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IDENTIFIKASI SENYAWA YANG BERPOTENSI SEBAGAI ANTIDIABETES PADA EKSTRAK ETANOL 30% DAUN KUMIS KUCING (*Orthosiphon aristatus*) HASIL KULTUR JARINGAN DAN BUDIDAYA

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ABSTRAK

Orthosiphon aristatus (kumis kucing) merupakan tanaman obat tradisional yang banyak mengandung senyawa metabolit sekunder seperti flavonoid, asam fenolat, dan senyawa turunan diterpene dan dapat dimanfaatkan untuk mengatasi gangguan metabolik termasuk inflamasi, gangguan saluran kemih dan diabetes. Penelitian ini bertujuan untuk membandingkan profil metabolit sekunder dari ekstrak etanol 30% daun kumis kucing hasil kultur jaringan dan budidaya konvensional. Ekstraksi dilakukan menggunakan metode *Ultrasonic Assisted Extraction* (UAE), dan identifikasi senyawa menggunakan instrumen *Liquid Chromatography–High Resolution Mass Spectrometry* (LC-HRMS). Hasil analisis menunjukkan bahwa terdapat 5 jenis senyawa dominan dalam ekstrak kultur jaringan antara lain asam rosmarinat (20,46%), asam malat (6,34%), asam kuinat (5,52%) asam sikorat (4,52%), dan asam vanillylmandelik (3,4%). Ekstrak hasil budidaya mengandung asam rosmarinate (11,36%), asam malat (4,7%), asam ferulat (9,46%), asam vanillylmandelik (4,95%) dan asam metilmalonat (4,15%). Senyawa epigallocatechin gallate (EGCG), isokuersetin, kuercitrin dan carmine hanya terdeteksi pada kultur jaringan. Hasil ini menunjukkan bahwa teknik kultur jaringan dapat mempengaruhi biosintesis dan akumulasi metabolit sekunder tertentu dalam tanaman, sehingga berpotensi dimanfaatkan untuk menghasilkan bahan baku berkualitas tinggi dalam pengembangan produk fitofarmaka.

Kata kunci: *Orthosiphon aristatus*, LC-HRMS, Kultur jaringan, Metabolit sekunder, Asam rosmarinat, Asam ferulat, Asam malat

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ABSTRACT

Orthosiphon aristatus (cat's whiskers) is traditional medicinal plant that contains many secondary metabolite compound such as flavonoids, phenolics acids, and diterpene derivatives and can be used to treat metabolic disorder including inflammation , urinary tract disorders and diabetes. This study aims to compare the secondary metabolite profile of 30% ethanol extract of cat's whiskers leaves from tissue culture and conventional cultivation. Extraction was carried out using Ultrasonic Assisted Extraction (UAE) method and the compounds were identified using Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS). The analysis results showed that there were 5 types of dominant compounds in the tissue culture extract, including rosmarinic acid (20.46%), malic acid (6.34%), quinic acid (5.52%), chicoric acid (4.52%), and vanillylmandelic acid (3.4%). The cultivated extract contained rosmarinic acid (11.36%), malic acid (4.7%), ferulic acid (9.46%), vanillylmandelic acid (4.95%), and methylmalonic acid (4.15%). The compounds epigallocatechin gallate (EGCG), isoquercetin, quercitrin, and carmine were only detected in tissue culture. These results indicate that tissue culture techniques can influence the biosynthesis and accumulation of certain secondary metabolites in plants, thus potentially being used to produce high-quality raw materials in the development of phytopharmaka products.

Keywords: *Orthosiphon aristatus, LC-HRMS, Tissue culture, Secondary metabolite, Rosmarinic acid, Ferulic acid, Malic acid*

INTRODUCTION

Orthosiphon aristatus, commonly known as cat's whiskers, is a herbaceous plant recognized for its pharmacological properties and rich composition of secondary metabolites, such as flavonoids, phenolic acids, and diterpene derivatives. These compounds have attracted considerable interest due to their potential therapeutic benefits, particularly in managing metabolic disturbances, including inflammation, urinary tract disorders, and diabetes (Faramayuda, Mariani, et al., 2021a) (Faramayuda, Mariani, et al., 2022a) (Faramayuda, Mariani, et al., 2021b). The current research aims to assess and compare the profile of secondary metabolites derived from the 30% ethanol extract of cat's whiskers leaves under two distinct cultivation conditions: tissue culture and conventional cultivation methods.

The extraction of bioactive compounds from plants significantly depends on the solvent employed, as various solvents can differently affect the yield and composition of metabolites (Sugier et al., 2022) (Aziz et al., 2021). Ultrasonic Assisted Extraction (UAE) was utilized as it is known to enhance the extraction efficiency of bioactive compounds due to its ability to increase mass transfer and dissolution of particles

(Aziz et al., 2021) (Faramayuda, Riyanti, et al., 2021). The identification of these compounds was carried out through Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS), a technique that provides detailed information about the molecular structure and composition of metabolites present in plant extracts (Faramayuda et al., 2021; Faramayuda et al., 2022).

Preliminary findings indicate that the LC-HRMS analysis revealed a distinctive profile of secondary metabolites between the two extraction sources. The tissue culture extracts yielded five dominant compounds: rosmarinic acid (20.46%), malic acid (6.34%), quinic acid (5.52%), chicoric acid (4.52%), and sagerenic acid (3.9%). Conversely, the extracts from conventional cultivation exhibited lower levels of rosmarinic acid (11.36%), ferulic acid (9.46%), vanillic acid (4.95%), malic acid (4.7%), and methylmalonic acid (4.15%).

Interestingly, some unique compounds, such as sinensetin (0.92%) and carnosic acid (0.28%), were exclusively detected in the tissue culture extracts, indicating that in vitro cultivation conditions can significantly influence the biosynthesis and accumulation of specific metabolites in cat's whiskers (Faramayuda, Mariani, et al., 2022a). Notably, both samples maintained equal relative levels of epigallocatechin gallate (EGCG) and isoquercetin, suggesting consistent biosynthetic pathways for these compounds across both cultivation methods (Faramayuda et al., 2022).

The results underscore the efficacy of tissue culture techniques in engineering plants for enhanced metabolite production, thus opening avenues for producing high-quality standards in the development of phytopharmaka products (Faramayuda et al., 2022). Understanding the variation in the metabolite profiles can lead to improvements in extraction strategies and cultivation practices, ultimately aiding in the harnessing of cat's whiskers' therapeutic potential more effectively (Faramayuda, Mariani, et al., 2022b)

The comparison of secondary metabolite profiles from different cultivation methods reinforces the influence of these methods on the biosynthesis of key bioactive compounds in Orthosiphon aristatus. The findings indicate that optimized tissue culture techniques can yield higher concentrations of essential metabolites, making it an attractive approach for future biotechnological applications and formulation of herbal pharmaceuticals.

MATERIAL AND METHOD

The materials used in the research were cat's whiskers leaves (Orthosiphon aristatus) from cultivation conventional and tissue culture plant seeds. Solvent used is ethanol 30%. Equipment used are Glassware, Ultrasonic LC 30 H Elma (Elma Schmidbawer Gmbh, Gottlieb- DaimLer Straβ 17 78224, Singen, Germany), water bath, brown bottle, evaporating dish, sieve, oven, analytical balance, crucible, microlab, quartz cuvette (Merck), centrifugator, micropipettes, Eppendorf pipettes, Microplate Reader Elx 800, freeze dryer, rotary vacuum evaporator (Heidolph, Germany), LC-HRMS

1. Extraction Sample

10 grams of cat's whiskers leaf simplicia powder was poured into an Erlenmeyer flask and added 100 ml of 30% ethanol, then extracted using the sonication method for 3x20 minutes at a temperature of 40oC, repeated 3 times, then the filtrate was separated from the residue. The extracted solution was collected and the concentration process was carried out using a rotary vacuum evaporator, so that the extract was obtained.

2. Metabolite Profile Analysis using LC-HRMS

A. Extract Preparation

50 mg of extract was weighed and placed into a 2 mL microcentrifuge tube. Next, 1 mL of LC-MS grade methanol was added and vortexed for 60 seconds. The mixture was then ultrasonicated for 30 minutes at room temperature. After the sonication process, the sample was centrifuged at 5000 x g for 10 minutes at 4°C. The pellet was discarded and the supernatant was filtered using a 0.22 µm PTFE filter and placed into a glass HPLC vial. The sample was ready for injection into the LC-HRMS system. The sample was analyzed using an Acclaim Vanquish C-18 analytical column (15 mm x 2.1 mm x 2.2 µm). The sample injection volume was 3 µL. The mobile phase used for the analysis was water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) with a flow rate of 0.3 mL/min. The analysis was performed in gradient mode, as follows: Mobile phase B was set at 5% at initial conditions and then adjusted to 90% for 16 minutes. At 90% B was held for 4 minutes and continued to initial conditions (5% B) for up to 25 minutes. The MS parameter conditions were as follows: spray voltage 3.30 kV with a capillary temperature of 320ºC and an auxiliary gas heater temperature of 30ºC. The sheath gas flow rate was set at 32 arbitrary units (AU) with an auxiliary gas flow rate of 8 AU and a sweep gas flow rate of 4 AU. Measurements were performed in positive

and negative ionization modes with a resolution of 70,000 for full MS and 17,500 for dd- MS2.

B. Data Analysis

Raw total ion chromatogram (TIC) data from LC-HRMS measurements were used to identify metabolite composition. TICs were processed using Compound Discoverer software (Thermo Scientific, USA) using an untargeted metabolomics workflow. Data were filtered by compound name, aligned with Mz Cloud, and MS2 DDA for preferred ion.

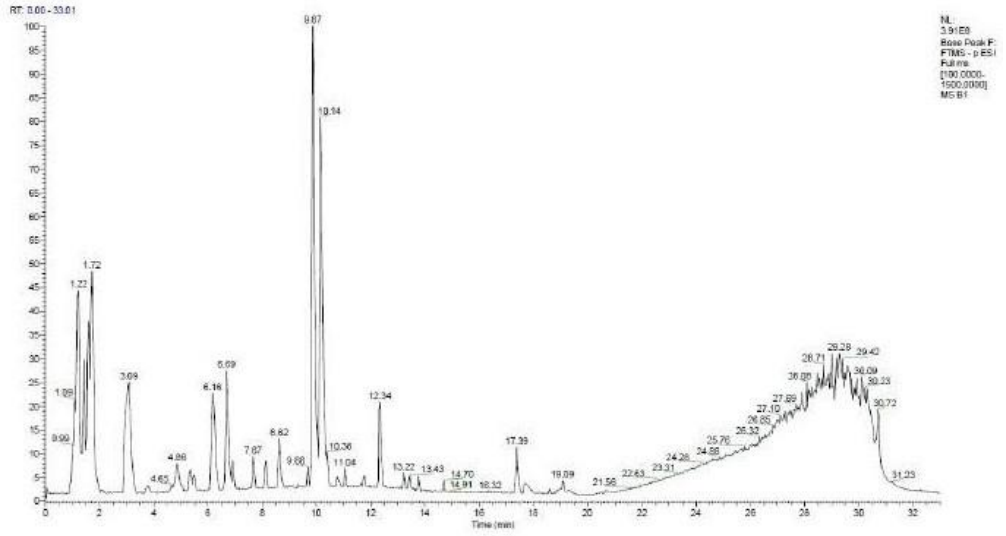

Figure 1. LC–HRMS chromatogram profile of the cultivation extract of Orthosiphon aristatus leaves

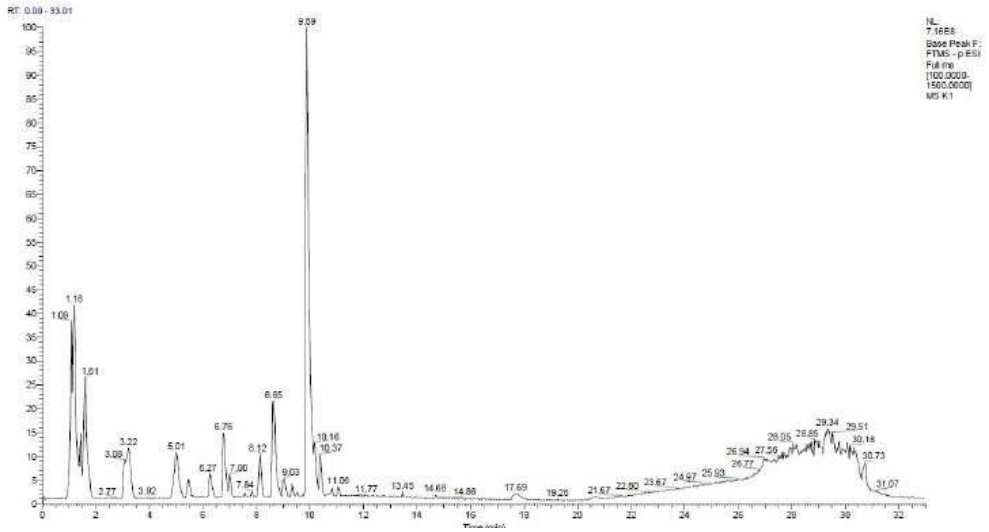

Figure 2. LC–HRMS chromatogram of tissue culture–derived Orthosiphon aristatus leaves extract.

Table 1. Differences in the % area of chemical compounds contained in cultivation cat whisker extract and tissue culture

No.	Compound Name	Molecular Formula	Molecular Weight	Area %	
				Tissue Culture	Cultivation
1.	(R)-(+)-rosmarinic acid	$C_{18}H_{16}O_8$	360,0846	20,46	11,36
2.	DL-Malic acid	$C_4H_6O_5$	134,0207	6,35	4,74
3.	Quinic acid	$C_7H_{12}O_6$	192,0629	5,53	2,27
4.	(−)-L-Chicoric acid	$C_{22}H_{18}O_{12}$	474,08	4,52	1,89
5.	Hex-2-ulose	$C^6H_{12}O_6$	180,0628	3,80	0,91
6.	Vanillyl mandelic acid	$C_9H_{10}O_5$	198,0524	3,40	4,95
7.	Methylmalonic acid	$C_4H_6O_4$	118,0257	3,34	4,15
8.	Caftaric acid	$C_{13}H_{12}O_9$	312,0484	2,93	1,60
9.	Glucoheptonic Acid	$C_7H_{14}O_8$	226,0687	2,39	0,52
10.	Aspirin	$C_9H_8O_4$	180,0416	2,09	3,00
11.	(E)-Ferulic acid	$C_{10}H_{10}O_4$	194,0574	1,78	9,46
12.	salvianolic acid	$C_{36}H_{30}O_{16}$	718,1541	1,67	1,30
13.	Citric acid	$C_6H_8O_7$	192,0266	1,32	1,88
14.	(±)-Tartaric acid	$C^4H_6O_6$	150,0157	1,17	2,10
15.	(±)-Usnic acid	$C_{18}H_{16}O_7$	344,0899	0,47	0,48
16.	D-Glucono-δ-lactone	$C_6H_{10}O_6$	178,0472	0,37	0,44
17.	Caffeoylmalic acid	$C_{13}H_{12}O_8$	296,0535	0,35	0,28
18.	Hept-2-ulose	$C_7H_{14}O_7$	210,0736	0,33	0,41
19.	4-Oxoproline	$C_5H_7NO_3$	129,0418	0,31	0,66
20.	Diacetin	$C_7H_{12}O_5$	176,068	0,28	0,65
21.	benzal chloride	$C_7H_6Cl_2$	159,9853	0,18	0,21

RESULT AND DISCUSSION

Based on the chromatographic analysis and tables documenting the compound contents in a 30% ethanol extract of Orthosiphon aristatus (commonly referred to as kumis kucing), this extract is rich in various phenolic and flavonoid compounds, including rosmarinic acid, chicoric acid, caffeic acid, and ferulic acid. The high relative abundance of rosmarinic acid (20.47% of total identified phenolic acids) highlights its potential primary contribution to the antidiabetic properties of this plant through various pharmacologically validated mechanisms (Hunaefi & Smetanska, 2013) (Zheng & Wang, 2001)

Rosmarinic Acid: A Key Compound

Rosmarinic acid is an ester of caffeic acid and is characterized by its phenolic hydroxyl groups (–OH ortho), contributing significantly to its antioxidant capacity. It is believed to mediate its antidiabetic effects by lowering plasma glucose levels and improving insulin sensitivity (Exarchou et al., 2002). Various studies have shown that rosmarinic acid exhibits potent antioxidant properties and inhibits key metabolic enzymes associated with type 2 diabetes, specifically α-glucosidase and α-amylase,

important targets in carbohydrate digestion and glucose management (Ariffin et al., 2011).

Mechanisms of Action

The mechanisms through which rosmarinic acid may exert its potential antidiabetic effects are multifactorial. Its role in reducing oxidative stress by scavenging free radicals is well-documented, directly influencing cellular responses to insulin (Katanić et al., 2020). Additionally, the modulation of metabolic pathways through its inhibitory action on alpha-amylase and alpha-glucosidase results in diminished absorption of carbohydrates, thereby lowering postprandial blood glucose levels (Sīle et al., 2022). Studies indicated metabolism enhancements with the ingestion of extracts high in rosmarinic acid (Exarchou et al., 2002).

Discussion on the Potential of Rosmarinic Acid in Diabetes Management

Rosmarinic acid, a polyphenol primarily found in various plants of the Lamiaceae and Boraginaceae families, is emerging as a significant compound in the management of diabetes due to its multifaceted biological activities. Characterized by its ester link between caffeic acid and other components, rosmarinic acid exhibits potent antioxidant capacities critical in counteracting oxidative stress—a condition often exacerbated in diabetic patients. The outlined mechanisms by which rosmarinic acid may mediate its antidiabetic effects include improving insulin sensitivity and decreasing plasma glucose levels through various enzymatic inhibitory actions (Neagu et al., 2023) (Noor et al., 2022).

Antioxidant Properties

Numerous studies underscore the strong antioxidant properties of rosmarinic acid. It scavenges reactive oxygen species (ROS) and upregulates antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Nechita et al., 2025). Its role as an antioxidant is vital in minimizing oxidative damage in diabetic conditions, thereby protecting pancreatic β-cells and enhancing insulin secretion (Luo et al., 2020). Moreover, rosmarinic acid has proven effective in both in vivo and in vitro contexts for mitigating oxidative stress—a critical contributor to diabetes and its complications (Baranauskaitė et al., 2020) (Chen et al., 2022).

Inhibition of Key Metabolic Enzymes

Key metabolic enzymes, particularly α- glucosidase and α-amylase, have been targeted for diabetic management due to their roles in carbohydrate digestion and glucose absorption. Rosmarinic acid demonstrates a strong inhibitory effect on these enzymes, significantly reducing postprandial blood glucose levels (Tshiyoyo et al., 2022) (Aryal et al., 2024). The action of rosmarinic acid on these enzymes can lead to improved glycemic control, making it a valuable component in dietary interventions aimed at managing diabetes. Furthermore, studies indicate that rosmarinic acid has a higher in-silico affinity for α-glucosidase than that of standard pharmaceutical agents such as acarbose, which supports its potential utility as a natural therapeutic alternative (Aryal et al., 2024)

Impact on Insulin Sensitivity

Rosmarinic acid also plays a crucial role in enhancing insulin sensitivity, which is essential for glucose management in diabetic patients. By activating the AMP-activated protein kinase (AMPK) pathway, rosmarinic acid improves glucose uptakeby musclecells, mimicking insulin action (Vlavcheski et al., 2017) (Gui et al., 2021). Enhanced AMPK activity is pivotal, as it mediates numerous metabolic processes that support regulated glucose homeostasis, reducing the insulin resistance characteristic of type 2 diabetes (Zhou et al., 2017).

Anti-inflammatory Effects

In addition to its antioxidant and enzyme- inhibitory properties, rosmarinic acid exerts notable anti-inflammatory effects. By downregulating pro-inflammatory cytokines and inhibiting related signal transduction pathways, it contributes to mitigating the chronic inflammation often linked to metabolic disorders such as obesity and diabetes (Grzegorczyk- Karolak et al., 2024). The reduction of inflammatory markers further enhances its efficacy in managing diabetes and presents a dual-action approach by combating both oxidative stress and inflammation-two key factors in diabetes pathology.

Comparative Analysis with Other Compounds

Comparatively, while rosmarinic acid is prominent due to its abundance and biological activities, other phenolics such as quercitrin and isorhamnetin contribute to the overall bioactivity of the extract. These compounds may enhance the efficacy of rosmarinic acid through synergistic interactions within the extract (Shan et al., 2005). For instance, quercetin is another flavonoid that has

shown potential in improving endothelial function and glucose metabolism by enhancing insulin sensitivity (Katanić et al., 2020). However, the focus on rosmarinic acid is substantiated by its significant proportions and pharmacological validations in various studies (Falé et al., 2011).

CONCLUSION

In summary, the diverse array of phenolic and flavonoid compounds within the 30% ethanol extract of *Orthosiphon aristatus*, particularly the significant presence of rosmarinic acid, suggests robust potential for antidiabetic applications. Future research should focus on conducting clinical trials and molecular studies to substantiate these findings and explore the full therapeutic potential of this extract in managing diabetes,

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