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OPTIMIZATION OF EXTRACTION WITH SOLVENT VARIATION BASED ON TOTAL FLAVONOID CONTENT IN WUNGU LEAVES (*Graptophyllum pictum (L.) Griff*): A LITERATURE REVIEW

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ABSTRACT
There are many studies analyzing the flavonoid content based on the total flavonoid content (TFC) of wungu leaves through the extraction process with different extraction solvents. So, this review using literature review methodology is aimed to find out the type of extraction solvent that has the most optimum condition based on the highest TFC. The databases for collecting data involved PubMed, Scopus, and Google Scholar. Temporary, the results based on some previous studies vary greatly. Two of four selected articles showed that the highest TFC was found in semi-polar solvents; ethyl acetate and acetone. It is suspected that the samples contain more aglycones (a subtype of flavonoids) that can be soluble in semi-polar solvents. Whereas, the two others showed that the nonpolar solvent (hexane) had the highest TFC. It is suspected that the samples contain more subtypes of flavonoids called isoflavones, flavanones, flavones, and flavonols that can be soluble in nonpolar solvents. To get the appropriate and best standardized extract of wungu leaves, re-examination of this optimization based on the highest TFC in further research is necessary by using different extraction solvents that are equally polar like water, methanol, and ethanol; because the flavonoids as secondary metabolites in plants are basically polar.

KEYWORDS: Antioxidant, Extraction, *Graptophyllum pictum*, Solvent, Total Flavonoid Content

INTRODUCTION

Wungu plant (*Graptophyllum pictum* (L.) *Griff*), a native caricature plant from Indonesia, is classified into the *Acanthaceae* family and known as a traditional Indonesian medicinal herb for treating some diseases naturally by Indonesian society (Kusumawati *et al.*, 2022; Makkiyah *et al.*, 2023). This plant is well-known as the Ungu (Purple) Plant or Handeuleum in Indonesia; Joseph's coat in English (Singh, 2015). The part of this plant that is often used and most beneficial as traditional medicine ingredient is the leaves. Wungu leaves had been widely used to treat various diseases and signs or symptoms such as constipation, hemorrhoid, urinary infection, rheumatism, scabies, ear diseases, swelling, smoothing skins wounds, and hepatomegaly (Goswami, Ojha and Mehra, 2021). The leaves can be popular as folk remedy because of its pharmacological activity such as antioxidant, immunomodulator, antiinflammatory, antidiabetic, antihemorrhoid, antibacterial, antifungal, analgesic, estrogenic, and wound healing. In addition, the leaves contain flavonoid, tannins, alkaloids, glycosides, anthraquinones, carbohydrates, coumarins, and saponins compounds (Jiangseubchatveera *et al.*, 2017).

Flavonoids are the largest group of phenolic or polyphenol compounds that are a natural source of antioxidants for the human body (Jiangseubchatveera *et al.*, 2017). The antioxidants are stable molecules that protect the body cell from the threat of free radicals, which are associated with diseases such as stroke, heart disease, and cancer (Sharifi-Rad *et al.*, 2020). Furthermore, flavonoids have the potential to prevent the occurrence of fat accumulation as well so that this compound is able to deal with the obesity problem that is the cause of diabetic mellitus (Anwar,

Fadlillaturrahmah and Sari, 2017). Flavonoids and their natural antioxidant activity are necessary because they contribute positively to human health (Carey, Lucey and Doyle, 2021; Gasmi *et al.*, 2022).

The secondary metabolite compounds, flavonoids, are obtained from plant extracts through the extraction process. Extraction is the process of separating a substance from a mixture using solvent. The extraction process is influenced by many factors; extraction time length, extraction temperature, speed of stirring, ratio between solvent ratio and dissolved sample, and solvent type (Anggista *et al.*, 2019). The type of solvent is one of the most important factors affecting the efficiency of separating flavonoid compounds from plant sources (Fadil, Lebrazi and Rais, 2022; Marliani, Artika and Nurcholis, 2022). It affects the obtaining of the active substance contents and the use of the best solvent will guarantee an optimal extraction process (Noviyanty, Salingkat and Syamsiar, 2019). The selection of extraction solvent is based on the polarity of the targeted compounds that will be extracted at the time of extraction. Polar compounds will be soluble in polar solvents such as ethanol, methanol, butanol, and water. Nonpolar compounds can be soluble in nonpolar solvents, such as n-hexane, chloroform, and ether (Leksono *et al.*, 2018). Another polarization characteristic of solvents is semipolar; such as acetone and ethyl acetate. It can dissolve polar compounds as well. The secondary metabolite compounds, such as flavonoids, can be soluble in polar and semipolar solvents (Afrellia and Puspitasari, 2023). So, the polar and semipolar solvents should be chosen to be used at the time of extraction when flavonoid compounds will be extracted, because flavonoid is polar (Tensiska *et al.*, 2020).

In terms of support the Indonesian government's national program in the development of the biopharmaceutical industry, plant exploration by determining standardized extracts of the wungu leaves is essential so it could be used as a raw material for traditional medicine for diseases that could potentially be treated by this plant as explained earlier. There are many studies investigating and analyzing the flavonoid content of wungu leaves (*Graptophyllum pictum (L.) Griff*) with different extraction solvents. Thus, this review was conducted to find out the type of extraction solvent that has the most optimum conditions based on the highest value of total flavonoid content for obtaining a standardized wungu leaves extract by summarizing the relevant studies.

MATERIALS AND METHODS

The methodology of this study is literature review. Databases of PubMed, Scopus, and Google Scholar were utilized for collecting data with keywords "*Graptophyllum pictum*" AND ("extract" OR "extraction") AND "flavonoid". The inclusion criteria of selected articles are Indonesian and English articles, published in the period 2014-2024, openly accessible in full text, not limited to specific regions or countries, using maceration method for extraction in study, and available for the result of total flavonoid content measurement. There were 691 articles totally screened by given keyword on the three databases. Then, four articles were selected and identified according to the inclusion criteria, which of these articles match with the topic and purpose of this review.

RESULTS

The findings of the article that discusses about flavonoid compounds in wungu leaves and the determination of the total flavonoid content are presented as follows.

Table 1. The Relevant Articles

No.	Authors, Year	Title	Method	Result
1.	(Makkiyah *et al.*, 2023)	Optimization of *Graptophyllum pictum* Leaves Extraction Using A Simplex Centroid Design Focused on Extracting Flavonoids with Antioxidant	True Experimental Method of extraction: maceration	Acetone extract reached the optimum condition for obtaining TFC with a TFC concentration of 14.7137 mg QE/g DW.

		Activity		
2.	(Makkiyah, Rahmi and Setyaningsih, 2022)	Phytochemical Test and Acute Safety Evaluation of Oral Purple Leaves (*Graptophyllum Pictum L. Griff*) Extract in Rats	True Experimental Method of extraction: maceration	TFC was higher in hexane extract (6.24%) than ethanol (2.66%).
3	(Salim *et al.*, 2021)	Kadar Fenolat Flavonoid Si Ungu Mentawai (*Graptophyllum pictum (L.) Griff*)	True Experimental Method of extraction: maceration	The highest TFC was found in ethyl acetate fraction 3000 µg/mL (48.47 mgRE/g extract); followed by ethanol extract, water fraction, and chloroform fraction.
4.	(Jiangseubchatveera *et al.*, 2017)	Phytochemical Screening, Phenolic and Flavonoid Contents, Antioxidant and Cytotoxic Activities of Graptophyllum pictum (L.) Griff	True Experimental Method of extraction: maceration	The highest TFC was found in hexane fraction (2821± 0.04 mg QE/g extract); followed by ethyl acetate, butanol, and aqueous fraction.

Abbreviations: TFC = total flavonoid content; mgQE/g DW = milligrams of quercetin equivalent per gram of dry weight; RE = routine equivalent.

DISCUSSION

The Findings of Included Articles

1. The Variety of Extraction Solvents

   There are many solvents used in each study during the process of extraction. A combination of solvents can increase the selectivity of flavonoid extraction (Fadil, Lebrazi and Rais, 2022; Marliani, Artika and Nurcholis, 2022). The extraction solvents were different in types of polarity. They consist of polar, semi-polar, and nonpolar solvents. The first article used polar solvents such as ethanol, methanol, and water; and semi-polar solvent, namely acetone. The second article used ethanol as polar solvent and hexane as non-polar solvent. The third one used ethanol and water as polar solvent, ethyl acetate as semipolar, and chloroform as nonpolar solvent. The last one used polar solvents like water and butanol, semipolar namely ethyl acetate, and nonpolar solvent namely hexane.

2. The Method of Total Flavonoid Content Determination

   Two studies from (Makkiyah *et al.*, 2023) and (Makkiyah, Rahmi and Setyaningsih, 2022) had determined the value of total flavonoid content in each extract after the extraction process. Both of these articles can be equally known which type of solvent is capable of attracting the flavonoid content maximally. Nevertheless, the way in determining the optimum solvent is different. The study in 2022, the optimum condition of the extraction solvent was viewed by the spectrophotometry UV/Vis measurement method based on the highest value of the extract absorbance and total flavonoid content that was contained in the extract. Spectrophotometry UV Visible is a technique that measures the light intensity as a beam of light passes through sample solution to determine how much a chemical compound absorbs light (Suhartati, 2017). Whereas, the study of 2023, the optimum condition was viewed by the simplex centroid method based on color contours; yellow to red indicates the higher response point. The simplex centroid technique is one of the most popular mixture designs that can be combined with a flavonoid extraction technique as a strategy to increase

the optimization and selectivity acquired for the target analysis of plants (Wibisono *et al.*,

2019).

According to the (Salim *et al.*, 2021) and (Jiangseubchatveera *et al.*, 2017), the determination of total flavonoid content was done after the process of fractionation. Its process was done gradually after the extraction. The first step was extraction using a maceration method by soaking in ethanol solvent until extract can be obtained. Then, the extract was fractionated by adding the selected solvents gradually according to the order of increasing polarity, starting from nonpolar until the least is polar solvent (Abubakar and Haque, 2020).

3. The Results

Two articles showed that the highest TFC was found in semi-polar solvents: ethyl acetate and acetone (Salim *et al.*, 2021; Makkiyah *et al.*, 2023). It is hypothesized that the samples contain more aglycones that are maximally attracted to semi-polar solvents. The others showed that hexane as the nonpolar solvent had the highest TFC. It is hypothesized that the samples contain more isoflavones, flavanones, flavones, and flavonols that are maximally attracted to non-polar solvents. There is a theory for supporting those hypotheses. Subtypes of less polar flavonoid compounds such as isoflavones, flavanones, flavones, and flavonols are better extracted with non-polar solvents (ether, chloroform and n-hexane), and the subtypes of more polar flavonoids such as glycosides and aglycones are better extracted using polar solvents such as alcohol and water-alcohol combination (Aryal *et al.*, 2019; Muflihah, Gollavelli and Ling, 2021). In addition, aglycone also can easily soluble in semi-polar solvents such as ethyl acetate, chloroform, and ether (Afrellia and Puspitasari, 2023).

CONCLUSIONS

Based on the gathered studies above, the results are variative, so the optimal extraction solvent type to be used in the wungu leaf extraction process is currently undetermined. This issue is needed to re-examine in further research about analysis of flavonoids subtypes that are contained in wungu leaves and the optimization of extraction solvent to get the best standardized extract of wungu leaves in the future. But, it is suggested that the extraction solvents should be given in a variety that are equally polar; for example is like using water, methanol, and ethanol as extraction solvents; because the flavonoids as secondary metabolites in plants are basically polar.

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Conflict of Interest

The authors affirm that no competing interests exist.

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