510 nm by UV-VIS spectrophotometer (Model SHIMADZU UV-2600). A calibration curve was prepared by using catechin and represented as μg CE g^{-1} DW, where, CE is catechin equivalent per gram of dry weight sample and equation was given as:

$$y = 0.0133x-0.11\ (R^2 = 0.9964)$$

Condensed Tannins Content (CTC): Determination of condensed tannin contents was accomplished by a method Makkar and Becker[20] with slight modification. The reading of extracted sample and control were taken at 500 nm by UV-VIS spectrophotometer (Model SHIMADZU UV-2600). A calibration curve was prepared by using catechin standard and represented as μg CE g^{-1} DW, where, CE is catechin equivalent per gram of dry weight sample and equation was given as:

$$y = 0.0114x-0.0713\ (R^2 = 0.9993)$$

All the determinations were carried out in triplicate and data were represented as mean of standard deviation.

Determination of antiradical capacity: One milliliter of extracted sample and 1 mL of methanol as control were mixed with 5 mL of reagents (4 mM ammonium molybdate+28 mM sodium phosphate+0.6 M sulfuric acid) and heated for 90 min at 95°C. The reading of extracted sample and control were taken at 695 nm by UV-VIS spectrophotometer (Model SHIMADZU UV-2600). The experiment was carried out in triplicate. A calibration curve was prepared by using ascorbic acid standard for calibration and results were represented as μg AAE g^{-1} DW, where AAE is ascorbic acid equivalent per gram of dry weight sample and equation was given as:

$$y = 0.0019x-0.1188\ (R^2 = 0.9963)$$

DPPH radical scavenging capacity: The antiradical capacity of vine tea extract was calculated as described by Miliauskas *et al.*[21] and Saha *et al.*[22]. The absorbance of crude extract and control were noted at 520 nm by UV-VIS spectrophotometer (Model SHIMADZU UV-2600) using ascorbic acid as a reference for calibration and results were expressed as μg ascorbic acid equivalent per g dry weight sample (μg AAE g^{-1} DW). Equation for ascorbic acid was given as:

$$y = 0.1511x-0.1794\ (R^2 = 0.9938)$$

Validation of the model: The optimized extraction parameters were confirmed for high antioxidant activities (antiradical capacity and DPPH) and phenolic content (TFA, CTC and TPC) depended on the results gained using single factor experiment. All responses were calculated under optimized extraction conditions to confirm or conclude the validity of the model.

Statistical analysis: All the statistical data were analyzed with the help of software Design Expert 7 (Stat-Ease, MN) with the experimental conditions explained in experimental design. The data were expressed as the Mean±Standard Deviation (SD). One way analysis of variance (ANOVA) was established followed by the Tukey's multiple comparison test[23], whereas, $p<0.05$ as significant of assays. Moreover, the correlation between antioxidant capacity and antioxidant compounds was studied with the help of Pearson correlation assay.

RESULTS AND DISCUSSION

Evaluation of extraction polarity: To study the impact of extractant polarity based on their polarity for preliminary experiment water and various concentration of ethanol were tested as the extraction solvents.

As data showed in Fig. 1a-e, extraction polarity had shown significantly effect ($p<0.05$) on phenolic compounds (TPC, TFA and CTC), anti-radical capacity assay and DPPH of crude extract. It was observed that the TFA and anti-radical capacity of crude extract increases as ethanol increases but after 40% of ethanol concentration, anti-radical capacity start to decrease but TFA continue to increase up to 60% and then decreases. On the other hand increased in ethanol concentration play a significant role on TPC, CTC and DPPH. These results of TPC and CTC were found in agreement with earlier reported studies[24]. Nawaz *et al.*[24] described that the binary-extractant solvent system was found to be best and

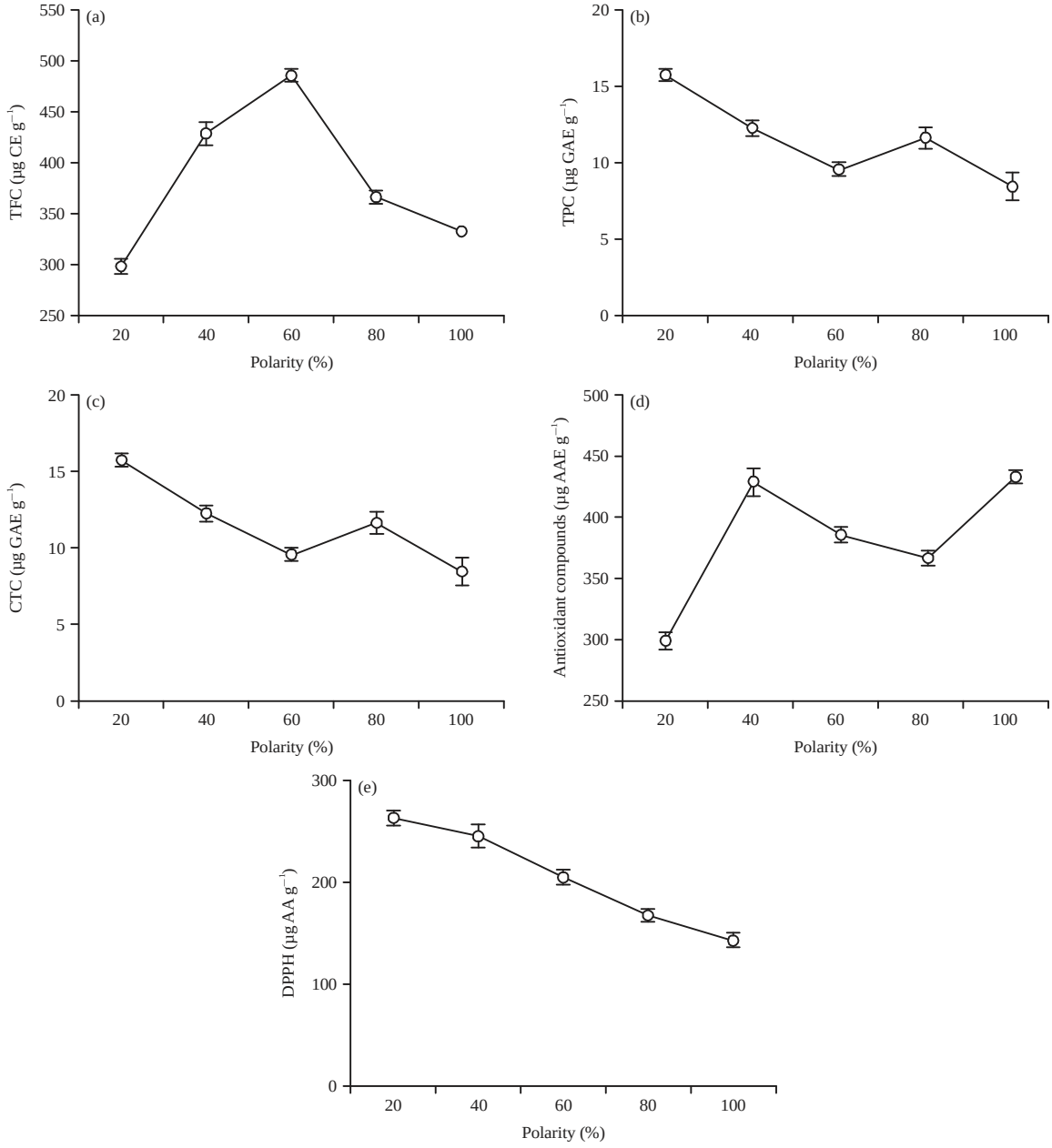

Fig. 1(a-e): Effect of extraction polarity on (a) TFC, (b) TPC, (c) CTC, (d) Antiradical capacity and (e) DPPH assay from vine tea (n = 3)

Data were expressed as Mean±Standard Deviation of assays. Error bars sign in graphs expressed about standard deviation

more suitable for phenolic compound extraction from plant material than that of single or mono extraction solvent system[24-27]. But in the case of TFA, the optimized extraction polarity was found to be entirely different with other two extracting compounds, which was optimized as 60% of ethanol concentration. This situation might be possible because mostly polyphenols were liked to be dissolved in non or weak polar extracting solvent system as compared to polar medium[28], consequently, TFA was optimized with weak polar extraction solvent system. methanol concentration and the liquid/solid ratio had significant effects whereas $p<0.05$ as significant of assays.

It is said that "Like dissolve like", extracting solvents medium were able to extract only those compounds that possessed similar polarity with extraction medium[27-30]. Moreover, all the phenolic compounds that were being extracted from vine tea might be possible that they all possessed similar polarity with extracting mediums. Keeping in view our findings of optimized extraction of antioxidant compounds (TPC, TFA, CTC), there was no single polarity obtained that can produce the maximum quantity of all these considered responses. Therefore, it is strongly recommended that phenolic compounds presented in vine tea were diverse in nature so that they have different polarities. But on the other hand, TPC and CTC both were optimized at 40% that also indicate that the phenolic compounds presented in vine tea possessed weak or non-polar characteristics.

To elucidate the effect of extraction polarity on the pharmacological activity (antioxidant capacities) of vine tea crude extract, it was found that, antioxidant capacities of vine tea were also affected by the concentration of ethanol in extracting substance because the rise of ethanol concentration was linked with the minimum level of antioxidant capacities (antiradical capacity and DPPH) of vine tea extract. As the concentration reached up to 80% the antioxidant capacity decreased to a lower levels. These findings also revealed that phenolic compounds presented in vine tea were moderately non polar in nature. However, further increase in the concentration resulted in a significant ($p<0.05$) rise in anti-radical capacity but no effect on DPPH. This phenomenon might be because of the diversity of molecular weight of extracting phenolic compounds. As it is reported in earlier research that phenolic compound with low molecular weight is more suitable to interact with DPPH as compared to large molecular weight[31]. Therefore, it is also suggested that binary solvent with 40% ethanol concentration are more efficient than that of pure ethanol (100%) for extracting phenolic compounds (low molecular weight) and their pharmacological activites. In consideration of cost effective approach, primarily and focused requirements of industry for operating producers, thus 40% extractant polarity was taken to be as best concentration of ethanol for extraction of phenolic compounds and their phamacological activities.

Evaluation of extraction time: To evaluate the impact of extraction time on the extraction of phenolic compounds from vine tea, it was examined that the extraction time plays an important role for extraction of phenolic compounds (TPC, TFA and CTC) and their antioxidant activity of vine tea extract. Results were depicted in Fig. 2a-e. As data showed that, the extraction time had significant ($p<0.05$) effect on the extracting compounds (TPC, TFA, CTC) as well as on their antioxidant activities. It was seen that all extractant compounds were remarkably changed during first 180 min and after that, there was no significant changes observed. This phenomenon might be depicted by Fick's second law of diffusion, which anticipates that with the passage of time, a stable equilibrium will be established in between the solute of the plant material (solid matrix) and the extraction solvents[16]. Therefore, further increase or rise in time period had no effect on the extraction of phenolic compounds. It was also observed that antioxidant capacities and DPPH value of vine tea extract were significantly ($p<0.05$) decreased after antioxidant and DPPH value reached to its maximum levels at 240 min. This is because of oxidation of phenolic compounds may occur because prolonged extraction would increase the possibility of oxidation which may lead towards the decreased of antiradical capacity or free radical scavenging ability of vine tea extract, these results were in accordance with the previously reported results[32].

Keeping in view the industrial requirement and economical point of view and also depending upon the quantification (yield) of antioxidant compounds and antioxidant activities of vine tea extract, 180 min was selected as the optimal extraction time because at this time period all responses showed the highest values or a values which had no significant difference. For the successive experiment in optimizing extraction parameters, it is recommended that the extraction time will be vary depending upon the particular element or compounds which were being to be optimized. So, data showed that optimized time for extraction of TFA and CTC were 120 min while in the case of TPC, antioxidant capacity and DPPH assay time was 240 min.

Evaluation of extraction temperature: To evaluate the impact of extraction temperature on extraction yield of phenolic compounds from vine tea, results showed that the extraction temperature plays an important role for extraction

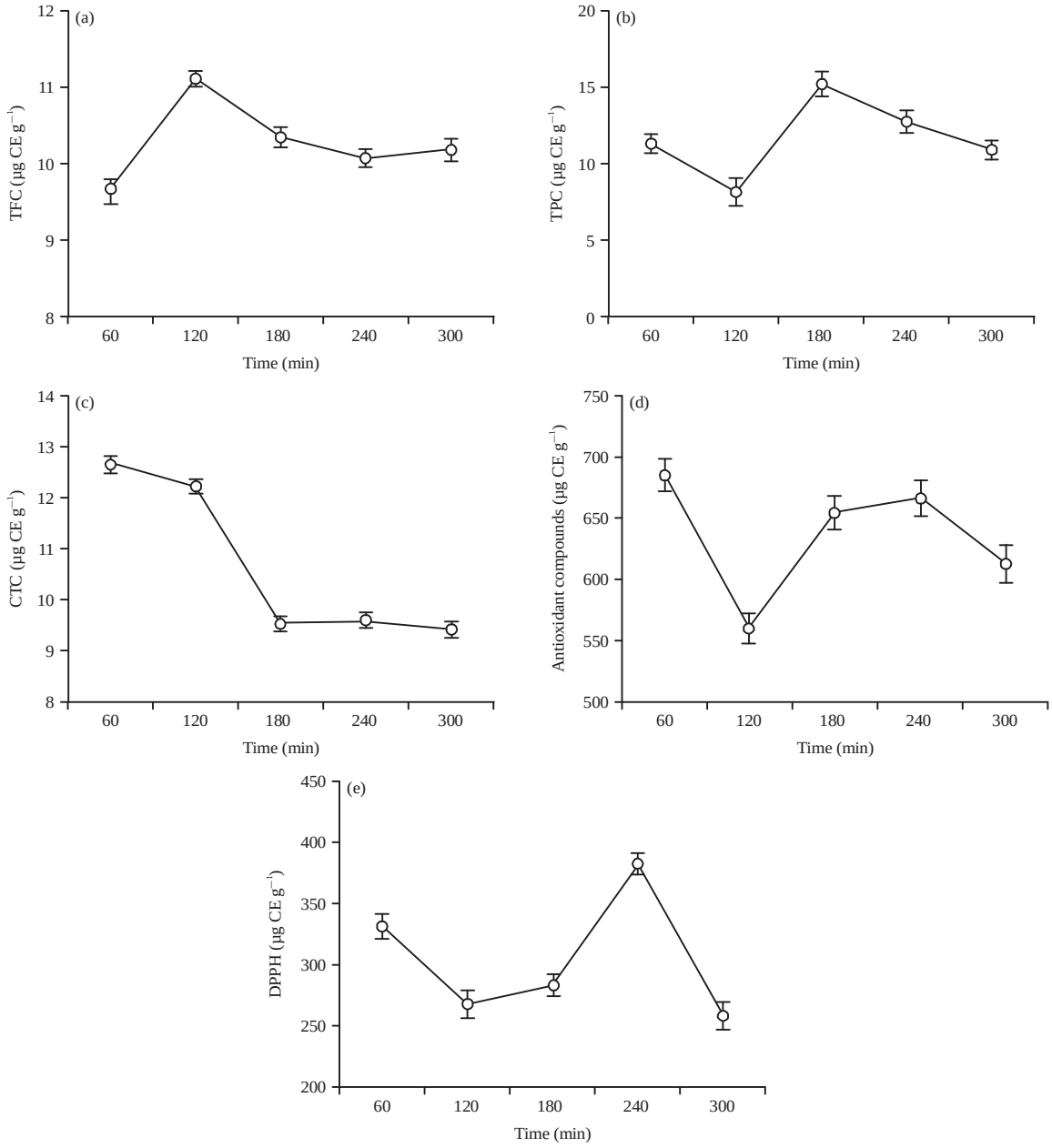

Fig. 2(a-e): Impact of extraction time on antioxidant compounds (a) TFC, (b) TPC, (c) CTC, (d) Antiradical capacity and (e) DPPH values from vine tea (n = 3)

Data were expressed as Mean±Standard Deviation of assays. Error bars sign in graphs expressed about standard deviation

of phenolic compounds (TPC, TFA and CTC) and their antioxidant activity of vine tea extract. Figure 3a-e, CTC and TFA had the highest values which was not significantly different with the value optimized extraction temperature 45°C. In contrast, the TPC, DPPH and antioxidant capacity of vine tea extract showed different behaviors than that of other phenolic compounds, in which antioxidant capacity and DPPH were increased with the raised in temperature and were found to be maximum in yield at 50 and 55°C, respectively. Finally, TFA and CTC were optimized by using the highest values at extraction temperature which was 45°C and for TPC it was at 50°C.

The increases of TFA and TPC with the increase of temperature were in concordance with earlier reports that concluded that the positive correction between the yield of extracted phenolic compounds and temperature involved in extraction[33,34]. Because extraction temperature could increase the recovery of extractant, by increasing the diffusivity of

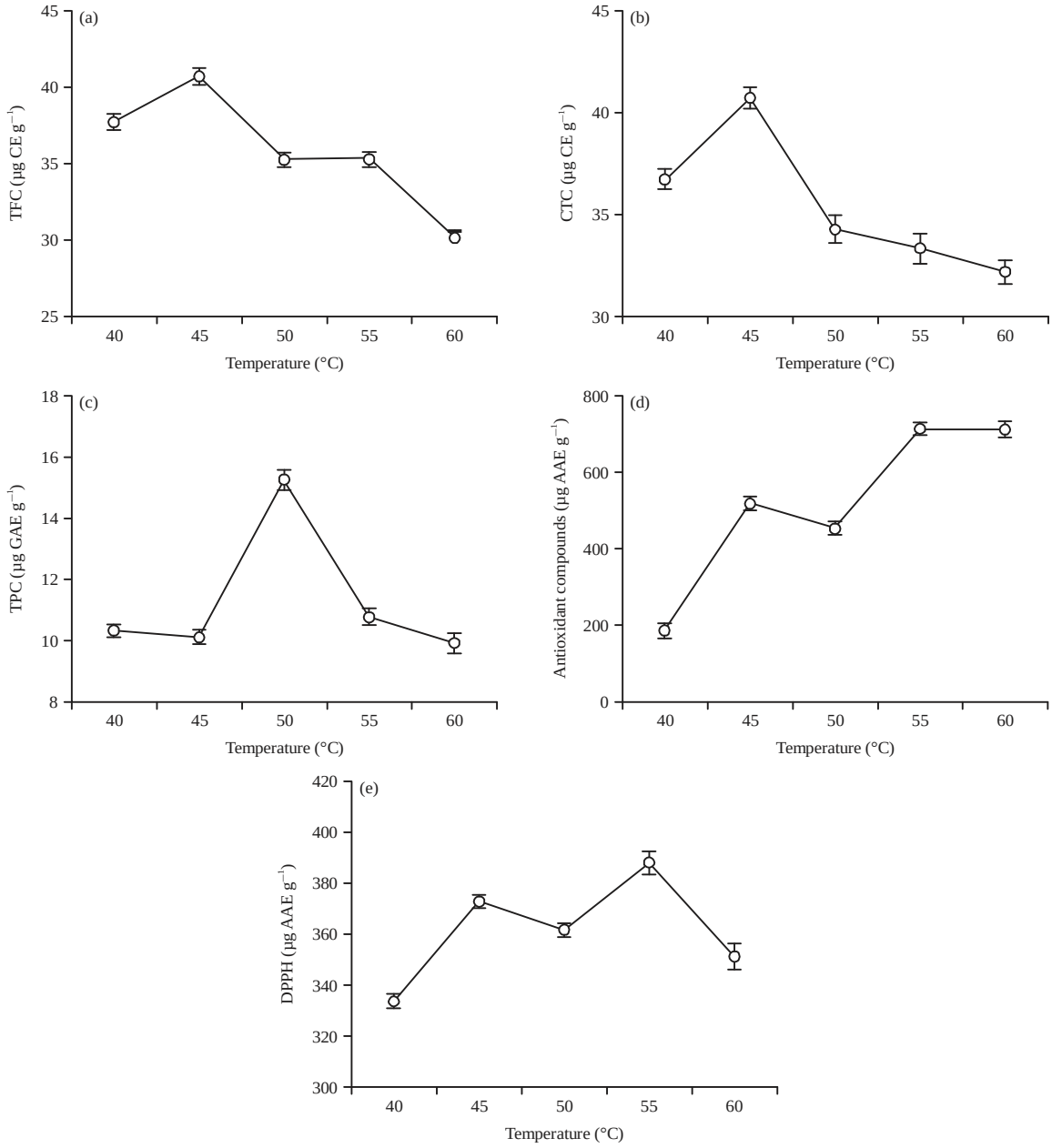

Fig. 3(a-e): Effect of extraction temperature on antioxidant compounds (a) TFC, (b) TPC, (c) CTC, (d) Antiradical capacity and (e) DPPH values from vine tea (n = 3)

Data were expressed as Mean±Standard Deviation of assays. Error bars sign in graphs explained the standard deviation

solvent and improving the solubility of phenolic compounds in solvents[25,26]. Additionally, Juntachote *et al.*[15] had described that raised in temperature could increase the mass transfer of phenolic compounds by reducing the viscosity and surface tension which might be helpful to improve the extraction of phenolic compounds. Furthermore, bound phenolic compounds can also be released by mild heating because mild heating could soften the tissues of the plant's cells that affect the integrity of the cell wall and make it more weaker, thus helped to release bound phenolic compounds as well[15,29]. Antiradical activity and DPPH values were also increased with the rise of temperature and after 55°C starts to decrease. The main reason for these results might be because of comparatively high temperature because the high temperature had a negative impact on the extraction of phenolic compounds due to degradation and bioavailability of functional compounds. The high temperature could enhance the extraction efficiency by enhancing both, diffusion

Table 1: Correlation coefficient among antioxidant compound and antioxidant activities with an effect of extraction polarity

Assays	TPC	TFC	CTC	Antioxidant capacity
TPC	0.002	-	-	-
CTC	0.134	0.037	-	-
Antioxidant capacity	0.135	0.010	0.003	-
DPPH	0.06	0.153	0.000	0.131

p<0.05, TPC: Total phenolic content, TFC: Total flavonoid content, CTC: Condense tannin content, DPPH: 2,2-diphenyl-1-picrylhydrazyl. All the determinations were carried out in triplicate and the data were represented as Mean±SD

Table 2: Correlation coefficient among antioxidant compound and antioxidant activities with an effect of extractant time

Assays	TPC	TFC	CTC	Antioxidant capacity
TPC	0.001	-	-	-
CTC	0.003	0.041	-	-
Antioxidant capacity	0.029	0.030	0.000	-
DPPH	0.068	0.036	0.600	0.391

p<0.05, TPC: Total phenolic content, TFC: Total flavonoid content, CTC: Condense tannin content, DPPH: 2,2-diphenyl-1-picrylhydrazyl. All the determinations were carried out in triplicate and the data were represented as Mean±SD

Table 3: Correlation coefficient among antioxidant compound and antioxidant activities with an effect of extractant temperature

Assays	TPC	TFC	CTC	Antioxidant capacity
TPC	0.446	-	-	-
CTC	0.028	0.940	-	-
Antioxidant capacity	0.078	0.586	0.320	-
DPPH	0.917	0.079	0.561	0.013

p<0.05, TPC: Total phenolic content, TFC: Total flavonoid content, CTC: Condense tannin content, DPPH: 2,2-diphenyl-1-picrylhydrazyl. All the determinations were carried out in triplicate and the data were represented as Mean±SD

coefficient along with solute solubility. On the other hand, increase in temperature might cause thermal destruction of phenolic compounds that could decrease the antioxidant activities of vine tea extract[29,35]. Depending upon the results depicted above, it is concluded that most of the antioxidant compounds present in vine tea had high antioxidant activities but they were heat sensitive. Because as the temperature reached to its highest level 60°C, vine tea heat sensitive phenolic compounds destroyed that led towards the reduction of antioxidant capacities. So in all consideration of these results, it was not only focused on the yield but also their antioxidant activities. These results were also in accordance with the previously reported work which reported that the antioxidant activities of phenolic compounds can be affected by shape and their interaction of phenolic compounds[36,37]. Polyphenols are bioactive natural molecules biogenerated through secondary metabolic pathways[38]. Therefore, these aspects are required further research and debates to explain the actual phenomenon behind the role of interaction and type or character of the shape of phenolic compounds that how it interact and play role in antioxidant activities.

Pearson correlation analysis: To determine the correlation between antioxidant compounds and antioxidant capacities assay, Pearson correlation analysis was established. In this correlation, we would have a better understanding and clearer concept on the interrelationship among the antioxidant activities and extracted phenolic compounds from vine tea extract under the influence of different extraction parameters (Table 1).

Data (Table 1) showed the significantly ($p<0.05$, $r=0.000$) positive correlation of CTC for DPPH. However, for antioxidant capacity, TPC and CTC were found significantly ($p<0.05$, $r = 0.01$, 0.003) positive correlation, this kind of correlation might be because of vine tea phenolic compounds were responsible for the radical scavenging ability of extract under the impact of extractant polarity. However, this correlation among CTC and DPPH would be well described in term of molecular weight of the substance. As it was reported earlier the condensed tannins compounds are high molecular weight polymers[39]. Thus, they were more efficiently responsive to DPPH radical scavenging capacity.

Under the influence of extraction time (Table 2), all antioxidant compounds (TPC, TFA, CTC) were showed significant ($p<0.05$, $r = 0.03$, 0.02 and 0.00) correlation with antioxidant capacity while in case of DPPH only TPC showed significant ($p<0.05$, $r = 0.03$) correlation. It was again suggested that vine tea phenolic compounds were mainly responsible for the antioxidant and phamacological activities. This finding is in line with previously reported results in the literature[31].

However, under the influence of extraction temperature, there was no significant positive or negative correlation had been observed. All tested antioxidant capacity assays and antioxidant compounds assays with an effect of extraction temperature were showed in Table 3. These results

recommended that rise in temperature could enhance the recovery of phenolic compounds but at the same time, antioxidant capacity will be first increase and then start to decrease. This is because of vine tea phenolic compounds bearing high antioxidant capacity was affected by heating. That caused degradation of these heat sensitive phenolic compounds. Consequently, vine tea phenolic compounds could easily lose their free radicals scavenging property. These results were in the same line as described in previous researches[40]. As Durling *et al.*[40], who have described that heating of phenolic compounds at high temperature could affect the nature of phenolic compounds and their antioxidant capacity (Table 3). The health promising effects of the medicinal plant has increased the interest of scientists and industrialists to focus on such natural medicinal plants. Extracts of vine tea appears to be a natural antioxidant, so further research should be addressed to identify these functional compounds and their structure in more details and role of vine tea extract as an attractive material, leading to possible drug development and their limitations.

CONCLUSION

All the extraction parameters (extractant polarity, extraction time and extraction temperature) exhibited a significant effect on the extraction efficacy of phenolic compounds from vine tea and their phamacological activities. The optimal parameters for phenolic extraction from vine tea were 40% ethanol with an extraction time of 180 min at an extraction temperature of 50°C, with values of 40.01 μg CE g^{-1} DW for TFA, 15.12 μg GAE g^{-1} DW for TPC and 12.70 μg CE g^{-1} DW for CTC. Under the impact of extractant polarity, DPPH exhibited strong positive correlation with CTC and TPC whereas, CTC showed a positive correlation with antioxidant capacity as a function of extraction temperature. However, CTC and TPC antioxidant compounds assays were non-significant with antioxidant capacity and for DPPH only TPC was significant as a function of extraction temperature. Single factor approach was used, to optimize vine tea phenolic compounds extractions. Hence, these results concluded that all three independent variables had great influence on isolation of phenolic compounds and had significant impact on the pharmacological activities of vine tea extract.

SIGNIFICANCE STATEMENT

This study discovers that the extraction variables (extraction polarity, time and temperature) had great influence in the isolation and extraction of phenolic compounds from vine tea. Furthermore, Impact of these variables on pharmacological activities especially antioxidant activity was studied using single factor experiment. These findings can help to determine the range of factors that affect recovery of functional compounds and provide key informative and preliminary test to develop central composite designs (CCRD) for Response Surface Methodology (RSM) in the optimization process.

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