



Comparative Assessment of Antioxidant Properties Between In-Vitro Cultured Cat's Whiskers Leaves (*Orthosiphon stamineus*) and Quercetin

Fahrauk Faramayuda ^{a,b,*}, Dinda Choirunnisa ^b, Yenni Karlina ^b

^a Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

^b Pharmaceutical Biology Department, Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

Abstract. The present study investigates the antioxidant properties of in-vitro cultured leaves of cat's whiskers (*Orthosiphon stamineus*) compared to the well-known antioxidant quercetin. Antioxidants are crucial for neutralizing free radicals and play a significant role in protecting cells from oxidative stress, a contributing factor to various diseases and aging processes. The research aims to quantify and compare the radical scavenging activity of extracts from the in vitro cultured leaves and quercetin, using the DPPH method, a widely recognized assay in antioxidant research. Extraction involved macerating in-vitro cultured cat's whiskers leaves with 96% ethanol, which facilitates the dissolution of active phytochemicals. Antioxidant activity was subsequently assessed through the DPPH assay, where colorimetric change indicates the extract's scavenging ability towards free radicals. The findings revealed that the in vitro cultured leaves extract exhibited a potent antioxidant activity, with an IC₅₀ value of 0.74 µg/mL, significantly lower than quercetin's IC₅₀ value of 7.51 µg/mL. These results highlight the potential of in-vitro cultured cat's whiskers as a natural antioxidant source, suggesting possible applications in the food and pharmaceutical industries, where combating oxidative stress is essential for health maintenance and disease prevention. Further research is necessary to elucidate the specific biochemical pathways and mechanisms underpinning the antioxidant capacity of the in vitro cultured leaves extracts.

Keywords: *O. stamineus*; In vitro culture; antioxidant; plant biotechnology; micropropagation.

Type of the Paper: Regular Article.



1. Introduction

Antioxidants play a pivotal role in neutralizing free radicals, thereby protecting against oxidative damage, which is increasingly recognized as a contributor to various diseases and aging. The plant *Orthosiphon stamineus*, commonly known as cat's whiskers, has garnered attention for its health benefits, particularly its antioxidant capabilities. It contains significant bioactive compounds exhibiting strong antioxidant activities [1]. This study aims to comparatively assess the antioxidant properties of in vitro-cultured cat's whiskers leaves and quercetin, a well-established antioxidant, utilizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to quantify their effectiveness.

The extraction of antioxidants from plant material is critical for assessing their potential health benefits. Extraction methods can significantly influence the yield and activity of bioactive

compounds [2]. Cold maceration has been found to better preserve antioxidant capacities compared to hot extraction methods, which may degrade sensitive phytochemicals [3]. In this study, an ethanol maceration method was employed, recognized for its effectiveness in extracting phenolic and flavonoid compounds—both renowned for their antioxidant properties. The utilization of 96% ethanol is corroborated by research that highlights its efficacy in maximizing the yield of antioxidant compounds from various plant sources [4].

The DPPH radical scavenging assay is a widely utilized method for evaluating the antioxidant activity of extracts from natural products [5]. This assessment provides crucial insights into the therapeutic potential of plant extracts, particularly pharmacological evaluations and nutraceutical applications. Previous studies have shown that various extraction methods, including maceration and ultrasonic methods, can yield varying antioxidant capacities from plant materials [6,7].

Research indicates that specific growth regulators, such as 2,4-D, can significantly influence callus formation, leading to increased accumulation of beneficial phytochemicals [8]. This enhancement is crucial, as it allows more efficient extraction processes that optimize antioxidant and antimicrobial properties [9,10]. Furthermore, optimizing extraction techniques, such as through response surface methodology (RSM), has successfully increased the antioxidant activity of cat's whiskers extracts [10].

Research has demonstrated that cat's whiskers extracts exhibit significant antioxidant activity, as assessed through various assays, reflecting their strong capacity to scavenge free radicals and prevent oxidative stress-related health issues [11,12]. This capacity is largely attributed to the phenolic compounds and flavonoids in the leaves [11,12]. In vitro culture of cat's whisker leaves, like that of other plant tissue, serves multiple vital purposes in plant propagation, conservation, and biochemical research. A primary advantage of in vitro culture is the regeneration of plant tissues in a controlled environment, enabling rapid multiplication of plant material without dependency on traditional soil-based agriculture. This method is especially beneficial for exotic or endangered plants, as it preserves genetic material while promoting biodiversity.

Furthermore, current research robustly supports the notion that in-vitro cultured extracts of cat's whiskers serve as formidable natural antioxidants, outperforming established antioxidants such as quercetin, as elucidated by the DPPH assay. Given the serious health implications of oxidative stress on, these findings highlight the need for further scientific exploration into the bioactive properties of *Orthosiphon stamineus*. Such research is imperative for substantiating its application in preventive health strategies and could lead to significant advancements in dietary recommendations and therapeutic interventions. The compelling evidence of its antioxidant qualities encourages a deeper understanding of the underlying mechanisms. This approach could

pave the way for innovative food products and underscore the relevance of harnessing natural plant-derived antioxidants in the search for effective therapy against oxidative stress-related disorders. By connecting these two perspectives, emerging scientific insights highlight cat whiskers as a significant contributor to both preventive health and nutritional strategies, demonstrating its dual potential to inform dietary innovations while addressing critical health issues.

2. Material and Methods

2.1 Instrument

Rotary evaporator (Heidolph, Germany), analytical balance (Metler toledo, German), analytical balance (Metler toledo, German), water bath (lab companion, korea), and UV-Visible spectrophotometer (Shimadzu, Japan, UV 1900i) were used.

2.2 Plant Materials

Cat whiskers plants from in vitro culture were obtained from micropropagation and acclimatization in the medicinal plant garden of the Faculty of Pharmacy, Jenderal Achmad Yani University (Fig. 1). The media and growth regulators used in micropropagation were Murashige and Skoog (MS) + 6-Benzylaminopurine (BAP) 2ppm + 1-Naphthaleneacetic acid (NAA) 3 ppm [12].



Fig. 1. Cat's whiskers plants from in vitro culture [12]. a = five month old, b = nine month old

2.3 Chemicals

Ethanol 96% (Merck, Germany), quercetin (Merck, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl; Merck, Germany) were used.

2.4 Quantitative Antioxidant Activity Test

2.4.1. Preparation of Reagent Solution and Determination of DPPH Wavelength

10 mg of DPPH powder were weighed and dissolved in pro-analysis methanol to a final volume of 100 mL to obtain a stock solution of 100 µg/mL. Then, 40 mL DPPH solution was diluted with pro-analysis methanol to a final volume of 100 mL, achieving a DPPH solution with a concentration of 40 µg/mL. The solution was subsequently incubated for 30 minutes, protected

from to prevent photodegradation of DPPH, which could otherwise compromise measurements accuracy. *Preparation and Measurement of Quercetin Solution.*

5 mg of quercetin were weighed and dissolved in pro analysis methanol up to a final volume of 50 mL, shaken until homogeneous, yielding a quercetin stock solution of 100 µg/mL. Working solutions of 6 concentrations (2, 4, 6, 8, 10, and 12 µg/mL) were prepared. The solution was incubated for 30 minutes, protected from the light, and their absorbance was measured using a UV Visible spectrophotometer at the maximum wavelength of the DPPH solution.

2.4.2. *Preparation and Measurement of Sample Solutions for Cat's Whiskers Leaves from In Vitro Culture (Orthosiphon stamineus)*

5 mg of extract was weighed and dissolved in methanol pro analysis up to a final volume of 50 mL, shaken until homogeneous, yielding a stock solution of 100 µg/mL. Working solutions were prepared at 6 concentrations (0.2; 0.4; 0.8; 1.2; 1.6; 2 µg/mL). The solution was incubated for 30 minutes, being protected from light, and their absorbance was measured using a UV-Visible spectrophotometer at the maximum wavelength of the DPPH solution.

2.4.3. *Determination percentage of DPPH radical scavenging activity*

The percentage of free radical reduction is calculated using equation (1).

$$\% \text{ Free radical reduction} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100\% \quad (1)$$

notes:

Control absorbance: Absorption of DPPH solution

Sample absorbance: Absorption of the sample solution.

3. Result and Discussion

3.1 *Measurement long wave maximum DPPH*

The maximum wavelength of DPPH used was 516.0 nm (Fig. 2). This wavelength provides the maximum absorbance for the test solution and ensures the greatest sensitivity.

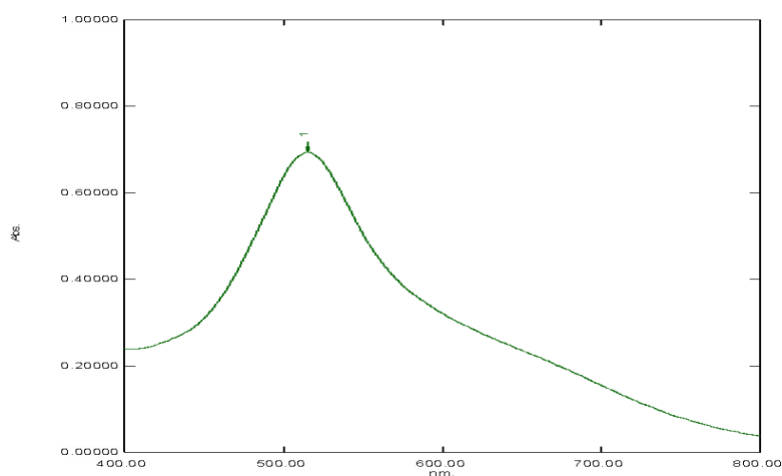


Fig. 2. Maximum wavelength of DPPH solution 40 µg/mL in methanol pro analysis

Evaluating antioxidant capacity is crucial for understanding the health benefits of natural medicine ingredients, particularly those rich in polyphenols and flavonoids. The established

maximum absorbance wavelength for the DPPH radical is approximately 517 nm, as reported in multiple studies and literature reviews [13]. This wavelength ensures sensitivity in detecting antioxidant activities, making it a standard in food science research.

Antioxidants interact with DPPH, exhibiting radical scavenging activity and producing a distinctive color change from deep purple to yellow, which can be quantitatively assessed. This change reflects not only the presence of antioxidants but also the extent to which they reduce DPPH, indicating their effectiveness. Studies have shown that the gradient of color change aligns with the antioxidants concentration in the tested solution, providing reliable data on antioxidant capacity [14,15]. This method is further validated by studies showing that absorbance changes correlate significantly with antioxidant levels across various experimental setups [16,17].

Flavonoids such as quercetin and kaempferol exhibit strong antioxidant properties, enabling them to scavenge free radicals and alter the color of the DPPH solution at or near 517 nm [18]. The absorption spectrum at this wavelength allows researchers to compare the radical scavenging potentials of various compounds, enriching our understanding of dietary antioxidants, and their health implications.

As noted in the literature, certain colored extracts may absorb at similar wavelengths, potentially confounding results [19,20]. For instance, pigments such as carotenoids in some plant extracts can overlap with DPPH absorbance at around 517 nm, leading to underestimation of free radical scavenging capability [20]. To address these interferences, advanced techniques such as HPLC coupled with DPPH assays have been recommended. This approach effectively separates pigments from antioxidant compounds, allowing more accurate measurement of antioxidant activities [21].

The reproducibility and accuracy of the DPPH assay depend on proper calibration of spectrophotometers and consistent use of the optimal wavelength at 517 nm [21]. Researchers emphasize standardizing conditions and invoking controls to ensure comparability across studies. Such standardization is vital for systematic reviews and meta-analyses intending to draw quantitative conclusions about the health benefits associated with antioxidant-rich diets [22].

The method exploits the hydrogen atom transfer (HAT) mechanism, in which antioxidants neutralize free radicals and inhibit oxidative stress [23]. This mechanistic understanding is crucial for pharmacological applications, particularly in developing natural antioxidants as therapeutic agents [24,25]. There are increasing concerns about oxidative stress-related diseases, including neurodegenerative disorders and cancer [26,27].

The widespread use of DPPH as a benchmark assay reflects its robustness and versatility. Its simplicity makes it suitable for high-throughput screening in the nutraceutical industries, where rapid evaluation of antioxidant potential can guide product formulation [28].

3.2 Activity test cat 's whiskers antioxidant in vitro culture and quercetin

The Inhibitor Concentration 50 (IC₅₀) value is the concentration of extract required to attenuate 50% of DPPH, reflecting the sample's antioxidant potency. A lower IC₅₀ value indicates activity. Absorbance values are used to calculate the percentage inhibition of DPPH free radicals (% reduction). The concentration curve (µg/mL) against % reduction is illustrated in Fig. 3 and Fig. 4. The linear regression equation is expressed as $y = bx + a$, and the IC₅₀ is calculated by substituting y with 50. The resulting antioxidant activity is presented in Table 1.

Table 1. IC₅₀ Values extract cat whiskers ethanol in vitro culture results and quercetin

No.	Sample	IC ₅₀ (µg / mL)
1.	Quercetin	7.509 µg / mL
2.	Ethanol extract of cat's whiskers from in vitro culture	0.735 µg / mL

Antioxidant activity analysis shows that the ethanol extract of in vitro–cultured cat's whiskers leaves (*Orthosiphon stamineus*) has an IC₅₀ of 0.735 µg/mL (Table 2), while quercetin has an IC₅₀ of 7.509 µg/mL (Table 3). These results indicate that in vitro–cultured cat whiskers has very strong antioxidant activity and greater antioxidant potential compared to quercetin.

Table 2. Antioxidant activity of cat's whiskers from in vitro culture

Concentration (µg/mL)	Absorbance of Quercetin			Average Absorbance	Absorbance of DPPH control	% free radical scavenging	IC ₅₀ (µg/mL)
	I	II	III				
0.2	0.30	0.314	0.308	0.307	0.581	47.139	0.735
0.4	0.299	0.306	0.295	0.300	0.581	48.400	
0.8	0.283	0.29	0.285	0.286	0.581	50.808	
1.2	0.273	0.281	0.276	0.277	0.581	52.414	
1.6	0.265	0.273	0.266	0.268	0.581	53.904	
2	0.254	0.257	0.263	0.258	0.581	55.624	

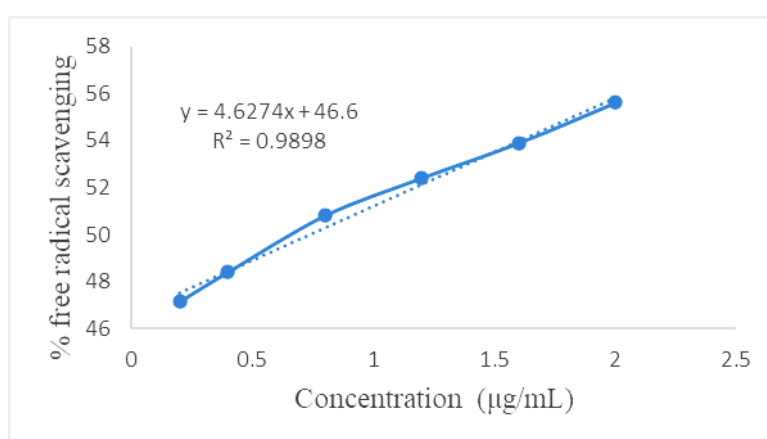


Fig. 3. Linear regression curve of the percentage of DPPH free radical scavenging against the concentration of cat's whiskers from in vitro culture

Radical scavenging activity is a pivotal metric in evaluating the antioxidant activity of extracts, particularly their ability to scavenge free radicals such as DPPH. It indicates the concentration of extract required to reduce DPPH activity by 50%, illustrating the sample's

efficacy against oxidative stress. A lower IC₅₀ value indicates greater antioxidant capability, making it a critical parameter for comparative analyses among different substances.

Table 3. Antioxidant activity of Quercetin

Concentration (µg/mL)	Absorbance of Quercetin			Average Absorbance	Absorbance of DPPH control	% free radical scavenging	IC ₅₀ (µg/mL)
	I	II	III				
2	0.503	0.538	0.606	0.549	0.692	20.665	7.509
4	0.412	0.489	0.495	0.465	0.692	32.755	
6	0.355	0.411	0.434	0.40	0.692	42.197	
8	0.28	0.312	0.32	0.304	0.692	56.069	
10	0.213	0.302	0.254	0.256	0.692	62.958	
12	0.207	0.211	0.204	0.207	0.692	70.039	

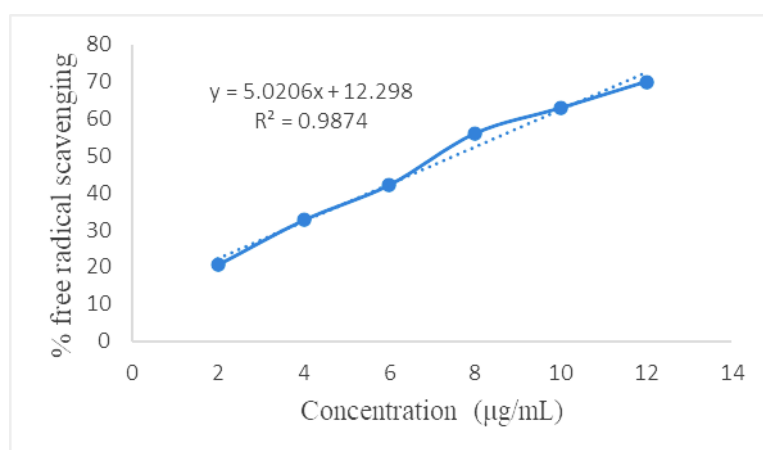


Fig. 4. Linear regression curve of the percentage of DPPH free radical scavenging against quercetin concentration

The IC₅₀ value is determined by plotting extract concentration against the percentage inhibition of the DPPH radical. Applying linear regression to these data generates an equation from which IC₅₀ can be calculated by substituting the response corresponding to 50% inhibition. This analytical approach is supported by several studies on the antioxidant activities of plant extracts.

Research has indicated that extracts with lower IC₅₀ values have a superior ability to donate hydrogen atoms to the DPPH radical, underscoring the impact of chemical composition on antioxidant performance. This correlation is primarily attributed to antioxidant components, particularly polyphenols and flavonoids, which are prevalent in many medicinal plants [29,30].

Absorbance measurements at 517 nm, as used in the DPPH assay, enable quantification of radical-scavenging activity. Each measurement is vital, as it directly influences the calculated IC₅₀ values. Reductions in absorbance correlate inversely with the effective concentration of antioxidant extracts, illustrating the interaction between DPPH radicals and hydrogen-donating antioxidants.

The antioxidant activity of plant extracts has attracted considerable interest due to their potential health benefits, particularly in mitigating oxidative stress-related diseases. This

discussion focuses on the effective antioxidant properties of *Orthosiphon stamineus* (cat's whiskers plant). In vitro studies have determined the IC₅₀ values of ethanol extracts of cat's whiskers leaves, demonstrating significant antioxidant activity. Specifically, the IC₅₀ of the ethanolic extract of *O. stamineus* was 0.735 µg/mL, while quercetin, a well-known antioxidant, had an IC₅₀ of approximately 7.509 µg/mL [31,32]. This suggests that in vitro cultured *O. stamineus* exhibits remarkably potent antioxidant capabilities, surpassing that of quercetin.

The utilization of plant tissue culture techniques has gained momentum in enhancing the antioxidant activity of medicinal plants, including the cat's whiskers plant (*Orthosiphon stamineus*). Antioxidant activity in plants primarily involves complex enzyme systems such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which play pivotal roles in neutralizing reactive oxygen species (ROS) that can damage cellular structures [33,34]. In cat whiskers, as in several other plant species, tissue culture has been shown to significantly enhance the production of these antioxidant enzymes. Environmental factors, nutrient availability in the culture media, and hormonal regulations strongly influence the antioxidant profile [35]. When cultured in vitro under optimized conditions, including proper light intensity and nutrient concentrations, the production of phytochemicals with antioxidant properties, such as flavonoids and phenolic compounds, is markedly increased. These compounds are crucial for mitigating oxidative stress in plant tissues [36,37]. Modulation of these antioxidants in response to stress is essential for plant survival and productivity, and tissue culture effectively enhances these responses.

The adoption of tissue culture techniques enables the selection of cell lines or explants with high antioxidant production. These optimized strains can be continuously cultivated to maintain consistent and enhanced yields of bioactive compounds. Findings from Ayyappan reinforce the notion that antioxidant potential correlates positively with therapeutic properties [38]. This principle can be applied to the tissue culture of cat's whiskers, resulting in superior antioxidant profiles.

4. Conclusions

The integration of plant tissue culture with the enhancement of antioxidant activity, particularly in cat's whiskers, is a growing field with the potential to advance the medicinal value of plants. Effective use of tissue culture techniques can improve the production and quality of phytochemical sources, ensuring the sustainable use of this plant and similar species. As studies further clarify the relationship between culture parameters and antioxidant synthesis, practical applications in agriculture, pharmaceuticals, and nutraceuticals are expected to expand, ultimately leading to improved health outcomes.

Abbreviations

Not applicable

Data availability statement

Data will be made available on request

CRedit authorship contribution statement

Fahrauk Faramayuda: Conceptualization, Writing – original draft and Formal analysis.

Dinda Choirunisa: Data Curation. **Yenni Karlina:** Investigation and Supervision

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest

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