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Efficacy of ethanol extraction time and temperature on phytochemical constituents extracted from *Callisia fragrans*

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*Callisiafragrans*is a wonderful herb with different therapeutic properties. Ethanol has been considered as an excellent solvent for polyphenol extraction by its safety for human consumption. This research optimized the ethanol-water proportions (25, 30, 35, 40, 45%) by different extraction time and temperature (90/25, 75/30, 60/35, 45/40, 30/45 minutes/^{o}C) on the total phenolic (mg GAE/100g), flavonoid (mg QE/100g), DPPH (mM TE/g) and FRAP (mM TE/g) extracted from *Callisiafragrans* leaves. Our results revealed that the highest phytochemical constituents extracted by 40% ethanol at temperature 35^{o}C in 60 minutes. This finding contributes important evidence to exploit the best functional constituents in this valuable herb.

Keywords: Callisiafragrans, ethanol, extraction time, temperature, phenolic, flavonoid, DPPH, FRAP

INTRODUCTION

Callisiafragrans is a cultivated perennial succulent herbaceous herb (Olennikov et al., 2008). *C. fragrans*leaves contain carbohydrates, ascorbic acids, amino acids and different biologically active constituents such as phenolic, flavonoid, coumarin, anthraquinone, triterpene, alkaloid, choline and fatty acid (Chernenko et al., 2007; Nguyen and Trinh, 2019). Antioxidant, anti-hypoxic, antiherpetic, antimutagenic and other healing properties beneficial for liver, pancreas, gastrointestinal tract, skin, bronchial asthma, radioprotective, hypersensitivity, dental cary from *Callisiafragrans*were clearly literatured (Ludmila et al., 2010; Mairapetyan et al., 2014; Susan et al., 2006; Malakyan et al., 2015; Thom et al., 2018).

Extraction of antioxidant constituents from herb is affected by numberous key variables like solvent, temperature, solid-liquid ratio, flow rate, extraction duration, particle size, extraction protocol, sample, duration and status of sample storage, interfering substances (Ana et al., 2009). Solvent extraction like water, acetone, methanol and ethanol is a common approach to extract biologically active constituents from herbs. Solvent polarity acts an important role in increasing phenol solubility. Methanol and ethanol have different polarities effectively extract various elements due to their chemical properties in numberous matrix (Boeing et al., 2014). Methanol is generally more efficient in extraction of lower molecular weight polyphenols, meanwhile aqueous acetone is ideal for extraction of higher molecular weight flavanols (Dai and Mumper, 2010). Methanol has higher efficiency to ethanol. However, ethanol is highly valued due to its food safety concerns. The effect of five extraction solvents (distilled water, 80% methanol, 80% ethanol, 80% ethyl acetate, and 80% chloroform) on yield, total phenolic content (TPC) and total flavonoid content (TFC) of *Callisia* leaves was determined (Thom et al., 2018). There is a food safety concerns related to organic solvents.

Ethanol is a cheap, safe and available solvent. Objective of our study examined the efficiency of ethanol-water proportions, extraction time and temperature on total phenolic, flavonoid, DPPH, FRAP extracted from *Callisiafragrans.*

MATERIALS AND METHODS

Material

Callisiafragrans leaves were collected from TienGiang province, Vietnam. After collecting, they must be kept in dry cool place and quickly conveyed to laboratory for experiments. It was washed carefully under tap water to remove foreign matters. All standards and reagents such as Folin-Ciocalteu reagent, Na_2CO_3, gallic acid, $Al(NO_3)_3$, potassium acetate, DPPH, methanol, ethanol, acetate buffer, 2,4,6- tripyridyl-s-triazine, HCl , $FeCl_3.6H_2O$ were analytical grade and purchased from Sigma-Aldrich.Lab utensils and equipments included weight balance, blender, vacuum pump, spectrophotometer.

Researching method

*Callisiafragrans*leaves was grinded in a blender, squeezed and filtered to collect the filtrate through Whatman paper under vacuum. The filtrate was then extracted successively with ethanol-to-water proportions (25, 30, 35, 40, 45%) by different extraction time and temperature (90/25, 75/30, 60/35, 45/40, 30/45 minutes/^oC). The ethanol extract was analyzed by spectrophotometer in respect of total phenolic (mg GAE/100g), flavonoid (mg QE/100g), DPPH (mM TE/g) and FRAP (mM TE/g).

Chemical analysis

Total phenolic content (mg GAE/100g) was evaluated using Folin–Ciocalteu assay (NizarSirag et al., 2014). Total flavonoid content (mg QE/100g) was avaluated by the aluminium calorimetric method (Formagio et al.,, 2015).DPPH (mM TE/100g) assay and FRAP (mM TE/100g) were performed according to Ivanov et al., (2014).

Statistical analysis

The experiments were run in triplicate with three different lots of samples. The data were presented as mean±standard deviation. Probability value of less than 0.05 was considered statistically significant. Statistical analysis was performed by the Statgraphics Centurion XVI.

RESULTSAND DISCUSSION

Effect ofsolvent-to-water proportions to antioxidant properties of phytochemical components

Solubility of polyphenolic elements and their diffusion to solvent depends on their chemical structure that vary from simple to highly polymerised constituents; therefore selection of appropriate solvent is very important in extraction. The most common solvents with different efficiency in extraction of plant phenolics are methanol, ethanol, propanol, acetone, ethyl acetate, dimethylformamide, and their mixture with water (Ana et al., 2009). Ethanol is normally preferred for food and pharmaceutical applications compared to other solvents due to its safety and affordability (Hemwimon et al., 2007; Guo et al., 2001). In our research, we examined the effect of different ethanol-to-water proportions (25, 30, 35, 40, 45%) to total phenolic, flavonoid, DPPH, FRAP extracted from*Callisiafragrans.* Results revealed that there was no significant difference at 40% and 45% ethanol-to-water in respect of phytochemical constituents extracted (table 1). Therefore we selected 40% ethanol for next experiment. According to Nguyen et al., (2019), methanol 40% was ideal to extract the antioxidant elements from tamarind seeds. In another report by Thom et al., (2018), significant differences were observed among total phenolic and total flavonoid obtaining by 80% methanol compared to other solvents. Total phenolic and total flavonoid of *C. fragrans* extracts increase in the following order: distilled water < 80% chloroform < 80% ethyl acetate < 80% ethanol < 80% methanol. Similarly, methanol, ethanol, and acetone in various concentrations (50%, 75%, and 100%) were used as solvent in the extraction of L. *aromatica*. Results revealed that 100% ethanol showed the highest total antioxidant activity, reducing power and DPPH (2, 2- diphenyl-1-picrylhydrazyl) radical scavenging activity (Quy et al., 2014). For extracting flavonoids from tea, ethanol performed higher efficiency than methanol and acetone (Wang and Helliwell, 2001). The existing water in the extraction supports the release of hydrophilic antioxidants (Aziz et al., 2003). Chew et al., (2011) optimized conditions for phenolic extraction of *Orthosiphonstamineus* extracts at 40% ethanol in 120 min at 65^oC. In another finding, total phenolic recovery increased for water content of ethanol from 10% to 30% and remained constant from 30% to 60%, while total phenolic concentration of extracts decreased for water content above 50% (Giorgia et al., 2007).

Table 1: Effect of ethanol-to-water proportions (%) to antioxidant properties of phytochemical components extracted from *Callisiafragrans*

Ethanol-to-water proportion (%)	Total phenolic (mg GAE/100 g)	Total flavonoid (mg QE/100 g)	DPPH (mM TE/100 g)	FRAP (mM TE/100 g)
25	585.32±0.02^{c}	257.95±0.02^{b}	18.45±0.03^{b}	20.71±0.01^{c}
30	712.45±0.03^{b}	289.36±0.01^{ab}	19.91±0.01^{ab}	24.65±0.02^{b}
35	737.83±0.01^{ab}	301.23±0.03^{ab}	20.45±0.02^{ab}	24.98±0.00^{ab}
40	763.28±0.00^{a}	340.38±0.01^{a}	20.87±0.00^{a}	25.42±0.01^{a}
45	765.40±0.03^{a}	342.05±0.00^{a}	20.90±0.03^{a}	25.50±0.02^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 2: Effect of extraction time (minutes) and temperature (°C) to antioxidant properties of phytochemical components extracted from *Callisiafragrans*

Time/ temperature (minutes/°C) of extraction	Total phenolic (mg GAE/100 g)	Total flavonoid (mg QE/100 g)	DPPH (mM TE/100 g)	FRAP (mM TE/100 g)
90/25	737.83±0.03^{c}	301.23±0.03^{c}	20.45±0.02^{c}	24.98±0.00^{c}
75/30	794.25±0.00^{bc}	337.84±0.02^{bc}	20.87±0.03^{bc}	25.63±0.02^{bc}
60/35	903.26±0.01^{a}	455.29±0.00^{a}	21.95±0.01^{a}	26.92±0.03^{a}
45/40	854.63±0.01^{ab}	408.32±0.03^{ab}	21.67±0.02^{ab}	26.47±0.00^{ab}
30/45	811.58±0.01^{b}	379.63±0.02^{b}	21.23±0.00^{b}	26.03±0.02^{b}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Effect of extraction time and temperature to antioxidant properties of phytochemical components

In our research, we examined the effect of different extraction time and temperature (90/25, 75/30, 60/35, 45/40, 30/45 minutes/°C) to total phenolic, flavonoid, DPPH, FRAP extracted from *Callisiafragrans.* Results revealed that the highest phytochemical constituents could be extracted from *Callisiafragrans* at 35°C in 60 minutes. Our results were similar to other literatures. According to Nguyen et al., (2019), the highest total phenolic value was obtained by methanol 40% at 40°C. Meanwhile, DPPH were highest by methanol 40% at 30°C. Ana et al., (2009) investigated the impact of solvent (water and ethanol in proportion: 50%, 70% and 96%) and extraction temperature (25–80°C) on polyphenols extraction of grape seed. The best results were achieved by using 50% ethanol at 80°C. In another report, Elena et al., (2019) proved that the optimum extraction conditions for extraction of phenolics from tiger nuts by-products were 50% ethanol, 35◦C. Intan et al., (2017) proposed an extraction temperature of 60 °C, an extraction time of 120 min and a water:ethanol solvent ratio of 90:10 v/v% on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthusnutans* Lindaul eaves. Jahangiri et al., (2011) used ethanol 80% as solvent at temperature 80◦C to extract the total phenolic compounds from leaves of *Ficuscarica*

CONCLUSION

Many parameters probably affect the recovery of antioxidant phenolic extractions from *Callisiafragrans*. Due to the unstable nature of phenolic components, it's necessary to optimize the appropriate conditions for extraction of these phytochemical constituents. Therefore it is very essential to optimize extraction yields and enrich the phenolic components. Solvent type and ratio, extraction temperature and time are the most key factors to ensure a complete extraction of antioxidant compounds. In this research, we realized that 40% ethanol, 60 minutes, 35°C were ideal for extraction of phytochemical constituents from *Callisiafragrans.*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Minh Phuoc Nguyen arranged the experiments and also wrote the manuscript.

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