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Short Communication

Chemical Constituents and Antioxidant Activity of Ultrasonication-Assisted Aqueous Extract of Pumpkin (*Cucurbita moschata*) Seeds

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ABSTRACT

Plants are widely used in alternative medicine, and hold significant potential for novel drug discovery and development. A notable example of such plant is the pumpkin plant (*Cucurbita moschata*) which offers numerous health benefits due to its antioxidants properties, especially the fruits and seeds. Therefore, this study aimed to identify the chemical constituents of pumpkin seeds and evaluate ots antioxidant activity. Pumpkin seeds were extracted by ultrasonication-assisted extraction (UAE) method using water as the extraction solvent. The chemical composition of the extract was identified by gas chromatographic-mass spectrometric (GC-MS) analysis. The extract was evaluated for its antioxidant activity using the 1-1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. GC-MS analysis revealed numerous chemical constituents including; Squalene, Stigmasterol, Hexadecanoic acid methyl ester, 2-Pentylcyclopropyl cyclopropanebutanoic acid, Hexadecanoic acid ethyl ester, 9,12-Octadecadienoic acid (*Z, Z*)-methyl ester, 16-methyl-Heptadecanoic acid methyl ester, Ethyl 9-cis,11-trans-octadecadienoate, and several forms of Ethyl iso-allocholate and its derivatives. Other compounds identified are 3-(octadecyloxy)propyl ester of oleic acid, 1-Heptatriacotanol, 8,14-Seco-3,19-epoxyandrost derived from Ethyl iso-allocholate, Tocopherol, Tris(acetyloxy) derivative of 3-Pyridinecarboxylic acid, 4,4-Dimethyl cholesta-22,24-dien-5-ol, Triol derivative of 9,10-Secocholesta-5,7,10(19)-triene presented as the 3*β*,5Z,7E isomer, and 3-hydroxy Spirost-8-en-11-one with specific stereochemistry of 3*β*,5*α*,14*β*,20*β*,22*β*,25R. the aqueous extract of pumpkin seeds exhibited potent antioxidant activity with IC_{50} value of 8.625 ± 0.169 µg/mL. In conclusion, pumpkin seed aqueous extract obtained through UAE method contains several compounds that may act as antioxidants.

Keywords: Gas Chromatography-Mass Spectrometry, Antioxidant, *Cucurbita moschata*, Ultrasonification-Assited extraction.

Introduction

Plants are among the most essential natural sources used as medicine for humans and animals. Over the years, plants have made significant contributions to the development of new drugs, and drug products. For example, the yellow pumpkin plant (*Cucurbita moschata*) is a rich source of antioxidant compounds with numerous nutritional benefits. Pumpkin is a climbing plant commonly cultivated as a vegetable in Central Java, Indonesia. In Indonesia, a number of processed foods are made from pumpkin plant. Studies have shown that pumpkin seeds, although contain many substances with numerous health benefits, are underutilized.[1]

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The plant has woody stems that are soft, rectangular, hairy, and light green, with nodes and a length of approximately 25 meters.[2] The foliage of the pumpkin plant is characterized by its singular and circular form, spaning 7-35 cm in length and 6-30 cm in width, featuring a wavy margin, and a round, hairy base. Its flowers, yellow and funnel-shaped, consist of bell-shaped petals. In contrast, the fruit exhibits a spherical shape with a pale yellow mesocarp; the seeds within are milky white, flat, and rigid, measuring roughly 0.5 cm in width and 1.5 cm in length.[2] Pumpkin seeds have antioxidant, hypoglycemic, and hypolipidemic effects.[3] These effects have been attributed to the presence of bioactive compounds like carotenoids and tocopherols in the seed oil.[3] A study revealed that 100 grams of organic pumpkin seeds are composed of several nutritional elements: 127 calories, 15 grams of carbohydrates (0 grams of sugar and 17.9 grams of fiber), 5 milligrams of protein, 21.43 grams of fat, 20 milligrams of calcium, and 0.9 grams of iron.[4] Pumpkin seeds are used as an alternative non-pharmacological therapy for many conditions, and has been found to have antianemia, anthelmintic, anticarcinogenic, antidepressant, antidiabetic and hypoglycemic, cytoprotective, as well as antimicrobial activities. It is important to note that the properties of this plant are due to the presence and powerful effects of the flavonoid active ingredients. There is a need to investigate the relationship between the chemical profile of pumpkin seeds and their biological activities, especially their antioxidant effect. Antioxidants are divided into two categories based on their source: natural antioxidants and synthetic antioxidants. Natural antioxidants are

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antioxidant compounds that occur naturally in the body as a mechanism of normal body defenses or originating from natural sources outside the body. Meanwhile, synthetic antioxidants are chemically synthesized compounds. One source of antioxidants are plants containing high polyphenolic compounds. A commonly used model for measuring the antioxidant ability of plant extract is the 1-1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. DPPH is a stable free nitrogen radical used as a reagent for free radical scavenging tests, it is freely soluble in organic solvent like methanol, and exhibit maximum absorbance in the wave length range 515-520 nm. The DPPH free radical scavenging assay is based on the reduction of DPPH free radicals in methanol solution by free radical inhibitors. In this assay, a potential radical inhibitor or antioxidant compound donates an electron to DPPH, causing it to be reduced. This is normally visualized as a change in the purple colour of DPPH solution in methanol into a yellow colour.[5] The intensity of these colour is usually measured spectrophotometrically at 515 – 520 nm, less intense colour has lower absorbance reading which translate to higher antioxidant effect of the test substance. The present study aim to identify the chemical constituents of pumpkin seeds extract through GC-MS analysis and evaluate the antioxidant activity of the extract to serve as a preliminary investigation upon which further research on the chemical profile and biological activities of pumpkin seeds can be nased.

Materials and Methods

Plant collection and identification

Yellow pumpkin (*Cucurbita moschata*) seeds were harvested from the Pakem area, Cangkringan, Yogyakarta (7°37'41"S 110°25'33" E) in October 2023. The plant material was identified and authenticated in the Merapi Farma Herbal Yogyakarta, Indonesia.

Preparation of plant extract

The pumpkin seeds were manually removed, cleaned, peeled, and dried in the oven at 45°C. The dried seeds were blended, and sifted until 1 kg of fine powder was obtained. The powdered seeds were extracted by ultrasonic waves (Bransonic® merk Branson CPX, USA) at a temperature of 35°C for 60 min using water as the extraction solvent. The extract solution was filtered, placed in a suitable plastic container, and frozen at -20°C. Subsequently, the frozen material was freeze dried for 24 h using a freeze dryer. Finally, a green dry extract with a characteristic odour was obtained.

Gas chromatographic-mass spectrometric (GC-MS) analysis

GC-MS analysis was conducted using the HP-5MS UI GCMS system, with Cromeleon 7 chromatogram software and, NIST 2014 library database. The system was equipped with a 30-meter-long column using helium gas as a carrier. The injector was maintained at a temperature of 230°C, 50 mL/min split flow, 50 split ratio, and 1.00 mL/min front inlet flow. The test sample was dissolved in ethanol and 1.5 mL of the solution was placed in a microtube, and vortexed in a centrifuge at 9500 rpm for 3 min. The supernatant was collected in a GC vial, and then injected into the GC-MS system.

Evaluation of the antioxidant activity

A 50 ppm DPPH stock solution was prepared by dissolving 5 mg of DPPH in 100 mL of absolute methanol. A blank/control solution containing 2 mL of methanol and 1 mL of 50 ppm DPPH solution was also prepared. For the test sample, 2 mL of sample solution and 2 mL of 50 ppm DPPH solution were mixed. The test sample and blank solutions were incubated at 27°C for 30 min. The absorbance of the resulting solution was measured at 517 nm using a UV-Vis spectrophotometer.[5]

Results and Discussion

Chemical constituents of pumpkin seed extract

Figure 1 shows the chromatogram obtained from the GC-MS analysis of pumpkin seed extract. The GC-MS analysis identified eighteen (18) distinct chemical constituents. The compounds, particularly those with a peak area exceeding 10%, are detailed in Table 1. It is important to note that pumpkin seeds contain Squalene and Stigmasterol as major components. A previous study of the GC-MS analysis of ethanol extract of pumpkin seeds revealed the presence of squalene, which was suggested as a likely contributor to the antioxidant activity of pumpkin seeds.[6]

Squalene has been shown to act as an antioxidant by protecting the body against oxidative stress induced by environmental stressors such as free radicals.[7] Squalene is often used to provide positive pharmacological effects, including use as an antioxidant,[8,9] anti-cancer,[10] anti-aging,[11] chemopreventive,[12] anti-bacterial, adjuvant for vaccines and drug carriers,[13] as well as detoxification.[14] In Japan, squalene is known by a special term "Tokubetsu no Miyage," meaning a precious gift.

Table 1: Chemical Constituents of Pumpkin Seeds

Peak Number	Compound	Retention time (min)	Relative Area %
1	Hexadecanoic acid, methyl ester	16.70	1.25
2	Cyclopropanebutanoic acid	16.84	0.01
3	Hexadecenoic acid, ethyl ester	17.35	0.77
4	9,12-Octadecadienoic acid (*Z,Z*)-methyl ester	18.28	7.17
5	Heptadecanoic acid, 16-methyl-methyl ester	18.57	0.53
6	Ethyl 9-cis,11-trans-octadecadienoate	18.88	3.18
7	Ethyl iso-allocholate	21.16	0.91
8	Oleic acid, 3-(octadecyloxy)propyl ester	21.36	0.66
9	Squalene	24.11	49.33
10	Ethyl iso-allocholate	24.31	1.14
11	Ethyl iso-allocholate	24.95	1.72
12	Ethyl iso-allocholate	25.04	0.62
13	Ethyl iso-allocholate	25.54	0.72
14	Tocopherol	26.13	8.79
15	3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7	29.06	0.55
16	Cholesta-22,24-dien-5-ol,4,4-dimethyl, Stigmasterol	29.58	13.67
17	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3*β*,5Z,7E)	30.30	3.69
18	Spirost-8-en-11-one, 3-hydroxy (3β,5a,14β,20β,22β,25R)	30.91	5.26

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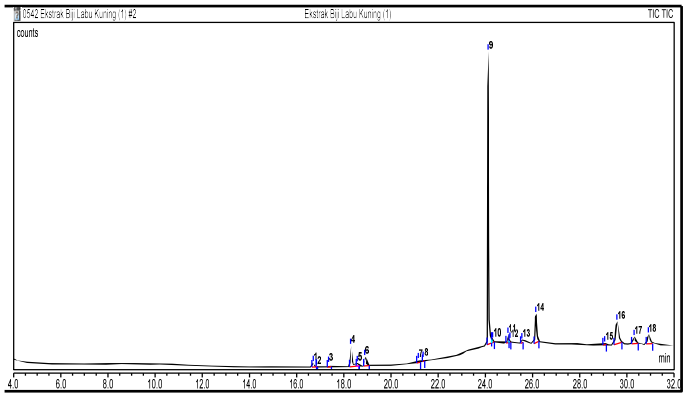

Figure 1: GC Chromatogram of Yellow Pumpkin Seed Extract

Residents of the Izu peninsula have used it to cure various ailments. Therefore, the organic compound is termed "Samedawa" which implies healing everything.[15] There is a considerable increase in the demand for squalene as a functional nutrient due to the discovery of its important pharmacological effects in preventing diseases resulting from heart dysfunction, such as high blood pressure.[16] The use of this functional nutrients in diet has multiple benefits. These benefits include prevention of obesity, reduction of metabolic disorders, increase blood oxygen levels, sharpen eyesight and cataracts prevention, increase in the production of insulin, prevention of genetic mutations due to exposure to carcinogenic UV radiation, acceleration of inflammatory wound healing, as well as increase in body immunity and fitness.[8,10,12,17]

It has been observed that certain compounds, including squalene, diminish the activity of COX-2, which is typically overexpressed in human monocytes and neutrophils subjected to lipopolysaccharide (LPS) treatment. Additionally, the expression of the genes MMP-1 and MMP-9 in LPS-treated human monocytes is downregulated by squalene. Antioxidant activities of squalene on the expression of the MMP-1 and MMP-3 genes in human neutrophils treated with LPS are also evident.[18]

The constituents involved in the squalene-squalene epoxidation reaction, specifically oxygen, NADPH, and NADPH-dependent reductase are co-localized with hemoglobin, and these play a pivotal role in sterol biosynthesis. The structural and molecular makeup of this assembly is analogous to that of squalene. In the process of sterol biosynthesis, this organic molecule (squalene) undergoes epoxidation and cyclization, culminating in the formation of a sterol ring.

Stigmasterol, identified chemically as stigmasta-5,22-dien-3-ol with the formula $C_{29}H_{48}O$ and commonly referred to as wulzen anti-stiffness factor or stigmasterin, is classified within the tetracyclic triterpenes group of phytosterols. This plant-derived sterol is not synthesized endogenously by the human body. Instead, they must be obtained exogenously through various dietary sources, including vegetable oils such as rapeseed and soybean oils, along with calabar nuts, cereals, unpasteurized milk, grains, medicinal plants, as well as nuts and legumes.[19] *In vivo* investigations have demonstrated that extracts from plants, abundant in stigmasterol, possess significant immunomodulatory and anti-inflammatory properties.[20] Such extracts can lead to a decreased secretion of pro-inflammatory cytokines, nitric oxide (NO), and tumor necrosis factor-gamma (TNF-ϒ), as well as an inhibition of cyclooxygenase-2 (COX-2).[21,22] Drugs that contain stigmasterol are highly valued in Traditional Chinese Medicine for their efficacy in managing immune-inflammatory reactions.[23,24]

Antioxidant activity of pumpkin seeds extract

The antioxidant activity of pumpkin seed extract is presented in Figure 2. The results are shown as percentage radical scavenging activity. As shown in Figure 2, pumpkin seed extract exhibited a concentration-dependent DPPH radical scavenging activity. The IC_{50} value for the antioxidant activity of the extract is shown in Table 2. The IC_{50} value is

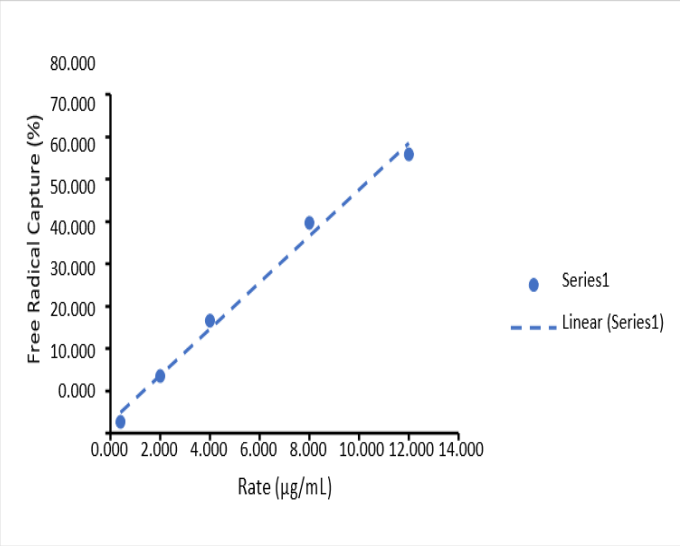

Figure 2: Free Radical Scavenging Activity of Pumpkin Seed Extract

the effective concentration of the extract required to reduce 50% of DPPH radical. Theoretically, the strength of antioxidant compounds is measured by their IC_{50} values. Compounds with IC_{50} values below 50 µg/mL are categorized as very strong antioxidants, compounds with IC_{50} values between 50-100 µg/mL are regarded as strong antioxidants, compounds with IC_{50} values between 100-150 µg/mL are moderate antioxidants, and compounds with IC_{50} values between 151 and 200 are weak antioxidants. The smaller the IC_{50} value, the stronger the antioxidant activity.[25] From Table 2, it can be seen that the IC_{50} value of the test sample (pumpkin seed extract) is less than 50 µg/mL, which indicates that pumpkin seed extract has very strong antioxidant activity. Pumpkin seed extract (*Cucurbita moschata*), obtained by ultrasonication-assisted extraction (UAE) has been shown to inhibit lipid peroxidation and increase the activity of catalase, super oxide dismutase (SOD), and glutathione. The mechanism by which the extract exhibit this antioxidant effect was proposed to be by electron donation to free radicals, stabilized them, thereby decreasing the lipid peroxidation reactions induced by these free radicals.[26-28]

Table 2: IC_{50} Value for the Antioxidant Activity of Pumpkin Seed Extract

Mean IC_{50} Value (µg/mL)	Standard Error of Mean (SEM)
8.625	0.169

Conclusion

In conclusion, pumpkin (*Cucurbita moschata*) seed aqueous extract obtained through ultrasonication-assisted extraction contains several compounds that act as antioxidants. The extract demonstrated very strong antioxidant activity with IC_{50} value of 8.625 µg/mL. GC-MS analysis identified 18 compounds in the extract with some of the compounds identified as Hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, Ethyl 9-cis,11-trans-octadecadienoate, Squalene, Ethyl iso-allocholate, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3*β*,5Z,7E), and 3-Hydroxy spirost-8-en-11-one. The presence of these compounds may have contributed to the potent antioxidant activity of pumpkin seed extract.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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