Malaysian Journal of Chemistry, 2024, Vol. 26(1), 247-256
(Special Issue: *International Conference on Natural Products, ICNP 2023*)

Evaluation of Phytochemicals, Antioxidant and Antibacterial Properties of *Tradescantia spathacea* Leaf Extracts

Xin-Yi Keng[1], Chi Wah Bryan Lim[1], Khye-Er Loh[2], Hui-Yin Tan[3] and Siow-Ping Tan[1]*
[1]Department of Physical Science, Faculty of Applied Sciences, Tunku Abdul Rahman University Management and Technology, Kuala Lumpur, Malaysia
[2]Department of Bioscience, Faculty of Applied Sciences, Tunku Abdul Rahman University Management and Technology, Kuala Lumpur, Malaysia
[3]Faculty of Applied Sciences, Tunku Abdul Rahman University Management and Technology, Kuala Lumpur, Malaysia
*Corresponding author (e-mail: tansp@tarc.edu.my)

Tradescantia spathacea Sw. (*T. Spathacea*) belongs to the Commelinaceae family and has a wide range of pharmacological activities. This study aims to identify the classes of phytochemicals through phytochemical screening, quantify the antioxidant content via total phenolic content (TPC) and total flavonoid content (TFC) analyses, evaluate the antioxidant activity via DPPH free radical scavenging activity and ferric-reducing antioxidant power (FRAP) assays and analysis of the antibacterial properties of leaf extracts via disc diffusion (DF) and broth microdilution (MD) assays. Phytochemical screening of the extracts revealed the presence of terpenes, alkaloids, flavonoids, glycosides, and quinones. The ethyl acetate extract showed the best TPC and TFC values of 84.5 ± 0.6 mg GAE/g DW and 33.6 ± 3.0 mg CE/g DW, respectively. Ethyl acetate leaf extracts also exhibited good antioxidant properties in the DPPH assay and FRAP assay with EC_{50} values of 288 ± 36 µg/mL and 12.18 ± 0.12 mmol Fe^{2+}/g DW, respectively. The results of the antibacterial assay revealed that ethyl acetate leaf extracts were active against *S. aureus, E. coli*, and *A. baumannii* with the MIC value of 5 mg/mL, respectively. To date, the antibacterial activity of *T. spathacea* leaf extract against *E. faecium* and *A. baumannii* has not been reported. The results showed that *T. spathacea* is a potential source of natural antioxidants and antibacterial agents.

Keywords: Commelinaceae; *Tradescantia spathacea*; phytochemical study; antioxidant; antibacterial

Received: September 2023; Accepted: November 2023

T. spathacea, also known as *Rhoeo spathacea*, *Rhoeo discolor*, *Tradescantia discolor*, Moses in the cradle, boat lily, and oyster plants [1]. It is a plant species belonging to the Commelinaceae family, the second-largest family of its kind [2]. This invasive plant is commonly cultivated in tropical and temperate regions such as Malaysia, India, Mexico, and Central America [3]. The plant has stable underground stems and lance-shaped green leaves with a glossy purple underside. *T. spathacea* is known for its richness in bioactive compounds including polyphenols [4], flavonoids [5], alkaloids, tannins, and terpenoids [6]. Therefore, it exhibits a wide spectrum of pharmacological activities, including antiviral [7], anticancer [8], antidiabetic [4], antimicrobial and antioxidant activities [5]. Additionally, it has been used as a traditional medicine for colds, sore throats, whooping cough, nasal bleeding, haemoptysis, cancer, anxiety, and respiratory diseases [3].

In recent years, there has been growing concern regarding the prevalence of non-communicable diseases (NCDs) such as obesity, hypertension, cardiovascular disease, neurodegenerative disease, and cancer [9]. NCDs are often attributed to oxidative stress caused by the excessive formation of reactive oxygen species (ROS) in the body and insufficient production of anti-oxidants to scavenge harmful free radicals, resulting in tissue damage [10]. Therefore, antioxidants are essential in reducing the risk of NCDs. Toxicology studies have shown that butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) may cause liver damage, skin allergies, gastrointestinal tract problems, and increase the risk of cancer [11, 12]. As a result, researchers have focused their efforts on exploring natural sources of antioxidants.

Numerous studies have shown that phyto-chemicals play a crucial role in pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimutagenic, and anti-allergenic effects [13, 14]. Continuing our previous study on the biological activities of *T. spathacea* roots [15], *T. spathacea* leaves were extracted with hexane and ethyl acetate to identify their phytochemical constituents and evaluated for their antioxidant and antibacterial properties. A phytochemical screening

†Paper presented at the International Conference on Natural Products 2023 (ICNP 2023)

will be performed to identify various phytochemical constituents present in the *T. spathacea* leaf extracts. Additionally, TPC and TFC assays were conducted to determine the content of phenolic compounds. Antioxidant activity was assessed by DPPH radical scavenging and FRAP assays, while antibacterial activity was investigated using disc diffusion (DF) and microdilution (MD) assays.

EXPERIMENTAL

Chemicals and Materials

All chemicals used were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH; Batch #0000119382), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%, Lot #BCCF0260), 2,4,6-Tripyridyl-S-triazine (TPTZ, Lot #BCBH5087V) and catechin (Lot #WXBC6250V) were purchased from Sigma-Aldrich, USA. Ascorbic acid (Batch #POTL310709), anhydrous sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$), sodium acetate (CH_3COONa), sodium nitrite ($NaNO_3$) ferric chloride hexahydrate ($FeCl_3.\ 6H_2O$), ferrous sulfate heptahydrate ($FeSO_4.7H_2O$), Folin-Ciocalteu reagent (Batch #PHAO051115) and Dragendorff's reagent were purchased from R&M Marketing, Essex, U.K. Gallic acid, mercuric chloride, potassium iodide, iodine, bromine, hexane (Hex), ethyl acetate (EA), concentrated hydrochloric acid (HCl), concentrated sulphuric acid (H_2SO_4), dimethyl sulfoxide (DMSO), dichloromethane (DCM), chloroform, ammonium hydroxide, glacial acetic acid, and sodium hydroxide were purchased from Merck, Darmstadt, Germany. Nutrient agar (R&M chemicals TM431), nutrient broth (Merck 1.05443.0500), tetracycline disk (Bio basic TB0504), two Gram-positive bacteria *Staphylococcus aureus* (ATCC9144), and *Enterococcus faecium* (ATCC6569), and two Gram-negative bacteria *Escherichia coli* (ATCC11775) and *Acinetobacter baumannii* (ATCC19606).

Plant Materials

The leaves of *T. spathacea* were freshly harvested in October 2021 from a plantation in Kepong, Kuala Lumpur, Malaysia. The identity of the plant has been authentically identified and confirmed by the Phytochemistry Group of Universiti Pendidikan Sultan Idris (UPSI). The voucher specimen (TM1053) is deposited in the herbarium of UPSI, Malaysia. Plant samples were cleaned with distilled water to remove dirt and subsequently freeze-dried to remove moisture. Dried leaf samples were ground to a fine powder with a mechanical grinder and stored in hermetic plastic bags for further use.

Extraction of Plant

In this study, Soxhlet extraction was used to extract the dried leaves of *T. spathacea*. All solvents used were of analytical grade. The leaves were first extracted with 1 L of hexane at 45°C for three hours, and the extraction was repeated three times to give crude hexane (Hex) extract. The leaves were then extracted with 1 L of ethyl acetate three times for three hours at 45°C to obtain crude ethyl acetate (EA) extract. Lastly, the crude Hex and EA extracts were concentrated by using a rotary evaporator (Heidolph, Germany). The weights of both crude extracts were weighed and transferred to glass vials for further analysis. The percentage yields of crude extracts were determined as the percentage of the weight of the crude extracts to the dry weight of leaves, using the following formula:

$$\text{Percentage yield} = \frac{Weight\ of\ leaf\ extracts}{Dry\ weight\ of\ leaves\ samples} \text{ x } 100\%$$

Qualitative Phytochemical Screening

In the current study, preliminary phytochemical tests were used to investigate the presence of phytochemicals in Hex and EA extracts obtained from *T. spathacea* leaves. 1 mg/mL of each crude extract was prepared and tested according to the standard protocols for the presence of various phytochemicals such as terpenes, steroids, alkaloids, tannins, saponins, flavonoids, glycolsides, phenols, quinones, and anthraquinones [16].

For the analysis of terpenes and steroids, Salkowski's test was used. The extract was treated with chloroform and filtered. The filtrates were treated with a few drops of concentrated sulphuric acid, shaken, and then allowed to stand. The formation of a reddish-brown colour indicates the presence of terpenes, while the formation of a brown ring indicates the presence of steroids.

For the detection of alkaloids was performed. The extract was treated with 1 mL of Dragendorff's reagent (solution of potassium bismuth iodide). The formation of a reddish-brown precipitate indicates the presence of alkaloids.

For the detection of tannins, Braymer's test was used. The extract was treated with 2 mL of distilled water followed by a few drops of 10% ferric chloride. The appearance of a dark blue or greenish-black colour indicates the presence of tannins.

For the analysis of saponins, a foam test is performed. The extract was treated with 2 mL of distilled water and shaken for 15 minutes in a graduated cylinder. The formation of a thick layer of foam indicates the presence of saponins.

For the detection of flavonoids, an alkaline reagent test is done by adding 1 mL of 2 M of sodium hydroxide to the extracts. The formation of an intense yellow colour indicates the presence of flavonoids.

For the detection of glycosides, a bromine water test is used. The extract was treated with 10 mL

of bromine water. Decolouration of bromine indicates the presence of glycosides.

For the detection of phenols, the ferric chloride test is done by adding a few drops of 5% ferric chloride solution into 1 mL of the extract. The formation of dark green or bluish-black colour indicates the presence of phenols.

For the analysis of quinones, a concentrated HCl test was used by treating the extracts with 1 mL of concentrated hydrochloric acid. The appearance of green colour indicates the presence of quinones.

For the detection of anthraquinones, an ammonium hydroxide test was conducted by dissolving the extracts in isopropyl alcohol and giving a drop of concentrated ammonium hydroxide solution. The formation of a red colour after 2 minutes indicates the presence of anthraquinones.

Determination of Total Phenolic Content (TPC)

Determination of TPC in Hex and EA leaf extracts was performed according to the standard method published by Phuyal and co-workers and was determined using the Folin-Ciocalteu's reagent [17]. 1 mL of crude extract (100 µg/mL) was mixed with 5 mL of 10% Folin-Ciocalteu reagent and 4 mL of 7% sodium carbonate in a test tube. The reaction mixture was then shaken well and incubated in a 40℃ water bath for 30 minutes. The absorbance was measured at 765 nm with a Hitachi UH5300 UV-Vis spectrophotometer. The TPC was expressed as milligrams of gallic acid equivalents per gram of dry weight extract (mg GAE/g DW), and calculated from a standard curve of gallic acid. The calibration line was established using the concentrations of gallic acid of 15.6 to 500 µg/mL. The results of total phenolic content were calculated from the mean of three measurements using the following formula:

$$\text{TPC} = \frac{(conc.\ of\ gallic\ acid\ equivalent \times volume\ of\ extract)}{mass\ of\ extract}$$

Where the concentration of gallic acid equivalent was calculated in mg/mL; Volume of extract expressed in mL; Mass of extract expressed in g.

Determination of Total Flavonoid Content (TFC)

TFC was determined using the aluminium chloride colourimetric method according to the standard method published by Phuyal and co-workers [17]. 1 mL aliquot of each extract at a concentration of 100 µg/mL was mixed with 0.3 mL of 5% sodium nitrite and 4 mL of distilled water in a test tube and allowed to react for 5 minutes. Then, 0.3 mL of 10% aluminium chloride, 2 mL of 1 M sodium hydroxide, and 2.4 mL of distilled water were added to the reaction mixture. After 30 minutes of reaction, the absorbance of the mixture was measured at 510 nm with a Hitachi UH5300 UV-Vis spectrophotometer. The TFC was expressed as milligrams of catechin equivalents per gram of dry weight extract (mg CE/g DW) and was calculated from a standard curve for catechin. The calibration line was established using the concentrations of catechin of 15.6 to 500 µg/mL. The results of TFC were calculated from the mean of three measurements using the following formula:

$$\text{TFC} = \frac{(conc.\ of\ catechin\ equivalent \times volume\ of\ extract)}{mass\ of\ extract}$$

Where the concentration of catechin equivalent was calculated in mg/mL; Volume of extract expressed in mL; Mass of extract expressed in g.

Determination of DPPH Free Radical Scavenging Activity (DPPH)

The DPPH free radical scavenging assay was performed using the 96-well microplate method according to the Prietto microplate protocol with some minor modifications [18]. The antioxidant activity of each crude extract and standard reference was evaluated in terms of the reduction of stabilized DPPH (1,2-diphenyl-2-picrylhydrazyl) free radicals. In this study, Trolox and gallic acid were used as standard references. Crude extracts and standard reference were prepared by serial dilution of different concentrations range from 4 to 500 µg/mL in 96-well microplates. A 0.2 mM of DPPH solution was freshly prepared in DMSO. 100 µL of DPPH solution was added to various concentrations of crude extracts and standard references, followed by incubation at room temperature for 30 minutes in the dark. The absorbance was measured at 517 nm using a microplate reader (Tecan Infinite M200 Pro). The DPPH radical scavenging activity was calculated using the following equation:

$$\%\ \text{DPPH radical scavenging} = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}}\ x\ 100\%$$

Where $A_{control}$ was the absorbance of the negative control (DPPH solution without sample); A_{sample} was the absorbance of the sample with DPPH and A_{blank} was the absorbance of the sample without DPPH. Antioxidant activity results are expressed as EC_{50} (µg/mL) and were determined by plotting the DPPH radical scavenging activity against the concentration of leaf extracts. The experiment was performed in triplicate.

Determination of Ferric-reducing Antioxidant Power (FRAP)

In the FRAP assay, the antioxidant capacity of leaf extracts was determined based on the reduction of ferric tripyridyl triazine complex (Fe^{3+}-TPTZ) to ferrous tripyridyl triazine complex (Fe^{2+}-TPTZ). It was measured spectrophotometrically according to a modified standard method described by Kenny [19]. The FRAP reagent working solution containing TPTZ was freshly prepared by mixing 300 mM acetate

buffer, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio. In this study, the crude extracts were at a concentration of 1000 µg/mL, and the standard reference, ascorbic acid, and gallic acid were prepared at 100 µg/mL. 180 µL of FRAP reagent working solution was added to 20 µL of crude extracts and standard reference. The reaction mixture was incubated in an oven at 37°C for 30 minutes. The absorbance was measured at 593 nm using a microplate reader (Tecan Infinite M200 Pro) and all measurements were done in triplicates. FRAP results are expressed as mmol of Fe^{2+} per gram of dry weight extract, and the antioxidant values were obtained from the calibration curve of absorbance at 593 nm against the concentration of $FeSO_4.7H_2O$ at 0.2, 0.4. 0.6, 0.8 and 1.0 mM.

Bacterial Culture

The antibacterial activities of Hex and EA extracts were evaluated against four bacterial strains. The two Gram-positive bacteria used were *Staphylococcus aureus* (ATCC9144) and *Enterococcus faecium* (ATCC 6569), while the two Gram-negative bacteria used were *Escherichia coli* (ATCC11775) and *Acinetobacter baumannii* (ATCC19606). The bacteria were purchased from Biomedia Holdings Pte Ltd, the distributor of American Type Culture Collection (ATCC) cultures and products. Each bacterial strain was pre-cultured in nutrient agar medium on agar plates, and the inoculated plates were incubated in a 37°C incubator for 24 hours. After incubation of the inoculated plate, a single colony formed on the plate was inoculated into a centrifuge tube containing 10 mL of sterile nutrition broth. The bacterial suspension was further incubated in an incubator for 24 hours at 37°C.

Disk Diffusion (DF) Assay

The disk diffusion (DF) assay was carried out based on the method described by Razmavar with minor modification against *S. aureus* (ATCC9144), *E. faecium* (ATCC6569), *E. coli* (ATCC11775) and *A. baumannii* (ATCC19606) [20]. Each crude extract was prepared in 10% DMSO at concentrations of 40, 20, 10, and 5 mg/mL by serial dilution. 20 µL of each extract concentration was loaded onto a 6 mm blank disk and placed on top of Mueller-Hilton agar plates. Gentamicin and Kanamycin disc (10 µg/disc) were used as the positive controls, while the disc containing 20 µL of 10% DMSO was used as a negative control. The bacteria spread Muller-Hilton agar plates were incubated in a 37°C incubator for 24 hours. The antibacterial activity of plant extracts was assessed by measuring the zone of inhibition (mm) around the discs and the DF assay was performed in triplicate.

Broth Microdilution (MD) Assay

Broth microdilution (MD) assay was conducted to determine the minimum inhibitory concentration (MIC) values of plant extracts using a standard modified protocol developed by the Clinical and Laboratory Standards Institute (CLSI) protocol against *S. aureus* (ATCC9144), *E. coli* (ATCC11775) and *A. baumannii* (ATCC19606) [21]. The turbidity of bacterial stock cultures was determined using a UV-Vis spectrophotometer by measuring its absorbances at 625 nm. It was then adjusted to 0.5 McFarland's standard when its absorbance showed a reading of 0.08 to 0.13. Plant extracts and the antibiotic tetracycline-HCl were prepared in DMSO at 10 mg/mL and 1 mg/mL, respectively. Two-fold serial dilutions of crude extracts were performed in sterilized 96-well plates from the first column to the tenth column in the same row. 100 µL of nutrient broth medium was added to each well, and 100 µL was transferred to the next column using a multichannel micropipette. Lastly, 100 µL of the mixture in the tenth column was discarded and the final concentrations the crude extracts and positive control were now one-half the original concentration in each well, ranging from 5 to 0.002 mg/mL. The plates were then incubated at 37°C incubator for 24 hours. MIC is defined as the minimum extract concentration required to inhibit the visible growth of a microorganism after overnight incubation. It was determined by an average of three sets of triplicate experiments.

RESULTS AND DISCUSSION

Percentage Yields

The Soxhlet extraction method was chosen for this study to obtain the leaf extracts due to its high extraction efficiency and the ability to reuse solvents [22]. Hexane was used in plant extraction to extract low-polarity bioactive compounds such as terpenoids, steroids, oils (Feng et al., 2019), and certain phenolic terpenes [23]. However, ethyl acetate was used to extract slightly more polar chemical constituents such as polyphenols, carbohydrates, amino acids, and chlorophylls [24]. In the Soxhlet extraction of 1.12 kg of *T. spathacea* dried leaves, the percentage yield of EA leaf extract was higher (1.34 %, 13.424 g) than that of Hex leaf extract (0.99 %, 9.929 g) (Table 1). Low percentage yields of EA and Hex leaf extracts were obtained, which is typical in most natural product extraction studies due to the low concentration of bioactive compounds present in the plant [25].

Table 1. Yields and percentage yields of crude extracts of *T. spathacea* leaves.

Crude extracts	Yields (g)	Percentage yields (%)
Hex	9.929	0.99
EA	13.424	1.34

Table 2. Phytochemical screening of *T. spathacea* leaf crude extracts.

Tests	*T. spathacea* leaf extracts	
	Hex	**EA**
Terpenes	+	+
Steroids	-	-
Alkaloids	+	+
Tannins	-	-
Saponins	-	-
Flavonoids	-	+
Glycosides	-	+
Phenols	-	-
Quinones	-	+
Anthraquinones	-	-

Hex: Hexane; EA: Ethyl acetate; +: presence, -: absence

Phytochemical Screening

Phytochemical analyses of Hex and EA leaf extracts were performed to investigate the presence of secondary metabolites, and the results were summarized in Table 2. The results showed that terpenes and alkaloids were detected in both leaf extracts. Certain terpenes and alkaloids are commonly used as natural folk medicines because they are natural compounds with various medicinal properties, such as anticancer, antifungal, antiviral, and others [26]. This study also revealed that EA leaf extract showed the presence of flavonoids, glycosides, and quinones, whereas Hex leaf extract showed the absence of these phytochemicals. This is due to the extraction of phytochemicals being largely dependent on their polarity and the nature of the solvent [23]. Flavonoids, glycosides, and quinones have higher polarity and are thus more efficient to be extracted with ethyl acetate solvent. Several studies reported that flavonoids were found to be very effective free radical scavengers, and they exhibit potent anti-oxidant activities that could protect organisms from oxidative damage [27]. As a result, EA leaf extracts may show promising antioxidant activity. Lastly, steroids, tannins, saponins, phenols, and anthraquinones have shown negative responses in both extracts of *T. spathacea* leaves.

Total Phenolic and Flavonoids Contents

Phenolic compounds and flavonoids represent the largest and most significant group of plant metabolites with a wide range of medicinal properties [23]. The total phenolic content of the extracts was calculated from the regression equation of the calibration curve ($y= 0.0058x + 0.1149$, $R^2= 0.995$) and expressed as milligram gallic acid equivalents per gram of dry weight extract (mg GAE/ g DW). The TPC values for Hex and EA leaf extracts were 70.4 ± 1.2 and 84.5 ± 0.6 mg GAE/ g DW, respectively (Table 3). The results of the study demonstrated that the EA leaf extract contained more phenolic compounds compared to the Hex leaf extract. This indicates that ethyl acetate is a more effective solvent for extracting phenolic compounds compared to hexane. This finding is consistent with previous studies, which also reported that ethyl acetate was more effective in extracting substantial amounts of phenolic compounds [28]. In addition, the antioxidant properties of the crude extracts were positively correlated with their phenolic content due to the ability of phenolic compounds to scavenge free radicals [17]. In short, EA leaf extract is expected to have higher antioxidant and anti-obesity activity.

Studies have shown that there is a positive correlation between the antioxidant properties of the crude extracts and their flavonoid content, implying that extracts with higher TFC values are more efficient at scavenging free radicals [17]. The total flavonoid content of the extracts was calculated from the regression equation of the calibration curve ($y= 0.0011x + 0.1055$, $R^2= 0.992$) and expressed as milligram of catechin equivalents per gram of dry weight extract (mg CE/ g DW). The TFC values for Hex and EA leaf extracts were 20.3 ± 3.1 and 33.6 ± 3.0 mg CE/g DW, respectively (Table 3). In conclusion, the TFC value of the EA leaf extract was slightly higher than that of the Hex leaf extract, which was consistent with the TPC values of both leaf extracts.

Table 3. Total phenolic content (TPC), total flavonoid content (TFC), DPPH scavenging activity (DPPH), and ferric reducing antioxidant power (FRAP) of *T. spathacea* leaf crude extracts.

Sample	TPC (mg GAE/g DW)	TFC (mg CE/g DW)	DPPH EC_{50} (µg/mL)	FRAP (mmol Fe^{2+}/g DW)
Hex leaf extract	70.4 ± 1.2	20.3 ± 3.1	384 ± 41 [a]	7.72 ± 0.03 [a]
EA leaf extract	84.5 ± 0.6	33.6 ± 3.0	288 ± 36 [b]	12.18 ± 0.12 [a]
Ascorbic acid	-	-	12 ± 1 [c]	119.29 ± 0.33 [b]
Trolox	-	-	6 ± 0 [c]	-
Gallic acid	-	-	-	292.82 ± 0.91 [b]

Hex: Hexane; EA: Ethyl acetate; GAE: Gallic acid equivalent; CE: Catechin equivalent; EC_{50}: Half maximal effective concentration; Data are expressed as mean ± SD; All experiments were performed in triplicate (n = 3); For each column, values followed by the same letter are significantly different at $p < 0.05$ as measured by the post-hoc Tukey HSD test.

DPPH Free Radical Scavenging (DPPH) Assay

The DPPH radical scavenging assay was implemented to evaluate the antioxidant activity of *T. spathacea* leaf extracts by measuring the ability of the extracts to donate hydrogen to the DPPH radical. The ability of crude extracts to donate hydrogen and scavenge free radicals can be measured by their capacity to gradually transform purple radicals into stable DPPH radicals of yellow or colourless appearance [18]. In this study, ascorbic acid and Trolox were used as standard references as they are known for their strong anti-oxidant activity [12]. The mean percentage of DPPH free radical scavenging activity of the crude extracts and standards at different concentrations was presented (Figure 1). The antioxidant activities of the crude extracts were evaluated using EC_{50}, which refers to the concentration of crude extracts required to scavenge 50% of DPPH free radicals. The EC_{50} of Hex extract, EA extract, ascorbic acid, and Trolox were 384 ± 41, 288 ± 36, 12 ± 1, and 6 ± 0 µg/mL, respectively (Table 3). The DPPH free radical scavenging activity of EA leaf extract was slightly higher than that of Hex leaf extract because of its lower EC_{50} value. The significant difference observed between the mean EC_{50} values of the crude extracts of Hex and EA was determined by the Tukey HSD test at $p < 0.05$, indicating that the antioxidant activities of the two crude extracts were not comparable and that the EA extract exhibited a better antioxidant potential. Several studies have reported that phenolic compounds are potent natural antioxidants known to prevent NCDs associated with oxidative stress [23, 29]. In this study, EA extract was found to have higher TPC and TFC values compared to Hex extract, which indicated that the EA extract contained more phenolic compounds. Therefore, the results demonstrated that EA extract had a greater antioxidant capacity than Hex extract. Nevertheless, the antioxidant activity of the standard references was found to be significantly higher than that of the two crude extracts. The reason for the lower antioxidant activity of the crude extract compared to the standard reference is that the crude extract is a mixture of different compounds, which may have varying effects on the antioxidant activity. According to Sannigrahi and members, the extract fraction was found to be more effective than the crude extract [30]. Thus, fractions of the crude extracts are expected to have higher antioxidant activity and further isolation of the crude extracts will be performed.

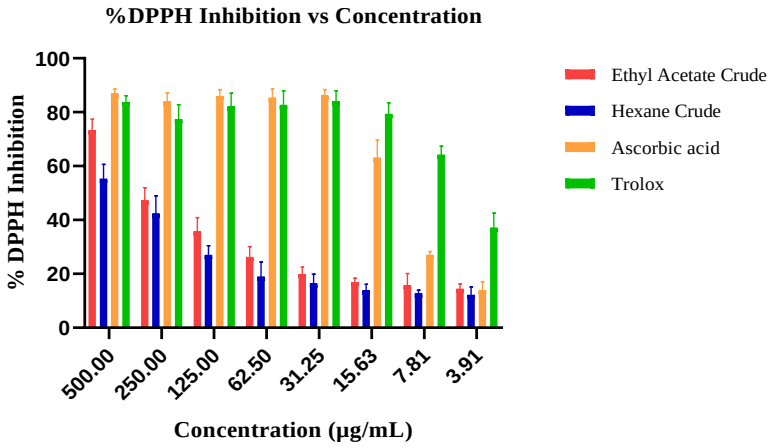

Figure 1. Free radical scavenging activity of *T. spathacea* leaf crude extracts.

Ferric (III) Reducing Antioxidant Power (FRAP) Assay

The antioxidant activity of the crude extracts was determined using the FRAP assay by measuring the ability of the extracts to donate electrons, based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) [19]. In this study, ascorbic acid and gallic acid were used as standard references and a calibration curve was constructed by plotting absorbance values against various concentrations of $FeSO_4.7H_2O$. The results of the FRAP assay were reported as the concentration of Fe^{2+} per gram of dry weight extract (mmol/g DW), which was calculated from the regression equation of the calibration curve ($y= 1.2403x + 0.0113$, $R^2= 0.9952$). The Fe^{2+} concentrations of the Hex extract, EA leaf extract, ascorbic acid, and gallic acid were determined to be 7.72 ± 0.03, 12.18 ± 0.12, 290.26 ± 5.92, and 293.56 ± 5.85 mmol/g DW, respectively (Table 3). The results showed that the iron(II) concentration produced by the EA extract is slightly higher than that of the Hex extract, indicating that EA extract has a higher reducing antioxidant capacity than Hex extract. The Tukey HSD test revealed no significant difference between the mean FRAP values of Hex and EA crude extracts at $p < 0.05$, indicating that the antioxidant activities of the two leaf extracts in the FRAP assay were comparable.

Disk Diffusion (DF) Assay

The antibacterial properties of the Hex and EA extracts were evaluated by DF and MD assay against *S. aureus*, *E. faecium*, *E. coli*, and *A. baumannii*. These bacterial strains were chosen because there are no previous reports on the antibacterial activity of *T. spathacea* leaf extract against *E. faecium* and *A. baumannii*. Furthermore, antimicrobial resistance is increasing in Malaysia due to widespread misuse and overuse of antibiotics, especially in the most commonly studied microorganisms, *S. aureus* and *E. coli* [31]. In the DF assay, the results obtained showed that the EA leaf extract was active against all bacteria except *E. faecium* with a zone of inhibition varying from 7 to 11 mm (Table 4). Hex leaf extract was inactive against all bacteria tested except *E. coli* with a zone of inhibition 7 to 8 mm in diameter. The results were consistent with the antimicrobial data reported by Garcia-Varela, showing that *T. spathacea* extract was effective against *E. coli* [32]. Moreover, the results indicated that the EA leaf extract had a stronger inhibitory effect on the growth of bacteria strains and the highest antibacterial activity against the tested bacteria was obtained with the EA extract with inhibition zone diameters of 11 mm against *S. aureus* (Table 4). In this study, 5 mg/mL of EA leaf extract inhibited *S. aureus* with an 8 mm inhibition zone, which is consistent with data reported by Parivuguna [33]. Furthermore, the antibacterial properties of the positive control used, gentamicin and kanamycin were relatively more active on selected bacteria as compared to the crude extracts. There is a shred of evidence from previous studies that crude extracts generally have been reported to have lower antibacterial activity than fractions, pure compounds, or positive controls [34]. A study by Awouafack showed that the *Eriosema robustum* crude extract (1.25 mg/mL) had significantly lower MIC values than compounds isolated (125-250 µg/mL) from the same extract.

Table 4. Zone of inhibition against four bacteria strains for *T. spathacea* leaf crude extracts.

Species	Concentration (mg/mL)	Inhibition zone (mm) ± Standard Error (mm)			
		Gram (+ve) bacteria strain		Gram (-ve) bacteria strain	
		S. aureus	***E. faecium***	***E. coli***	***A. baumannii***
Hex leaf extract	5	NI	NI	7 ± 0	NI
	10	NI	NI	7 ± 0	NI
	20	NI	NI	7 ± 0	NI
	40	NI	NI	8 ± 0	NI
EA leaf extract	5	8 ± 0	NI	7 ± 0	7 ± 0
	10	9 ± 0	NI	7 ± 0	8 ± 1
	20	10 ± 0	NI	8 ± 1	8 ± 1
	40	11 ± 0	NI	8 ± 0	9 ± 1
Gentamicin	10 µg/disc	23 ± 1	16 ± 0	20 ± 1	12 ± 0
Kanamycin	10 µg/disc	22 ± 1	NI	18 ± 1	18 ± 1

Hex: Hexane; EA: Ethyl acetate; NI: No inhibition zone was detected.

Table 5. MIC of *T. spathacea* leaf crude extracts and tetracycline-HCL against *S. aureus*, *E. coli*, and *A. baumannii*.

Subjected bacterial	MIC (mg/mL) ± RSD (%)		
	***T. spathacea* plant leaf extracts**		**Positive control**
	Hex	**EA**	**Tetracycline-HCl**
S. aureus	-*	5 ± 0	2 µg/mL ± 0
E. coli	5 ± 0	5 ± 0	4 µg/mL ± 0
A. baumannii	-*	5 ± 0	4 µg/mL ± 0

Hex: Hexane; EA: Ethyl acetate; *: MD assay was not conducted on hexane leaf extract against *S. aureus* and *A. baumannii* due to there being no activity against these bacteria in the DF assay. The microdilution was performed in triplicate.

Broth Microdilution (MD) Assay

In the MD assay, the results showed that Hex and EA leaf extracts had a weak inhibitory effect against *S. aureus*, *E. coli*, and *A. baumannii* with a MIC value of 5 mg/mL (Table 5). Antibacterial activity is considered significant if MIC values are below 25 µg/mL, moderately active when MIC values are in the range of 25 to 100 µg/mL, and weakly active when MIC values exceed 100 µg/mL [35]. Both crude extracts were considered weak inhibitors against selected bacteria by comparison with an antibiotic (tetracycline-HCL). To date, the antibacterial activity of *T. spathacea* leaf extract against *E. faecium* and *A. baumannii* has not been reported. Furthermore, the results of the MD assay against *S. aureus* and *E. coli* were consistent with previous research findings [5]. Although the extracts showed weak antibacterial activity, the isolated compounds generally demonstrated higher antibacterial activity [36]. Njateng and colleagues showed that the antimicrobial activities of fractions (MIC = 0.5 to 1 mg/mL) and pure compounds (MIC = 6.25 to 100 µg/mL) were relatively more potent than crude extracts (MIC = 2 to 8 mg/mL). Collectively, these findings have sparked interest in the isolation of secondary metabolites for the discovery of natural antimicrobial agents against bacterial infections.

CONCLUSION

In conclusion, phytochemical screening of *T. spathacea* leaf extracts revealed that they contain a wide variety of secondary metabolites, including terpenes, alkaloids, flavonoids, glycosides, and quinones. TPC and TFC assays confirmed the presence of significant amounts of polyphenols and flavonoids in both crude extracts. Additionally, statistical analysis showed that the antioxidant activity of EA extract was significantly higher than that of Hex leaf extract ($p < 0.05$). Furthermore, EA extract showed higher antibacterial activity against *S. aureus* compared with Hex leaf extract, exhibiting an inhibition zone of 8 mm at a concentration of 5 mg/mL and a MIC value of 5 mg/mL. These findings suggest that *T. spathacea* leaves have great potential as a valuable source of natural antioxidants and antimicrobials. Given the diverse biological activities of phytochemicals, further studies are required to isolate and identify the specific bioactive compounds responsible for the observed pharmacological effects.

ACKNOWLEDGEMENTS

This study was supported by Tunku Abdul Rahman University College Publication Incentive (86000-4923).

REFERENCES

1. Pulipaka, S., Suttee, A., Kumar, M. and Sriram, P. (2022) Review on phytopharmacological activities of *Alpinia mutica* and *Tradescantia spathacea*. *Plant Archives*, **2**, 901–918.

2. Tan, J. and Yee, M. (2020) The biological activities of the spiderworts (*Tradescantia*). *Food Chemistry*, **1**, 1–44.

3. Prajapati, T., Shukla, A. and Modi, N. (2020) *Tradescantia spathacea* Sw: A review of its pharmacological and ethnopharmacological properties. *International Journal of Research Culture Society*, **4**, 52–56.

4. Vo, Q., Nguyen, P., Zhao, B., Ali, M. and Choi, J. (2015) Protein tyrosine phosphatase 1B (PTP1B) inhibitory constituents from the aerial parts of *Tradescantia spathacea* Sw. *Fitoterapia*, **103**, 113–121.

5. Tan, J., Lim, Y. and Lee, S. (2013) Antioxidant and antibacterial activity of *Rhoeo spathacea* (Swartz) stearn leaves. *Journal of Food Science and Technology*, **52**, 2394–2400.

6. Kadam, P. and Kakde, N. (2017) Phytochemical study of *Tradescantia Spathacea*. *International Journal of Research in BioSciences*, **6**, 48–51.

7. Chan, Y., Khoo, K. and Sit, N. (2016) Investigation of twenty selected medicinal plants from Malaysia for anti-Chikungunya virus activity. *International Microbiology*, **19**, 175–182.

8. García, V., Fajardo, R., Serna, S., Altamirano, J. and Cardineau, G. (2016) Cancer cell specific cytotoxic effect of *Rhoeo discolor* extracts and solvent fractions. *Journal of Ethnopharmacology*, **190**, 46–58.

9. Institute for Public Health (2020) National health and morbidity survey (NHMS) 2019: Non-communicable diseases, healthcare demand, and health literacy—Key Findings. *National Health and Morbidity Survey* (NHMS).

10. Sehwag, S. and Das, M. (2013) Antioxidant Activity: An Overview. Research & Reviews. *Journal of Food Science & Technology*, **2**, 1–10.

11. Jacob, J., Thomas, S., Loganathan, S. and Valapa, R. (2020) Antioxidant incorporated biopolymer composites for active packaging. *Processing and Development of Polysaccharide-based Biopolymers for Packaging Applications*, **10**, 239–260.

12. Lourenco, S., Moldão-Martins, M. and Alves, V. (2019) Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*, **24**, 4132.

13. Koparde, A., Doijad, R. and Magdum, C. (2019) Natural products in drug discovery. *IntechOpen*, **14**, 1–19.

14. Dincheva, I., Badjakov, I. and Galunska, B. (2023) New insights into the research of bioactive compounds from plant origins with nutraceutical and pharmaceutical potential. *Plants*, **12**, 258.

15. Lim, B., Keng, X., Loh, K. and Tan, S. (2023) Determination of antioxidant and antibacterial properties of *Tradescantia spathacea* root extracts. *Vegetos*.

16. Shaikh, J. and Patil, M. (2020) Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, **8**, 603–608.

17. Phuyal, N., Jha, P., Raturi, P. and Rajbhandary, S. (2020) Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum*. *The Scientific World Journal*, **1**, 1–7.

18. Prieto, J. (2012) Procedure: Preparation of DPPH radical, and antioxidant scavenging assay. *Dr Prieto's DPPH Microplate Protocol*, **1**, 1–3.

19. Kenny, O., Brunton, N. and Smyth, T. (2015) *In vitro* protocols for measuring the antioxidant capacity of algal extracts. *Methods in Molecular Biology*, **1308**, 375–402.

20. Razmavar, S., Abdulla, M., Ismail, S. and Hassandarvish, P. (2014) Antibacterial activity of leaf extracts of *Baeckea frutescens* against methicillin-resistant *Staphylococcus aureus*. *Biomed Research International*, 521287.

21. Clinical and Laboratory Standards Institute (CLSI) (2012) Performance standards for antimicrobial disk susceptibility tests: Approved standard–Eleventh edition. Wayne. *Clinical and Laboratory Standards Institute*, 19087.

22. Patel, K., Panchal, K. and Ingle, P. (2019) Review of extraction techniques. *International Journal of Advanced Research in Chemical Science*, **6**, 6–21.

23. Galanakis, C., Goulas, V., Tsakona, S., Manganaris, G. and Gekas, V. (2013) A knowledge base for the recovery of natural phenols with different solvents. *International Journal of Food Properties*, **16**, 382–396.

24. Feng, W., Li, M., Hao, Z. and Zhang, J. (2019) Analytical methods of isolation and identification. *Phytochemicals in Human Health*, 1–27.

25. Gonfa, T., Teketle, S. and Kiros, T. (2020) Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and *in-vitro* antioxidant activity evaluation of *Cadaba rotundifolia* Forssk leaf extracts. *Cogent Food and Agriculture*, **6**, 1853867.

26. Heinrich, M., Mah, J. and Amirkia, V. (2021) Alkaloids used as medicines: Structural phytochemistry meets biodiversity—an update and forward look. *Molecules*, **26**, 1836.

27. Carmona, H., Ceballos, L. and González, C. (2018) Polyphenols and flavonoids in Colombian fruit and vegetables - applications and benefits: a review. *Journal of Food and Nutrition Research*, **6**, 176–181.

28. Myint, Y., Yadanar, H., Zin, H. and Thew, O. (2020) Determination of total phenolic contents and total flavonoid contents of crude extracts from selected three medicinal plants. *3rd Myanmar Korea Conference Research Journal*, **3**, 2035–2041.

29. Fan, M., Chen, G., Zhang, Y., Nahar, L. and Sarker, S. D. (2020) Antioxidant and anti-proliferative properties of Hagenia abyssinica roots and their potentially active components. *Antioxidants*, **9**, 143–149.

30. Sannigrahi, S., Kanti, M., Kumar, P., Parida, S. and Jain, S. (2010) Antioxidant potential of crude extract and different fractions of Enhydra

fluctuans Lour. *Iranian Journal of Pharmaceutical Research*, **9**, 7–82.

31. Naeemmudeen, N., Mohd-Ghazali, N., Bahari, H., Ibrahim, R., Samsudin, A. and Jasni, A. (2021) Trends in antimicrobial resistance in Malaysia. *Medical Journal of Malaysia*, **76**, 698–705.

32. García-Varela, R., García-García, R., Barba-Dávila, B., Fajardo-Ramírez, O., Serna-Saldívar, S. and Cardineau, G. (2015) Antimicrobial activity of *Rhoeo discolor* phenolic rich extracts determined by flow cytometry. *Molecules*, **20**, 18685–18703.

33. Parivuguna, V., Gnanaprabhal, R., Dhanabalan, R. and Doss, A. (2008) Antimicrobial properties and phytochemical constituents of *Rheo discolor* Hance. *Ethnobotanical Leaflets*, **12**, 841−845.

34. Awouafack, M., McGaw, L., Gottfried, S., Mbouangouere, R., Tane, P., Spiteller, M. and Eloff, J. (2013) Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from *Eriosema robustum* (Fabaceae). *BMC Complementary and Alternative Medicine*, **13**, 289.

35. Kalli, S., Araya-Cloutier, C., Hageman, J. and Vincken, J. (2021) Insights into the molecular properties underlying antibacterial activity of prenylated (iso)flavonoids against MRSA. *Scientific Reports*, **11**, 14180.

36. Njateng, G., Du, Z., Gatsing, D., Mouokeu, R., Liu, Y., Zang, H., Gu, J., Luo, X. and Kuiate, J. (2017) Antibacterial and antioxidant properties of crude extract, fractions and compounds from the stem bark of *Polyscias fulva Hiern* (Araliaceae). *BMC Complementary and Alternative Medicine*, **17**, 99.