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Green extraction technique: Subcritical water extraction of *emilia sonchifolia* (L.)

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Abstract

An environmental kindly technique, subcritical water extraction (SWE) are based on using water as extraction solvent at temperatures between 100 °C and 374 °C. Increasing the temperature at moderate pressure also reduces the surface tension and viscosity of water causes the polarity of subcritical water is comparable to organic solvents. Therefore, the subcritical water could be improved the competency for the extraction. The aim of this work was to study the flavonoid content of *Emilia sonchifolia* (L.) using different extraction procedures (SWE and the traditional extraction or ethanolic extraction). The results revealed that quercetin, a plant-derived flavonoid, was a major component in both extraction procedures. The use of SWE provided higher quercetin content and antioxidant activity. Quercetin content by SWE and traditional extraction were 45.92 μg/ml and 39.94 μg/ml, respectively. The EC_{50} (Effective Concentration, 50%) of SWE and traditional extraction were 496 and 555.67 μg/ml, respectively. Additionally, this work demonstrated that the traditional time-consuming techniques for 12 hours of the extraction of flavonoids could be substituted for the SWE technique within 1 hour. Consequently, the capability of SWE technique was elaborately evaluated and revealed on this work.

Keywords: Subcritical water, Emilia sonchifolia (L.)

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1. Introduction

E.sonchifolia is a branching, perennial herb up to 40 cm. Leaves are lyrate-pinnatilobed, up to 10 cm. It is widespread in tropical regions around the world, apparently native to Asia (China, India, Southeast Asia, etc.) and naturalized in Africa, Australia, the Americas, and various oceanic islands. The main components in *E.sonchifolia* were identified as flavonoids, Chirumbolo (2010). Flavonoids are secondary plant metabolites in various medicinal plants. Flavonoids show important pharmacological activities, such as anti-allergy, anti-inflammatory, anti-viral, anti-microbial activities, Priya, & Sharma (2013), anti-cancer, Al-Oqail, Al-Rehaily, Hassan, Ibrahim, Ahmad, Ebada, & (2012), antimicrobial activities, Haq Ihsan, Ullah, Bibi, Kanwal, Ahmad, & Mirza (2012) and antioxidant activity, Cacace, & Mazza (2006).

The extraction method is a significant step for the isolation and recovery of high added valued compounds from medicinal plants, in particular flavonoids. The traditional extraction method has various steps, Ramos, Kristenson, & Brinkman (2002). These steps are disadvantaged by the consumption of organic solvents which are environmental problems, whereas subcritical water extraction (SWE) can play a significant role to overcome the drawbacks.

SWE is a green technique. Since this technique uses water as the extraction solvent. The extraction temperatures by SWE technique are between of the boiling point to the critical point temperatures of water (100 °C to 374 °C) with a high pressure (22 MP). This pressure is high enough to keep the water as the liquid solvent, Kronholm, Hartonen, & Riekkola (2007). Increasing the temperature at the moderate pressure also reduces the surface tension and viscosity of water. Therefore, the subcritical water could be compared to organic solvents, Nelson (2008).

The objective of this work was to compare the efficacy of SWE with that of conventional extraction (ethanolic 95% v/v) regarding the flavonoid content and antioxidant activity from *E.sonchifolia*.

2. Methods

2.1. Extraction method

Subcritical extraction 100 g of powdered *E.sonchifolia* sample was soaked in water (1:50 w/v) and conducted at the temperature of 160 °C, under sufficient pressure (enough to maintain water in the liquid state) in a homebuilt apparatus for 60 min. The apparatus was slightly modified with the report from, Cacace and Mazza (2006).

Soxhlet extraction 100 g of powdered *E.sonchifolia* sample was extracted with 95% ethanol for 12 h. at temperatures of 60 °C in a Soxhlet apparatus. The extract was filtered and alcoholic content in extract was evaporated using Rota vapour at 70 °C.

2.2. Identification and Quantitation of flavonoids

The separation was performed by Reversed Phase High Performance Liquid Chromatography (RP-HPLC) using an ACE-129-2546 ® C18 column (25 cm x 4.6 mm i.d; 5 μm). The extracts were automatically injected (10.0 μL). The column was thermostatically controlled at 25 °C and a 1.0 ml/min flow rate was applied. The chromatogram was monitored at 270 nm, and UV spectra of individual peak was recorded in the range of 200–400 nm. A simple and gradient elution-based RP-HPLC method was developed for the analysis of typical flavonoids (*i.e*., catechin (1), rutin (2), quercetin (3), apigenin (4), hesperitin (5)) in the extracts. For the development of an effective mobile phase, various solvent

systems, including 0.1% glacial acetic acid (solvent A) and acetonitrile (ACN) of HPLC grade (solvent B) were investigated.

2.3. Determination of antioxidant activity

For 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) assay, an aliquot of samples will be mixed with 4.5 mL DPPH reagent (0.006 mM of DPPH redical in methanol) and the final volume was adjusted to 5.0 ml with water. After 10 minutes incubation, the disappearance of DPPH radical colour upon radical reduction was monitered by measuring the absorbance at 517 nm using UV/VIS spectrophotometer with deionized water in reagent as a control blank. The percentage of the remaining DPPH radical was calculated and plotted against sample and represented the results as the effective concentration at 50 % (EC_{50}).

3. Results

3.1. Identification and Quantitation of flavonoids

The optimized gradients of Reversed Phase High performance Liquid Chromatography (RP-HPLC) employed in *E.sonchifolia* extracts was: 0–2 min, 50% B (ACN) in A (0.1% glacial acetic acid); 2–10 min, 50-20% B in A and 10-20 min, 50% B in A. Method identification of compounds was performed on the retention time, coinjections, spectral matched with 100 μg/mL investigated standards (catechin (1), rutin (2), quercetin (3), apigenin (4), hesperitin (5)).

As results, quercetin was found in both extracts from the different extraction procedure under spiking technique and comparing with the spectrum of quercetin standard. It is evident that, quercetin is a major component in the both extracts. For the preparation of the calibration curve, standard stock solutions of the both extracts at the concentration of 1,000 μg/ mL were prepared in ethanol, filtered through 0.22 μm filters (Millipore), and appropriately diluted (10–100 μg/mL) to obtain the desired concentrations in the quantification range. The calibration curves were plotted of the peak areas versus concentrations.

The results were shown that amount of quercetin in *E.sonchifolia* extract by SWE (45.92 $\pm$ 3.22 μg/g, of dry raw material) was higher than soxhlet extraction (39.94$\pm$ 0.63 μg/g, of dry raw material) (Table 1).

Table 1. Contents of quercetin in E.sonchifolia extracts from the different extraction

No.	Quercetin (mg/g, of dry raw material)	
	Subcritical water extraction	Ethanol extraction
1	45.16	39.96
2	44.98	39.68
3	47.63	40.18
AVG	45.92	39.94
SD	1.48	0.25
%RSD	3.22	0.63

3.2. Determination of antioxidant activity

Quercetin has been found as a major component in the extracts of *E.sonchifolia* and may acts as an antioxidant. Quercetin has a higher reduction potential compared with curcumin and comparable to trolox, Zhang, Swarts, Yin, Liu, Tian, Cao, Swarts, Yang, Zhang, Zhang, Olek, Schwartz, Keng, Howell, Zhang, & Okunieff (2011). The addition of *E.sonchifolia* extract infusions the DPPH solution induced a decrease in the optical density at 517 nm. The effective concentration for being an antioxidant at 50 percent (EC_{50}) of the subcritical water extract and an ethanolic extract were at 496 and 556 μg/ml, respectively. Obviously, subcritical extraction was comparable to ethanolic extraction.

Table 2. The effective concentration for being an antioxidant at 50 percent (EC_{50}) in *E.sonchifolia* extracts from the different extraction

Concentration (mg/mL)	Absorbance No.1	No.2	No.3	Average	%RSD
100	0.296	0.295	0.295	0.295	0.195
200	0.252	0.252	0.251	0.252	0.229
300	0.214	0.213	0.214	0.214	0.270
400	0.184	0.184	0.185	0.184	0.313
600	0.109	0.109	0.109	0.109	0.000
800	0.052	0.052	0.052	0.052	0.000
DPPH	0.348	0.347	0.348	0.348	0.166

4. Conclusion

This current study is one of Thai National Researches with emphasis on the standardization and quality control of Natural products. In addition, this is the first report on the analysis of quercetin content and antioxidant activity of *E.sonchifolia*. The results of this work demonstrated that subcritical water extraction of *E.sonchifolia* was comparable to soxhlet with ethanol. The amounts of quercetin by subcritical water and traditional extraction were 45.92 μg/ml and 39.94 μg/ml, respectively. The EC_{50} of subcritical water extract and traditional extract were 496 and 556 μg/ml, respectively. This work confirmed the capability of safety SWE as a substitute for the traditional time-consuming techniques for the extraction of flavonoids as antioxidative compounds.

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