The effects of different extraction conditions on the polyphenol, flavonoids components and antioxidant activity of Polyscias fruticosa roots

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Abstract. Polyscias fruticosa is an herbal plant having a myriad of medicinal purposes, especially in antioxidant activities, anti-inflammatory and antipyretic. This study was carried out to evaluate the effects of different extraction conditions on the polyphenol, flavonoids content extraction and antioxidant activity of the Polyscias fruticosa root extract. DPPH and ABTS methods were employed to measure free radical scavenging activities. The total polyphenol, flavonoids content was calculated by gallic acid and quercetin equivalent, respectively. The absorbance is measured by using UV-Visible spectrophotometry. The results show that the conditions in which the highest extraction yield was attained consisted of 90\% ethanol, the ratio between material and ethanol of 1:20 g/mL, extraction time of 3 hours and extraction temperature of 30\textdegree C. This extract showed the highest polyphenol content (96,09 µg gallic acid equivalent/mg) and the highest flavonoid content (58,30 µg quercetin equivalent/mg of dry extract). Moreover, DPPH and ABTS antioxidants activity results showed that Polyscias fruticosa root extracts exhibited the IC\textsubscript{50} value of 96,14 µg/mL and 38,76 µg/mL, respectively. These results indicate that Polyscias fruticosa roots can be used in dietary applications with the potential to reduce oxidative stress.

1. Introduction
Nowadays, natural extraction from medicinal plants has been receiving a great deal of public attention due to the abundant content of bioactive molecules including vitamins, proteins, phenolic compounds [1-4]. These bioactive compounds play a vital role in different industrial fields such as cosmetics, pharmaceutical, food industries due to antimicrobial, antioxidant, anticancer activities[5-7]. Moreover, bioactive mixtures may cure different fatal diseases caused by Reactive Oxygen Species (ROS). Polyscias fruticosa belongs to the Araliaceae family which consisted of about 159 species and
cultivated mainly in tropical India, Malaysia, and Polynesia [8]. In recent years, cultivating Polyscias fruticosa have been receiving a great deal of public attention due to the essential oil extracted from its flowers and leaves.

The previous study reports that *P. fruticosa* essential oils play an important role in the treatment of different diseases such as ischemia, inflammation and increase blood in the brain [9]. *P. fruticosa* is one of spice and medicinal herb which used as a vegetable on soup and also enhance the flavor and organoleptic properties. There are different highly valuable compounds in the essential oil of Polyscias fruticosa such as, alkaloids, saponins, vitamins B₁, B₂, B₆, C, 20 amino acids [10]. *Polyscias fruticosa* essential oil is powerful antibacterial, analgesic, wound healing, and anti-inflammatory [11]. Human diseases may results in oxidative stress due to the imbalance between the neutralization and formation of pro-oxidants [12][13]. Moreover, antioxidants with free radical scavenging activities have the function in the prevention and therapeutics of diseases. The previous study demonstrated that the antioxidant efficiency of the extracts against lipid peroxidation initiated by Fe²⁺/ ascorbate. [14]. Polyphenolic compounds including flavonoids and phenolic acids are usually located in plants which have antioxidants activity [15][16].

In recent years, cultivating *Polyscias fruticosa* have been receiving a great deal of public attention due to the essential oil extracted from its flowers and leaves [17]. The present study was carried out to evaluate chemical components and determine the effects of different extraction conditions on the total polyphenol, flavonoids content, and antioxidant activity of the roots of *P. fruticosa*.

### 2. Materials and methods

#### 2.1 Materials and chemicals

The roots of *Polyscias fruticosa* was collected from Ho Chi Minh Biotech, Viet Nam in March 2019. The *Polyscias fruticosa* roots were dried at 40°C, following blending to remove the water content and powdering.

#### 2.2 Procedures

##### 2.2.1. Determination of polyphenol, flavonoids components of *P. fruticosa* roots in different factorst.

A set of experiments was carried out for evaluating the effects of different organic solvents on polyphenol, flavonoids content extraction including organic solvents (ethanol, methanol and acetone), concentrations of solution (50, 70 and 90 %), ratio between material and extracting (1:4, 1:10 and 1:20), the extraction time (2, 3 and 4 hours) and the temperature (30, 50 and 70°C).

The parameters of solvent concentration, ratio of material to solvent, time and temperature were initially fixed with the respective values of 90%, 1:20 (g/ml), 2 hours and 30°C. Suitable extraction solvent is selected based on the highest total polyphenol and flavonoid content. Total polyphenol and flavonoid contents of the extracts were determined using modified method of Vuong et al. with some modifications [18]. Then use this solvent to evaluate other parameters to find the best extraction conditions.

Extracts exhibited the highest polyphenol content and the highest flavonoids content was evaluated the ability to capture free radicals DPPH and ABTS.

##### 2.2.2. Total polyphenol content determination (TPC)

The level of total polyphenol in the crude extracts was determined by using Folin–Ciocalteu reagent and external calibration with gallic acid [18]. First, the 0.5 mL extract was pipetted into a test tube containing 2.5 mL Folin-Ciocalteu reagent 10% (v/v). After 5 minutes, 2 mL Na₂CO₃ 20% (w/v) was added to the sample. Next, the mixture was vigorously shaken and incubated for 60 minutes in the dark. Finally, the absorbance was spectrophotometrically measured at 765 nm and the results were shown in μg of gallic acid equivalents per dry weight of sample (μgGAE/mg).
2.2.3. Total flavonoids content (TFC)

Based on the aluminum chloride colorimetric method, the total flavonoid content was determined [18]. Mixing 0.5 mL of the extract with 0.3 mL of 5% NaNO₂. After 5 minutes, mixing with 0.3 mL of 10% AlCl₃. Then, 2mL of 1M NaOH and 1.9 mL distilled water was added and vigorously shaken. The absorbance was spectrophotometrically measured at 510 nm.

2.2.4. DPPH Scavenging Activity

The antioxidant activity of the individual essential oil was tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [18]. 1.5mL DPPH (OD 517 nm = 1.1 ± 0.02) into 500 µL solution sample. The sample solution with pre-concentration, and the mixed the stable at room temperature in the dark within 37 min. The optical measurement of mixture by UV/VIS - 1800 Shimadzu Spectrometer at 517 nm. Blank sample, but 500 µL solution replaced EtOH 99.7%. Standard sample: Vitamin C (0.1g ÷ 0.01) was dissolved ethanol 99.7% into volume flask 100mL, in the dark (C = 100 µL/mL).[19]

The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or percent inhibition (%I) [20]. The IC50 value was the concentration of the sample which inhibited percentage reaches 50% [21]

\[ \%I = \frac{Ab - As}{Ab} \times 100 \]  

In there: Ab - Absorbance of blank sample, As - Absorbance of sample, %I - Percent inhibition

2.2.5. ABTS Scavenging Activity.

Based on Thaipong and Kamonwannasit [21][22], ABTS scavenging activity was used. First, adding 10 mL of 2.6 mM K₂S₂O₈ in 10 mL of 7.4 mM ABTS solution in 15 hours. Next, preparing the working solutions by putting 1ml of stock solution into 60 mL of methanol to take the absorbance value of 1.1 ± 0.02 at 734 nm. Then, 0.5 mL of sample added with 1.5 mL of the working solution for 30 minutes RT. Using UV-VIS spectrophotometer measured the mixture at 734 nm. The percentage of ABTS decolorization of the sample was determined according to the equation:

\[ \%\text{decolorization} = [1-(ABS\text{sample}/ABS\text{control})] \times 100 \]

2.2.6. Statistical analysis

All determinations were carried out in triplicate and the results were expressed as mean values and standard deviation. One-way analysis of variance (ANOVA) was performed using SPSS statistics software (version 20, IBM, USA) and differences between samples were compared using Tukey’s test with a significance of 0.05 (P < 0.05).

3. Results and discussions

3.1 The affects of different organic solvents on the extraction of total polyphenol, flavonoids

Extraction solvent is one of the significant factors which affect extraction efficiency. Figure 1 shows the effects of extraction solvent on polyphenol and flavonoid content. The parameters of between solvent concentration, ratio material and solvent, time, and temperature were fixed with the respective values of 90%, 1:20 (g/ml), 2 hours and 30°C. The results showed that the ethanol extraction solvent gave higher polyphenol (28.56 µgGAE/mg) and flavonoid (26.26 µgGAE/mg) content than the methanol and acetone extraction solvent. As most phenolic compounds are polar permitting, so they have been efficiently extracted in high yield, in higher polarity solvent like ethanol [19]. Beside, ethanol is a common solvent extraction solvent in industry due to its non-toxic, relatively inexpensive and available.
3.2 The effects of concentrations of solvent on the total polyphenol, flavonoids content extraction

Figure 2 indicates the total polyphenol and flavonoids content in different concentration solvents. Effect of concentration of solvent on the total polyphenol and flavonoids content was studied at 50%, 70% and 90%. Other parameters were fixed include ethanol, ratio between material and solvent (1:20 g/ml), extraction time (2 hours) and temperature (30°C). Suitable extraction solvent concentrations are selected based on polyphenol and flavonoids content. The results showed that the extract of ethanol 90% gave higher total polyphenol (28.56 µgGAE/mg) and flavonoid content (26.26 µgQE/mg) than ethanol 70% and 50% with significantly difference (p<0.05).

Figure 2. The total polyphenol and flavonoids content in different concentration solvents

Ethanol 90% is the most suitable solvents for extract from *P.fruticosa* root due to its ability to dissolve not only many polar but also nonpolar compounds. Moreover, ethanol has been known as a suitable solvent for polyphenol extraction and is safe for human consumption. From the results obtained, we selected ethanol 90% extraction solvent for further experiments.

3.3 The affects of ratio between sample and solvent on the total polyphenol, flavonoids content extraction

From the previous results obtained, we selected ethanol 90% extraction solvent for this experiment. The parameters of extraction time and extraction temperature were fixed with the respective values of...
2 hours and 30°C. Figure 3 illustrates the total polyphenol and flavonoids content in the different ratio (1:2, 1:1, 1:4) between sample and solvent. The solvent extraction procedure was carried out according to the extraction procedures described by Tan et al. (2011) with slight modifications [25]. The total polyphenol and flavonoids content in the ratio between material and ethanol (1:20 g/mL) was significantly higher than ratio 1:4 and 1:10 (g/mL). Further increase in the ratio between sample and solvent (1: 20 to 1: 4) was not significant in increasing of the concentration of both total polyphenol and flavonoids content (p>0.05). According to Tan et al. (2011), a high solvent ratio is considered favorable in extracting polyphenol and flavonoid compounds. This result is in line with a previous study which proved that Pegaga (Centella Asiatica) compounds yields were increased gradually with the increase of the solid-to-solvent ratio and achieved the highest polyphenol and flavonoid content at 1:15 [25].

![Figure 3](image3.png)

**Figure 3.** The total polyphenol and flavonoids content in different ratio between sample and solvent

### 3.4 The effects of extraction time on the total polyphenol, flavonoids content extraction

Figure 4 shows the total polyphenol and flavonoids content in different extraction time. The highest total polyphenol (96.09 µgGAE/mg) and flavonoids (58.3 µgQE/mg) content was in 3-hour-extraction following 2-hour-extraction and 4-hour-extraction. Base on The Fick’s second law of diffusion anticipation. There is a balance between in the solid matrix and the bulk solution in the solute matrix after a particular time. So, over time is not valuable to extract more polyphenols.

![Figure 4](image4.png)

**Figure 4.** The total polyphenol and flavonoids content in different extraction time
3.5 The effects of extraction temperature on the total polyphenol, flavonoids content extraction

The total polyphenol and flavonoids content in 30°C were significantly higher than the extraction temperature 50°C and 70°C. Extraction temperature affects solubility, mass transfer rate and stability of polyphenol compounds. Within a certain limit, high temperatures enhance the extraction efficiency by enhancing the degree of diffusion and solubility of analytes in solvents [6]. Beyond that limit, high extraction temperatures will reduce total polyphenol and flavonoids content.

![Graph showing the total polyphenol and flavonoids content in different extraction temperature](image)

**Figure 5.** The total polyphenol and flavonoids content in different extraction temperature

3.6 Antioxidant activity of Polyscias fruticosa roots in the most suitable extraction solvent

There are different techniques for estimating the antioxidant activity of both synthetic compounds and natural. The DPPH assay and ABTS were a rapid and low-cost method, which usually used for evaluation of the antioxidative potential of different natural stocks. The results showed that ethanol (90%); ratio between material and ethanol (1:20 g/mL); time (3 hours); temperature (30°C) to extract total polyphenol and flavonoids content in *Polyscias fruticosa* roots is highest. This extract was evaluated antioxidant activity by method DPPH radical scavenging activity and ABTS inhibition activity.

![Graph showing DPPH radical scavenging activity](image)

**Figure 6.** DPPH radical scavenging activity
Antioxidants can remove the radical by hydrogen donation, which results in a decrease of DPPH absorbance at 515 nm. The IC50 value was the concentration of the sample which inhibited percentage reaches 50% [25]. Therefore, IC50 values are negatively correlated to antioxidant activity, the lower IC50 value means the highest antioxidant activity of the tested sample. Figure 6,7 shows the IC50 values in the DPPH and ABTS radical scavenging activity assay of the extracts IC50 = 96.14 µg/mL and 38.76 µg/mL, respectively.

4. Conclusions

Polyscias fruticosa essential oil has been widely used in folk medicine for treatment of anxiety, memory deficit, and cancer thanks to its high antioxidant activity and antibacterial properties. The parameters between different organic solvents, solvent concentration, ratio material and solvent, extraction time and extraction temperature effect significantly on the extraction process of Polyscias fruticosa roots. The results showed that ethanol (90%); the ratio between material and ethanol (1:20 g/mL); time (3 hours); temperature (30°C) to extract total polyphenol and flavonoids content in Polyscias fruticosa roots is highest. Total phenolics result was calculated as (96.09 µg gallic acid equivalent/mg), total flavonoids as (58.30 µg quercetin equivalent/mg of dry extract) and the ability to capture free radicals DPPH of this extract (IC50 = 96.14µg/ml) and anti-oxidants activity ABTS (IC50 = 38.76 µg/ml). According to the results of the present investigation, the plant showed significant antioxidant activity that can be used for the medical purpose for the treatment of various diseases.

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