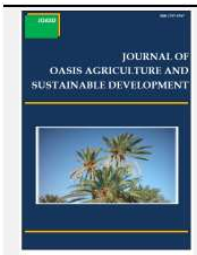

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Impact of Solvent Selection on the Extraction Efficiency of Antioxidant Compounds from a local population of *Pisum sativum* Cultivated in Arid Southern Tunisia

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Abstract

This study evaluates the effect of different solvents (methanol, water, acetate, chloroform, and hexane), on the extraction of antioxidant compounds from a local pea population (*Pisum sativum*) cultivated in the arid region of southern Tunisia. The extraction efficiency was assessed by determining the total phenolic content (TPC), total flavonoid content (TFC), condensed tannins (TC), anthocyanin content, and antioxidant capacity. A one-way ANOVA analysis revealed a significant influence of solvent type on the extraction yield of these bioactive compounds. Among the five tested solvents, methanol and acetate demonstrated the highest extraction efficiency, yielding the highest concentrations of TPC, TFC, anthocyanins, and antioxidant activity. In contrast, chloroform was particularly effective for extracting condensed tannins, whereas water and hexane exhibited the lowest extraction efficiency. These findings emphasize the crucial role of solvent selection in optimizing the extraction of bioactive compounds from *Pisum sativum* seeds, which can enhance their nutritional and medicinal value.

1. INTRODUCTION

Pisum sativum, commonly known as pea, is an annual herbaceous plant from the Fabaceae family that originated in the Mediterranean region. It has become one of the most widely cultivated and consumed legumes globally due to its rich nutritional profile, versatility in food preparation, and contribution to sustainable agriculture (McPhee, 2003). Peas are highly valued for their nutrient-rich composition, providing high-quality proteins, essential amino acids, dietary fiber, and a variety of vitamins and minerals such as vitamin C, vitamin K, folic acid, and iron (Zhao et al., 2014). Additionally, their low glycaemia index and of bioactive compounds richness, including polyphenols, flavonoids, and saponins, contribute to numerous health benefits, such as improved blood glucose regulation, cholesterol reduction, oxidative stress mitigation, and anti-inflammatory effects (Kumari et al., 2021). Regular consumption of peas has also associated with a lower risk of chronic diseases, including cardiovascular diseases, type 2 diabetes, and certain types of cancer (Dahl et al., 2012). Despite these benefits, pea cultivation, research, and industrial applications remain underdeveloped in some areas, limiting its full potential as a dietary and agricultural resource.

Beyond their nutritional value and health benefits, peas are also rich in bioactive compounds, particularly phenolic compounds, which contribute to their antioxidant and protective properties. Therefore, the choice of extraction solvent significantly influences the yield, composition, and bioactivity of phenolic compounds in peas (*Pisum sativum* L.). Different solvents, such as water, ethanol, methanol, acetone, and their mixtures, differ in polarity, affecting the solubility and extraction efficiency of phenolics. Polar solvents such as methanol and ethanol are commonly used for the

extraction of a wide range of phenolic compounds, including flavonoids and phenolic acids, due to their ability to dissolve both hydrophilic and moderately lipophilic compounds. Acetone and ethyl acetate, on the other hand, are more effective for less polar phenolics (Hapsari et al., 2022; El Mannoubi et al., 2023). Additionally, aqueous-organic solvent mixtures enhance extraction efficiency by improving solubility and reducing degradation of thermo sensitive phenolics. The solvent type also impacts the antioxidant activity of the extracted phenolics, as different phenolic compounds exhibit varying solubility in different solvents. Therefore, optimizing the solvent composition is crucial for maximizing the recovery of bioactive phenolic compounds from peas for potential applications in food and pharmaceutical industries (Kaczorová et al., 2021; Lee et al., 2024).

The objective of this study is to extract phenolic compounds from local population of pea (*Pisum sativum* L.) cultivated in the arid region of Southern Tunisia using different solvents, including hexane, chloroform, methanol, acetate, and water, to evaluate their extraction efficiency and determine the most effective solvent for maximizing phenolic yield and bioactivity.

2. MATERIAL AND METHODS

2.1. Plant material

In 2019, seeds from a local *Pisum sativum* L. population were collected from the Arid Lands Institute in Medenine, Tunisia (33°29'58.00"N, 10°38'30.00"E, 16 m elevation). Prior to initiating the experiments, a germination test was conducted for each seed lot to assess viability. Only seed lots with germination rate above [e.g., 85%] were used in the study to ensure experimental reliability. After being thoroughly washed, the seeds were dried in an oven at 50 °C for 72 hours. Once dehydrated, they were ground into a fine powder and stored in airtight glass containers at room temperature (24 ± 2 °C) until further analysis.

2.2. Preparation of extract

To extract phenolic compounds, five solvents with different polarities (water, hexane, chloroform, ethanol, and methanol) were used. Initially, 2 g of powdered pea seeds were macerated in 20 mL of the selected solvent. The mixture was then incubated in a water bath at 40 °C for 20 minutes. After extraction, the solution was filtered through Whatman No. 1 filter paper and subsequently centrifuged at 2500 rpm for 15 minutes. To enhance extraction efficiency, the procedure was repeated. The extraction was performed separately for each solvent, not successively. After each extraction, the corresponding solvent was evaporated to dryness using a rotary evaporator under reduced pressure to obtain the crude extract. Finally, the yield was stored in amber bottles at 4 °C for further analysis.

The extraction yield of each solvent was calculated by weighing the dried extract obtained after evaporation and expressing it as a percentage of the initial dry weight of the plant material using the formula: Yield (%) = (Weight of extract / Weight of dry sample) × 100.

2.3. Total polyphenols content (TPC)

The determination of total polyphenol content (TPC) followed the method described by Singleton and Rossi (1965). Initially, 20 µL of the plant extract was mixed with 100 µL of Folin-Ciocalteu's reagent and allowed to react in the dark for 3 minutes. Subsequently, 300 µL of sodium carbonate solution (20% m/v) was added, and the final volume was adjusted to 2 mL with deionized water. The mixture was then thoroughly stirred and incubated in the dark at room temperature for 2 hours. After the incubation period, the absorbance was measured at 765 nm using a blank sample, prepared in the same way but with the corresponding solvent replacing the plant extract. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry plant material.

2.4. Total flavonoid content (TFC)

To determine the total flavonoid content, the colorimetric method described by Djeridane et al. (2006) was employed. A standard calibration curve was prepared using quercetin at a concentration of 5 g/L, with a range of 5 to 200 mg/mL. A volume of 1 mL from either the plant extract or the quercetin standard solution was combined with 1 mL of a freshly prepared 2% aluminum chloride ($AlCl_3$) solution. After allowing the reaction to proceed at room temperature for 10 minutes, absorbance measurements were recorded at 430 nm using a Shimadzu UV-1600 spectrophotometer. A blank sample, prepared by replacing the plant extract with the corresponding solvent, served as a reference. The total flavonoid content was quantified and expressed as milligrams of

quercetin equivalent per gram of dry weight (mg QE/g DW).

2.5. Condensed tannin content (TC)

The condensed tannin content was assessed using the vanillin assay, as outlined by Broadhurst and Jones (1978). In this method, 250 µL of the extract was mixed with 3 mL of a 4% methanolic vanillin solution, followed by the addition of 150 µL of concentrated sulfuric acid (HCl). The reaction mixture was then incubated at 30°C for 20 minutes. After the incubation period, absorbance was measured at 500 nm using methanol as a blank. Catechin was used as a reference standard for constructing the calibration curve (25–300 mg/L). The tannin content in the extracts was expressed in terms of catechin equivalents (mg CE/g DW) relative to the dried plant material.

2.6. Total Anthocyanin content (TAC)

To determine the anthocyanin content (TAC) in pea seed extracts, the pH differential method is employed. First, two buffers are prepared: one at pH 1.0 (0.025 M potassium chloride) and another at pH 4.5 (0.4 M sodium acetate). In separate test tubes, 100 µL of pea seed extract is combined with 900 µL of each buffer and left to equilibrate for 15 minutes at room temperature, shielded from light. After equilibration, the absorbance of both solutions is measured at 520 nm and 700 nm using a spectrophotometer. The absorbance difference is calculated by subtracting the absorbance at pH 4.5 from that at pH 1.0. The total anthocyanin content is then determined using the following formula:

$$\text{TAC (mg cyanidin} - 3 - \text{glucoside equiv per } gDW) = \frac{A \times MW \times DF \times 1000}{L \times \varepsilon}$$

Where A is the absorbance difference, MW represents the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, ε is the molar extinction coefficient for cyanidin-3-glucoside (26,900 L/mol·cm), and L is the path length of the cuvette (1 cm). The result is expressed as mg cyanidin-3-glucoside equivalent per gram of dry weight of the pea seed extract.

2.7. Assay of antioxidant activity DPPH

The DPPH radical scavenging activity of the extracts was assessed using a modified version of the Brand-Williams et al. (1995) method. To initiate the reaction, 1 mL of the extract at varying concentrations was combined with 2 mL of a methanolic DPPH solution (10^{-4} M). The concentrations tested for each solvent extract were as follows: water (50, 100, 150, 200 µg/mL), methanol (25, 50, 75, 100 µg/mL), ethyl acetate and chloroform (20, 40, 60, 80 µg/mL), and hexane (10, 20, 30, 40 µg/mL). The mixture was then gently stirred and allowed to stand in the dark for 30 minutes. Following incubation, the absorbance was recorded at 515 nm using a blank as a reference. A lower absorbance value corresponded to a higher free radical scavenging activity. The percentage of DPPH inhibition was calculated to determine the antioxidant potential of the extracts:

$$\% \text{ inhibition} = [(A0 - A1) / A0] \times 100$$

where A0 represents the control absorbance, and A1 represents sample absorbance. The calibration curve between the inhibition percentage and the concentration of Trolox (100–2000 µM) was then formed, and the results are expressed in µmoL TEAC/g DW.

2.8. Statistical analysis

The data obtained were statistically analyzed using the SPSS 20 software package. Statistical significance was considered at $p < 0.05$, and Duncan's new multiple range test was applied. The results for all analyzed compounds were expressed as the mean values with standard deviations (SD), and the data are presented as Mean ± SE.

3. RESULTS

3.1. Total polyphenol content

The extraction yields (Table 1) varied significantly among solvents, with methanol (17.8 ± 0.2%) and water (16.2 ± 0.1%) showing the highest yields, while hexane had the lowest (10.5 ± 0.15%). The Fig. 1 illustrated the total polyphenol content extracted from a local pea population using different solvents: water, acetate, methanol, chloroform, and hexane. The results revealed significant differences in extraction efficiency between these solvents. Methanol exhibited the highest extraction efficiency, yielding significantly greater polyphenol content (29.23 mg GAE/100g DW) than the other solvents. Acetate (26.18 mg GAE/100g DW) and chloroform (24.66 mg GAE/100g DW) displayed intermediate extraction capacities, with no significant difference between them. In contrast, water (19.27 mg GAE/100g DW) and hexane (21.17 mg GAE/100g DW) showed the lowest polyphenol extraction yields. These findings highlighted methanol as the most effective solvent for

extracting polyphenols from peas, likely due to its high polarity and ability to dissolve a broad range of phenolic compounds.

Table 1. Extraction yield of pea extracted with different solvents.

Solvent	Yield (%)
Methanol	17.8±0.2 [a]
Water	16.2±0.1 [ab]
Acetae	14.8±0.05 [c]
Chloroform	13.1±0.1 [cd]
Hexane	10.5±0.15 [d]

The data are presented as mean values ± standard error (n = 3). Superscript lowercase letters (a–d) indicate homogeneous sub-classes as determined by ANOVA (p < 0.05) using Duncan's new multiple range test.

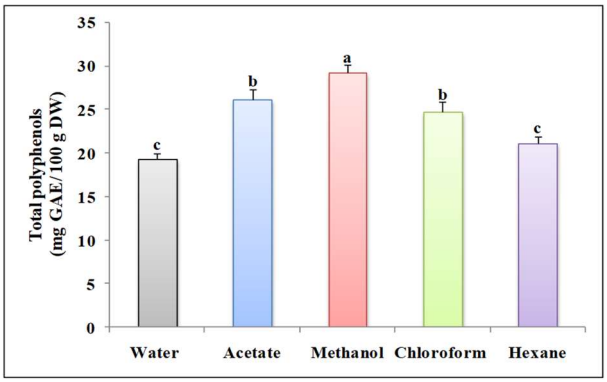

Fig. 1. Total polyphenol content extracted from a local pea population cultivated in the arid region of southern Tunisia using different solvents.

The data are presented as mean values ± standard error (n = 3). Superscript lowercase letters (a–d) indicate homogeneous sub-classes as determined by ANOVA (p < 0.05) using Duncan's new multiple range test. GAE: Gallic Acid Equivalent.

3.2. Total flavonoid content

The Fig. 2 presented the total flavonoid content extracted from a local pea population cultivated in the arid region of Southern Tunisia using five different solvents. Among these, acetate demonstrated the highest extraction efficiency, yielding the greatest flavonoid content (13.45 mg QE/100 g DW). Methanol also showed a strong extraction capacity (11.18 mg QE/100 g DW), followed by chloroform (10.22 mg QE/100 g DW), which exhibited an intermediate efficiency. In contrast, hexane (8.43 mg QE/100 g DW) and water (7.84 mg QE/100 g DW) were the least effective, with water yielding the lowest flavonoid content. These results suggested that acetate is the most suitable solvent for flavonoid extraction, likely due to its high polarity and strong affinity for flavonoid compounds. Furthermore, the observed variations in extraction efficiency highlighted the influence of solvent properties on the solubility and extraction of flavonoids from pea samples.

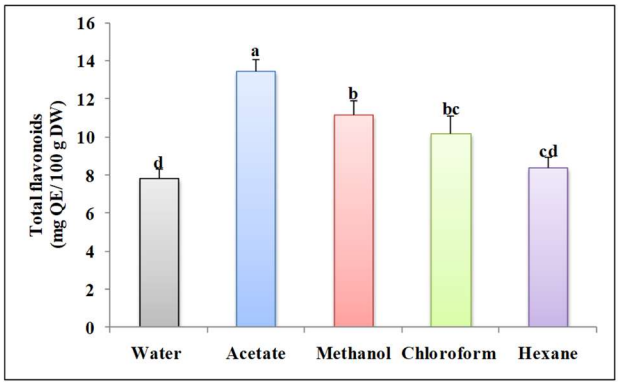

Fig. 2. Total flavonoid content extracted from a local pea population cultivated in the arid region of southern Tunisia using different solvents.

The data are presented as mean values ± standard error (n = 3). Superscript lowercase letters (a–d) indicate homogeneous sub-classes as determined by ANOVA (p < 0.05) using Duncan's new multiple range test. QE: Quercetin Equivalent.

3.3. Condensed tannin content

The Fig. 3 presented the condensed tannin content extracted from the local pea population using different solvents. The results revealed that chloroform was the most efficient solvent, yielding the highest tannin concentration (17.41 mg CE/100 g DW). Acetate (14.82 mg CE/100 g DW) and methanol (14.11 mg CE/100 g DW) showed comparable extraction capacities, with no significant difference between them. Hexane (11.36 mg CE/100 g DW) extracted a moderate amount of tannins, whereas water yielded the lowest concentration (9.11 mg CE/100 g DW). These variations highlighted the impact of solvent effect on tannin extraction, with chloroform proving to be the most effective due to its strong ability to dissolve tannin compounds.

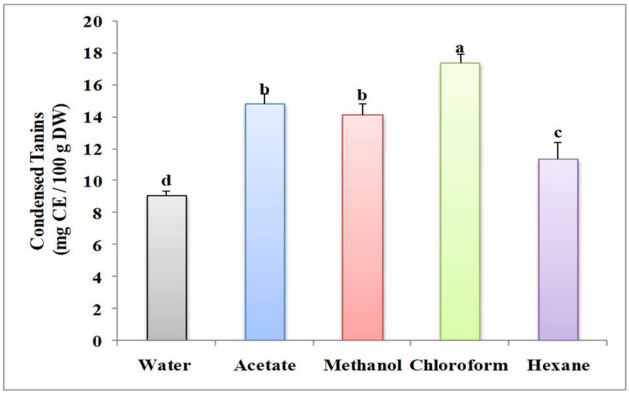

Fig. 3. Condensed tannin content extracted from a local pea population cultivated in the arid region of southern Tunisia using different solvents.

The data are presented as mean values ± standard error (n = 3). Superscript lowercase letters (a–d) indicate homogeneous sub-classes as determined by ANOVA (p < 0.05) using Duncan's new multiple range test. CE: catechin equivalent.

3.4. Anthocyanin content

The Fig. 4 illustrated the anthocyanin content extracted from the local pea population using different solvents. Acetate showed the highest extraction efficiency (7.42 mg cyanidin-3-glucoside/ g DW), followed by methanol (6.55 mg cyanidin-3-glucoside/ g DW), which exhibited a slightly lower but comparable yield. Chloroform (5.69 mg cyanidin-3-glucoside/ g DW) and hexane (4.74 mg cyanidin-3-glucoside/ g DW) showed moderate extraction capacities, while water resulted in the lowest anthocyanin content (4.23 mg cyanidin-3-glucoside/ g DW). The observed differences highlighted the influence of solvent polarity on anthocyanin solubility, with acetate emerging as the most effective solvent, likely due to its ability to extract a broader range of anthocyanin compounds. These findings emphasized the importance of selecting an appropriate solvent for optimizing anthocyanin extraction from plant materials.

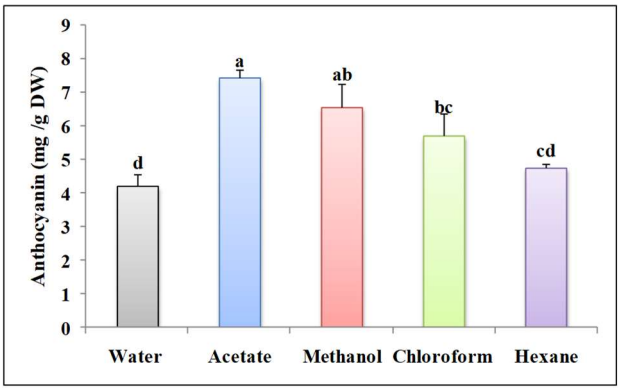

Fig. 4. Anthocyanin content extracted from a local pea population cultivated in the arid region of southern Tunisia using different solvents.
The data are presented as mean values ± standard error (n = 3). Superscript lowercase letters (a–d) indicate homogeneous sub-classes as determined by ANOVA (p < 0.05) using Duncan's new multiple range test.

3.5. Antioxidant activity (DPPH)

The Fig. 5 presented the DPPH radical scavenging activity (expressed as µmol TEAC/g DW) of the obtained extracts from the local pea population using different solvents. Methanol exhibited the highest antioxidant activity (7.58), closely followed by acetate (6.82), with no statistically significant difference between them. Chloroform demonstrated a moderate antioxidant potential (6.11), while hexane (5.33) and water (4.25) showed the lowest DPPH scavenging activity. These results suggested that methanol and acetate are the most effective solvents for extracting antioxidants from peas, likely due to their ability to dissolve a wide range of bioactive compounds with strong radical-scavenging properties.

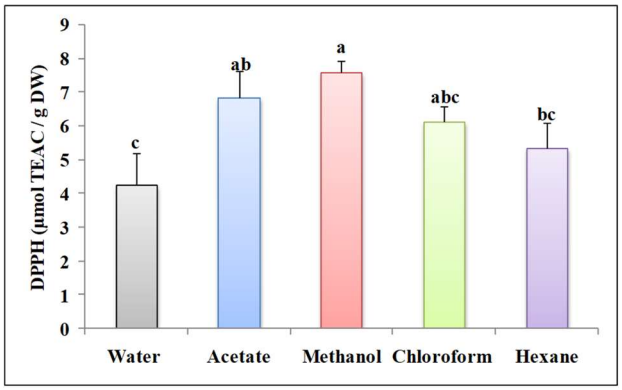

Fig. 4. DPPH radical scavenging activity extracted from a local pea population cultivated in the arid region of southern Tunisia using different solvents.
The data are presented as mean values ± standard error (n = 3). Superscript lowercase letters (a–d) indicate homogeneous sub-classes as determined by ANOVA (p < 0.05) using Duncan's new multiple range test. TEAC: Trolox Equivalent Antioxidant Capacity.

4. DISCUSSION

The richness of polyphenols in pea legumes has generated interest in their potential use as natural food additives. However, optimizing the extraction process is essential to maximize yield, maintain polyphenol stability, and ensure cost-efficiency. Polyphenol extraction from plants is a crucial step in their recovery and can be carried out using various methods, solvents, and extraction parameters. Common extraction techniques include solvent extraction, ultrasound-assisted extraction, enzyme-assisted extraction, and supercritical extraction, all of which have been employed for isolating polyphenols from peas (Alara et al., 2021; Lungade et al., 2024; Palos-Hernández et al., 2025). Several solvents, such as water, methanol, acetate, chloroform, and hexane, have been widely used in different studies (Al-Alwani et al., 2014; Hepsibah et al., 2016; Jacobsen et al., 2019; Truong et al., 2021). The choice of solvent depends primarily on the chemical nature of the polyphenols being extracted (Naczk and Shahidi, 2004; Ignat et al., 2013). Given that polyphenols vary from simple molecules to complex polymerized compounds and often form complexes with other food components, their polarity and solubility differ significantly (Reis Giada et al., 2013; Chen et al., 2022; Palaiogiannis et al., 2023). Consequently, the most effective solvent is one that ensures maximum solubility of the targeted polyphenols.

Additionally, factors such as sample particle size, pH, extraction time, and temperature also influence the extraction efficiency (Gil-Martín et al., 2022; Shi et al., 2022).

This study assessed the efficiency of various solvents in extracting antioxidant compounds from a local pea population grown in the arid region of southern Tunisia. Five solvents were used for the extraction: methanol, water, acetate, chloroform, and hexane to determine their effectiveness in isolating total phenolic content (TPC), total flavonoid content (TFC), total condensed tannins (TC), total anthocyanins (TAC), and DPPH radical scavenging activity. The results indicated that methanol and acetate achieved the highest extraction yields, followed by chloroform, while hexane and water exhibited the lowest efficiency across all analyses. The extraction efficiency therefore dependsprimarily depends on the polarity of the solvent and the solubility of the compounds within it. Our results are consistent with the findings of Zhao et al. (2006), who showed that methanol and ethyl acetate performed better than other solvents in extracting phenolic compounds. This is likely due to their higher polarity and superior solubility for phenolic constituents in plant materials. In this study, methanol exhibited the highest extraction efficiency for phenolic compounds from the local pea population. Similarly, Hadrich et al. (2014) reported that the antioxidant activity of pea extracts varied depending on solvent polarity, with methanolic extracts displaying the strongest antioxidant potential. Methanol is commonlyused for extracting medium-polar and polar phenolic compounds, including flavonoid glycosides and phenolic acids (Chávez-González et al., 2020).

Furthermore, our findings demonstrated that chloroform was particularly effective in extracting condensed tannins. This observation was in line with previous studies indicating that chloroform, as a moderately polar solvent, plays a crucial role in the extraction of specific antioxidant compounds, especially condensed tannins and other non-polar or semi-polar phenolic compounds (Lahmar et al., 2023). While it is less efficient than highly polar solvents like methanol and ethanol for extracting total phenolic content (TPC), it has been shown to selectively isolate specific bioactive compounds with antioxidant properties. Its capacity to dissolve lipophilic antioxidants makes it particularly useful for targeting plant extract fractions that may not be effectively extracted by polar solvents (Fatiha et al., 2012; Rajauria et al., 2019; Kebu et al., 2024). However, due to its toxicity, chloroform is often combined with other solvents to enhance both extraction efficiency and safety (Mitra and Mishra, 2019; Kumar et al., 2023). Conversely, water and non-polar solvents such as hexane showed lower extraction efficiency, likely due to the limited solubility of phenolic compounds in these solvents (Carré et al., 2024).

In practical applications, the selection of a suitable solvent should not only consider extraction efficiency but also factors such as toxicity, cost, and environmental impact (Hashemi et al., 2024). Methanol, while highly effective, poses toxicity concerns, whereas water presents a safer alternative for food and pharmaceutical industries. Water-based extraction methods, though less efficient, are preferable for sustainable and green extraction approaches (Awad et al., 2022).Therefore, choosing the right solvent is crucial for optimizing the extraction of bioactive compounds and harnessing their full antioxidant potential.

5. CONCLUSIONS

The efficiency of bioactive compound extraction strongly dependson the choice of solvent. In this study, polar solvents such as methanol and acetate yielded the highest amounts of polyphenols and flavonoids, along with the strongest antioxidant activities. This can be attributed to their polarity, which enhances the solubility of phenolic acids and other polar bioactive compounds. Conversely, water and non-polar solvents like hexane demonstrated significantly lower extraction efficiencies. These findings highlight the crucial role of solvent selection in optimizing the extraction process. To further improve the phenolic compound yield while maintaining environmental sustainability and health safety, future research should focus on refining extraction parameters and exploring solvent combinations.

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