



RESEARCH ARTICLE

Assessment of bioactive compounds and antioxidant properties of *Schumannianthus dichotomus* Roxb. obtained via a microwave-assisted extraction method

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Abstract

This study focuses on assessing the bioactive compounds, particularly regarding their antioxidant capabilities. The research utilized microwave-assisted extraction (MAE) to obtain 80 % ethanolic and aqueous extracts from the leaves, offshoots and rhizomes of *Schumannianthus dichotomus* Roxb. A phytochemical analysis was conducted on the three extracts to identify secondary metabolites, including alkaloids, flavonoids, phenolics, glycosides, phytosterols and tannins. Total phenolic content was assessed using the Folin-Ciocalteu method, whereas the total flavonoid content was measured employing the aluminium chloride colorimetric technique. Concurrently, the antioxidant capacity was evaluated utilizing DPPH, ABTS and FRAP assays. The results revealed that all plant extracts demonstrated the presence of phenols, flavonoids, glycosides and phytosterols. The leaves extracted with 80 % ethanol yielded the greatest amount of phenolics, recorded at 2.797 ± 0.117 mg GAE/g sample, while the aqueous extract of the leaves had the highest concentration of flavonoids, measuring 0.544 ± 0.062 mg CE/g sample. Additionally, the most antioxidant activities, particularly for DPPH and ABTS, were obtained in the 80 % ethanol extract from the rhizomes, exhibiting trolox equivalent values of 0.212 ± 0.003 and 2.044 ± 0.031 mg TE/g sample, respectively. Meanwhile, the highest reducing power was observed in the 80 % ethanol extract from the offshoots, measuring 3.864 ± 0.292 mg TE/g sample. Findings also indicated a strong positive correlation between TPC and FRAP, closely followed by the correlation between TPC and DPPH. The findings suggest that *S. dichotomus* is a reliable source of antioxidant qualities, highlighting its potential use in treating many disorders, especially those associated with oxidative stress.

Keywords: antioxidants; microwave-assisted extraction; phytochemicals; phenolics; *Schumannianthus dichotomus* Roxb.

Introduction

Throughout various time periods, a wide array of plants has been employed in the development of medicinal products and most contemporary medications originate from these natural botanical sources. Treatments derived from plants play an essential role in the healthcare of both people and animals. Besides their healing properties, these therapies are vital for promoting overall health. Phytochemicals found in plants serve as strong antioxidants that combat reactive oxygen species and offer numerous health advantage (1). It is acknowledged that free radicals lead to oxidative stress, which can result in damage to DNA, lipids and proteins in living organisms, contributing to a range of health issues including cancer, diabetes, aging, cardiovascular diseases, rheumatism, inflammatory bowel diseases and other degenerative conditions (2). Certainly, these plants have historically served as reliable, efficient and eco-friendly providers of natural antioxidants or agents that neutralize free radicals, especially phenolic substances, which

include phenolic acids, flavonoids, tannins, stilbenes and anthocyanins (3). Phenolics are primarily recognized for their role in enhancing the antioxidant properties of both medicinal and culinary plants. Their activities play a significant role in combating various health issues that include anti-inflammatory, antimicrobial and anticancer properties (2).

Plants belonging to the Marantaceae family often thrive in damp or marshy tropical woodlands and are commonly characterized as perennial herbs with rhizomes. Three distinct species are recognized within the *Schumannianthus* genus, which are *S. virgatus*, *S. monophyllus* and *S. dichotomus*. In Thailand, the plant *S. dichotomus* Roxb., locally referred to as “Khla” is a type of shrub that grows from rhizomes and is mainly utilized in the production of handicrafts, especially for its stems. Typically, the height ranges from 3 to 5 m, accompanied by a basal diameter between 2 and 5 cm. This species is primarily found across regions in South and South-East Asia, including countries like India,

Bangladesh, Myanmar, Vietnam, Malaysia, Indonesia and Thailand (4, 5). *S. dichotomus* is a recently introduced medicinal plant in Thailand, with the leaf, stem, rhizome and root being valuable components for phytochemical applications. Conventional applications of the rhizome involve alleviating fever and addressing skin ailments, while the stem is utilized for treating ear pain (5). Investigation into the medicinal characteristics of *S. dichotomus* has revealed scant records concerning its stem and rhizome, which displayed antinociceptive and antipyretic effects in mice studies. A qualitative analysis of the methanolic extract from the rhizome revealed the existence of phenolic compounds, tannins, flavonoids, alkaloids, carbohydrates, terpenes and phytosterols. However, glycosides, saponins and gums were not detected in the rhizome (6). The content of total phenolics and flavonoids found in the rhizome exceeds that in the stem. A recent study highlighted that the methanolic extract derived from the rhizome of *S. dichotomus* exhibited both hepatoprotective and hypoglycemic effects in mice experiencing hepatotoxicity induced by CCl_4 and hyperglycemia induced by dextrose (7). In traditional Thai medicine, the rhizome is noted for its mild taste and is often decocted to help reduce fever and alleviate skin (8). A traditional Thai herbal recipe for managing diabetes includes eight medicinal components, one of which is the rhizome of *S. dichotomus*, as documented in King Rama V's writings on traditional medicine (9).

Typically, Thai herbal remedies are commonly made by either steeping them in water or soaking them in ethanol. A notably effective and eco-friendly method known as microwave-assisted extraction (MAE) shortens the extraction duration and minimizes the use of organic solvents. Preferably, it employs safer solvents like water. Due to its importance, enhancing the extraction method is crucial, since the effectiveness relies heavily on the solvent selected (10). Finding the ideal solvent can increase

both the phenolic concentration and the antioxidant properties of the extract. Consequently, the goal of our study was to evaluate the bioactive components and antioxidant properties present in various sections such as the leaves, offshoots and rhizomes of *S. dichotomus*. These were extracted using a microwave-assisted technique with different solvents. Emphasize the limited studies works employing MAE on this plant species. This information can be utilized in various sectors, including food, pharmaceuticals and dietary supplements, supporting the development of high-quality functional items.

Materials and methods

Chemicals

The Folin-Ciocalteu reagent, along with catechin, gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (trolox) and 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were provided by Sigma Chemical Co., located in St. Louis, Missouri, United States. All the solvents and reagents utilized in this study were of analytical purity.

Collection and preparation of plant samples

S. dichotomus plants were collected from the Ton Khla handicraft community enterprise, Thung Fon district, Udon Thani province (17°29'06.6"N 103°11'49.6"E). A botanist at the Bangkok Herbarium within the Department of Agriculture in Thailand utilized taxonomic keys to determine the species of the plant. The reference sample as assigned a voucher number labelled SN244290. The leaves, offshoots and rhizomes of the plant (Fig. 1)



Fig. 1. *Schumannianthus dichotomus* Roxb. (A) Whole plants; (B) Leaves; (C) Offshoots and (D) Rhizomes.

were carefully separated, thoroughly cleaned and then cut into thin slices. Following this, they were dried in a heated air oven for a duration of 48 hr at 50 °C.

Preparation of plant extracts

A modified microwave-assisted extraction (MAE) technique was applied for the extraction of *S.dichotomus* (11). About 20 g of each dried sample were milled and placed them using 300 mL of 80 % ethanol and distilled water for 15 min. This was succeeded by extraction through a microwave (Samsung, MS23F300EEK/ST, Thailand) set at a power level of 600 W for 60 sec. The extraction was conducted three times, allowing a resting interval that matched the extraction time for every cycle. Subsequently, all the extracts underwent filtration and the solvents were removed using a vacuum rotary evaporator. The resulting crude extracts were reconstituted in dimethyl sulfoxide (100 % DMSO) at a concentration of 100 mg/mL and stored in an amber glass bottle at 4 °C for subsequent analysis. The percentages of crude extract yields, expressed as % w/w based on dry plant materials, can be found in Table 1. The percentage yield of the extract was calculated by utilizing the dry weight of the extract (a) and the weight of the soaked sample material (b) according to the formula: percentage yield (%) = $a/b \times 100$.

Phytochemical screening analysis

The qualitative analysis of extract samples aimed to utilize the previously outlined techniques for identifying various categories of chemicals, such as carbohydrates, alkaloids, phenols, flavonoids, glycosides, saponins, phytosterols or steroids and tannins. This identification was carried out through color reaction techniques. The solution was prepared with 50 mg of plant extract dissolved in 10 mL of 80 % ethanol and distilled water and was subjected to a phytochemical assessment following recognized standard protocols (12).

Determination of total phenolic content

The assessment of the total phenolic content (TPC) in the extract samples was conducted utilizing a modified Folin-Ciocalteu method (13). To summarize, an aliquot of 100 µL of freshly prepared 10 % Folin-Ciocalteu reagent was mixed with 20 µL of 30 mg/mL extract solution within 96-well microplates, allowing the reaction to proceed for 5 min at approximately 25 °C. Following this, an 80 µL portion of a 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added to the mixture, which was then incubated at 25 °C for 30 min. The absorbance of the resulting mixture was measured at 765 nm using a microplate reader, specifically the SpectraMax M2. Each trial was conducted in triplicate, enabling the

quantification of total phenolic content, which was expressed as mg GAE/g sample, representing milligrams of gallic acid equivalent per gram of sample. This quantification was facilitated through a calibration curve derived from a dilution series of gallic acid ranging from 5 to 25 µg/mL.

Determination of total flavonoid content

The total flavonoid content (TFC) of the extract sample was evaluated using a modified aluminum-chloride colorimetric method (13). Initially, 0.5 mL of extracts at a concentration of 30 mg/mL was combined with 0.15 mL of 5 % sodium nitrite and 2 mL of distilled water. This mixture was incubated for a period of 6 min. Following this incubation, 0.15 mL of 10 % aluminum chloride was added. After another 6 min of standing, 1 mL of 1 M sodium hydroxide was included and the total volume was adjusted with distilled water to reach 5 mL. The solution was then mixed thoroughly using a vortex mixer and 200 µL of this final solution was transferred to a 96-well plate. Absorbance was measured at a wavelength of 510 nm using a microplate reader, specifically the SpectraMax M2. Each sample underwent testing in triplicate, with the results reported as milligrams of catechin equivalent per gram of sample (mg CE/g sample). The quantification process relied on a calibration curve generated from varying concentrations of catechin ranging from 30 to 180 mg/L.

DPPH radical scavenging activity

The assessment of the DPPH radical scavenging activity from the sample extract can be achieved using a modified version of a conventional method (13). To start, a DPPH stock solution with a concentration of 0.1 mM was created by dissolving 0.4 mg of DPPH powder in 9.6 mL of 80 % ethanol. From this stock, 20 µL from the sample extracts at a concentration of 30 mg/mL was combined in a 96-well microplate with 180 µL of 0.1 mM DPPH solution. This mixture was thoroughly shaken and then allowed to sit in the dark at approximately 25 °C for 30 min. After this incubation period, the absorbance of the reaction mixture was measured at a wavelength of 515 nm against a blank sample of ethanol, using a microplate reader (SpectraMax M2). The percentage of DPPH radical scavenging inhibition for the examined samples was calculated using the provided formula:

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

In which, A_{control} = DPPH absorbance without samples, A_{sample} = DPPH absorbance with samples.

Values of relevance were established by referencing the

Table 1. The percent yield and phytochemical constituents of *S. dichotomus* extracts

Phytochemicals	<i>S. dichotomus</i> extracts					
	Leaves		Offshoots		Rhizomes	
	80 % Ethanol	Aqueous	80 % Ethanol	Aqueous	80 % Ethanol	Aqueous
Carbohydrates	–	+	–	+	–	+
Alkaloids	+	–	+	–	+	–
Phenols	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Saponins	+	–	+	–	+	–
Phytosterols	+	+	+	+	+	+
Tannins	+	+	–	–	–	–
% Yield	20.80	4.70	42.25	26.50	30.50	22.80

(+) Present, (–) Absent.

trolox standard curve, which was developed with concentrations between 1 and 10 mg/L. These values are expressed as mg TE/g sample, indicating the milligrams of trolox equivalent per gram of sample. The data provided reflects the mean results from three tests conducted in parallel.

ABTS radical cation scavenging activity

The method for scavenging ABTS radical cations was adapted from the procedure described in previous study (13), with some modifications. A stable stock solution of ABTS radical cation was prepared by mixing an aqueous solution of ABTS at a concentration of 4 mM with 2.45 mM potassium persulfate ($K_2S_2O_8$) in a 1:1 (v/v) ratio. This reaction took place at room temperature in the dark for a duration of 12-16 hr before further use. The resulting ABTS solution was then diluted with distilled water until an absorbance of 1.0 ± 0.02 was reached at a wavelength of 734 nm. After this, 20 μ L of sample extracts at a concentration of 30 mg/mL were combined with 150 μ L of the working solution in a 96-well microplate. The mixture was incubated in the dark at approximately 25 °C for 15 min, following which absorbance readings were recorded at 734 nm using a microplate reader (SpectraMax M2). The percentages of inhibition for ABTS radical cation scavenging were calculated using the following formula:

$$\% \text{ ABTS radical cation scavenging inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

In which A_{control} = ABTS absorbance without samples, A_{sample} = ABTS absorbance with samples.

All results were established using a calibration curve for trolox across a concentration spectrum of 5-100 mg/L. The findings were expressed in milligrams of trolox equivalent per gram of sample (mg TE/g sample). The experiments were performed three times for accuracy.

Ferric reducing antioxidant power assay

The assay for ferric-reducing antioxidant power (FRAP) was modified from an established methodology (13), which utilizes antioxidants as reducing agents within a colorimetric framework connected to redox reactions. To prepare the FRAP reagent, a mixture was created consisting of 0.2 M acetate buffer at pH 3.6, made by combining 46.3 mL of 0.2 M acetic acid and 3.7 mL of 0.2 M sodium acetate, along with 1 mM of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1. This mixture was then incubated at 37 °C for 20 min prior to its application. The assay procedure was conducted in 96-well microplates, where 20 μ L of extract samples (at a concentration of 30 mg/mL) were combined with 150 μ L of the FRAP reagent and allowed to stand at room temperature (25 °C) for 10 min. The conversion of ferric ions (Fe^{3+}), which are colorless, to ferrous ions (Fe^{2+}), which appear blue, was monitored by taking absorbance measurements at 595 nm using a microplate reader (SpectraMax M2) after a duration of 30 min. The resulting FRAP values were expressed as milligrams of trolox equivalent per gram of sample (mg TE/g sample), utilizing a trolox dilution series for calibration that ranged from 25 to 500 μ M.

Statistical analysis

All analyses were performed three times ($n = 3$). Results were expressed as mean \pm standard deviation and were further analyzed using the Statistical Package for Social Sciences (SPSS version 21). A one-way ANOVA was utilized to assess significant discrepancies

between means, while Duncan's new multiple range tests facilitated the detection of the least significant differences at a 95 % confidence level ($p < 0.05$).

Results and Discussion

Extraction yields

In this study, the experiments were structured to assess how different extraction solvents influence the response variables, which include extraction yield, total phenolic content, total flavonoid content and antioxidant activities. The selected solvents were 80 % ethanol and aqueous to gather the leaves, offshoots and rhizomes of *S. dichotomus*, as shown in Table 1. The extract yields, represented as percentages, varied between 20.80 % and 42.25 % (w/w) for the 80 % ethanol extractions, while for aqueous extractions, the range was from 4.70 % to 26.50 % (w/w). The highest yield of crude extract was achieved from the offshoots using 80 % ethanol extraction, producing 42.25 %. This was closely followed by the rhizomes extracted with 80 % ethanol, which yielded 30.50 %. In contrast, the lowest yield was found in leaves extracted with aqueous solution, with a yield of only 4.70 %. The extraction yield is influenced by various factors, including the water proportion and polarity. The results demonstrated that polar protic solvents produced superior extraction yields (13). Notably, hydro-ethanol stood out among all the solvents tested, achieving a significantly greater extraction yield. This suggests that an 80% ethanol solution is an effective solvent for maximizing extraction yields from *S. dichotomus* (15). It is widely recognized that the yield of active compounds obtained from plant materials is significantly influenced by the water-to-raw material ratio, making it a crucial consideration. Additionally, the polarity of the extracting agent is instrumental in enhancing the recovery of phenolic and flavonoid substances (14).

Phytochemical screening of *S. dichotomus*

Phytochemical analysis serves not only to identify the various components of plant extracts but also highlights which ones are most prevalent. Additionally, it plays a critical role in the discovery of bioactive compounds that may contribute to the development of effective pharmaceuticals (16). Table 1 illustrates the phytochemical constituents present in various solvent extracts derived from the leaves, offshoots and rhizomes of *S. dichotomus*. Each of the plant extracts demonstrated the presence of phenols, flavonoids, glycosides and phytosterols. The 80% ethanol extracts showed no presence of carbohydrates, while the aqueous extracts did not contain alkaloids and saponins. Additionally, tannins were absent in both the offshoot and rhizome extracts. The findings of this research align with those of Zahra et al. (12), who identified phytochemicals present in extracts from the leaves and rhizomes of *S. dichotomus* using various solvents. The identification of these phytochemical compounds within *S. dichotomus* indicates their potential to offer health benefits through antioxidant activities and to decrease the risk of chronic diseases that are not infectious (17). With their extensive health advantages, phenolic and flavonoid compounds are regarded as key categories of phytochemicals (18). These substances are acknowledged for their antioxidant properties and have been examined for their effectiveness in neutralizing free radicals and safeguarding against oxidative damage (19). Flavonoids possess a degree of polarity, which makes it possible to extract them using polar solvents like water, methanol

and ethanol (20). Alkaloids derived from the beta-carboline category exhibit potent effects against microbes, HIV and parasites (21). Saponins have biological activities such as antioxidants and antiglycation (22). Tannins demonstrate properties that combat viruses, bacteria and tumors (23).

Total phenolic and flavonoid content of *S. dichotomus*

The levels of phenolics and flavonoids found in natural products serve as crucial indicators for assessing both the quantitative aspects of the extract and its biological potency, as these compounds are vital to various physiological functions (24). Earlier research has shown that the selection of solvent plays a crucial role in determining how effectively polyphenols can be extracted from various sources (25). This research evaluates the total phenolic and flavonoid levels present in 80 % ethanol and aqueous extracts obtained from the leaves, offshoots and rhizomes of *S. dichotomus*, as illustrated in Table 2. In 80 % ethanol, the total phenolic content (TPC) exhibited values between 1.846 ± 0.015 and 2.797 ± 0.117 mg GAE/g sample, while in an aqueous solution, it ranged from 0.473 ± 0.007 to 2.386 ± 0.032 mg GAE/g sample. Conversely, the total flavonoid content (TFC) showed a variation of 0.264 ± 0.046 to 0.532 ± 0.022 mg CE/g sample in 80 % ethanol and from 0.303 ± 0.013 to 0.544 ± 0.062 mg CE/g sample in aqueous. It has been noted that the 80 % ethanol extracts of *S. dichotomus* generally exhibited greater TPC and TFC in comparison to the aqueous extracts, with the exception of the leave extract, which showed a higher TFC in the aqueous form than in the 80 % ethanol extract. The findings from the TPC analysis indicated that the leaves of *S. dichotomus* extracted with 80 % ethanol demonstrated the highest concentration, recorded at 2.797 ± 0.117 mg GAE/g sample. This was closely followed by the rhizome extracts using the same ethanol concentration, which yielded a TPC of 2.532 ± 0.152 mg GAE/g sample. In contrast, the TFC results revealed that the aqueous extract from the leaves had the highest level, measuring 0.544 ± 0.062 mg CE/g sample, while the rhizome extracts obtained with 80 % ethanol presented a TFC of 0.532 ± 0.022 mg CE/g sample. The results obtained by Zahra et al. (12) were in strong alignment with our results, indicating that leaf extracts possess a higher of phenolic compounds compared to those from rhizomes. The levels of these compounds differ depending on the specific parts of the plant, the seasons in which they are planted, the various stages of growth and the types of solvents employed for extraction (26). Furthermore, the selection of botanical material affects the levels of bioactive compounds, which in turn influences both the total phenolics and flavonoids present (27). The results proved that the solvents selected for extraction and separation play a crucial role in dissolving the natural compounds found in plants. Consequently, the varying polarity of phenolics and flavonoids in each extract correlated with how well these compounds dissolved in the solvents employed during the extraction process (28). These findings were in agreement with a previous study (29), indicating that aqueous alcoholic solvents, specifically 80 % methanol and ethanol, proved to be more effective than water in extracting total

phenolic content from peanut shells. Previous studies have shown that polyphenols and flavonoids dissolve more easily in solvents that are less polar compared to water (30, 31). Nonetheless, incorporating water into the solvent can aid in the extraction of phenolic compounds, as demonstrated by our findings, which are consistent with the results presented in earlier research (32, 33).

Antioxidant activities of *S. dichotomus* extracts

The assessment of antioxidant potential can be conducted using various techniques, each with its own advantages and disadvantages. In our research, we examined the antioxidant capabilities of *S. dichotomus* extracts through three specific *in vitro* chemical assays which are DPPH, ABTS and FRAP.

The DPPH assay is a popular method for evaluating antioxidant activity *in vitro* due to its simplicity, affordability, speed and the reproducibility of its results. DPPH radicals are nitrogen-centered organic free radicals that display a purple color in ethanolic solutions. When these radicals encounter proton-donating substances, known as antioxidants, they undergo a transformation that changes their color from purple to yellow, indicating the conversion to DPPH (diphenyl-picrylhydrazine) (13). In this research, the antioxidant effectiveness of extracts from the leaves, offshoots and rhizomes of *S. dichotomus* was examined in both 80 % ethanol and aqueous solvents, with results compared to those of ascorbic acid, as detailed in Table 3. The levels of DPPH radical scavenging activities observed *in vitro* differ significantly depending on the solvent used ($p < 0.05$), indicating that the antioxidant characteristics of the extract are substantially influenced by the type of extraction solvents. All the extracts demonstrated strong antioxidant properties, although they were less effective than ascorbic acid. The DPPH test of *S. dichotomus* exhibited a rise from 0.061 ± 0.007 to 0.212 ± 0.003 mg TE/g sample. The rhizome's 80 % ethanol extract demonstrated the highest DPPH scavenging capacity, with a trolox equivalent measuring 0.212 ± 0.003 mg TE/g sample. This was followed by the aqueous extract from the leaves and the 80 % ethanol extract from the offshoots (0.206 ± 0.0050 and 0.205 ± 0.001 mg TE/g sample), respectively. The increased ability of rhizomes, leaves and offshoots to donate hydrogen could be attributed to their higher concentrations of total polyphenols.

Regarding the antioxidant capabilities of *S. dichotomus* extracts assessed through the ABTS assay, the findings were in alignment with the outcomes of the DPPH radical scavenging tests. As illustrated in Table 3, there were significant differences in the scavenging activity against the ABTS cationic radical by *S. dichotomus* extracts obtained using various solvents from different tissues, which ranged from 1.276 ± 0.089 to 2.044 ± 0.031 mg TE/g sample. The extract from the rhizome using 80 % ethanol showed the most effective ABTS scavenging, achieving a value of 2.044 ± 0.031 mg TE/g sample. This was closely followed by the aqueous and 80 % ethanol extracts from the offshoots, which measured at 2.023 ± 0.040 and 1.965 ± 0.036 mg TE/g sample, respectively.

Table 2. Total phenolic and total flavonoid contents was detected in *S. dichotomus* extracts

Treatments	Total phenolic content (mg GAE/g sample)		Total flavonoid content (mg CE/g sample)	
	80 % Ethanol	Aqueous	80 % Ethanol	Aqueous
Leaves	2.797 ± 0.117^e	2.386 ± 0.032^d	0.264 ± 0.046^a	0.544 ± 0.062^b
Offshoots	1.846 ± 0.015^c	0.876 ± 0.100^b	0.314 ± 0.005^a	0.303 ± 0.013^a
Rhizomes	2.532 ± 0.152^d	0.473 ± 0.007^a	0.532 ± 0.022^b	0.497 ± 0.048^b

The values are presented as mean \pm standard deviation ($n = 3$). Different superscript lowercase letters in column denote significant differences at $p < 0.05$ using one-way ANOVA followed by Duncan's new multiple range test (DMRT). GAE = gallic acid equivalent; CE = catechin equivalent.

Table 3. The antioxidant activities of *S. dichotomus* extracts obtained using various solvents were compared to ascorbic acid with the concentration set to 0.5 mg/mL

Treatments	DPPH scavenging activity (mg TE/g sample)		ABTS scavenging activity (mg TE/g sample)		FRAP value (mg TE/g sample)	
	80 % Ethanol	Aqueous	80 % Ethanol	Aqueous	80 % Ethanol	Aqueous
Leaves	0.145 ± 0.007 ^c	0.206 ± 0.005 ^d	1.495 ± 0.045 ^b	1.688 ± 0.122 ^c	3.119 ± 0.087 ^b	3.119 ± 0.164 ^b
Offshoots	0.205 ± 0.001 ^d	0.074 ± 0.006 ^b	1.965 ± 0.036 ^d	2.023 ± 0.040 ^d	3.864 ± 0.292 ^c	0.767 ± 0.087 ^a
Rhizomes	0.212 ± 0.003 ^d	0.061 ± 0.007 ^a	2.044 ± 0.031 ^d	1.276 ± 0.089 ^a	3.595 ± 0.245 ^c	0.898 ± 0.085 ^a
Ascorbic acid	–	0.214 ± 0.001 ^d	–	2.238 ± 0.017 ^e	–	17.767 ± 0.261 ^d

The values are presented as mean ± standard deviation ($n = 3$). Different superscript lowercase letters in column denote significant differences at $p < 0.05$ using one-way ANOVA followed by Duncan's new multiple range test (DMRT). TE = trolox equivalent.

The ferric reducing ability of different parts of *S. dichotomus* extracts is displayed in Table 3. This ferric reducing power assay measures the reduction of the Fe^{3+} /ferricyanide complex into the Fe^{2+} /ferrous form, which serves as an indicator of the antioxidant strength of the plant extracts. As the extracts demonstrate their reduction capability, the initial yellow color transforms into either a blue or green color (34). The findings from this research indicated that different extracts of *S. dichotomus* exhibited a capacity for reducing power. Furthermore, the extracts obtained using 80 % ethanol demonstrated a greater reducing power, ranging from 3.119 ± 0.087 to 3.864 ± 0.292 mg TE/g sample, in contrast to the aqueous extracts, which yielded values from 0.767 ± 0.087 to 3.119 ± 0.164 mg TE/g sample. In a similar manner, the sequence of reducing power in descending order was identified as the 80 % ethanol derived from offshoots, followed closely by the 80 % ethanol obtained from rhizomes. The FRAP values recorded for these were 3.864 ± 0.292 and 3.595 ± 0.245 mg TE/g sample, respectively. The significant ferric reducing antioxidant capability of *S. dichotomus* extracts obtained from 80 % ethanol and aqueous suggests that polar solvents are efficient in retrieving electron-donating biomolecules from plant sources. These extracts have the potential to function as both primary and secondary antioxidants (35).

However, the scavenging activities assessed through the DPPH, ABTS and FRAP assays were lower than ascorbic acid, the positive control, yielding values of 0.214 ± 0.001 , 2.238 ± 0.017 and 17.767 ± 0.261 mg TE/g sample, respectively. Notably, the antioxidant capacity in leaves, offshoots and rhizomes exhibited a remarkable increase when utilizing 80 % ethanol extracts. The differences in the antioxidant strength of various extracts from *S. dichotomus* could be because of the marked differences in the amounts of phenols and flavonoids present. Previous studies support this idea, indicating that the antioxidant effect of phenolic substances is greatly affected by the kind of solvent used (36–38). Highly polar solvents like hydroethanolic, water, acetone and methanol are commonly utilized to extract phenolic substances and extracts that are polar tend to show a stronger ability to provide antioxidant benefits (39). Notably, the hydroethanolic extracts derived from *S. dichotomus* is recognized as a valuable resource owing to its capacity to neutralize free radicals and reduce oxidative stress. Moreover, the elevated levels of phenolics and flavonoids present in *S. dichotomus* enhanced its antioxidant capabilities. Our research support previous studies that highlight two critical factors influencing the antioxidant capability of phenolic compounds: the presence of hydroxyl groups and the level and type of conjugation. Generally, more effective antioxidants tend to exhibit a higher degree of conjugation and

possess an increased number of hydroxyl groups, enhancing their ability to neutralize free radicals (28, 40). In addition, flavonoids inhibit the creation of reactive oxygen species by forming chelates with trace elements that play a role in the generation of free radicals. This process aids in neutralizing reactive species and enhances the activity and protection of antioxidant defenses (41).

Correlation between TPC and TFC with antioxidant activities

To explore the relationships between total phenolic content (TPC) and total flavonoid content (TFC) with antioxidant activities in extracts from various parts of *S. dichotomus* using multiple solvents, Pearson's correlation coefficient was employed, as illustrated in Table 4. The results of this investigation align with earlier research, revealing the best positive correlation between TPC and FRAP ($R^2 = 0.852$), followed closely by the correlation between TPC and DPPH ($R^2 = 0.804$). Furthermore, while other phenolic compounds exhibited either moderate or weak positive correlations with antioxidant activities, TFC demonstrated a negative correlation with ABTS. Many investigations have reported a favorable relationship between the levels of phenolic compounds and antioxidant activities (42, 43). The leaves,

Table 4. The correlation coefficient was determined for the association between the total phenolic content (TPC), total flavonoid content (TFC) and the antioxidant properties of *S. dichotomus* extracts obtained using different solvents

Assays	DPPH	ABTS	FRAP	TPC	TFC
DPPH	1				
ABTS	0.536	1			
FRAP	0.535	0.577	1		
TPC	0.804	0.202	0.852	1	
TFC	0.245	-0.147	0.050	0.007	1

offshoots and rhizomes of *S. dichotomus* have been recognized as powerful sources of antioxidants, indicating that secondary metabolites play a crucial role in their antioxidant capabilities. These findings correspond to our study, highlighting the existence of phenols and flavonoids, which demonstrate strong hydrogen-donating antioxidant abilities that serve to protect against oxidative damage caused by hydroxyl radicals (44).

Conclusion

Based on the findings related to the phytochemicals, phenolic compounds and antioxidant properties derived from 80 % ethanol and aqueous extracts of various parts of *S. dichotomus*, it can be determined that all the extracts from this plant showed the presence of phenols, flavonoids, glycosides and phytosterols. The analysis indicated that the 80 % ethanol extracts were greater in yielding total phenolic content and antioxidant capacities,

specifically with regard to DPPH, ABTS and FRAP assays when compared to the aqueous extracts. The extracts from the leaves using 80 % ethanol notably contained the highest levels of total phenolic content, while the rhizome extracts in 80 % ethanol along with the aqueous extracts from leaves and rhizomes exhibited the most total flavonoid content. Furthermore, the extracts from rhizomes and offshoots with 80 % ethanol demonstrated the strongest antioxidant activities. The high antioxidant activity observed in the extracts may be due to the large amounts of phenolic and flavonoid compounds they contain. Future work should explore *in vivo* antioxidant efficacy or pharmaceutical applications of *S. dichotomus* extracts

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Authors' contributions

CW participated in the study design, carried out the experiments and drafted the manuscript. NB and JC revised the manuscript. PW performed the statistical analysis and SP participated in the plant collection and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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