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Comparative study of bioactive compound content and antioxidant activity in different extraction methods of *Syzygium Polyanthum* leaves

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ABSTRACT

Background: Syzygium Polyanthum is classified among the 5,000 medicinal plant species documented in the Indonesian Medicinal Herb Index. It is utilised for a variety of purposes in traditional medicine and has potential health benefits. Extraction of medicinal plants is crucial to separate active plant materials or secondary metabolites. The aim of the study was to compare extracts of *Syzygium Polyanthum* Leaves using different methods: maceration and UAE in extracting flavonoids, phenolic compounds and antioxidant activity.

Methods: the bioactive component content and antioxidant activity of Syzygium polyanthum leaves extracted using two different methods—maceration and ultrasound-assisted extraction (UAE)—are compared. 96% ethanol was employed as the extracting solvent in the maceration and UAE extraction processes for S. polyanthum.The bioactive substances in both extracts were determined using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS).

Result: The amount of total phenolic, flavonoid, and IC50 in S. polyanthum leaves was evaluated between the maceration extraction method and UAE. Liquid chromatography-high Resolution Mass Spectrometry (LC-HRMS) analysis was utilized to detect the presence of bioactive compounds, then classified according to the PubChem database's taxonomy. There were comparable amounts of flavonoids in both extracts (60.194 mg QE/g and 60.074 mg QE/g). According to the DPPH assay, the total phenol for the extracts obtained by maceration and UAE was 94.63 ± 2.18 mg GAE/g and 122.87 ± 5.99 mg GAE/g extracts respectively, and the IC50 for the extracts obtained by maceration and UAE were 88.21 ± 1.38 µg/mL and 41.23 ± 6.11 µg/mL.

Conclusion: The findings of the study indicated that the ultrasonic-assisted extraction (UAE) approach yielded significantly greater quantities of total phenolic and flavonoid compounds, as well as exhibited stronger antioxidant activity, when compared to the maceration extraction method. The utilisation of the UAE approach resulted in a significant rise in the antioxidant activity of S. polyanthum leaves.

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INTRODUCTION

Syzygium polyanthum is a tropical plant distributed all over Asian Countries, including Indonesia, Malaysia, and Thailand. The Indonesian local name of *S. polyanthum varies* such as salam leaves, gowok, manting, kastolam, etc. depending on where it grows. The leaves of *S. polyanthum* are rich in therapeutic values for diarrhea, cataracts, hypercholesterolemia, diabetes mellitus, cardiovascular disease[1]. Besides, the fruits, roots, and barks of this plant are also commonly used for various traditional medicinal and non-medicinal purposes. The pharmacological properties of *S. polyanthum* are antioxidant, antidiabetic, antihypertensive, antimicrobial, antidiarrheal, anticancer, lipid-lowering, and dental plaque inhibition.[1,2]

There has been a lot of research showing the versatile application of *S. polyanthum* especially in promoting health benefits. The pharmaceutical effect of this plant is related to the content of the bioactive compounds which influence by several factors, such as extraction methods, solvent, and part of the plant used for extraction.[3] Using a suitable solvent and accepted extraction technique, extraction is a process to separate active substances or metabolites such as alkaloids, flavonoids, terpenes, and saponins.[4] Other than the plant component and extraction solvent, the extraction process is one of the fundamental factors that affect the quality of an extract that may best collect bioactive chemicals.[3]

Extraction is an important step in the process of obtaining phytochemical components from plant sources. Several extraction procedures, including maceration, percolation, soxhlet, supercritical extraction, ultrasonic extraction, and microwave extraction, have been previously developed with the purpose of identifying bioactive chemicals derived from plants.[4,5] Certain chemicals have the potential to undergo degradation when exposed to elevated temperatures and intense light. Hence, the careful choice of appropriate extraction techniques is crucial for the standardisation of herbal products, as it facilitates the separation of desirable soluble constituents. Polyphenols, flavonoids, and phenolic compounds are highly prevalent in natural bioactive substances, exhibiting notable antioxidant properties. Multiple studies have demonstrated that the extraction procedures employed significantly impact the overall phenol and flavonoid content, as well as the antioxidant activity of these compounds.[5,6]

There are a number of techniques for extracting medicinal plants, including the traditional ones like maceration, reflux, and Soxhlet, which have a likelihood of compound degradation, a relatively long duration, and a considerable amount of solvent employed.[5] These traditional extraction techniques are frequently superseded by more environmentally friendly ones, such ultrasound-assisted extraction (UAE). The mechanical effect of UAE increases internal diffusion and eddy currents, which has an impact on the mass transfer of the solvent into the sample matrix and raises the content to release.[6] The extraction effectiveness and extractability of bioactive substances are improved using the UAE approach.[7]

Numerous flavonoids, including rutin, myricetin, epicatechin, catechin, and genistein, have demonstrated important biological properties, such as anti-inflammatory, anti-tumor, and anti-bacterial properties.[8,9] By preventing lipid peroxidation, scavenging free radicals, or chelating metal ions, phenolic acids such protocatechuic acid, chlorogenic acid, ferulic acid, and cinnamic acid also provide various therapeutic benefits for disorders caused by oxidative stress.[10-13] Therefore, it is possible that some plants may serve as an excellent source of functional food ingredients in industrial applications, particularly in the pharmaceutical and nutraceutical industries, due to the availability of phenolic chemicals generated under favourable UAE circumstances. Therefore, the purpose of this work is to compare the bioactive component content and antioxidant activity of S. *polyanthum* leaves that have been extracted using maceration and the UAE method

MATERIAL AND METHODS

Plant Material & Extraction

The botanical specimens of S. *polyanthum* that were collected were sourced from UPT. Balai Materia Medika in East Java, Indonesia (7°52'01.2"S and 112°31'13.2"E) With the determination of taxonomic identification number 074/629/102.7-A/202. At room temperature, the leaves of S. *polyanthum* were powdered and air-dried. Two distinct extraction techniques, such as maceration and ultrasound-assisted extraction, were used to remove the S. *polyanthum* leaves (UAE). The S. *polyanthum* leaves powder was macerated by soaking it in 96% ethanol (1:10, m:v) for 24 hours at room temperature while stirring occasionally. The mixture was then evaporated using a rotary evaporator at 50 degrees Celsius and 70 revolutions per minute. Additionally, the UAE procedures followed the guidelines outlined in the earlier work.[8] SONICA Ultrasonic Cleaner, Model SONICA® 2400EP S3 (Soltec Soluzioni Technologiche, Italy), was used to clean the UAE after it had been soaked in 96% ethanol for 30 minutes at room temperature (1:10, m,v). A rotary evaporator (50°C, 70 rpm) was used to evaporate the mixture after filtering it through paper. Prior to usage, both extracts were then kept in a 4°C refrigerator.

Bioactive Compounds Identification

Liquid chromatography-High Resolution Mass Spectrometry (LC-HRMS) Analysis was used to identify the presence of bioactive chemicals, according to Nafisah et al.[14,15] to the extracted sample, 0.1% ethanol was added before being fed into the LC-HRMS device (Thermo Fisher Scientific Inc.; USA). Comparing the components in both extracts allowed for the identification to be completed. The retention time (RT) values for the top 5 compounds were given in this investigation. Following that, the bioactive substances were categorized using the PubChem database's taxonomy (https://pubchem.ncbi.nlm.nih.gov/).

Total Phenolic and Flavonoid Analysis

The determination of total phenolic and flavonoid content was done based on Christina et al[10] The phenolic content was measured using Folin-Ciocalteu methods. Briefly, each extract (20 µl) was mixed with 10% Folin-Ciocalteu reagent (100 µl) in a 96-well plate for 5 min. After that, the mixture was added with 75 µl sodium carbonate (75 g L-1) and incubated at 25°C for 120 min in the dark. Besides, the total flavonoid was determined by mixing 50 µl with distilled water and a 5% solution of sodium nitrate (70 µl and 15 µl, respectively) in a 96-well plate and incubated for 5 min at 25°C. After that, each sample was added with 15 µl and allowed to stand for 6 min and followed by the addition of 100 µl of sodium hydroxide (1 M). Gallic acid and quercetin were used as standard in both analyses and measured using a microplate reader with a wavelength 510 nm (BioTek Instrumens, Inc., USA). All the sample was done in triplicate.

Antioxidant Analysis

By using the DPPH assay, the antioxidant activity was assessed and compared to ascorbic acid, the industry standard. In a nutshell, 40 l of each extract dose—which ranged from 0 to 150 mg/ml—was combined with 50 M DPPH reagent in 200 l of ethanol and incubated at room temperature for 15 min in the dark. The microplate reader absorbance at 517 nm, which was used to measure the antioxidant activity, was used to make this determination. The sample was completed three times overall. We calculated the extract inhibitory activity using the following formula:

(Ac - As)/Ac x 100% for inhibition activity (%) (1)

Where As denotes the sample extract's absorbance and Ac denotes the absorbance of the control. The regression value created from the value of the inhibitory activity was then used to determine the IC50.

RESULTS

Table 1 displays the retention time/RT [min] of each chemical. This study revealed 5 bioactive compounds with the highest area based on the identification of the bioactive compounds utilizing LC-HRMS analysis. Deoxyphomalone, (+)-ar-turmerone, and 318237-79-5, also known as 1-(3-hydroxy-3-methylpent-4-en-1-yl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol, were among the bioactive substances

Table 1. **Bioactive contained in *S. polyanthum leaves* maceration extract and UAE**

Maceration			UAE		
Compound	**Area**	**RT [min]**	**Compound**	**Area**	**RT [min]**
Deoxyphomalone	3.39E+09	14.688	**Deoxyphomalone**	1.49E+09	14.707
Nootkatone	2.93E+08	17.633	**(+)-ar-Turmerone**	1.08E+08	17.418
(+)-ar-Turmerone	1.17E+08	17.422	**318237-79-5**	9.84E+07	22.231
318237-79-5	1.08E+08	22.225	**Oleanolic acid**	4.93E+07	20.784
Caryophyllene oxide	4.41E+07	16.566	**CHEBI:189401**	4.72E+07	0.923

Table 2. **Classification of bioactive compounds**

Extraction method	Name	Classification
Maceration and UAE	Deoxyphomalone	Ketone
	(+)-ar-Turmerone	Terpenoid
	318237-79-5	Terpenoid
Maceration	Nootkatone	Terpenes
	Caryophyllene oxide	Terpenes
UAE	Oleanolic acid	Terpenes
	CHEBI:189401	Alkaloid

Table 3. **Total phenolic, flavonoid, and antioxidant activity**

	Maceration	UAE
Total Phenol (mg GAE/g)	94.63 ± 2.18	122.87 ± 5.99
Total Flavonoid (mg QE/g)	60.194 ± 3.44	60.074 ± 2.66
DPPH IC50 (μg/ml)	88.21 ± 1.38	41.23 ± 6.11

present in both the maceration and the UAE extract. Additionally, the maceration extract was the only source of nootkatone and caryophylleneoxide. Oleanolic acid and CHEBI:189401, also known as (1R,9R)-5-Cyclohexyl-11-(propylsulfonyl) Only the UAE extract contains -7,11-diazatricyclo[7.3.1.02,7] trideca-2,4-dien-6-one (Table 1). Deoxyphomalone was relatively the most prevalent chemical found in S. *polyanthum* leaves that were extracted via maceration and UAE, according to the area determined by the analysis.

Table 2 displays the categorization of S. *polyanthum* leaves extract bioactive chemicals based on the PubChem database.

In Table 2, The bioactive substance was categorized as an alkaloid, terpenoid, ketone, and terpene. Due to the varied solvents and characteristics of the *S. polyanthum leaves.* The classification is based on the active compounds in maceration in the form of terpenes (nootkatone and caryophyllene oxide), terpenoids ((+)-ar-turmerone and 318237-79-5) and ketone (deoxyphomalone). The classification is based on the active compounds in UAE, terpenes (Oleanolic acid) and Alkaloids (CHEBI:189401). Meanwhile, the same compounds obtained from both extraction methods are Ketone (Deoxyphomalone) and Terpenoid ((+)-ar-Turmerone and 318237-79-5).

Table 3 showed Total phenolic, flavonoid, and antioxidant activity, The maceration extraction method and UAE were compared for the amount of total phenolic and flavonoid in S. *polyanthum* leaves. When compared to maceration extraction, the total phenolic content was increased in the UAE approach (122.87 mg GAE/g and 94.63 mg GAE/g, respectively). However, the amount of flavonoids in both extracts (60.194 mg QE/g and 60.074 mg QE/g) was comparable (Table 3). Based on the DPPH assay, UAE method enhanced antioxidant activity of *S. polyanthum* leaves compared to maceration method indicated by the lower IC50 value of DPPH assay (Table 3). The IC50 was obtained from the regression value of the inhibition percentage of DPPH assay. The IC50 of *S. polyanthum* leaves with the maceration method was 88.21 μg/ml, whereas with the UAE method was 41.23 μg/ml.

DISCUSSION

The extraction of most phytochemicals in prior studies was conducted through the utilisation of solvent maceration and steam distillation techniques. The current investigation has employed the UAE approach, which differs from the extraction methods employed in other investigations. This study involved the extraction of S. Polyanthum leaves by two distinct methods: maceration and ultrasound-assisted extraction (UAE). The bioactive constituents obtained from both extraction methods were analysed using liquid chromatography-high resolution mass spectrometry (LC-HRMS). The utilisation of LC-HRMS offers several notable advantages, including its capacity to effectively manage a wide range of chemicals, its precision in identifying compounds, and its heightened sensitivity in comparison to alternative methodologies.[9,10]

Table 1 displays a number of analogous compounds that are present in both extraction procedures, specifically deoxyphomalone and turmerones. Numerous in vitro biological activities of deoxyphomalone and turmerones have been documented, encompassing anti-inflammatory, immunomodulatory, antiproliferative, and antifungal properties. The bioactive compound investigated in this study was classified into four categories: alkaloid, terpenoid, ketone, and terpene. The chemical composition of the plant material in this study exhibited variations in solvents and properties, resulting in differences from a previous study that employed GCMS analysis. The analytical technique known as Gas Chromatography–Mass Spectrometry (GC-MS) is utilised to evaluate a variety of compounds including hydrocarbons, aldehydes, terpenoids, phenolics, fatty acids, monoterpenes, diterpenes, triterpenes, and sesquiterpenes. Terpenes, including squalene, an isoprenoid molecule, and phytol, a cyclic diterpene alcohol, were identified as significant constituents in all of the extracts. Oleanolic acid (OA), which is present in the United Arab Emirates (UAE), has been recognised for its pharmacological properties, including antioxidant, anti-tumor, anti-inflammatory, anti-diabetic, and antimicrobial actions. Currently, there is ongoing research investigating the potential of OA (oleic acid) in mitigating the oxidative stress and inflammation caused by excessive fructose consumption, as well as its potential in preventing metabolic syndrome.[12,13] The UAE (Ultrasound-Assisted Extraction) technique has been widely employed in many research studies to improve the extractability of phenolic, thermo-labile, and unstable chemicals. The major compound detected in the extracts in this study was not detected in these previous studies. However, the observed variations in phytochemical content can also be due to changes in the extraction methods utilised. The plant materials utilised in this investigation were sourced from S. polyanthum leaves cultivated in Malaysia, whereas prior studies predominantly collected leaves from S. polyanthum plants produced in Indonesia. Basically, UAE method enhances solvent penetration through plant cell with the aid of sound wave.[9] Its mechanistic action includes fragmentation, erosion, and destruction-detexturation of plant structures, thus, this will enhance the diffusion process by increasing the solute transfer rate.[10,11] This method is usually employed for its higher extraction efficiency and its shorter extraction time as compared to maceration and soaking with the less amount of solvent application.[9,10]

This study demonstrates that the UAE approach has the potential to enhance the overall phenolic content and antioxidant properties. Both techniques possess identical antioxidants. The antioxidant derived through the utilisation of the UAE approach exhibits an antioxidant capacity of less than 50, indicating a high level of antioxidant activity. This study is in accordance with the research conducted by Gajic et al. (2019), whereby it was shown that the UAE approach yielded a greater total phenolic content and exhibited superior antioxidant activity in comparison to the maceration and soxhlet extraction methods. These findings are compatible with the increased phenolic content observed in the UAE method. Furthermore, it has been asserted that the UAE method was more efficacious in the removal of phenolic compounds from plants.[9,10]

Previous research indicated that UAE can increase the extract's antioxidant activities.[5,6,8] Several extraction techniques have been proposed for isolating naturally occurring physiologically active components depending on the intended application. Conventional extraction techniques include hot reflux extraction, maceration, and soxhlet extraction. These approaches do, however, have several drawbacks, including time consumption, inefficiency, and lack of economic viability. UAE has been developed as a solution to these issues, allowing for the extraction of physiologically active chemicals.[16,17] UAE shortens the extraction periods while increasing the extract yield. By breaking down the cell wall and reducing the size of the particles, the sonic cavitation produced by UAE improves the contact between the target chemicals found in the cell wall and the solvents utilised in UAE.[18,19]

In recent years, UAE has been utilised in place of many traditional extraction techniques. Among the benefits of UAE are the decreases in energy, solvent usage, and extraction time. The expansion and compression cycles produced by ultrasonic waves while utilising UAE cause bubbles to develop.[20-22] When these bubbles collapse, a localised pressure is created in the matrix that breaks down the cell walls and improves the release of intracellular materials into the solvent.[23-26] This shortens the extraction time and increases the extraction yield. Nevertheless, other factors including the solvent-to-solid ratio, the kind and concentration of the solvent, the duration of contact, and temperature still have an impact on the extraction process yield.[26]

UAE versus traditional extraction comparison, the enhanced yield of extract may be ascribed to the transmission of ultrasonic waves and cavitation. Plant cell walls are attacked by cavitation bubbles, which promotes solvent passage through cell walls. The ultrasonication process boosted the mass transfer of the original material into each extract solution by producing shear force due to the ultrasound vibrations. Ultra-sonication helps to decrease the raw material's particle size, increasing its surface area the extraction yield was enhanced by the mass transfer increase.[27-29]

In the UAE the dispersion of ultrasonic waves in a liquid media destroys plant wall, improving solvent penetration and enabling the extraction of bioactive components in a matter of minutes.[30,31] Consequently, UAE offers the benefit of lowering energy consumption and extraction process time while maintaining high efficiency as compared to conventional approaches.[32,33] Cell walls ruptured during ultrasonication. Common plants that have had ultrasonic treatment develop a loose structure and their surface erodes due to cavitation that happens close to the surface.[34] This can cause the solvent to seep into the plant material more quickly and thoroughly. Others came to a similar conclusion when they discovered that ultrasonic causes mechanical mixing, promotes cavitation events, and can alter the structural properties of cellulose by increasing its surface area and decreasing its crystallinity.[35,36] The UAE extraction time is shorter than in previous studies, which enables the creation of an energy-efficient procedure. The application of the sophisticated mathematical methodology with ultrasound led to a reduction in energy consumption when compared to other accessible processes.[37]

An investigation was conducted on the viability of enhancing the extraction rate of antioxidants from using ultrasonic treatment. The application of ultrasonic treatment significantly changed the cellular structure, as demonstrated by scanning electron microscopy (SEM) and spectrum Fourier transform infrared spectrophotometer (FT-IR) analysis. This resulted in increased surface area, eroded cell walls, and greater exposure of cellulose and hemicellulose. These conditions produced the maximum antioxidant activity. UAE has approximately seven times higher extraction efficiency than conventional solvent extraction (CSE) when it comes to the extraction rate of common bean antioxidants. In this term, ultrasonic treatment is a great potential technique to increase the rate at which common bean antioxidants are extracted.[38,39] These findings suggest that the lignin network structure partially decomposes when subjected to ultrasonic treatment this considerable increase in intensity following ultrasonic treatment, this also suggests that applying ultrasonic treatment can assist increase the release of antioxidants by breaking down the lignin to some extent and releasing more cellulose and hemicellulose on their surface.[34]

The maceration method depends on the natural diffusion of the solvent into the solid over time in order to extract the desired chemicals. The method is characterised by its simplicity and cost-effectiveness; however, it is important to note that it may be time-consuming and may not achieve optimal extraction of all chemicals. UAE is well-known for its proficiency in expediting the extraction process and enhancing yields, particularly for chemicals that present difficulties. The greater penetration of the solvent into the solid material can lead to increased efficiency and effectiveness within a shorter timeframe. Nevertheless, an effective completion of the extraction may necessitate the utilisation of specialised equipment and the possession of professional expertise. The selection of the methodology is contingent upon the particular specifications of the extraction procedure. If optimising time efficiency, increasing yields, and effectively extracting problematic chemicals are of paramount importance, it may be more appropriate to consider the utilisation of UAE. Nevertheless, if the primary considerations are simplicity, cost-effectiveness, and overall extraction requirements, maceration may be the ideal method. The selection of an appropriate methodology necessitates the careful evaluation of various aspects, including the inherent characteristics of the compounds involved, the availability of resources, the limitations imposed by time, and the final objective of the extraction procedure. In numerous instances, conducting a comparative study or trial of both strategies might assist in ascertaining the best suitable methodology for a specific application. The selection of extraction methods is reliant upon several factors, including the characteristics of the chemicals being extracted, temporal limitations, and the availability of resources.[34]

UAE is one of the easiest and least expensive extraction techniques when compared to conventional procedures. It may be used for a variety of solvent systems in industrial scale preparations. In addition to speeding up the extraction process, UAE may lessen reliance on a specific solvent and permit the use of substitutes, which could have greater advantages for the economy, the environment, and public health and safety. The molecular and structural properties of bioactive substances are maintained by UAE.[34]

UAE offers better low-processing-temperature extraction of food ingredients and heat-sensitive bioactive substances. UAE produces highly reproducible food processes that take only a few minutes or seconds to finish, need less energy than traditional extraction procedures, increase end product purity, and save processing costs.[34,35] The UAE has been given additional impetus by the emphasis on waste utilisation, component recovery, environment protection, and green technology.[35,36] It has been demonstrated that the UAE is more capable to removing the bioactive substances from fruit and vegetable leftovers without altering the volatile bioactive components' structural integrity. The UAE supply of bioactive compounds derived from fruit and vegetable byproducts can meet the growing demand for natural bioactive compounds by means of targeted research and ongoing technological advancements.[36] The present study possesses both strengths and limitations. One notable strength is its ability to reveal the potent antioxidant properties of S Polyanthum, which holds promise for medicinal uses. However, it is important to note that further investigation is necessary to fully explore and understand these findings.This study exclusively examines the comparison between United Arab Emirates (UAE) and conventional extraction methods. However, further research investigations are needed to gain a more comprehensive understanding of the bioactive chemicals found in S. *polyanthum* leaves. These studies should be undertaken on larger scales of extraction to yield more accurate and reliable results. This will enable an improved understanding of the bioactive compounds present in S. *polyanthum* leaves.

CONCLUSION

The bioactive compounds found in S. *polyanthum* leaves are altered by the extraction techniques; according to the study's findings, at least two bioactive chemicals differ in the maceration and UAE processes. Additionally, when compared to the maceration extraction process, the UAE approach shows higher total phenolic content and antioxidant activity. Therefore, the UAE is better suited for extracting phenolic components and the antioxidant activity of S. *polyanthum* leaves.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

This study was explanatory research with true experiments. The study protocol was approved by the Ethics Committee of the Veterinary Faculty, Airlangga University, Surabaya, Indonesia (No: 2.KE.133.11.2021).

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AUTHOR CONTRIBUTIONS

Conceptualization and design R.A., B.S., W.W; definition of intellectual content B.S., W.W; Literature search R.A.; Clinical studies R.A., B.S., and W.W; Experimental studies R.A., B.S., and W.W;; Data acquisition R.A., B.S., and W.W; Data and Statistical analysis R.A. and W.W; Manuscript-original draft preparation R.A., W.W; manuscript editing, manuscript review and supervision R.A., W.W. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Abdurrahman MD. Biological Evaluation and Chemical Composition of Syzygium polyanthum (Wight) Walp. Plant Science Today. 2022;9(1):167–177. Available from: https://doi.org/10.14719/pst.1386
2. Uddin ABMN, Hossain F, Reza ASMA, Nasrin MS, Alam AHMK. Traditional uses, pharmacological activities, and phytochemical constituents of the genus Syzygium: A review. Food Sci Nutr. 2022 Mar 4;10(6):1789-1819. PMID: 35702283; PMCID: PMC9179155.
3. Rahim EN, Ismail A, Omar MN, Rahmat UN, Ahmad WA. GC-MS Analysis of Phytochemical Compounds in Syzygium polyanthum Leaves Extracted using Ultrasound-Assisted Method. Pharmacognosy Journal. 2018;10(1):110-119.
4. Wahjuni S, Wita IWH. Hypoglycemic and antioxidant effects of Syzygium polyanthum leaves extract on alloxan-induced hyperglycemic Wistar Rats. Bali Medical Journal. 2017;3(3):S113-S116.
5. Ismail A, Ahmad WANW. Syzygium polyanthum (Wight) Walp: A Potential Phytomedicine. Pharmacognosy Journal. 2019;11(2):429-438.
6. Thakur M, Singh K, Khedkar R. Phytochemicals: Extraction process, safety assessment, toxicological evaluations, and regulatory issues. Environmental Science. 2020;2020:341-61.
7. Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020 Jan-Mar;12(1):1-10.
8. Yang QQ, Gan RY, Ge YY, Zhang D. Ultrasonic treatment increases extraction rate of common bean (Phaseolus vulgaris L.) antioxidants. Antioxidants. 2019;8:83.
9. Zoumpoulakis P, Sinanoglou V, Siapi E, Heropoulos G, Proestos C. Evaluating modern techniques for the extraction and characterisation of sunflower (Helianthus annuus L.) seeds phenolics. Antioxidants. 2017;6:46.
10. Savic Gajic I, Savic I, Boskov I, Žerajić S, Markovic I, Gajic D. Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds from Black Locust (Robiniae Pseudoacaciae) Flowers and Comparison with Conventional Methods. Antioxidants (Basel). 2019;8(8):248.
11. Zhou T, Xu DP, Lin SJ, Li Y, Zheng J, Zhou Y, et al. Ultrasound-assisted extraction and identification of natural antioxidants from the fruit of Melastoma sanguineum Sims. Molecules. 2017;22(2):306.
12. Chen X, Li L, Liu X, Luo R, Liao G, Li L. Oleic acid protects saturated fatty acid mediated lipotoxicity in hepatocytes and rat of non-alcoholic steatohepatitis. Life Sciences. 2018;203:291-304.
13. Aditya R, Santoso B, Widjiati W. Anti-inflammatory and antioxidant potential of Syzygium polyanthum (Wight) Walp. bioactive compounds in polycystic ovary syndrome: An in silico study. J Pharm Pharmacogn Res. 2022;(10)4:725–36.
14. Nafisah W, Fatchiyah F, Widyananda MH, Christina YI, Rifa'i M, Widodo N, Djati MS. Potential of bioactive compound of Cyperus rotundus L. rhizome extract as an inhibitor of PD-L1/PD-1 interaction: An in silico study. Agriculture and Natural Resources. 2022;56:751-60.
15. Yusoff IM, Mat Taher Z, Rahmat Z, Chua LS. A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. Food Res Int. 2022;157:111268.
16. Riyadi PH, Atho'illah MF, Tanod WA, Rahmawati IS. Tilapia viscera hydrolysate extract alleviates oxidative stress and renal damage in deoxycorticosterone acetate-salt-induced hypertension rats. Vet World. 2020;13(11):2477-83.
17. Um M, Han TH, Lee JW. Ultrasound-assisted extraction and antioxidant activity of phenolic and flavonoid compounds and ascorbic acid from rugosa rose (Rosa rugosa Thunb.) fruit. Food Sci Biotechnol. 2017;27(2):375-82.
18. Vilkhu K, Mawson R, Simon L, Bates D. Applications and opportunities for ultrasound assisted extraction in the food industry - A review. Innov. Food Sci. Emerg. Technol. 2008;9:161–9.
19. Saleh IA, Vinatoru M, Mason TJ, Abdel-Azim NS, Aboutabl EA, Hammouda FM. A possible general mechanism for ultrasound-assisted extraction (UAE) suggested from the results of UAE of chlorogenic acid from Cynara scolymusL. (artichoke) leaves. Ultrason. Sonochem. 2016;31:330–6.
20. Machado-Carvalho L, Martins T, Aires A, Marques G. Optimization of phenolic compounds extraction and antioxidant activity from Inonotus hispidus using ultrasound-assisted extraction technology. Metabolites. 2023;13(4):524.
21. Naqvi SAR, Akbar N, Shah SMA, Ali S, Abbas A. Green approaches for the extraction of bioactive from natural sources for pharmaceutical applications. In: Inamuddin, Boddula R, Ahamed MI, Asiri AM, editors. Green Sustainable Process for Chemical and Environmental Engineering and Science. Elsevier: Amsterdam, The Netherlands: 2021. p. 269–91.
22. Wen L, Zhang Z, Sun DW, Sivagnanam SP, Tiwari BK. Combination of emerging technologies for the extraction of bioactive compounds. Crit. Rev. Food Sci. Nutr. 2020;**60**:1826–41.
23. Liu Y, Liu X, Cui Y, Yuan W. Ultrasound for microalgal cell disruption and product extraction: A review. Ultrason Sonochem. 2022;87:106054.
24. Muhammad DS, Salim HM, Virlliana CD, Donastin A, Adriansyah AA. The effect of syzygium polyanthum (wight) extract in histological changes of kidney in diabetic mice model. Bali Med J. 2022;11(3):1301-4.
25. Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, et al. Techniques for extraction of bioactive compounds from plant materials: A review. J. Food Eng. 2013;**117**:426–36.
26. Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason. Sonochem. 2017;**34**:540–60.
27. Kumar K, Srivastav S, Sharanagat VS. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrason Sonochem. 2021;70:105325.
28. Adetunji LR, Adekunle A, Orsat V, Raghavan V. Advances in the pectin production process using novel extraction techniques: a review. Food Hydrocolloids. 2017;62:239–50.
29. Vilkhu K, Manasseh R, Mawson R, Ashokkumar M. Ultrasound technologies for food and bioprocessing. Springer; New York, NY: 2011. Ultrasonic recovery and modification of food ingredients; p. 345–68.
30. Vinatoru M, Mason TJ, Calinescu I. Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. TrAC Trends in Analytical Chemistry. 2017;97:159-78.
31. Medina-Torres N, Ayora-Talavera T, Espinosa-Andrews H, Sánchez-Contreras A, Pacheco N. Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. Agronomy. 2017;7:47.
32. Palmieri S, Pellegrini M, Ricci A, Compagnone D, Lo Sterzo C. Chemical composition and antioxidant activity of thyme, hemp, and coriander extracts: A comparison study of maceration, soxhlet, UAE, and RSLDE techniques. Foods. 2020;9(9):1221.
33. Skenderidis P, Petrotos K, Giavasis I, Hadjichristodoulou C, Tsakalof A. Optimization of ultrasound-assisted extraction of goji berry (Lycium barbarum) fruits and evaluation of extracts' bioactivity. J. Food Process Eng. 2017;40:e12522.
34. Ji HF, Du AL, Zhang LW, Xu CY, Yang MD, Li FF. Effects of drying methods on antioxidant properties in Robinia pseudoacacia L. flowers. J. Med. Plants Res. 2012;6:3233–9.
35. Ramic M, Vidovic S, Zekovic Z, Viadic J, Cvejin A, Pavlic B. Modeling and optimization of ultrasound-assisted extraction of polyphenolic compounds from Aronia melanocarpa by-products from filter-tea factory. Ultrason. Sonochem. 2015;23:360–8.
36. Dranca F, Oroian M. Total monomeric anthocyanin, total phenolic content and antioxidant activity of extracts from eggplant (Solanum melongena L.) peel using ultrasonic treatments. J. Food Process Eng. 2017;40.
37. Shi W, Jia J, Gao Y, Zhao Y. Influence of ultrasonic pretreatment on the yield of bio-oil prepared by thermo-chemical conversion of rice husk in hot-compressed water. Bioresour. Technol. 2013;146:355–362.
38. Maran JP, Manikandan S, Nivetha CV, Dinesh R. Ultrasound-assisted extraction of bioactive compounds from Nephelium lappaceum L. fruit peel using central composite face-centered response surface design. Arab. J. Chem. 2017;10:S1145–S1157.
39. Sarikurkcu C, Kocak MS, Tepe B, Uren MC. An alternative antioxidative and enzyme inhibitory agent from Turkey: Robinia pseudoacacia L. Ind. Crops Prod. 2015;78:110–115.