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Phytochemicals Compound and Antioxidant Activity of *Tradescantia spathacea* Sw. Leaf Extracts

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Abstract

Tradescantia spathacea Sw. is commonly used as a traditional medicine plant to treat several diseases. This study aims to identify phytochemical constituents using phytochemical screening, quantify the total phenolic (TPC) and flavonoid content (TFC) analysis, and evaluate the antioxidant activity of various extracts via DPPH free radical scavenging activity. The phytochemical constituent revealed the presence of bioactive compounds such as flavonoid, alkaloid, saponin, steroid, triterpenoid, and tannin in different extracts of Tradescantia spathacea Sw. leaf. The methanolic leaf extract showed the best total phenolic and flavonoid content values of 83.35±0.00 mgGAE/g and 33.79±0.13 mg/g, respectively. Methanolic extracts also showed potent antioxidant activity in the DPPH assay with IC_{50} values of 80,835 µg/mL. The results suggest that Tradescantia spathacea Sw. could be an alternative source of natural antioxidant agents.

Keywords: phytochemical compound; *Tradescantia spathacea* Sw.; antioxidant.

INTRODUCTION

The epidemiological transition has increased in degenerative diseases. According to the World Health Organization, up to 17 million deaths occur yearly due to degenerative diseases (Di Renzo et al., 2021). Oxidative stress plays a role in various diseases, particularly degenerative ones. This stress is caused by an imbalance between reactive oxygen species (ROS) or free radicals and antioxidants in the body (Pizzino et al., 2017). However, antioxidant compounds can inhibit or prevent oxidative stress (Dhalaria et al., 2020). Synthetic antioxidants have been used for their higher stability, low cost, and wide availability (Xiu-Qin et al., 2009). The most commonly used synthetic antioxidants in the industry include butyl hydroxytoluene (BHT), butyl hydroxy anisole (BHA), propyl gallate (PG), and ter-butyl hydroquinone (TBHQ) (Lourenço et al., 2019).

Synthetic antioxidants have been widely used, but several published studies indicate adverse effects such as skin allergies (Velázquez-Sámano et al., 2019), tumors, and carcinogens (Felter et al., 2021). Therefore, the search for natural materials to replace synthetic antioxidants is actively pursued. Natural antioxidants can be derived from medicinal plants like fruits, vegetables, flowers, spices, and traditional herbs (Xu et al., 2017). These natural antioxidants contain secondary metabolite compounds, including polyphenols (such as phenolic acids, flavonoids, anthocyanins, lignans, and stilbene), carotenoids (xanthophyll and carotene), and vitamins (E and C) (Baiano & Del Nobile, 2016). Some polyphenolic compounds with hydroxyl groups are reported to act as antioxidants that can prevent free radicals through various mechanisms like reducers, free radical capturers, metal chelators, and electron donors (Kumar et al., 2013). Furthermore, flavonoid groups are known for their high antioxidant properties both in vitro and in vivo. Natural antioxidants are safe, relatively affordable, and have fewer adverse health effects (Dewage et al., 2022).

Indonesia has a rich biodiversity that was used as raw material to produce natural medicine plants (Rahayu & Andini, 2019). Local plants can serve as an alternative source of medicine for treating various diseases. One such plant is the leaves of *Tradescantia spathacea* Sw, commonly found in tropical climates. Despite being considered ornamental plants, they have potential medicinal properties (Russo et al., 2017). The *Tradescantia spathacea* Sw leaves, flowers, and stems are often used to make tea in Western countries (Pulipaka et al., 2020). Leaves of *Tradescantia spathacea* Sw contain a range of bioactive compounds including flavonoids, carotenoids, alkaloids, saponins, anthocyanins, tannins, terpenoids, steroids, ferulic acid, chlorogenic acid, vanillic acid, and p-cumaric acid (García-Varela et al., 2015; Rosales-Reyes et al., 2007). Many of these compounds exhibit pharmacological activities, with particular emphasis on their antioxidant properties. Overall, the diverse compounds found in pineapple leaves have the potential to be utilized in the production of herbal medicines for various health benefits (Tan et al., 2015).

Bioactive compounds found in *Tradescantia spathacea* Sw leaf can be extracted using the maceration method (Amalia Rachmawati et al., 2020). Several factors, such as the type of solvents, temperature, time, and pH, influence the extraction process. The solvent used in the process is essential for extracting bioactive compounds in plants (Xu et al., 2017). Most phenolic compounds, flavonoids, and anthocyanins are polar antioxidants, so the solvents used are water, ethanol, methanol, and acetone (Ngo et al., 2017). Meanwhile, nonpolar antioxidants such as carotenoids are soluble in organic solvents (Strati & Oreopoulou, 2011).

Ethanol extract from *Tradescantia spathacea* Sw roots has been reported to have antioxidant activity equivalent to α-tocopherol and stronger than ascorbate acid (González-Avila et al., 2003). Using the infusion method, the water extract of *Tradescantia spathacea* Sw leaves has shown potential as a chemoprevention agent against liver cancer in mice in vivo(Rosales-Reyes et al., 2007). Other studies suggest that the ethanol extract contains higher levels of flavonoids compared to the water extract of *Tradescantia spathacea* Sw leaves (Ramesh et al., 2021). The ethanol extract's phytochemical test indicates alkaloids, glycosides, and flavonoids, while the water extract shows the presence of alkaloids, tannins, phenolics, and flavonoids. However, there was no report regarding the antioxidant activity of the extract generated in this study. Research on bioactive compounds from various solvents to extract *Tradescantia spathacea* Sw leaf has not been carried out. This research aimed to identify the phytochemical constituent, total phenolic and flavonoid content, and antioxidant activity with IC_{50} values of ethanol, methanol, acetone, water, n-hexane, and ethyl acetate extracts.

MATERIALS AND METHODS

Chemicals and Materials

All materials used were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) from Sigma Aldrich, ascorbic acid, ethanol, methanol, ethyl acetate, hexane, acetone, Folin-Ciocalteu reagent, Dragendroff-s reagent, Liembermann-Burchard, Quercetin, Gallic acid, anhydrous sodium carbonate, Aluminium chloride, Sodium nitrite, Ferric chloride hexahydrate, Sulphuric acid, and Hydrochloric acid were purchased from Merck

Plant Materials

Tradescantia spathacea Sw. Leaves were collected from Kalasan village, Sleman, Yogyakarta, in January 2023. The plant confirmed the authenticity of the plant material in the Pharmacy Department Laboratory, Gadjah Mada University. Samples were collected and examined for infection, spores, damage, and distortion. The leave samples were washed with tap water to avoid contamination and dried for about ten days. The dried leaves were cut into small pieces and ground into fine powder.

Preparation of Extracts

In this study, maceration was used to extract the leaves of *Tradescantia spathacea* Sw. All solvents used were analytical grade. The dried powder was extracted using different solvents, such as ethanol, methanol, acetone, water, n-hexane, and ethyl acetate. The solvents and dried leaves were incubated at room temperature and constant stirring for three days. After incubation, extracts were filtered with a rotary evaporator using Whatman filter paper. The crude extracts were determined by comparing the percentage of the weight of the crude extracts to the dry weight of the leaves.

Phytochemical Screening

Phytochemical screening was used to investigate the presence of flavonoids, alkaloids, saponins, steroids, terpenoids, and tannins from all solvents.

Determination of Total Phenolic Content (TPC)

The total phenolic content of *Tradescantia spathacea* Sw leaf was determined by spectrophotometry using the Folin-Ciocalteu method. In the analysis, an extract solution was dissolved in ethanol with a 1 mg/ml concentration. 2.5 mL of 10% Folin-Ciocalteu reagent and 2.5 mL of 7% sodium carbonate solution were mixed with 0.5 mL of extract solution.All samples were incubated for 45 minutes in the dark at room temperature. The absorbance was measured using a spectrophotometer at 765 nm. The standard solution of gallic acid was determined using the same procedure. The phenolic content was measured in milligrams of gallic acid equivalent per gram of extract (mg of GA/g).

Determination of Total Flavonoid Content (TFC)

The total flavonoid contents of extracts were determined using the aluminum chloride colorimetric method and the standard solution of Quercetin. 0.25 ml of extract dissolved in methanolic solvent was added to 0.1 mL of 5% $NaNO_2$. After 6 min, 0.15 ml of aluminum chloride solution (10%) was added. The solution was mixed well and incubated at room temperature for 5 min. The absorbance was determined using a spectrophotometer at 510 nm. The total flavonoid content was expressed as mg Quercetin per 100 gr dry weight of the sample **(**mg QE/100 g).

Antioxidant Activity using DPPH Method

The antioxidant activity of crude extracts was measured using a DPPH free radical scavenging assay. 3,8 mL of

DPPH solution was added to 0,2 mL of various concentrations of crude extracts and standard solution (extract concentration 40, 80, 120, 160, 200 ppm and ascorbic acid as standard solution 2, 4, 6, 8, 10 ppm). The solution was incubated at room temperature for 5 minutes in the dark, and the absorbance was measured at 515 nm using UV-Vis Spectroscopy (González-Avila et al., 2003). The DPPH radical scavenging activity was calculated using the following equation:

$$\textbf{\% DPPH radical scavenging} = \frac{A\ control - A\ sampel}{A\ control} x\ 100\%$$

The antioxidant activity resulted in IC50 (µg/mL), and each concentration was replicated 3 times.

RESULTS AND DISCUSSION

Results

Extracts

The maceration extraction method was chosen for this study to produce efficient extraction, low-cost extraction, and simple-to-use equipment (Mohammed Golam Rasul, 2018). This study uses solvents with different polarities to extract *Tradescantia spathacea* Sw. leaf. Polar solvents used were water, methanol, ethanol, aprotic polar solvents such as acetone, and non-polar solvents such as ethyl acetate and n-hexane. In the maceration extraction of 100 grams of *Tradescantia spathacea* Sw. dried leaf. The percentage yield of 100 g *Tradescantia spathacea* Sw. Dried leaves had ethanol leaf extract higher (22,36%) than other solvents such as methanol (16,04%), water (11,97%), ethyl acetate (7,51%), n-hexane (13,43%), and acetone (5,08%) (Table 1).

Table 1. Percentage yields of *Tradescantia spathacea* Sw. leaves extract.

Extracts	ethanol	methanol	water	etyl acetate	n-hexana	aceton
Percentage yields (%)	22,36	16,04	11,97	7,51	13,43	5,08

Phytochemical screening

The result of phytochemical test showed that flavonoids, alkaloids, saponins, steroids, terpenoids, and tannins were detected in all extracts. This result showed that n-hexane extract showing the least diverse phytochemical composition and methanolic extract showed all phytochemical except steroid.

Table 2. Phytochemical screening of *Tradescantia spathacea* Sw in various extracts.

Phytochemical compounds	Ethanol extract	Methanol extract	Water extract	Etyl acetate extract	n-hexana extract	Aceton extract
Flavonoids	++	++	+	+	+	++
Alkaloids	++	++	+	+	-	++
Saponin	++	++	+	-	-	++
Steroid	-	-	+	+	+	++
Terpenoid	-	+	-	-	-	-
Tannin	++	++	-	+	-	++

Total Phenolic Content (TPC), Total Flavonoids Content (TFC), and Antioxidant Activity

The total phenolic content, total flavonoids content, and antioxidant activity were determined. According to Table 3, methanol leaf extracts contained higher TPC, TFC, and antioxidant activity than another extracts.

Table 3. Total phenolic content (TPC), total flavonoid content (TFC), and DPPH scavening activity (DPPH) of *Tradescantia spathacea* Sw. leaf crude extracts.

Sample	TPC (mg GAE/g DW)	TFC (mg /g DW)	DPPH (µg/mL)
Ethanol	$78.47{\pm}0.89^{e}$	$25.74{\pm}0.00^{e}$	99,5286
Methanol	$83.35{\pm}0.00^{f}$	$33.79{\pm}0.13^{f}$	80,8346
Water	$59.27{\pm}0.38^{c}$	$19.88{\pm}0.25^{d}$	126,2964
Etyl acetate	$67.27{\pm}0.00^{d}$	$29.57{\pm}0.00^{b}$	149,5571
N-hexane	$45.96{\pm}0.00^{b}$	$24{\pm}0.13^{a}$	226,1243
Aceton	$63.35{\pm}0.00^{a}$	$18.70{\pm}0.22^{c}$	143,7940
Ascorbic acid	-	-	36,4916

Discussion

Phytochemical screening is used to evaluate the bioactive constituents from plant extracts. In the current study, the qualitative phytochemical analysis of ethanol, methanol, water, ethyl acetate, n-hexane, and acetone extracts of *Tradescantia spathacea* Sw is carried out, as shown in Table 2. The result showed that flavonoids, alkaloids, saponins, steroids, terpenoids, and tannins were detected in all extracts, with the n-hexane extract showing the least diverse phytochemical composition. The presence of these phytochemicals has therapeutic potential for *Tradescantia spathacea* Sw. Flavonoids show multiple pharmacological activities, such as antioxidants, anti-inflammatory, antiviral, and antibacterial (Melrose, 2023). Terpenoids have biological activities such as anticancer, antioxidant, antiallergic, and anti-inflammatory (Masyita et al., 2022). Tannins show various pharmacological effects such as antimicrobial, antioxidant, anticancer, anti-nutritional, and cardio-protective properties (Smeriglio et al., 2017). Alkaloids have pharmacological activities, such as antiviral, antibacterial, anti-inflammatory, and anticancer properties (Adamski et al., 2020).

Several studies show that *Tradescantia spathacea* Sw. Leaf contains phenolic, tannins and flavonoids (Tan et al., 2015), saponin, carotenoids, anthocyanins, terpenoids, ferulic acid, chlorogenic acid, vanillic acid, p-coumaric acid and steroids (García-Varela et al., 2015). *Tradescantia spathacea* Sw. leaf contains alkaloids, flavonoids, tannins, phenolic compounds, glycosides, terpenoids, anthocyanins, carotenoids, coumarins and steroids (Pulipaka et al., 2020). This study shows that flavonoids were found in all extracts, but the strongest ones were detected in methanol, ethanol, and acetone in a yellowish-red color. The detection of these bioactive compounds supports the pharmacological potential of *Tradescantia spathacea* Sw., as corroborated by studies identifying phenolic compounds, saponins, carotenoids, anthocyanins, terpenoids, and various organic acids (Fraga-Corral et al., 2020; Pulipaka et al., 2020).

Phenolic and flavonoids are commonly important in plants and human health. The effect of phenolic is currently interest to antioxidative, reducing agents, free radical scavengers and anticarcinogenic activities (Ghasemzadeh & Ghasemzadeh, 2011). The phenolic compounds can serve as an antioxidant via different mechanistic reaction pathways, such as inhibition of lipid peroxidation, metal chelation, quenching of singlet oxygen and radical scavenging (Wang et al., 2021). The types of extraction solvents have been shown to affect extraction yields (Venkatesan et al., 2019). The total phenolic contents of the extract of *Tradescantia spathacea* Sw. leaf was expressed as miligram gallic acid equivalents per gram of dry weight extract (mgGAE/g DW). In case of leaves, the higher value of TPC (83.35 ± 0.00^{f} mgGAE/g**)** was extract with methanol and lower **(**45.96 ± 0.00^{b} mgGAE/g) with n-hexane extract (Tabel 3). Additionally, the methanol extract exhibited a higher value of TPC value than the ethanol extract. However, despite the higher percentage yield of the ethanol extract, this may be attributed to its higher tannin content (Suharni et al., 2021).

The result showed that methanol leaf extract contained more phenolic compounds than the other leaf extract. The result indicates that methanol is more effective solvent for extracting phenolic compouns than another leaf extract. These results indicate methanol's superior efficacy as a polar solvent for extracting phenolic and flavonoid compounds, consistent with findings before (Yadav et al., 2011). In previeous studies, which also reported that methanol was effective as solvent in extracting amounts of phenolic compound (Wang et al., 2021). In addition, the phenolic content of crude extracts was positively correlated with the antioxidant properties (Osman et al., 2020). Concisely, methanol leaf extract is expected to have higher antioxidant activity from

***Tradescantia spathacea* Sw. leaf.**

In this study showed that there is a positive correlation both their flavonoids content and the antioxidant properties. Flavonoids are the major group of phenolic compounds with biological activities such as radical scavening, antimicrobial activities, antiviral, anti-infammatory, anticancer, and anti-allergic (Ayele et al., 2022)**.** In this study, flavonoid content showed the same trend as the total phenolic compounds. The total flavonoid~~s~~ contents were expressed as mg Quercetin Equivalents per g of dry weight extract (mg QE/g DW) (Sembiring et al., 2018)**.** Total flavonoid content was determined using the aluminium chloride method. Aluminium chloride will form stable complex with carbonyl group at C4 and hydroxyls at C3 (flavonols) and C5 in flavonols and flavones. It could also form labile acid complexes with hydroxyls in the ortho position in B rings of flavonoids (Sembiring et al., 2018). The result showed that total flavonoids content of the methanol leaf extract was higher than another solvent, which was consistent with phenolic content values.

The antioxidant activity of *Tradescantia spathacea* Sw leaf extracts was evaluated using the DPPH radical scavenging assay, which measures the extracts' capacity to provide the DPPH radical hydrogen. Crude extracts ability to donate hydrogen and scavenge free radicals can be measured by gradually converting purple radicals into stable DPPH radicals with a yellow or colorless appearance (Gulcin & Alwasel, 2023). This study used ascorbic acid as a standard reference due to its potent antioxidant properties (Lourenço et al., 2019). The antioxidant activity of the crude extracts was determined using the IC_{50} value, which indicates the concentration of the extracts needed to scavenge 50% of DPPH radicals.

According to several studies, phenolic compounds are potent natural antioxidants that can stop oxidative stress-related NCDs (Fan et al., 2020). In this study, the methanol extract had higher TPC and TFC values than the other extract, suggesting it contained more phenolic compounds. Thus, the findings showed that the methanol extract was more capable of generating antioxidants than the other extract. However, the standard references' antioxidant activity was noticeably higher than than of the six crude extracts. In the case of leaves, the higher value of IC_{50} of antioxidant (80,8346 µg/mL) was extracted with methanol and lower (226,1243 µg/mL) with n-hexane extract (Table 3).

Phenolic compounds are key contributors to this activity, as they inhibit lipid peroxidation, scavenge radicals, and chelate metals(Fan et al., 2020; Wang et al., 2021). The findings align with previous study, which validated the antioxidant potential of phenolic-rich extracts using FRAP assays (Russo et al., 2017). These results support the role of methanolic extracts in mitigating oxidative stress-related non-communicable diseases (NCDs) and suggest their application in pharmaceutical development for natural antioxidant agents. Based on these findings, indigenous plants may serve as valuable assets in conventional medicine. It could lead to the development of products with similar characteristics and additional medicinal benefits that warrant further research.

CONCLUSIONS

In conclusion, six crude extracts of *Tradescantia spathacea* Sw leaf contain secondary metabolites such as flavonoids, alkaloids, saponins, steroids, terpenoids, and tannins. Significant amounts of flavonoids and polyphenols were present in all crude extracts. The methanol extract exhibited the highest total phenolic and flavonoid content, with 83.35±0.00 mgGAE/g and 33.79±0.13 mg/g, and showed an antioxidant activity of 80,8346 µg/ml. The study suggests that *Tradescantia spathacea* Sw leaf has the potential to be a natural source of antioxidants. The isolation of specific bioactive compounds is recommended as further research to elucidate the observed pharmacological effect.

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