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ULTRASOUND-ASSISTED EXTRACTION OF *Pinus merkusii* NEEDLES YIELDS HIGHER TOTAL PHENOLICS, FLAVONOID AND STRONG ANTIOXIDANT ACTIVITY COMPARED TO CONVENTIONAL AND KINETIC MACERATION

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ABSTRACT

Pine (*Pinus merkusii*) needles are an abundant forest biomass that remains largely underutilised despite containing high-value phenolic compounds with antioxidant potential. This study systematically compared three extraction methods conventional$_i$ maceration, kinetic maceration, and ultrasound-assisted extraction(UAE) to obtain extracts with optimal yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. Dried pine needles were extracted using each method, followed by quantitative evaluation of TPC (Folin Ciocalteu), TFC (aluminium chloride colourimetry), and DPPH radical scavenging activity (IC_{50}). The results showed that the UAE produced the highest extraction yield (12.33%), followed by kinetic maceration (9.50%) and conventional maceration (8.72%). UAE also exhibited superior TPC (36.488 ± 3.134 mg GAE/g) and TFC (333.118 ± 45.772 mg QE/g) compared to kinetic maceration (TPC: 30.616 ± 1.964; TFC: 262.151 ± 26.074) and conventional maceration (TPC: 27.737 ± 3.390; TFC: 206.237 ± 29.565). Antioxidant activity was very strong for UAE (IC_{50} = 39.255 ± 4.468 ppm) and kinetic maceration (43.250 ± 2.567 ppm), whereas conventional maceration showed weak activity (193.045 ± 12.614 ppm). These findings demonstrate that UAE is the most efficient method for recovering bioactive phenolics and flavonoids from P. merkusii needles, producing extracts with high yield and strong antioxidant capacity. This study provides valuable data for optimizing the utilisation of pine needle waste into value-added natural antioxidant products.

Keywords: Ultrasound-Assisted Extraction (UAE), Phenolic, Flavonoid, Antioxidant, Pinus Merkusii

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INTRODUCTION

Pine (*Pinus merkusii*) is a plant species belonging to the Pinaceae family and is widely distributed in tropical and subtropical regions, including Indonesia.[1,2] This plant is primarily utilised as a source of timber, oleoresin, and resin, which possess high economic value.[3,4] Pine needles contain a variety of secondary metabolites, including flavonoids, tannins, saponins, alkaloids, phenolic compounds, and essential oils, which exhibit notable biological activities.[5,6] Phenolic compounds represent the largest class of natural antioxidants in plants.[7] These compounds possess one (phenols) or more (polyphenols) phenolic rings, characterized by hydroxyl groups attached to aromatic rings, enabling them to donate hydrogen atoms and readily neutralise free radicals through oxidation reactions.[8,9] Naturally occurring phenolic compounds are predominantly polyphenols, commonly found in the form of ethers, esters, or glycosides, including flavonoids, tannins, tocopherols, coumarins, lignins, cinnamic acid derivatives, and multifunctional organic acids. In pine needles, phenolic constituents are classified into several groups: (1) phenolic acids such as gallic acid, caffeic acid, ferulic acid, and p-coumaric acid; (2) flavonoids including quercetin, kaempferol, rutin, and catechin; (3) condensed tannins (proanthocyanidins); and (4) lignans.

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These phenolic$_i$ compounds contribute to various bioactivities, including antioxidant, anti-inflammatory, and antibacterial effects, and exhibit potential protective roles in cardiovascular and nervous$_i$ systems.[8,10] Several studies have$_i$ reported a strong correlation between total phenolic content in pine needle extracts and their antioxidant capacity, indicating that the extraction method plays a crucial role in determining extract quality.[8,11] Despite the known bioactivity of pine needles, the comparative effectiveness of different extraction methods specifically for Pinus merkusii needles remains insufficiently elucidated. While ultrasound-assisted extraction (UAE) has been studied for other plant materials, there is a lack of direct comparison between conventional maceration, kinetic maceration (without heating), and UAE for maximizing yield, total phenolics, total flavonoids, and antioxidant activity from P. merkusii needles. This research directly supports two Sustainable Development Goals. First, SDG-3 (Good Health and Well-being) by developing natural antioxidant extracts from pine needles as potential pharmaceutical and nutraceutical ingredients. Second, SDG-12 (Responsible Consumption and Production) by valorizing an abundant agricultural waste product (pine needles) into a high-value resource, thereby reducing environmental burden and promoting sustainable natural resource management. Extraction methods significantly influence extraction yield and total phenolic content obtained from dried pine needle materials. Three extraction techniques are evaluated in this study: ultrasound-assisted extraction (UAE), kinetic maceration without heating, and conventional cold maceration. UAE employs ultrasonic waves to enhance cell wall permeability and accelerate solvent penetration, often resulting in higher extraction yields and phenolic content within a shorter extraction time compared to conventional methods.[8,12] Kinetic maceration without heating employs variations in extraction time and phase dynamics to enhance diffusion while avoiding elevated temperatures, thereby making it suitable for thermolabile compounds. Conventional cold maceration, although widely used as a traditional technique, generally requires longer extraction durations and tends to produce lower extract yields. Recent comparative studies have demonstrated that Ultrasound-Assisted Extraction (UAE) offers higher efficiency in the extraction of phenolic compounds.[8] Based on these considerations, the present study aims to systematically evaluate variations in pine needle extraction using ultrasound-assisted extraction, kinetic maceration without heating, and conventional maceration techniques. In addition to their bioactive potential, the abundance of pine needles constitutes a significant rationale for this investigation. In pine forest ecosystems, substantial quantities of fallen needles are generated annually and are frequently regarded as low-value organic waste. Therefore, the valorization of pine needles not only has the potential to enhance their economic value but also contributes to sustainable natural resource management, in alignment with Sustainable Development Goal-12.

EXPERIMENTAL

Material

Pine needles (*Pinus merkusii*) were collected from the Wonosobo highland region, Indonesia. All chemicals and reagents used in this study were of analytical grade. Ethanol (70%), distilled water, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), glacial acetic acid (CH_3COOH), chloroform, zinc powder, magnesium powder, and ferric chloride ($FeCl_3$ 1%) were procured from Smart Lab (Indonesia Quercetin, used as a reference standard, was purchased from Sigma-Aldrich. Mayer's, Wagner's, and Dragendorff's reagents were employed for qualitative phytochemical screening.

Methods

This study was designed as an experimental investigation employing three extraction methods: conventional maceration, kinetic maceration, and ultrasound-assisted extraction. The extracts obtained from each method were evaluated based on extraction yield, total phenolic content, total flavonoid content, and antioxidant activity. Antioxidant activity was assesse$_i$ using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.[13] The results obtained from the different extraction methods were subsequently compared to identify the most effective extraction approach.

RESULTS AND DISCUSSION

The$_i$ results of pine needle extract obtained using three different extraction methods: conventional maceration, kinetic maceration, and ultrasound-assisted extraction (UAE) are presented as follows.

Organoleptic Evaluation

Organoleptic evaluation of the pine needle extracts was performed by assessing their color, texture, odour, and taste. The assessment aimed to provide a preliminary qualitative characterisation of the extracts obtained from each extraction method.[14] The results of the organoleptic evaluation are summarised in Table-1 and Fig.-1.

Table-1: Organoleptic Characteristics of Pine Needle Extracts

Extraction Method	Extraction Method	Extraction Method	Extraction Method	Extraction Method
Conventional maceration	Brown	Viscous	Characteristic	Bitter
Kinetic maceration	Brown	Viscous	Characteristic	Bitter
Ultrasound-assisted extraction(UAE)	Brown	Viscous	Characteristic	Bitter

a b c

Fig.-1: Pine Needle (*Pinus merkusii*) Extracts Obtained Using Different Extraction Methods: (a.) Conventional Maceration; (b.) Kinetic Maceration; and (c.) Ultrasound Assisted Extraction (UAE)

Extraction Yield

The extraction yields of pine needle extracts obtained through different extraction methods are presented in Table-2.

Table-2: Extraction Yield of Pine Needle Extracts

Extraction Method	Yield (%)
Conventional maceration	8.72
Kinetic maceration	9.50
Ultrasound-assisted extraction (UAE)	12.33

As shown in Table-2, the highest extraction yield was obtained using the ultrasound-assisted extraction (UAE) method. The yield obtained from the UAE exceeded 10%, indicating a favourable extraction efficiency compared to kinetic maceration and conventional maceration. This result suggests that variations in the extraction method and extraction time significantly influence extract mass and yield. The application of ultrasonic energy enhances solvent penetration and mass transfer, thereby improving extraction efficiency within a shorter processing time. Similar findings have been reported, indicating that extraction time efficiency plays a critical role in determining extract yield.[8]

Phytochemical Screening

Qualitative phytochemical screening was performed to identify the presence of major bioactive compound groups, including phenolics, flavonoids, tannins, alkaloids, terpenoids, and saponins. The results of the phytochemical screening are summarized in Table-3.

The phytochemical screening results demonstrated that all extraction methods successfully extracted the targeted classes of secondary metabolites. This finding indicates that variations in extraction techniques did not qualitatively alter the presence of major bioactive compound groups in pine needle extracts.[14]

Total Phenolic Content, Total Flavonoid Content, and Antioxidant Activity

The results of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity assays are presented in Table-4.

Table-3: Qualitative Phytochemical Profile of Pine Needle Extracts

Phytochemical Constituents	Conventional Maceration	Kinetic Maceration	UAE
Phenolics	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+

Note: (+) indicates the presence of the tested compound.

Table-4: Total Phenolic, Total Flavonoid Content, and Antioxidant Activity of Pine Needle Extracts

Extraction Method	Total Phenolics (mg GAE/g)	Total Flavonoids (mg GAE/g)	Antioxidant Activity (IC_{50}, ppm)
Conventional maceration	27.737 ± 3.390	206.237 ± 29.565	193.045 ± 12.614
Kinetic maceration	30.616 ± 1.964	262.151 ± 26.074	43.250 ± 2.567
UAE	36.488 ± 3.134	333.118 ± 45.772	39.255 ± 4.468

As shown in Table-4, significant differences were observed among the extracts obtained using different extraction methods. The extract produced by conventional maceration exhibited weak antioxidant activity, as indicated by a high IC_{50} value (193.045 ± 12.614 ppm), accompanied by lower total phenolic and flavonoid contents compared to the other methods.[13] In contrast, extracts obtained using kinetic maceration and UAE demonstrated very strong antioxidant activity, with IC_{50} values of 43.250 ± 2.567 ppm and 39.255 ± 4.468 ppm, respectively. UAE extract provides a natural alternative to synthetic antioxidants, supporting SDG-3 (Target 3.4) on reducing non-communicable diseases. Furthermore, converting discarded pine needles into a high-value extract reduces agricultural waste, aligning with SDG 12 (Target 12.5). These extracts also exhibited higher total phenolic and flavonoid contents. Antioxidant activity was evaluated using quercetin as a reference standard. Based on IC_{50} classification, antioxidant activity is considered very strong when $IC_{50} < 50$ ppm, whereas IC_{50} values > 100 ppm indicate weak or negligible activity.[13] These results suggest a strong correlation between total phenolic and flavonoid contents and antioxidant capacity. Consequently, pine needle extracts obtained via kinetic maceration without heating and ultrasound-assisted extraction can be classified as possessing very strong antioxidant activity. These findings are consistent with previous reports highlighting the effectiveness of optimized extraction techniques in enhancing the recovery of antioxidant compounds from plant materials.[13]

CONCLUSION

Based on the comparative evaluation of three extraction methods, it can be concluded that the pine needle (*Pinus merkusii*) extract obtained using ultrasound-assisted extraction (UAE) demonstrated the most optimal performance. The UAE method produced an extraction yield of 12.33%, a total phenolic content of 36.488 ± 3.134 mg GAE/g extract, and a total flavonoid content of 333.118 ± 45.772 mg GAE/g extract. In addition, the extract exhibited very strong antioxidant activity, with an IC_{50} value of 39.255 ± 4.468 ppm. This study supports SDG-3 (Good Health and Well-being) by providing a natural antioxidant source and SDG-12 (Responsible Consumption) by valorising pine needle waste into a high-value extract.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3:Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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