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PHYTOCHEMICAL SCREENING AND FREE RADICAL–SCAVENGING ACTIVITY OF *ORTHOSIPHON STAMINEUS* (CAT WHISKERS) LEAVES

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ABSTRACT

Orthosiphon stamineus, commonly known as "Cat's Whiskers" or "Java Tea," is a medicinal herb found in Southeast Asian nations, known for its historical use in treating various ailments. This study aimed to extract and analyze the phytochemical composition of *Orthosiphon stamineus* using the maceration technique, followed by an assessment of its antioxidant potential through the DPPH assay. The phytochemical screening revealed the presence of flavonoids, saponins, and tannins, which align with prior research. Notably, the plant contains polyphenolic compounds, with flavonoids like sinensetin and eupatorin, as well as terpenoids, including labdane diterpenes. Essential oils, mainly composed of monoterpenes and sesquiterpenes, were also identified. While alkaloids like orthosiphol C have been reported, they were not detected in this research. The DPPH assay demonstrated that the extract exhibited increasing inhibitory activity against DPPH radicals with higher concentrations, indicating its potential as a natural antioxidant source. The presence of rosmarinic acid and flavonoids suggests potential antioxidant effects that may be explored further. Overall, this study provides insights into the phytochemical profile of *Orthosiphon stamine*us and its potential benefits, particularly in skin anti-aging. Further research is needed to fully understand the pharmacological significance of its compounds.

Key words: O*rthosiphonstamineus*, Catwhiskers, Photochemical, Freeradicalscavenging

INTRODUCTION

Orthosiphon stamineus is a medicinal herb in the family Lamiaceae. It is widely found in Southeast Asian nations like Malaysia, Thailand, and Vietnam. In Indonesia, it is called Kumis Kucing. Kumis Kucing gets its peculiar local name from the abundance of long filaments in its blossoms that resemble cat whiskers. The plant has historically been used to treat renal disorders, diabetes, rheumatism, arthritis, and bladder inflammation (Chua et al., 2018). Cat whiskers also have the potential to be used as additives or active substances in cosmetics. (Adiwibowo, 2020; Lee et al., 2016; Madani, 2021). The plant *Orthosiphon stamineus*, commonly has been reported to contain various secondary metabolites, including flavonoids, saponins, tannins, and essential oils (Surahmaida & Umarudin, 2019). Within the spectrum of bioactive components, phenolic compounds stand out as a paramount group of bioactive constituents prominently found in *Orthosiphon stamineus* (Akowuah & Zhari, 2010). These bioactive constituents have been observed to significantly contribute to the antioxidant potential of the crude extract derived from *Orthosiphon stamineus*, antioxidants disrupt radical chain oxidation by providing a hydrogen atom from the hydroxyl group within a stable end product, thereby preventing the initiation or continuation of subsequent oxidative reactions (S.M.Khamsah et al., 2006) The foremost and pivotal initial stage in the examination of medicinal flora involves the process of extraction, as it is imperative for the isolation of the targeted bioactive compound from the plant material, facilitating subsequent investigations such as fractionation and purification. Maceration is a method involving the immersion of plant

materials, whether coarse or in powdered form, in a solvent, and permitting them to sit at room temperature for a minimum duration of three days with regular agitation. This technique is considered a straightforward and uncomplicated approach for the extraction of bioactive compounds (Abd Aziz et al., 2021). The choice of solvent type and its concentration represents a critical factor affecting the efficacy of the extraction process. Prior investigations have established the optimal solvent concentration for the extraction of *Orthosiphon stamineus* leaves, yet it is worth noting that these studies did not employ maceration as the chosen extraction method (Chew et al., 2011). According to the aforementioned protocol, the bioactive constituents within Orthosiphon stamineus will be extracted through the maceration technique, followed by a subsequent phytochemical analysis of the extract. To assess its antioxidant potential, an evaluation utilizing the DPPH assay will be performed on the ethanol extract obtained from *Orthosiphon stamineus*.

2. MATERIAL AND METHODS

Materials

Orthosiphon stamineus leaves were purchased from a local herb supplier (Bandung, West Java, Indonesia), ethanol, Mayer reagent, Wagner reagent, Dargerdroff reagent, $FeCl_3$, Pb acetate, aquadest and filter paper.

The instruments employed in this study encompassed maceration containers, a rotary evaporator, volumetric flasks, reaction tubes, vials, UV-Vis spectrophotometry, glass beakers, funnels, analytical balances, Erlenmeyer flasks, cheesecloth, sieves, measuring cylinders, dropper pipettes, and micro pipettes.

Methods

Sample Preparation

The leaves of *Orthosiphon stamineus* are detached from the stems and subsequently subjected to a thorough washing procedure to eliminate any adhering soil particles. Following this cleansing process, they are left to air-dry at ambient room temperature for a duration of two weeks. Subsequently, the Orthosiphon stamineus leaves were finely pulverized using a grinder. (Surahmaida & Umarudin, 2019)

Extraction Process

Fifty grams of crushed *Orthosiphon stamineus* leaves were deposited into a glass container, which was subsequently filled with 500 mL of 80% ethanol. Following an initial soaking period of one week, the solvent was replaced, and the leaves were subjected to a further week of immersion. The collected filtrate is subsequently concentrated using a rotary evaporator.

Phytochemical Screening

The initial phytochemical screening examination was conducted using the aqueous extract of *Orthosiphon stamineus*. Phytochemical testing methods were carried out according to previously research, with several modifications (Musman, 2013; Surahmaida & Umarudin, 2019)

The screening for alkaloids in *Orthosiphon stamineus* involves the utilization of three reagents: Dragendorff, Mayer, and Wagner. A change in color serves as an indicator of the presence of alkaloids in the sample. Flavonoid analysis was conducted by reacting the extract of *Orthosiphon stamineus* with 1 mL of 0.1 M lead acetate. The observation of color changes serves as an indication of the presence of flavonoids within the sample. The assessment for saponins was performed by introducing the extract to 5 mL of distilled water, followed by heating. The generation of foam serves as an indicator of the presence

of saponins in the sample. The assessment of tannins is conducted by combining the extract with 5 drops of a 0.1 M FeCl3 solution. A color shift to dark blue or greenish black signifies the presence of tannins in the sample.

Free Radical–Scavenging Activity

The DPPH method was used to determine the free radical scavenging activity of *Orthosiphon stamineus* leaf extracts (Hatano et al., 1988). 2 mg of DPPH were dissolved in a 50 mL measuring flask using 96% ethanol.

25 mg of solid extract were dissolved in 96% ethanol within a 25 mL volumetric flask. Subsequently, 5 mL of the resulting solution was transferred into a 50 mL volumetric flask and then filled to the mark using 96% ethanol. Extracts were prepared at various concentrations, specifically 15, 30, 45, 60, and 75 ppm, using a 100 ppm stock solution.

2 mL of each variant of extract concentration were aliquoted and transferred into opaque vials. Subsequently, 3 mL of a 40 ppm DPPH solution was added to each vial, allowing it to react for 30 minutes, after which the absorbance was measured using a UV-VIS spectrophotometer.

RESULT AND DISCUSSION

Tabel 1. Preliminary Phytochemical screening of the aqueous extract of *Orthosiphon stamineus* leaves

Test	Aqueous extract
Alkaloid	
Dragendorff	-
Mayer	-
Wagner	-
Flavonoid	+
Saponin	+
Tannin	+

In this research, the initial phytochemical examination indicated the existence of Flavonoids, Saponins and Tannins. The findings of this study align with prior research (Surahmaida & Umarudin, 2019).

Orthosiphon stamineus, commonly known as "Cat's Whiskers" or "Java Tea," is a valuable subject of botanical research due to its historical use for its diuretic and anti-inflammatory properties. As part of this study, we aimed to comprehensively profile the phytochemical composition of this plant to better understand its chemical constituents. *Orthosiphon stamineus*, a member of the Lamiaceae family and native to Southeast Asia, has been a focus of numerous phytochemical investigations (Ameer et al., 2012). In this study, we categorized the active compounds into several key groups. These phytochemical constituents contribute to the medicinal properties associated with *Orthosiphon stamineus. T*able 1 show the result of phytochemical assay of *Orthosiphon stamineus*

Polyphenolic compounds were found to be abundant in *Orthosiphon stamineus*, contributing significantly to its antioxidant properties. These compounds include flavonoids, phenolic acids, and tannins, with notable constituents such as rosmarinic acid, sinensetin, eupatorin, and salvianolic acid. These polyphenols are well-known for their potential health benefits and likely play a role in the plant's traditional medicinal uses. (Abdelwahab et al., 2011)

Among the polyphenols, flavonoids are particularly noteworthy. They are responsible for the plant's color and are associated with various health benefits. In *Orthosiphon stamineus*, flavonoids like sinensetin, eupatorin, and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone were identified. Their presence adds to the overall medicinal potential of the plant. (Al-Suede et al., 2014)

Orthosiphon stamineus was found to contain terpenoid compounds, with diterpenes being prominent. Notable among them are labdane diterpenes, including orthosiphol A and B. These terpenoids contribute to the plant's phytochemical profile and may have pharmacological implications that warrant further investigation. (Zakaria et al., 2023)

The leaves of *Orthosiphon stamineus* contain essential oils, with variations in their chemical composition based on the plant's origin. These essential oils typically consist of monoterpenes and sesquiterpenes, which may contribute to the overall biological activity of the plant. (Azizan et al., 2017)

While alkaloids, such as orthosiphol C, have been reported in *Orthosiphon stamineus*, it's worth noting that they were not detected by tests such as the Dragendorff, Wagner, and Mayer tests in this research. Further exploration of alkaloid presence and their potential effects may be required. (Hatano et al., 1988)

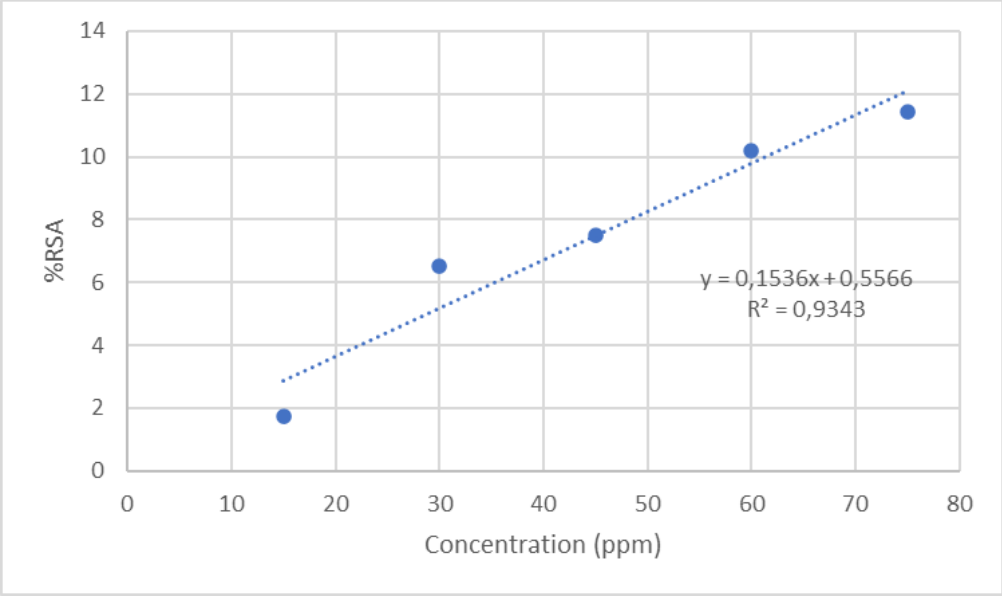

Figure 1. DPPH Free Radical Scavenging Activity

In scientific terms, DPPH, a stable free radical exhibiting a purple hue, undergoes reduction to form α,α-diphenyl-β-picryl hydrazine, characterized by a yellow coloration, when it interacts with an antioxidant. Antioxidants disrupt the chain oxidation process of free radicals by contributing hydrogen atoms from hydroxyl groups, resulting in the formation of a stable end product. This end product neither initiates nor perpetuates subsequent lipid oxidation reactions. (Sherwin, 1978). Figure 1 showed that as the concentration of the extract increases, the inhibitory activity against DPPH radicals also increases. This indicates that *Orthosiphon stamineus* has the potential to become a natural source of antioxidants.

Rosmarinic acid is one of the bioactive compounds that endow Orthosiphon stamineus with the potential as a natural antioxidant. This compound inhibits tyrosinase, hyaluronidase, elastase, and collagenase enzymes, serving to scavenge free radicals within skin cells, thereby preventing trans-epidermal water loss and contributing to the protection

of the skin against wrinkle formation. Further research is needed regarding the use of cat whiskers as anti-aging for the skin. (Tundis et al., 2015)

CONCLUSION

In conclusion, the phytochemical analysis of *Orthosiphon stamineus* reveals a diverse range of compounds, including polyphenols, flavonoids, and terpenoids. These phytochemical constituents play a pivotal role in the plant's medicinal properties, elucidating its traditional use for diuretic and anti-inflammatory purposes. The presence of rosmarinic acid, flavonoids, and diterpenes suggests potential antioxidant effects. This is supported by the increasing percentage of inhibition observed with higher concentrations of *Orthosiphon stamineus* extract. Nevertheless, further research is essential to comprehensively understand the pharmacological significance of these compounds in *Orthosiphon stamineus*, particularly in terms of its benefits for skin anti-aging.

REFERENCES

Abdelwahab, S. I., Mohan, S., Mohamed Elhassan, M., Al-Mekhlafi, N., Mariod, A. A., Abdul, A. B., Abdulla, M. A., & Alkharfy, K. M. (2011). Antiapoptotic and Antioxidant Properties of Orthosiphon stamineus Benth (Cat's Whiskers): Intervention in the Bcl-2-Mediated Apoptotic Pathway. *Evidence-Based Complementary and Alternative Medicine*, *2011*, 156765. https://doi.org/10.1155/2011/156765

Abd Aziz, N. A., Hasham, R., Sarmidi, M. R., Suhaimi, S. H., & Idris, M. K. H. (2021). A review on extraction techniques and therapeutic value of polar bioactives from Asian medicinal herbs: Case study on Orthosiphon aristatus, Eurycoma longifolia and Andrographis paniculata. *Saudi Pharmaceutical Journal : SPJ : The Official Publication of the Saudi Pharmaceutical Society*, *29*(2), 143–165. https://doi.org/10.1016/j.jsps.2020.12.016

Adiwibowo, M. T. (2020). ADITIF SABUN MANDI BERBAHAN ALAMI: ANTIMIKROBA DAN ANTIOKSIDAN. *JURNAL INTEGRASI PROSES*, *9*(1), 29. https://doi.org/10.36055/jip.v9i1.8397

Akowuah, G. A., & Zhari, I. (2010). Effect of Extraction Temperature on Stability of Major Polyphenols and Antioxidant Activity of *Orthosiphon stamineus* Leaf. *Journal of Herbs, Spices & Medicinal Plants*, *16*(3–4), 160–166. https://doi.org/10.1080/10496475.2010.509652

Al-Suede, F. S. R., Khadeer Ahamed, M. B., Abdul Majid, A. S., Baharetha, H. M., Hassan, L. E. A., Kadir, M. O. A., Nassar, Z. D., & Abdul Majid, A. M. S. (2014). Optimization of Cat's Whiskers Tea (Orthosiphon stamineus) Using Supercritical Carbon Dioxide and Selective Chemotherapeutic Potential against Prostate Cancer Cells. *Evidence-Based Complementary and Alternative Medicine*, *2014*, 396016. https://doi.org/10.1155/2014/396016

Ameer, O. Z., Salman, I. M., Asmawi, M. Z., Ibraheem, Z. O., & Yam, M. F. (2012). Orthosiphon stamineus: traditional uses, phytochemistry, pharmacology, and toxicology. *Journal of Medicinal Food*, *15*(8), 678–690. https://doi.org/10.1089/jmf.2011.1973

Azizan, N., Mohd Said, S., Zainal Abidin, Z., & Jantan, I. (2017). Composition and Antibacterial Activity of the Essential Oils of Orthosiphon stamineus Benth and Ficus deltoidea Jack against Pathogenic Oral Bacteria. *Molecules (Basel, Switzerland)*, *22*(12). https://doi.org/10.3390/molecules22122135

Chew, K. K., Khoo, M. Z., Ng, S. Y., Thoo, Y. Y., Wan Aida, W. M., & Ho, C. W. (2011).

Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphon stamineus extracts. *International Food Research Journal*, *18*(4), 1427–1435.

Chua, L. S., Lau, C. H., Chew, C. Y., Ismail, N. I. M., & Soontorngun, N. (2018). Phytochemical profile of Orthosiphon aristatus extracts after storage: Rosmarinic acid and other caffeic acid derivatives. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *39*, 49–55. https://doi.org/10.1016/j.phymed.2017.12.015

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chemical & Pharmaceutical Bulletin*, *36*(6), 2090–2097. https://doi.org/10.1248/cpb.36.2090

Lee, S. J., Seok, J., Jeong, S. Y., Park, K. Y., Li, K., & Seo, S. J. (2016). Facial pores: definition, causes, and treatment options. *Dermatologic Surgery*, *42*(3), 277–285. https://doi.org/10.1097/DSS.0000000000000657

Madani, F. N. (2021). *AKTIVITAS ANTIBAKTERI EKSTRAK DAUN KUMIS KUCING (Orthosiphon Stamineus) TERHADAP BAKTERI Propionibacterium acnes*.

Musman, M. (2013). *Kimia Bahan Alam Laut*. Syiah Kuala University Press.

S.M.Khamsah, G., Akowah, G., & Zahri, I. (2006). Antioxidant Activity and Phenolic Content of Orthosiphon Stamineus Benth From Different Geographical Origin. *Journal of Sustainability Science Anf Management*, *1*(2), 14–20.

Sherwin, E. R. (1978). Oxidation and antioxidants in fat and oil processing. *Journal of the American Oil Chemists' Society*, *55*(11), 809–814. https://doi.org/10.1007/BF02682653

Surahmaida, S., & Umarudin, U. (2019). STUDI FITOKIMIA EKSTRAK DAUN KEMANGI DAN DAUN KUMIS KUCING MENGGUNAKAN PELARUT METANOL. *Indonesian Chemistry and Application Journal*, *3*(1), 1. https://doi.org/10.26740/icaj.v3n1.p1-6

Tundis, R., Loizzo, M. R., Bonesi, M., & Menichini, F. (2015). Potential role of natural compounds against skin aging. *Current Medicinal Chemistry*, *22*(12), 1515–1538. https://doi.org/10.2174/0929867322666150227151809

Zakaria, N. H., Majid, F. A. A., Helmi, N. A. N. M., Fadhlina, A., & Sheikh, H. I. (2023). Medicinal Potentials of Strobilanthes crispus (L.) and Orthosiphon stamineus Benth. in the Management of Kidney Stones: A Review and Bibliometric Analysis. *Journal of Herbal Medicine*, *42*, 100773. https://doi.org/10.1016/j.hermed.2023.100773