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PHYTOCHEMICAL AND NUTRIENT ANALYSIS OF ETHANOL EXTRACT SALAK PONDOH (*Sallaca edulis Reinw) PEEL FOR DEVELOPMENT OF ANTI-OBESITY*

Studi Fitokimia dan Kandungan Nutrisi Ekstrak Etanol Kulit Salak Pondoh untuk Pengembangan Anti-Obesitas

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ABSTRAK

Insiden obesitas meningkat di seluruh dunia selama beberapa tahun terakhir. Diet dan aktivitas fisik saja pada penderita obesitas kurang memberikan hasil yang optimal, sedangkan penggunaan obat anti obesitas dalam jangka panjang dapat menyebabkan efek samping. Penelitian ini bertujuan untuk menganalisis nutrisi dan kandungan fitokimia kulit buah salak pondoh untuk mengembangkan pengobatan obesitas. Jenis penelitian ini merupakan eksperimental laboratorium dengan 3 tahap: ekstraksi kulit luar salak pondoh, analisis nutrisi, dan analisis Gas Chromatography and Mass Spectrometry yang selanjutnya disebut GC-MS. Kulit buah salak pondoh diperoleh dari pusat agribisnis di Sleman, Yogyakarta. Kulit buah salak pondoh kering diekstraksi dengan etanol 70% menggunakan metode remaserasi. Kandungan nutrisi yang terdapat pada ekstrak etanol dianalisis di Laboratorium Teknologi Pangan, Fakultas Pertanian, Universitas Sebelas Maret, sedangkan analisis fitokimia dilakukan di Laboratorium Riset Terpadu dan Analisis Pengujian Universitas Gadjah Mada, dengan menggunakan metode Gas Chromatography and Mass Spectrometry (GC-MS). Ekstrak kulit buah salak pondoh (EPP) mengandung persentase karbohidrat (41,33%), mineral (28,37%), dan air (25,31%) yang lebih tinggi dibandingkan persentase serat kasar (3,68%), lemak (2,62%), protein (2,37%), dan vitamin C (0,88%). Hasil GC-MS mengidentifikasi adanya 75 fitokimia di mana dua dari lima senyawa dengan proporsi tertinggi adalah metil ester asam heksadekanoat dan asam n-heksadekanoat. Kandungan fitokimia tersebut memiliki aktivitas biologis yang berpotensi untuk pengembangan terapi obesitas. Perlu dilakukan penelitian lanjutan secara in vitro dan in vivo untuk mengevaluasi sifat anti- obesitas EPP tersebut.

Kata kunci: *fitokimia, kulit salak pondoh, nilai nutrisi, obesitas*

ABSTRACT

The incidence of obesity has been increasing worldwide in recent years. Diet and physical activity alone in obese individuals do not yield optimal results, while long-term use of anti-obesity drugs may cause side effects. This study aims to analyze the nutrient and phytochemical content of salak pondoh fruit peel to develop obesity treatment. This type of research is a laboratory experimental with three stages: extraction of salak pondoh peel, nutritional analysis, and Gas Chromatography and Mass Spectrometry (GC-MS). The salak pondoh fruit peel was obtained from the

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agribusiness center in Sleman, Yogyakarta. The dried salak pondoh peel was extracted with 70% ethanol using remaceration method. The nutrient content of ethanol extract was determined by analysis at the Food Technology Laboratory, Faculty of Agriculture, Sebelas Maret University, while the phytochemical analysis was conducted at the Integrated Research and Testing Laboratory of Gadjah Mada University using the Gas Chromatography and Mass Spectrometry (CG-MS) method. The ethanol extract of salak pondoh peel (EPP) contains a higher percentage of carbohydrates (41.33%), minerals (28.37%), and water (25.31%) than crude fiber (3.68%), fat (2.62%), protein (2.37%), and vitamin C (0.88%). Result of GC-MS analysis showed 75 phytochemicals in the extract, where two of the five highest component are hexadecanoic acid methyl ester and n-hexadecanoic acid. These phytochemical content has biological activity that is beneficial for the treatment of obesity. Further in vitro and in vivo studies are needed to evaluate the anti-obesity properties of EPP.

Keywords: nutrient value, obesity, phytochemical, salak pondoh peel

INTRODUCTION

The World Health Organization (WHO) defines obesity as excess fat accumulation, which causes negative impacts on health [1]. The incidence of obesity has increased throughout the world over the last few years; at least 2.8 million people die every year due to obesity. By 2030, it is estimated that one in five women and one in seven men will suffer from obesity, meaning there will be more than one billion people struggling with obesity worldwide [2]. According to Riskesdas 2018, the prevalence of obesity among adults in Indonesia is 21.8%, while the prevalence of obesity among adolescents is 4.8% [3]. Obesity is associated with a high mortality rate because obesity is associated with other metabolic disorders such as dyslipidemia, hypertension, type 2 diabetes mellitus, and several types of cancer [4]. Diet and physical activity alone for obese sufferers do not provide optimal results [5].

Salak pondoh is one of the fruits with various benefits. Previous studies have proven that salak can help reduce body weight and blood sugar levels [6]. In another study the ethanol extract of salak peel with details of phytochemical screening containing tannins, polyphenols, alkaloids, saponins, and flavonoids can reduce body weight, but the content and the mecanism of action is unclear [7]. Salak peel has remarkable health benefits when processed into herbal tea. Salak peel contains natural compounds that can be benefit for various health issues, including diabetes. The peel is also rich in fiber and vitamin C. Furthermore, salak peel has other benefits, such as preventing constipation, controlling uric acid levels, improving heart performance, and maintaining stamina [8]. However, study to determine the phytochemical content of salak pondoh peel ethanol extract as an anti-obesity has never been carried out.

Physical activity still plays a very important role in weight loss and overall health improvement, including diabetes [9]. Physical activities, such as resistance training and aerobic exercises, have been proven to help reduce body weight, reduce blood sugar level, improve metabolism, and lower cholesterol levels in the body, all of which are directly related to obesity risk. As explained in studies related to physical activity, the combination of a controlled diet and regular physical exercise can lead to more optimal weight loss compared to diet intervention alone [10]. The same applies to the use of salak pondoh peel extract; it has potenty in lowering blood sugar levels, then it is possible that it can be useful for treating obesity because bioactive compounds in salak pondoh peel, such as pterostilbene, cinnamic acid, and other compounds that play a role in enhancing body metabolism [11].

Salak pondoh was chosen for this study due to its bioactive compounds, which

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possible to act as anti-obesity agents. The increasing prevalence of obesity in Indonesia necessitates the discovery of solutions to reduce this prevalence. Therefore, salak pondoh peel serve as an alternative solution to this issue. This study contributes to developing a natural alternative treatment for obesity by utilizing salak pondoh peel extract. The objective of this study was to analyze the phytochemical and nutrient content of ethanol extract of salak pondoh peel and identify key bioactive compounds with potential as anti-obesity agents.

METHODS

This study was conducted from December 2023 to January 2024. This type of study was laboratory experimental with 3 stages: salak pondoh peel extraction, nutrition analysis, and Gas Chromatography and Mass Spectrometry (CG-MS) analysis Salak pondoh peel in this study were obtained from the salak pondoh agribusiness center, Sleman, Yogyakarta. Sleman is one of the potential agricultural regencies in the Special Region of Yogyakarta. Salak pondoh Sleman is a superior product with the highest yield in the area. More over salak pondoh Sleman is also claimed to hava a distinctive quality characteristic that distinguishes it from similar cultivar in other regions. Directorate General of Intellectual Property (DJKI) has granted the Geographical Indication (GI) certificate to its product and its indicate that salak pondoh Sleman was impossibly found in other regions [12].

Salak pondoh peel extraction was carried out at the Phytochemical Laboratory of Universitas Setia Budi, Surakarta. The nutrient analysis was carried out at the Food Chemistry and Biochemistry Laboratory, Faculty of Agriculture of Universitas Sebelas Maret and the GC-MS analysis was carried out at the Integrated Laboratory for Research and Testing (LPPT) Universitas Gadjah Mada (UGM) Yogyakarta. No repetition was carried out in this study.

Proximate analysis is a method used to determine the basic components of a material, such as water, fat, protein, carbohydrates, minerals, and crude fiber. The purpose of this analysis is to provide a more comprehensive understanding of the nutritional value and energy content of a material or product. Proximate analysis was performed to determine the nutritional content of the Salak Pondoh peel extract (EPP).

Gas Chromatography and Mass Spectrometry (CG-MS) is a type of chromatography used in organic chemistry for separation and analysis. GC- MS is a gas chromatography technique used with mass spectrometry. The use of GC-MS is to look for compounds that easily evaporate under high vacuum and low-pressure conditions when heated. Meanwhile, mass spectrometry is used to determine molecular weight and molecular formula and produce charged molecules [13].

The extraction process in this research uses the remaceration method. The remaceration method was chosen because it is one of the most used extraction methods and can avoid the risk of damaging thermolabile target compounds [14]. The maceration method is an extraction method that uses soaking the ingredients. The material is soaked in a solvent suitable for the targeted active compound with a low-temperature or no-heating process [15]. In this research, maceration was carried out twice.

The maceration method is one of the most used extraction techniques because it is relatively simple and effective in obtaining active compounds from plant materials. This process involves soaking plant materials, such as Salak Pondoh peel, in a suitable solvent, such as ethanol. During soaking, the active compounds contained in the plant material dissolve into the solvent. The main advantage of the maceration method is its ability to avoid the degradation of heat-sensitive (thermolabile) compounds because the process does not involve heating, thus preserving the

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quality of these compounds. This process typically takes a considerable amount of time, depending on the type of material and solvent used, to ensure optimal extraction. In this study, maceration was carried out twice to improve the extraction efficiency. The first process aims to obtain an initial extract containing most of the active compounds, while the second process increases the extract concentration by capturing the remaining compounds that were not fully extracted in the first round. Through this method, the researchers hope to obtain Salak Pondoh peel extract with higher active compound content, which can be utilized more effectively, for example, in medicinal applications or other uses. Another benefit of the maceration method is its simplicity in execution, not requiring complex equipment and relatively minimal supervision, making it highly suitable for small to medium-scale research.

The solvent selection also has an important role in the extraction process. In this research, ethanol was chosen as the solvent. Ethanol is an organic solvent widely used because it is non-toxic, cheap, can be used in various extraction methods, and is safe when used as a solvent for extracts used in food or medicine [16]. The concentration results are calculated to determine the yield of the extract.

Extraction of Salak Pondoh Peel

Fresh peel of salak pondoh were cleaned under tap water and then dried under indirect sunlight for seven days until completely dry. We used 10-kilograms salak pondoh peel which then produces 1-kilograms of simplicia and produces 80-grams of extract. Dried salak pondoh peel were grounded into powder and sieved using a 60-mesh sieve. The procedure for extracting salak pondoh peel was adapted from an existing method conducted by Valentino [7]. Initially, the powder of salak pondoh peel was macerated using 70% ethanol with a 1:9 ratio for five days. The second maceration used the previous residues was dissolved in 70% ethanol with a 1:4 ratio for two days. Collected filtrates that evaporated using a vacuum evaporator at 50 — 56 °C, 70 rpm, for 7 — 8 hours. The ethanol extract of salak pondoh peel (EPP) was kept in a refrigerator before use. In this study, the yield of EPP was 8%. This result is greater than previous researchers [7]. Several factors, such as the length of maceration time and the number of repetitions, can influence the yield quantity produced [17].

Nutrient Analysis

The extraction of salak pondoh peel was carried out using 70% ethanol solvent through the maceration method. After the extraction was completed, the nutritional content of the salak pondoh peel extract was analyzed using several laboratory methods. The water content was tested by drying the sample in an oven at 105°C until a constant weight was achieved. The ash content was determined by incinerating the sample in a furnace at 550°C until a constant weight was reached. The protein content was measured using the Kjeldahl method, in which the nitrogen in the protein was measured after the sample was extracted with strong acid. The fat content was tested using the Soxhlet method, where ethanol solvent was used to extract the fat, which was then evaporated to determine its content. The carbohydrate concentration was calculated by subtracting the total percentage (100%) from the results of water, ash, protein, and fat tests. Finally, the vitamin C content was tested using a spectrophotometric method, where a specific reagent was used to form a color that could be measured to determine the vitamin C concentration in the sample [18]. All tests were conducted at the Food Technology Laboratory, Faculty of Agriculture, Sebelas Maret University, and the results of these analyses were used to determine the nutrient content of the salak pondoh peel extract.

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Phytochemical Analysis Using Gas Chromatography-Mass Spectrophotometer

The phytochemical analysis of the Salak Pondoh Peel Extract (EPP) was conducted at the Research and Testing Laboratory of Universitas Gadjah Mada, Yogyakarta, using the Thermo Fisher Scientific Gas Chromatography-Mass Spectrophotometer (GC-MS) version 7.2.10.24543. The extracted sample was dissolved in 1.5 mL of ethanol solvent and homogenized using a centrifuge at 9,500 rpm for 3 minutes. The resulting supernatant was then injected into the HP-5 MS UI column (30 m x 0.25 mm x 0.25 µm), with the maximum injector temperature set at 325/350°C. The injection mode was set to split at a flow rate of 50 mL/min, and the injection volume was 1 µL [19]. Phytochemicals were identified using the GC-MS Library available at the laboratory.

RESULTS

Nutrient Levels in Ethanol Extract of Salak Pondoh Peel (EPP)

Proximate analysis is the analysis of a material to determine its nutritional and energy value. Table 1 showed that the EPP contained water, minerals, fat, protein, carbohydrates, vitamin C, and crude fiber. Carbohydrates (41.33%) were higher than minerals (28.37%) and water (25.31%). Crude extract, fat, and protein concentration were comparable. The extract had 0.88% vitamin C (886.7 mg/100g).

Table 1. Proximate (Nutrition) Analysis of Salak Pondoh Peel Extract

Macro and Micronutrient	Concentration (%)
Water	25.31
Mineral	28.37
Fat	2.62
Protein	2.37
Carbohydrate	41.33
Vitamin C	0.88
Crude Fiber	3.68

Phytochemical Contents in Ethanol Extract of Salak Pondoh Peel (EPP)

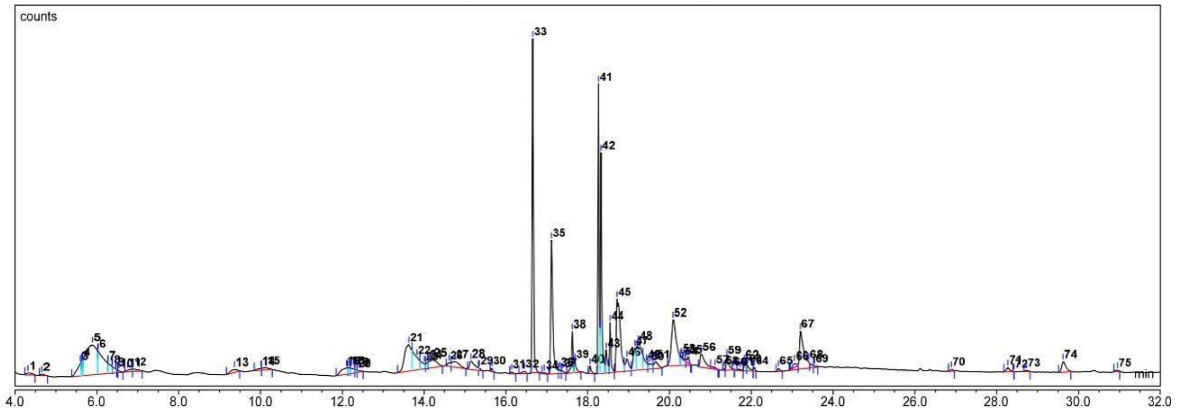

Figure 1. Results of GC-MS Analysis of EPP Bioactive Compound

We analyzed phytochemicals in the EPP using the GC-MS approach. The results of the GC-MS test showed that there were 75 peaks of bioactive compounds in EPP. The five compounds with the highest peaks were (33) *hexadecanoic acid, methyl ester*; (41) *9,12 – Octadecadienoic acid (Z,Z)-, methyl ester*; (42) *9,12,15- Ocadecatrienoic acid, methyl ester*; (35) *n – Hexadecanoic acid*; and (45) *Linoelaidic acid* (Figure 1).

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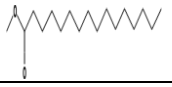
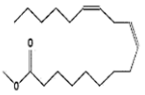
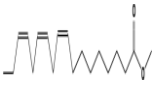
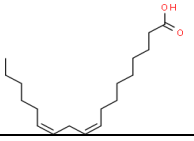
Table 2. Chemical Compounds Found in EPP and Their Biological Activities

No Peak	Bioactive compound	Chemical Formula	Molecular Weight (g/mol)	Area %	Molecular Structure
33	Hexadecanoic acid, methyl ester	C17H34O2	270	10.25	
41	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	7.39	
42	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	292	7.23	
35	n-Hexadecanoic acid	C16H32O2	256	7.00	
45	Linoelaidic acid	C18H32O2	280	7.08	

DISCUSSION

Proximate analysis of salak pondoh peel extract (EPP) showed that EPP contained carbohydrate, mineral, water, crude fiber, fat, protein and vitamin C. Each of these components has a different role in the body, such as protein which is essential for building and repairing body tissues [20], fat which provides energy [21], and carbohydrates which serve as the main fuel source for the body [22]. Additionally, the minerals and vitamin C contained in the material are vital for various body functions, such as maintaining electrolyte balance, strengthening the immune system, and supporting the function of other organs [23]. In this study, the results showed that the water content in EPP was 25.31%, which was higher compared to previous research that reported a water content of 13.71%. This higher water content can affect the stability of the material and its effectiveness in further processing. The water component is important because it influences the texture, solubility, and shelf life of the material. A higher water content can enhance the material's potential as a natural hydration agent in various health products [24]. Furthermore, the mineral content found in EPP was 28.37%, indicating that Salak Pondoh peel extract is rich in essential nutrients. These minerals play a role in supporting various physiological processes in the body, such as bone and teeth formation, oxygen transport, and vital enzymatic functions. The presence of minerals in significant amounts makes EPP a valuable source of additional nutrition, especially for those who need higher mineral intake. Thus, the water and mineral content found in EPP provides high nutritional value, making it a promising material for use in health product development or as a natural supplement.

Minerals play an important role in various metabolic processes, including the metabolism of carbohydrates and fats [25]. They act as cofactors for enzymes

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involved in these metabolic pathways, which are essential for the conversion of nutrients into energy and the regulation of fat storage. Specifically, certain minerals like magnesium, calcium, and zinc are critical for maintaining the body's energy balance and enhancing muscle mass [25]. These minerals help in activating enzymes that regulate fat breakdown and muscle protein synthesis. By supporting proper fat metabolism and muscle development, they contribute to reducing fat accumulation and promoting lean body mass. This is why a diet rich in minerals is beneficial for improving body composition and overall health.

However, the results in this study were limited due to the absence of more specific tests on mineral content and fat metabolism. Although we measured the overall mineral content in the Salak Pondoh peel extract (EPP), a more detailed analysis of individual minerals and their specific effects on fat and carbohydrate metabolism was not performed. Additionally, the study did not examine the direct impact of these minerals on fat reduction and muscle mass in vivo, which could provide more insight into their exact role. Future research should focus on conducting a more thorough investigation into the specific mineral content and its relationship with fat metabolism to better understand how minerals from Salak Pondoh peel extract could influence body composition.

EPP contained 0.88% of vitamin C. Vitamin C is an enzymatic antioxidant easily soluble in water. Vitamin C has a hydroxyl group that can react with free radicals to prevent oxidative damage [26]. Vitamin C also influences body weight by increasing neuroprotection in the ARC to regulate the body's basal metabolism and balance insulin sensitivity [27]. Vitamin C plays a crucial role in the body’s defense against oxidative stress due to its strong antioxidant properties [28]. As an enzymatic antioxidant, it reacts with free radicals, neutralizing them and preventing oxidative damage to cells and tissues. This is especially important in maintaining the health of various organs and tissues, including the skin, blood vessels, and muscles. The ability of Vitamin C to reduce oxidative stress can help prevent or manage conditions such as inflammation and aging-related damage, which are often linked to metabolic issues like obesity and insulin resistance. Additionally, Vitamin C has been shown to influence body weight regulation through its neuroprotective effects in the arcuate nucleus (ARC) of the brain [29]. The ARC plays a key role in controlling the body’s metabolism, appetite, and energy balance. Vitamin C can enhance the signaling pathways in the brain, improving the regulation of basal metabolism, and helping to balance insulin sensitivity. By supporting proper metabolic function and improving insulin sensitivity, Vitamin C can help prevent excessive fat accumulation and may assist in weight management. This explains why Vitamin C in the salak pondoh peel extract (EPP) could contribute to its potential anti-obesity effects.

Based on Table 2, we can see the five highest phytochemical content of the EPP were *Hexadecanoic acid, methyl ester*; *9,12 – Octadecadienoic acid (Z,Z)-, methyl ester*; *9,12,15- Octadecatrienoic acid, methyl ester*; *n – Hexadecanoic acid*; and *Linoelaidic acid*. Most of these phytochemicals are derivatives of *Hexadecanoic acid*. Meanwhile, the phytochemical compound in ethanol extract of snake fruit seeds contained *irganox 1076*, *9,12-octadecadienoic acid (Z, Z)*, *9-octadecadienoic acid (Z)*, *9-octadecenamide (CAS)* and *tetracontane* [30]. *Hexadecanoic acid* has activity as antioxidant and it can be found in Phaleria macrocarpa extract this compound has a higher binding affinity to pancreatic lipase than Positive group given orlistat [31]. *n- Hexadecanoic acid* from *Bipolaris axonopicola* extract also can bind with high affinity to human lipase enzyme compared to orlistat therapy [26]. *Hexadecanoic acid* from Allium cepa leaves shows antioxidant activity that can reduce visceral fat and central obesity [32].

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It was almost like the previous study if salak peel contained *gallic acid*, *linoelaidic acid*, *palmitic acid*, *α-tocopherol*, and *steric acid*, which may contribute to α- glucosidase inhibitor activity [33]. The limitation of our research was that there is no quantitative supporting data on the active compounds and the phytochemical content of the EPP. The advantage or novelty of this study is to examine the content of nutrients and phytochemistry salak pondoh peel extract and its potential for obesity that has never been researched before.

CONCLUSION

Based on the results of the study, salak pondoh peel extract (EPP) contains 28.37% minerals. Although the study did not detail the types of minerals it contains, in general, some minerals such as magnesium, calcium, and zinc are known to play a role in fat and carbohydrate metabolism and reduce fat accumulation. Regarding the vitamin C content (0.88%), this percentage is relatively small. However, vitamin C still has a role in improving insulin metabolism and sensitivity, which can contribute to weight management. *Hexadecanoic acid* can bind with high affinity to human lipase enzyme and have antioxidant activity so that can reduce visceral fat and central obesity. These phytochemical and nutrient content of EPP has biological activity that is possible beneficial for the treatment of obesity. Further in vitro and in vivo studies are needed to evaluate the anti-obesity properties of EPP.

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