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SHORT COMMUNICATION

Optimizing Polyphenol Content and Extraction Methods for Antioxidant Constituents from *Portulaca oleracea*: Comparing Reflux and Maceration Methods with Various Solvents

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Abstract

Background: The pursuit of developing effective drugs as antioxidants can be traced back to herbal ingredients, including purslane (*Portulaca oleracea*). The potential of purslane as a medicinal herb can be maximized by selecting the most suitable extraction method and type of solvent. **Objective:** To assess the levels of total phenolic compounds, total flavonoids, and radical scavenging capacity in *P. oleracea* obtained through various extraction methods and solvents. **Methods:** This study combines maceration and reflux methods with 96% ethanol, 80% ethanol, 96% methanol, and 80% methanol solvents to extract total phenolics, flavonoids, and radical scavenging activity from purslane. The folin-ciocalteu method was employed for measuring phenolic content, the $AlCl_3$ method for flavonoid content, and the DPPH method for radical scavenging activity determination. **Results:** The reflux method using 80% methanol produced the highest total phenolic content, 5.15 ± 0.07 mg GAE/g DW. The maceration method using the same solvent yielded the highest total flavonoid content, 5.74 ± 0.29 mg QE/g DW. Both extraction methods showed similar radical scavenging activity, 1.10 ± 0.003 mg AAE/g DW for maceration and 1.07 ± 0.04 mg AAE/g DW for reflux. **Conclusion:** The extraction method and solvent significantly impact the total phenolic and flavonoid content produced by *P. oleracea*.

Keywords: Flavonoids, Maceration, Phenolics, *Portulaca oleracea*, Reflux, Radical Scavenging Activity

1. Introduction

Pharmaceutical research today encompasses a wide range of scientific fields in the quest to treat a plethora of diseases. One of the root causes of many diseases is oxidative stress[1]. Oxidative stress is characterized by an imbalance in the production of free radicals such as Reactive Oxygen Species (ROS) and the body's capacity to neutralize them through endogenous antioxidant systems[2]. This imbalance can lead to a multitude of health problems due to the damage inflicted on cell components, including DNA, proteins, lipids, and cell membranes[3]. Some diseases, including cardiovascular disease, neurodegenerative disease, inflammatory disease, and cancer, are caused by oxidative stress[1, 4-6]. Oxidative stress can damage the artery walls, leading to cardiovascular diseases such as atherosclerosis, coronary heart disease, and stroke[7]. It can also

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trigger neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's disease[8], which can occur when Reactive Oxygen Species (ROS) damage nerve cells in the brain, disrupting their normal function. Furthermore, ROS can cause genetic mutations and uncontrolled cell growth, leading to tumors and cancer[9,10].

Health problems that result from oxidative stress can be alleviated by balancing the body's antioxidant levels. Antioxidants can be divided into two categories: Endogenous and exogenous[11]. Endogenous antioxidants are produced naturally in the body, such as glutathione, and antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, also exist in the human body[12]. On the other hand, exogenous antioxidants come from external sources to help neutralize ROS and prevent oxidative stress. Examples of exogenous antioxidants include vitamin C, E, and phenolic and flavonoid compounds[13].

The discovery of effective drugs as antioxidants can be traced back to natural herbal ingredients that are widely available in the environment. These ingredients, sourced from plants, contain various active compounds like polyphenols, flavonoids, alkaloids, and terpenoids[14-16]. One such well-known herbal ingredient is purslane, which is recognized for its medicinal properties in Central Europe, Asia, and the Mediterranean. Purslane is not only used as a herbal ingredient but also as a vegetable, which can be cooked or consumed raw. It contains phenolic compounds like caffeic acid, p-fumaric acid, scopoletin, ferulic acid, chlorogenic acid, and clonic acid[17].

The development of purslane as a herbal ingredient for medicine can be improved by selecting the most suitable extraction method and type of solvent. The objective of selecting methods and solvents for purslane is to produce the optimal levels of polyphenol and antioxidant content. Commonly used extraction methods include solvents such as reflux, maceration, soxhlet, and supercritical extraction[18,19]. Each method has unique characteristics and can yield different results in terms of extraction efficiency. Selecting the appropriate method can ensure optimal extraction outcomes[20]. Moreover, the solvent used can affect extract quality[21]. Some solvents are better at extracting higher concentrations of active compounds, while others are less efficient. Choosing a solvent suitable for extraction purposes can result in extracts with the desired quality, such as optimal active compound concentrations and minimal contaminants or other unwanted substances[22]. In this study, the TPC and TFC, as well as the RSA, were evaluated and compared for different extraction methods and solvent types. As a result, the appropriate extraction method and solvent for optimizing TPC and TFC extraction can be carefully determined.

2. Materials and Methods

2.1 Sampel Preparation

The purslane plant utilized in this study was collected from the IPB Biopharmaca Collection Park (-6.5475338,106.7162354) and is of the species *P. oleracea*. The research was carried out at the Tropical Biopharmaca Research Center (TropBRC) Laboratory from June 20 to August 10, 2022 and the aerial parts of the plant were analyzed. To prepare the plant samples, they were washed with running water and dried in an oven (EYELA NDO-700) at 50°C for 24 hours. The dried samples were then crushed using a blender and sieved through a 60-mesh sieve.

2.2 Maceration Method

Purslane powder was finely weighed to 18 g and dissolved in 180 ml of a solvent consisting of 96% ethanol (Merck KGaA), 80% ethanol (Merck KGaA), 96% methanol (Merck KGaA), or 80% methanol (Merck KGaA). The maceration process was carried out for 2×24 hours, with stirring and filtering every 24 hours. The resulting macerated filtrate was filtered using filter paper (Whatman No. 42) and evaporated using a rotatory evaporator heidolph G3 at 55°C until only 20 ml of extract remained.

2.3 Reflux Method

To prepare the purslane extract, the powder was weighed to 18 g and dissolved in 180 ml of a solvent consisting of 96% ethanol (Merck KGaA), 80% ethanol (Merck KGaA), 96% methanol (Merck KGaA), or 80% methanol (Merck KGaA). The mixture was then put into a water bath at 76°C for 2 hours. The resulting filtrate was filtered and evaporated using a rotary evaporator at 55°C until 20 ml remained.

2.5 Total Phenolic Analysis

The method for analyzing the total phenolic content was based on the modifications by Khumaida *et al*[23]. To determine the phenolic content, 20 µl of sample extract was added to 120 µl of folin-ciocalteu (10%) (v/v) in a microplate and incubated in a dark room for 5 minutes. Then, 80 µl of Na_2CO_3 solution (10%) (w/v) was added to the microplate and the mixture was incubated in the dark for an additional 30 minutes. The absorbance of the solution was measured using a nano spectrophotometer (SPECTROstarNano BMG LABTECH) at a wavelength of 750 nm. The standard used for comparison had a concentration range of 0-300 ppm gallic acid.

2.5 Total Flavonoids Analysis

The flavonoid content in the sample was determined by the method described by Calvindi *et al*., which involved adding 10 µl of the sample extract to 50 µl of ethanol solution, 10 µl of aluminum chloride (10% w/v), 10 µl of glacial acetic acid (CH_3COOH), and 120 µl of distilled water into a 96-well microplate[24]. The mixture was incubated in a dark room at room temperature for 30 minutes, and the absorbance of the sample extract was then measured using a nano spectrophotometer (SPECTROstarNano BMG LABTECH) at a wavelength of 415 nm. Quercetin was used as the standard, with concentrations ranging from 0-500 ppm.

2.6 Radical Scavenging Activity Measurement

Based on the methodology described by Calvindi *et al*., the radical scavenging activity of purslane extract was evaluated using the DPPH method[24]. To perform the assay, 100 µl of the extract was mixed with 100 µl of DPPH reagent (125 µM) in a microplate and incubated in the dark at room temperature for 30 minutes. The absorbance of the solution was then measured at 517 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH). As a standard, trolox was used with concentrations ranging from 0-50 µmol.

2.7 Data Analysis

The data were analyzed using the one-way ANOVA method and Tukey's follow-up test in IBM SPSS Statistics 25. The calculations were performed using microsoft office excel 2019. The Pearson correlation test was used to carry out correlation analysis in graph pad prism 8. The data were presented in tables and graphs using graph pad prism 8 software.

3. Results and Discussion

The phenolic content of various extracts of *P. oleracea* obtained through reflux and maceration methods is presented in Figure 1. The reflux method utilized 80% methanol and produced the highest TPC (5.15 ± 0.07 mg GAE/g DW), followed by 96% methanol, 80% ethanol, and 96% ethanol. Meanwhile, the maceration method resulted in 80% ethanol extract (5.11 ± 0.07 mg GAE/g DW) having the highest TPC, followed by 80% methanol, 96% ethanol, and 96% methanol. The results of this study differ from a previous study that utilized the same reflux method on *P. oleracea* leaf [25], likely due to the use of aerial parts. It is clear that the solvent, extraction process, and sample all play a role in determining the number of phenolic compounds extracted. Phenolic compounds, which are naturally found in plants and are known for their antioxidant

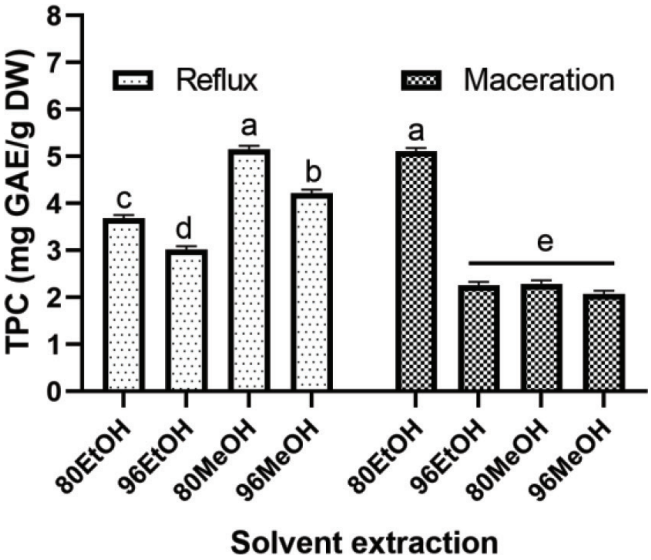

Figure 1. Total Phenolic Content (TPC) in different extract of *P. oleracea* from reflux and maceration methods. Each bar displays the mean ± SEM. Different letters denote statistically significant differences using the Tukey test and a p-value of less than 0.05. Solvents: 80EtOH = 80% ethanol; 96EtOH = 96% ethanol; 80MeOH = 80% methanol; and 96MeOH = 96% methanol.

properties and capacity to neutralize potentially harmful free radicals, have received significant attention in recent years[26-28].

The study found that the reflux and maceration techniques are the most effective for extracting polyphenolic compounds from *P. oleracea* tissues, with methanol and ethanol at 80% efficiency. The reflux technique tends to produce higher levels of phenol content compared to maceration. Heat present in the reflux process helps with the hydrolysis of glycosidic and ester linkages of condensed flavonoids and tannins, resulting in increased phenolic content[29]. Although there is no single solvent considered the best for polyphenol extraction, solvents with higher polarity tend to be more effective since polyphenols are more soluble in these solvents. It is important to note that the solubility of solutes is majorly influenced by their structure, specifically in the case of phenolic compounds[30].

The flavonoid content in this study, which was obtained through reflux and maceration techniques, showed a range of values (Figure 2). The flavonoid content obtained in the study varied from 0.04 ± 0.01 to 5.74 ± 0.03 mg QE/g DW. The highest flavonoid content was extracted using 80% methanol, which yielded 5.73 ± 0.06 mg QE/g DW. Meanwhile, the maceration technique using 80% methanol solvent produced the highest flavonoid content of 5.74 ± 0.03 mg QE/g DW. The method of extraction, the use of solvents, and the type of sample significantly affect the amount of extracted flavonoid compounds. Previous research by Uddin, *et al*. also demonstrated differences in flavonoid content using ethanol and methanol solvents[25]. Methanol is known to be more effective in extracting flavonoid compounds as it can inhibit polyphenol oxidase, resulting in more optimal flavonoid compounds[25, 31].

Both extraction methods, using 80% methanol, yielded high total flavonoid content. The maceration method produced a slightly higher flavonoid content than the reflux method. However, using an inappropriate temperature in the reflux method may result in faster oxidation of flavonoid compounds and lower yield[32]. Several flavonoid compounds are thermally unstable, and the maceration method at lower room temperature can help minimize their degradation, resulting in better recovery of flavonoid compounds[33]. Additionally, the duration of extraction affects the flavonoid content obtained, with a longer duration yielding higher flavonoid content in the maceration method[34].

The Radical Scavenging Activity (RSA) of the two extraction methods did not show a significant difference ($p>0.05$). However, the maceration method produced an average RSA that was slightly higher than the reflux method, with values of 1.10 ± 0.003 mg AAE/g DW for maceration and 1.07 ± 0.04 mg AAE/g DW for reflux (figure 3). These results suggest that the extraction method and type of solvent do not influence the RSA produced by *P. oleracea*. This plant has been known for a long time to have medicinal effects in the treatment of various diseases and has also been widely studied for pharmacological development[35]. Radical scavenging activity or antioxidant in plants can be produced by compounds from the flavonoid group and other types of compounds, such as ascorbic acid and α-tocopherol[36,37]. In this study, other compounds that are not polyphenols may have contributed to the antioxidant activity, which was not affected by the extraction method and solvents and resulted in almost the same antioxidant activity.

The parson correlation test was used to determine the polyphenolic compounds in *P. oleracea* that possess antioxidant activity. The analysis showed a negative correlation between the Total Phenolic Content (TPC)

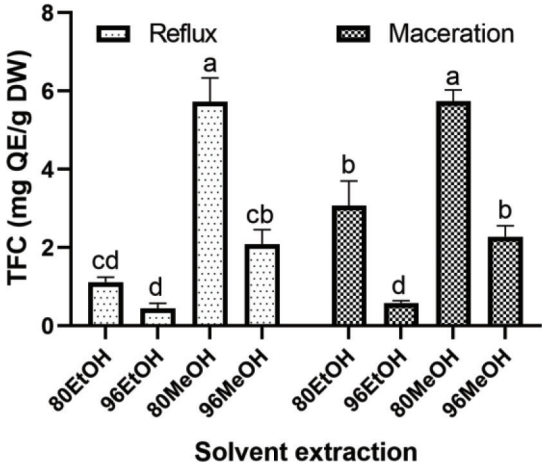

Figure 2. Total Flavonoid Content (TFC) in different extract of *P. oleracea* from reflux and maceration methods. Each bar displays the mean ± SEM. Different letters denote statistically significant differences using the Tukey test and a p-value of less than 0.05. Solvents: 80EtOH = 80% ethanol; 96EtOH = 96% ethanol; 80MeOH = 80% methanol; and 96MeOH = 96% methanol.

and antioxidant activity (RSA) (r = -0.17) (figure 4A), as well as between the Total Flavonoid Content (TFC) and RSA (r = -0.23) in all *P. Oleracea* extracts (figure 4B). The results suggest the presence of compounds other than those from the phenolic or flavonoid groups that contribute to DPPH radical scavenging activity[36-38]. This is consistent with the DPPH radical scavenging activity value, which showed no significant differences across all methods and solvents (figure 3). Furthermore, the insignificant positive correlation between TPC and TFC (r = 0.18) (figure 4C) suggests that only a small proportion of flavonoid compounds were obtained through both methods and solvents. Other phenolic compounds that may contribute to the total content include phenolic acids, stilbenes, lignans, tannins, hydroxybenzoic acids, curcuminoids, and hydroxycinnamates[39-42].

The study demonstrated that there was no notable relationship between polyphenolic compounds, such as total phenolics and total flavonoids, and antioxidant activity. This can be attributed to various factors, including an insufficient number of samples, the intricate nature of polyphenolic compounds, and diverse analytical methods[43-45]. A larger number of samples would allow for more repetitions of the experiment, resulting in more accurate data measurements and conclusions that align with theoretical predictions[46]. In this study, three replicate experiments were conducted for each solvent and extraction method, yielding a total of 24 data points (3x4x2). This data is considered adequate for further analysis, which suggests that using three repetitions does not lead to significantly deviating results and an insignificant relationship.

The intricate nature of polyphenolic compounds, particularly the phenolic and flavonoid categories, suggests that not every compound within these groups exhibits the same antioxidant properties or in some cases,

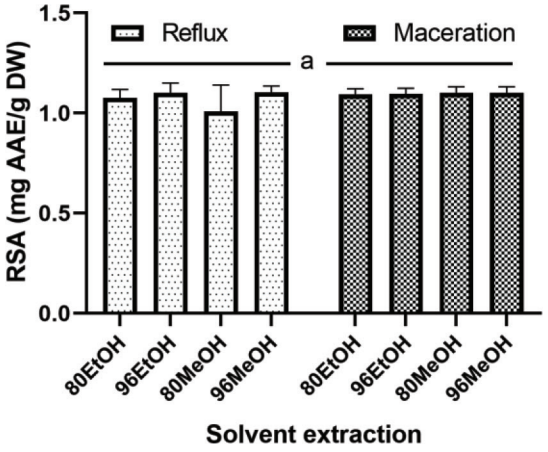

Figure 3. The antioxidant activity of the *P. oleracea* extract was different from the reflux and maceration methods. Solvents: 80EtOH = 80% ethanol; 96EtOH = 96% ethanol; 80MeOH = 80% methanol; and 96MeOH = 96% methanol.

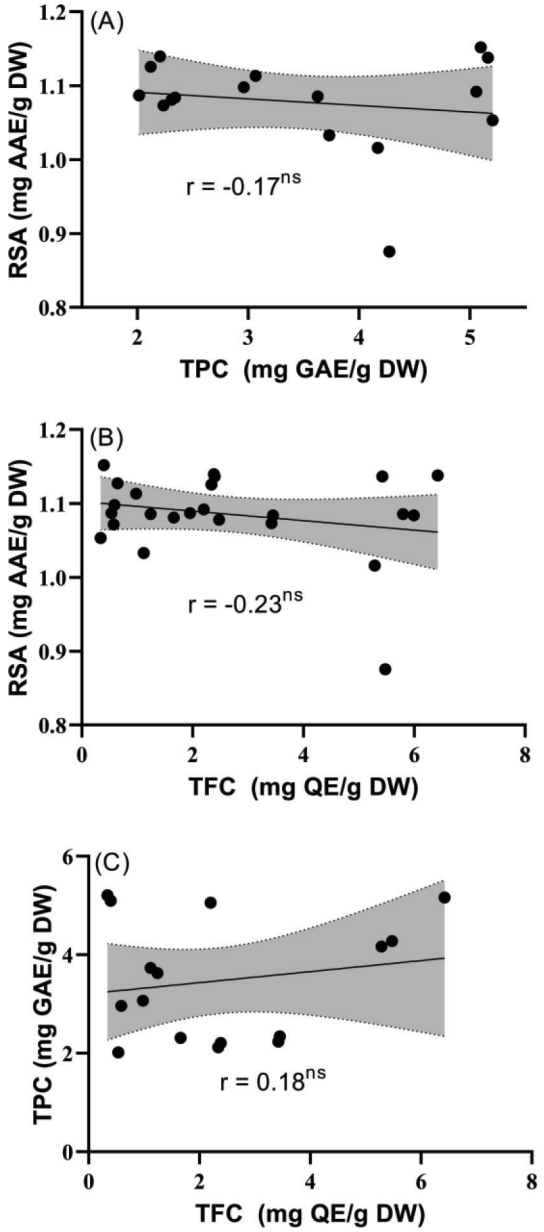

Figure 4. Simple linear correlation of Total Phenolic Content (TPC) with Radical Scavenging Activity (RSA); **(A).** Total Flavonoid Content (TFC) with RSA; **(B).** TPC with TFC in *P. oleracea* extracts; **(C).** Pearson's correlation coefficient value; ns, not significant at P < 0.001.

possesses no such properties at all[40,47]. This could result in non-significant correlations due to the diverse chemical structures of the polyphenols examined. Some phenolic compounds with documented antioxidant activity comprise gallic acid, caffeic acid, and curcumin[48-50]. On the other hand, flavonoid compounds known to exhibit antioxidant properties consist of quercetin, kaempferol, apigenin, and catechin[51-54]. Further research is warranted to determine if these compounds are present in minute amounts in *P. oleracea* samples, which could explain the insignificant relationship between total phenolic and flavonoid content and antioxidant activity. Additionally, the testing procedures for polyphenolic antioxidant activity vary greatly, encompassing both *in vitro* and *in vivo* methods that each possess their own advantages and disadvantages[44]. The variability in testing methods can result in disparate findings and, consequently, non-significant correlations[55].

4. Conclusion

The Total Phenolic (TPC) and Total Flavonoid (TFC) contents of *P. oleracea* varied depending on the extraction method and solvent used, with significant differences observed. The highest TPC of 5.15 ± 0.07 mg GAE/g DW was obtained through the reflux method using 80% methanol, while the highest TFC of 5.74 ± 0.03 mg QE/g DW was obtained through the maceration method using 80% solvent. However, the Radical Scavenging Activity (RSA) of *P. oleracea* did not show significant differences, suggesting that other factors may have influenced the RSA value. Additionally, the pearson correlation results indicated that the TPC and TFC of *P. oleracea* in this study did not directly contribute to RSA using the DPPH method.

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6. References

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