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Research Article

Comparative Phytochemical, Antioxidant, and Antibacterial Study of Different Solvent Extracts of *Cissus hastata* Leaves

Patcharin Singdam[1]*, Anussara Kamnate[2], On-Anong Somsap[3], Ruhainee Tohkayomatee[1]

ABSTRACT

Introduction: Plants have long been utilized in traditional medicine for treating a variety of diseases. Plant-derived extracts are a rich source of phytochemicals with documented antibacterial and antioxidant properties. **Objective:** This study aimed to investigate and compare the phytochemical profiles, antioxidant capacities, and antibacterial activities of various crude extracts from the leaves of *Cissus hastata*. **Methods:** Leaf extracts of *C. hastata* were prepared using hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE). These extracts were analyzed for preliminary phytochemical constituents, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH, ABTS, and FRAP assays), and antibacterial activity against five bacterial strains: *Staphylococcus aureus* (TISTR 517), methicillin-resistant *Staphylococcus aureus* (MRSA 142), *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ESBL 182), and *Salmonella typhimurium* (TISTR 292). **Results**: Phytochemical screening of different solvent extracts of *C. hastata* leaves revealed the presence of diverse bioactive compounds. The EtOAc and AqE extracts exhibited the highest TPC (65.31 ± 1.85 and 61.45 ± 3.34 mg GAE/g extract, respectively) ($p < 0.05$). In contrast, the EtOH extract showed the highest TFC (29.92 ± 3.42 mg QE/g extract) ($p < 0.05$). The EtOH and AqE extracts also demonstrated the strongest antioxidant activities in the DPPH (IC_{50}: 307.07 ± 7.18 µg/mL and 316.86 ± 11.78 µg/mL), ABTS (IC_{50}: 160.21 ± 5.43 µg/mL and 208.45 ± 3.84 µg/mL) ($p < 0.05$), and FRAP (4.69 ± 0.08 and 4.96 ± 0.07 mM $FeSO_4$/mg extract) assays. The antibacterial activity was observed exclusively against Gram-positive bacteria. Among all the extracts, the AqE extract at a concentration of 100 mg/mL exhibited significant antibacterial effects against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, and *Bacillus cereus*, with zones of inhibition measuring 15.43 ± 0.46 mm, 14.76 ± 0.58 mm, and 15.66 ± 1.04 mm, respectively. **Conclusion:** Ethanol-based extracts of *C. hastata* leaves demonstrate high antioxidant and antibacterial activities and represent a promising source of bioactive compounds for developing natural therapeutic agents.

Keywords: *Cissus hastata*, Leaves, Phytochemical, Antioxidant, Antibacterial

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INTRODUCTION

Plants have long been used as folk medicine to treat various disorders[1]. Traditional plant-based remedies remain prevalent due to their accessibility, affordability, and minimal side effects. Moreover, plant extracts are a valuable source of bioactive compounds[2] that serve as candidates for the development of new drugs. These extracts contain various phytoconstituents exhibiting a wide range of pharmacological properties, including antidiabetic, anticancer, antimicrobial, antihypertensive, antioxidant, antihyperlipidemic, cardioprotective, immunomodulatory, and anti-inflammatory activities[3]. In particular, plant-derived compounds such as polyphenols and flavonoids have demonstrated strong antioxidant activity by counteracting oxidative stress. When consumed, they may help protect against oxidative damage in humans[4]. Antioxidants inhibit or delay cellular damage by scavenging or neutralizing harmful free radicals. This can reduce the risk of non-communicable diseases, including aging-related disorders, inflammatory conditions, tumors, and renal or hepatic diseases[5].

Cissus hastata Miq., a member of the Vitaceae family, is native to the eastern coast of Australia and found in Southeast Asia, including Thailand. It is a climbing plant characterized by long, flexible red tendrils that scramble over low vegetation and tree branches. The lanceolate leaves taper to a reddish pointy tip. Small flowers appear in clusters along the stem and develop into round berries that turn black when ripe[6]. Compared to other species in the *Cissus* genus, the pharmacological potential of *C. hastata* remains relatively unexplored. For instance, *Cissus quadrangularis* has been extensively studied and is known to contain various bioactive compounds, including gallic acid derivatives, steroids, iridoids, flavonoids, stilbenes, and triterpenes. This species exhibits antioxidant, analgesic, anti-inflammatory, antipyretic, anticancer, and antibacterial activities[7]. Similarly, *Cissus cornifolia* contains alkaloids, steroids, triterpenoids, flavonoids, cardiac glycosides, coumarins, saponins, tannins, and terpenoids, and has demonstrated a wide range of pharmacological activities, including antifungal, antibacterial, anticonvulsant, antidiabetic, antidiarrheal, anti-inflammatory, antioxidant, and antiproliferative effects[8]. Recent studies on *C. hastata* reported that methanolic leaf extracts contain various phytochemicals. These include alkaloids, tannins, and steroids, with a total phenolic content of 21.3 mg gallic acid equivalents (GAE)/g of extract. The extract also demonstrated antioxidant activity, with an IC_{50} value of 7.27 µg/mL against 2,2-diphenyl-1-picrylhydrazyl (DPPH). Therefore, this study aimed to evaluate the phytochemical profile, total phenolic and flavonoid contents, antioxidant potential, and antibacterial activity of *C. hastata* leaf extracts using different solvent extractions[6].

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MATERIALS AND METHODS

Chemical reagents

Ascorbic acid, Folin-Ciocalteu reagent, gallic acid, quercetin, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma-Aldrich (Germany). Meuller Hinton agar media was purchased from HiMedia (Mumbai, India). All other chemicals and reagents used in this study were of analytical grade.

Plant materials

Fresh leaves of *Cissus hastata* were collected from their natural habitat in Nakorn Sri Thammarat Province, Thailand, between July and September 2023. The authenticity of the plant was confirmed, and the specimen was deposited in the Faculty of Medicine, Princess of Naradhiwas University. The leaves were washed with tap water, oven-dried at 50 °C for 72 hours, and then ground into coarse powder using a mechanical grinder for further use.

Preparation of plant extracts

Two hundred grams of dried leaf powder were sequentially macerated with solvents of increasing polarity: hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE). Each extraction was performed at room temperature for 72 hours and repeated three times with a fresh solvent to enhance yield. The extracts were filtered through Whatman No. 1 paper and concentrated under reduced pressure at 50 °C using a rotary evaporator or freeze-dried. Dried extracts were weighed, stored in dark containers at 4 °C, and used for subsequent analyses. The extraction yield (%) was calculated as:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the dried crude extract}}{\text{Weight of dried leaf powder}} x\ 100$$

Phytochemical screening

Preliminary phytochemical screening was conducted on the Hex, EtOAc, EtOH, and AqE extracts of *C. hastata* leaves to detect the presence (+) or absence (-) of alkaloids, anthraquinones, cardiac glycosides, coumarins, saponins, steroids, tannins, and terpenoids using modified procedures[9,10].

Determination of total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu method[11]. In brief, 25 µL of diluted extract was mixed with 100 µL of diluted Folin-Ciocalteu reagent in a 96-well plate and incubated for 5 minutes. Then, 75 µL of sodium carbonate (Na_2CO_3) (100 g/L) was added. After 2 hours at room temperature, absorbance was measured at 765 nm using a microplate reader (SPECTROstar Nano, BMG LabTech). Gallic acid was used as a standard, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/ g extract).

Determination of total flavonoid content (TFC)

The total flavonoid content was determined by the aluminum chloride colorimetric method[11]. In each well, 50 µL of diluted extract was mixed with 100 µL of ethanol and 50 µL of 10% aluminum chloride (10% $AlCl_3$). After 3 minutes of incubation at room temperature, 20 µL of 1 M sodium acetate (CH_3COONa) and 60 µL of ethanol were added. The mixture was incubated in the dark for 40 minutes, and absorbance was measured at 430 nm. Quercetin was used as the standard, and results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The DPPH radical scavenging activity, which reflects the free radical scavenging capacity of the extracts, was assessed following the method described by Singdam *et al*[11]. Briefly, 50 µL of each extract was mixed with 150 µL of 0.2 mM DPPH in a 96-well plate and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm. Ascorbic acid and quercetin were served as standards. DPPH radical scavenging inhibition (%) was calculated using the formula:

$$\text{DPPH radical scavenging inhibition (\%)} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \text{ x } 100$$

Where $A_{control}$ is the absorbance without the test sample, and A_{sample} is the absorbance with the test sample.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

The ABTS radical scavenging activity, which measures the ability of antioxidants to quench $ABTS^+$ radicals, was conducted using a method based on a previous study[11]. ABTS (7 mM) and potassium persulfate (2.42 mM) solutions were mixed in equal volumes and incubated in the dark for 12-16 hours to generate ABTS radicals. The resulting solution was diluted to an absorbance of 0.8 ± 0.1 at 743 nm. Extract samples (25 µL) were mixed with 180 µL of diluted ABTS solution in a 96-well plate and incubated in the dark at room temperature for 30 minutes. Absorbance was recorded at 743 nm. Ascorbic acid and quercetin were used as standards. The percentage of ABTS radical scavenging was calculated using the same formula as in the DPPH assay.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay primarily measures the reducing potential of antioxidants in a sample was performed following the method of Singdam *et al*[11]. The FRAP reagent was prepared by mixing 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in 40 mM HCl, 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$), and 300 mM acetate buffer (pH 3.6) in a 1:1:10 ratio. A diluted extract (20 µL) was added to 180 µL of FRAP reagent in a 96-well plate, and the mixture was incubated in the dark at 37 °C for 15 minutes. Absorbance was measured at 593 nm. FRAP values were calculated from the standard curve prepared from ferrous sulphate ($FeSO_4$) solution and were expressed as mM $FeSO_4$ equivalents per milligram of extract (mM $FeSO_4$/mg extract).

Antibacterial properties

The evaluation of antibacterial activity was conducted using the agar well diffusion method[12]. Five bacterial strains (Gram-positive; *Staphylococcus aureus* TISTR 517, Methicillin-resistant *Staphylococcus aureus* (MRSA 142), and *Bacillus cereus* ATCC 11778, and Gram-negative; *Escherichia coli* ESBL 182 and *Salmonella typhimurium* TISTR 292) were used in the study. Bacterial strains were initially grown on Mueller Hinton Agar (MHA) for 18 hours at 37 °C, followed by subculturing in Mueller Hinton Broth (MHB) for 4 hours. The bacterial suspension was then standardized to 0.5 McFarland turbidity (approximately 1.5×10^8 CFU/mL) using 0.85% NaCl solution. Each bacterial inoculum was evenly spread on MHA plates, and wells measuring 0.6 cm in diameter were created using a sterile pipette tip. Aliquots of 100 µL from different solvent extracts of *C. hastata* leaves, at concentrations of 25, 50, and 100 mg/mL, were tested. The plates were maintained at room temperature for 5 hours to allow diffusion, then incubated at 37 °C for 18 hours. Inhibition zone diameters were measured using a vernier caliper and recorded in millimeters (mm).

Gentamicin (10 µg/mL) and 10% DMSO were used as positive and negative controls, respectively.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). Significant differences were determined by a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test ($p < 0.05$) using GraphPad Prism 10.4.0.

RESULTS AND DISCUSSION

Extraction yields

The percentage yields of the crude extracts of *C. hastata* with hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE) solvents are shown in Table 1. The extraction yields (%w/w) of the extracts were 4.43% (Hex), 1.94% (EtOAc), 11.59% (EtOH), and 7.45% (AqE). The results revealed that ethanol extraction produced the highest amount of crude extract, whereas ethyl acetate extraction resulted in the lowest yield. The variations in extraction yields observed in this study may be attributed to several factors, including the choice of solvents, extraction method, plant material used, and the chemical composition of different plant parts[13,14]. Moreover, the extraction yields of *C. hastata* varied with solvent polarity, with ethanol giving the highest yield, indicating its superior ability to extract phytochemicals from *C. hastata*. This can be attributed to the polar nature of ethanol. Its polarity facilitates the extraction of a broad range of polar and semi-polar bioactive compounds, such as phenolic acids and flavonoids[15].

Table 1. Percentage of extraction yield from different solvent extracts of *C. hastata* leaves.

Extracts	Extraction yield (%)
Hex	4.43
EtOAc	1.94
EtOH	11.59
AqE	7.45

Extract abbreviations: Hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE).

Table 2. Phytochemical analysis of different solvent extracts of *C. hastata leaves*.

Phytochemicals	Test reagent (test performed)	Observation	Results			
			C. hastata leaf extracts			
			Hex	EtOAc	EtOH	AqE
Alkaloids	Wagner's reagent	Reddish-brown precipitate	-	-	+	+
Anthraquinones	Borntrager's test	Reddish-pink coloration	-	-	-	-
Cardiac glycosides	Keller-Killiani test	Brownish ring between the layers	+	-	+	+
Coumarin	Alkaline reagent test	Yellow coloration	-	+	+	+
Saponins	Foam test	Persistent froth	-	-	+	+
Steroids	Liebermann-Burchard test	Blue or bluish-green coloration	+	+	+	-
Tannins	Ferric chloride	Dark green or dark blue coloration	-	-	+	-
Terpenoids	Salkowski test	Brownish ring at the interface	-	-	+	+

(+) = Positive (-) = Negative. Extract abbreviations: Hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE).

Preliminary phytochemical screening

Phytochemical screening of *C. hastata* leaf extracts revealed the presence of alkaloids, cardiac glycosides, coumarin, saponins, steroids, tannins, and terpenoids. However, none of the extracts exhibited a color change in the anthraquinones test (Figure 1 and Table 2). The Hex extract showed only positive tests for cardiac glycosides and steroids. The EtOAc extract showed positive tests for coumarin and steroids. However, the EtOH extract showed positive tests for all tested phytoconstituents, except anthraquinones. Lastly, the AqE extract contained various chemical constituents, including alkaloids, cardiac glycosides, coumarin, saponins, steroids, and terpenoids. Our findings align with those of Muhamad *et al.* (2023), who found that the methanol leaf extract of *C. hastata* contained alkaloids, steroids, and tannins but lacked saponins. Also, their study did not assess the presence of cardiac glycosides, coumarins, or terpenoids[6]. The absence of saponin in the extracts of the prior study may be attributed to the different solvent extraction methods. Furthermore, the phytochemical composition of medicinal plants can be influenced by environmental factors during growth and development, including temperature, atmospheric CO_2, ozone levels, light exposure, and soil conditions[16].

Total phenolic content (TPC) and total flavonoid content (TFC)

Phenolic and flavonoid compounds play a vital role in scavenging free radicals and protecting the body from oxidative stress by supplying hydrogen atoms or electrons to neutralize the free radicals and prevent cellular damage. This process can help lower the risk of various health problems, including cancer, diabetes, and other degenerative diseases[17]. In this study, the total phenolic and flavonoid contents of different solvent extracts of *C. hastata* leaves were evaluated (Figure 2). Total phenolic content (TPC) was expressed as gallic acid equivalents (GAE), based on the standard calibration curve: $y = 0.0066x + 0.0064$ with $R^2 = 0.9993$ (Figure 2a). Similarly, total flavonoid content (TFC) was determined as quercetin equivalents (QE) using the equation: $y = 0.01x - 0.0562$, with $R^2 = 0.9998$ (Figure 2c). The EtOAc and AqE extracts had significantly the highest TPC (65.31 ± 1.85 mg GAE/g extract and 61.45 ± 3.34 mg GAE/g extract, respectively) ($p < 0.05$), while the lowest TPC was shown by the Hex extract (7.31 ± 0.29 mg GAE/g extract) (Figure 2b). The highest TFC was significantly observed in the EtOH extract (29.92 ± 3.42 mg QE/g extract) ($p < 0.05$). Moreover, the AqE extract contained the second-highest amount of total flavonoid (20.21 ± 1.07 QE/g extract), while the Hex and EtOAc extracts exhibited the lowest flavonoid amount (16.82 ± 3.53 and 17.42 ± 2.50 mg QE/g extract, respectively) (Figure 2d). However, a previous study found that the TPC value of the methanolic extract of *C. hastata* leaves was 21.30 mg GAE/g extract[6]. Due to their high solubility in organic solvents, polyphenols are often extracted from plant materials using solvents such as methanol, ethanol, ethyl acetate, acetone, or mixtures of solvents with water[18,19]. According to previous research, the combination of ethanol and water exhibits higher polarity compared to absolute ethanol, which can enhance the extraction efficiency of free phenolics[20]. Moreover, flavonoids, a type of phenolic compound, exhibit varying solubility based on their structural forms. The flavonoids of the aglycone type are more soluble in polar solvents, and the glycosides are more soluble in nonpolar solvents[21]. Ethanol, especially at high concentrations, is particularly effective at extracting aglycone flavonoids, which may account for the elevated TFC in the ethanol extract. This is supported by findings from Maulana *et al.* (2019), who reported that 96% ethanol yielded the highest TPC among various ethanol-water mixtures, highlighting ethanol's strong selectivity for flavonoids over other phenolic constituents[22]. In summary, the results demonstrated that the highest TPC was obtained

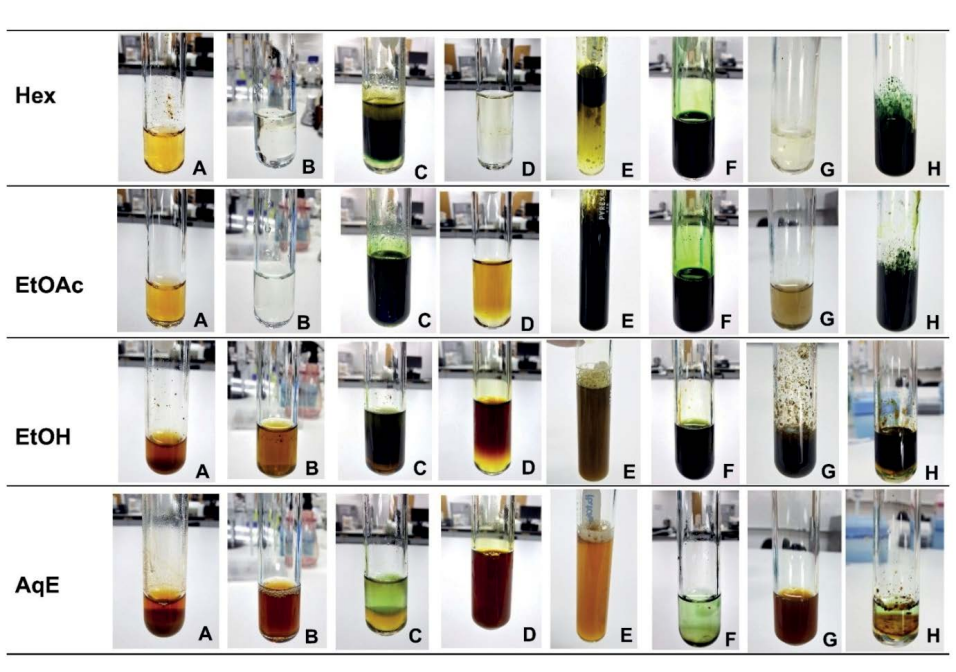

Figure 1. Phytochemical tests of *C. hastata* leaf extracts using different solvent extractions. (A) Alkaloids were detected in the EtOH and AqE extracts, as evidenced by the formation of a reddish-brown precipitate. (B) Anthraquinones were absent in all extracts, indicated by the lack of any visible color change. (C) Cardiac glycosides were present in the Hex, EtOH, and AqE extracts, as shown by a characteristic brownish ring between the layers. (D) Coumarins were detected in the EtOAc, EtOH, and AqE extracts, as indicated by the appearance of a yellow to orange coloration. (E) Saponins were observed only in the EtOH and AqE extracts, as evidenced by the formation of a persistent froth or foamy layer. (F) Steroids were detected in all extracts except the AqE extract, with a bluish-green coloration as a positive indication. (G) Tannins were present only in the EtOH extract, as shown by the formation of a dark green or dark blue color. (H) Terpenoids were observed in both the EtOH and AqE extracts, evidenced by the presence of a brownish ring at the interface. Extract abbreviations: Hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE).

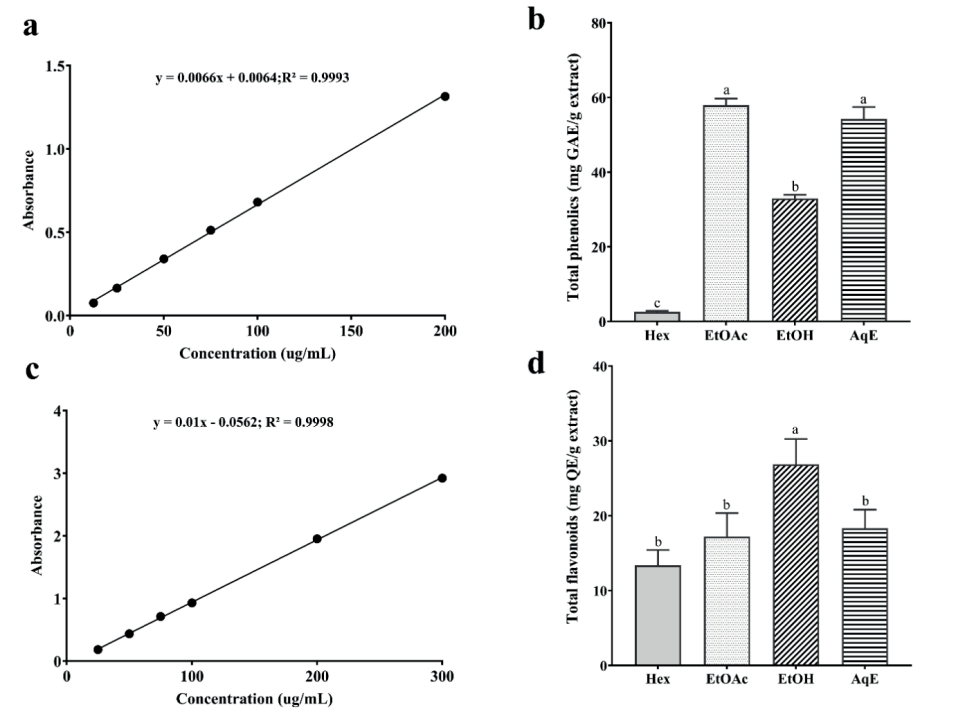

Figure 2. The total phenolic and flavonoid content (TPC and TFC) in different solvent extracts of *C. hastata* leaves. (a) The calibration curve of TPC; (b) TPC; (c) The calibration curve of TFC; and (d) TFC. The data are presented as mean ± SD based on three independent replicates ($n = 3$). Statistically significant differences among the extracts were indicated by different superscript letters ($p < 0.05$). Extract abbreviations: Hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE).

from polar and moderately polar solvents, specifically 50% ethanol and ethyl acetate, while the 95% ethanol extract yielded the highest TFC. These findings align with previous studies, suggesting that solvent polarity significantly influences the extraction efficiency of phenolics and flavonoids based on their solubility and structural forms[23].

Antioxidant activity analysis

In vitro antioxidant assays such as DPPH, ABTS, and FRAP assess an antioxidant's ability to donate electrons to convert free radicals into stable anions[24]. The DPPH assay evaluates antioxidant activity and operates on the principle of electron transfer. DPPH is a stable free radical that has a purple color and strongly absorbs light at a wavelength of 517 nm. In the presence of an antioxidant, DPPH undergoes reduction through the donation of an electron or hydrogen atom, leading to a visible color change from deep purple to yellow or colorless. This reduction in absorbance at 517 nm reflects the sample's antioxidant capacity[25]. Similarly, the ABTS assay evaluates antioxidant activity by measuring the reduction of the blue-green ABTS radical cation, which has a strong absorbance at 734 nm. When antioxidants are introduced, they donate electrons to ABTS, resulting in a reduction of the blue-green color to pale blue. This decolorization is directly proportional to the antioxidant capacity of the sample[26].

The percentage inhibition of DPPH and ABTS radicals at various concentrations ranging from 100 to 500 µg/mL is presented in Figure 3a-3b. Additionally, the DPPH and ABTS radical scavenging activity of the extracts was evaluated by calculating the IC_{50} values, which represent the concentration required to inhibit 50% of the free radicals. Among the tested extracts, the EtOH and AqE extracts exhibited significantly higher DPPH scavenging activity, with IC_{50} values of 307.07 ± 7.18 and 316.86 ± 11.78 µg/mL, respectively ($p < 0.05$). These values were notably lower than those of the EtOAc extract (843.45 ± 49.97 µg/mL) and the Hex extract (2229.00 ± 73.83 µg/mL), sµggesting that EtOH and AqE extracts possess superior DPPH scavenging potential compared to the other extracts (Figure 3c).

In the ABTS assay, as shown in Figure 3d, the EtOH extract displayed the strongest radical scavenging ability with an IC_{50} value of 160.21 ± 5.43 µg/mL, followed by the AqE extract (208.45 ± 3.84 µg/mL), EtOAc extract (507.16 ± 8.51 µg/mL), and the Hex extract (1162.83 ± 76.56 µg/mL). The significantly lower IC_{50} values of the EtOH and AqE extracts in the ABTS assay indicate their greater efficiency in neutralizing ABTS radicals ($p < 0.05$). When compared to ascorbic acid and quercetin, standard antioxidants, the IC_{50} values of all the extracts were higher, indicating that the extracts were less potent than ascorbic acid and quercetin. For the DPPH assay, ascorbic acid and quercetin exhibited an IC_{50} value of 12.37 ± 0.70 and 8.37 ± 0.13 µg/mL, respectively, while for the ABTS assay, the IC_{50} value of ascorbic acid and quercetin was 90.40 ± 6.27 and 9.09 ± 0.34 µg/mL, respectively (Figure 3c-3d).

Furthermore, the reducing potential of *C. hastata* leaves was evaluated through the FRAP assay, which quantifies the ability of antioxidants in the leaf extracts to reduce the Fe^{3+}-TPTZ complex to its ferrous (Fe^{2+}) form at a low pH.[24] The FRAP values were expressed as mM $FeSO_4$ equivalents per mg of extract. These values were determined using a standard calibration curve, with the equation: $y = 0.0771x + 0.0218$; $R^2 = 0.9983$ (Figure 3e).

As illustrated in Figure 3f, the reducing potential of *C. hastata* leaf extracts varied based on the solvent employed for extraction. The AqE and EtOH extracts exhibited the highest FRAP values (4.96 and 4.69 mM $FeSO_4$/mg extract, respectively), consistent with their high DPPH and ABTS activity. These results were markedly higher than those obtained for the EtOAc extract (1.08 ± 0.18 mM $FeSO_4$/mg extract) and the Hex extract (0.21 ± 0.05 mM $FeSO_4$/mg extract), indicating that polar solvents, particularly ethanol and ethanol-water mixture, are more effective in extracting reducing agents from *C. hastata* leaves. In comparison, the standard antioxidants ascorbic acid and quercetin demonstrated significantly greater reducing capacities, with values of 36.60 ± 3.16 and 238.03 ± 3.64 mM $FeSO_4$/mg extract, respectively ($p < 0.05$), underscoring their strong antioxidant activity. These findings suggest that *C. hastata* leaves contain bioactive compounds with reducing properties, particularly those extractable by polar solvents. The markedly higher activity observed in the AqE and EtOH extracts highlights the role of solvent polarity in maximizing the yield of antioxidant constituents.

Previous findings by Muhamad *et al.* (2023) indicated that the methanol extracts of *C. hastat*a leaves had potent DPPH scavenging activity, with an IC_{50} value of 7.27 µg/mL.[6] However, in the present study, although the EtOH and AqE extracts of *C. hastata* leaves exhibited higher total phenolic content, their IC_{50} values were substantially higher (307.07 ± 7.18 µg/mL and 316.86 ± 11.78 µg/mL, respectively). This discrepancy suggests that antioxidant activity may not correlate directly with total phenolic content, but rather with the presence and proportion of specific phenolic or flavonoid compounds[27]. Among the four different solvent extracts of *C. hastata* leaves, those obtained using 95% ethanol and 50% ethanol solvent also exhibited the most potent antioxidant activity.

Antioxidant activity can be attributed to the presence of phenolic and flavonoid compounds, which are well known for their electron-donating abilities and free radical scavenging properties. The efficiency of ethanol in polyphenol extraction has also been documented in earlier studies, providing further support for the observed antioxidant potential of these extracts[28,29]. These findings suggest that ethanol-based solvents are highly efficient at extracting potent antioxidant compounds, particularly polyphenols and flavonoids, from *C. hastata* leaves. Overall, the extracts obtained from 95% ethanol and 50% ethanol solvent showed the highest TPC, TFC, and antioxidant activities, confirming the efficiency of polar solvents in extracting bioactive compounds from *C. hastata* leaf. However, high TPC and TFC do not always correlate with antioxidant potency due to several factors, including compound specificity, synergistic effects, and the dual role of certain compounds[30,31]. Thus, although TPC and TFC are commonly used indicators of antioxidant capacity, they do not account for the complex interactions and mechanisms involved in antioxidant activity[32].

Antibacterial activity

The antibacterial activities of the hexane (Hex), ethyl acetate (EtOAc), ethanol (EtOH), and aqueous (AqE) extracts of *C. hastata* leaves were evaluated against five bacterial strains: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, and *Salmonella typhimurium*. These strains were selected to represent both Gram-positive and Gram-negative bacteria with clinical and food safety significance. *S. aureus* and MRSA are common causes of skin, soft tissue, and hospital-acquired infections[33], while *B. cereus* is a well-known foodborne pathogen[34]. *E. coli* and *S. typhimurium* are representative Gram-negative enteric pathogens associated with gastrointestinal infections[35]. Collectively, both Gram-positive and Gram-negative bacteria ensure a comprehensive evaluation of the broad-spectrum antibacterial potential of the plant extracts.

The results, presented in Table 3, revealed varying degrees of inhibition zones (mm) among different concentrations (25-100 mg/mL). The antibacterial activity of all extracts of *C. hastata* leaves was observed solely against Gram-positive bacteria at any tested concentration (25-100 mg/mL). Notably, the Hex extract exhibited inhibitory effects at 100 mg/mL against *S. aureus* (14.81 ± 0.77 mm), MRSA (12.63 ± 1.24 mm), and *B. cereus* (14.86 ± 0.52 mm). The EtOAc extract exhibited

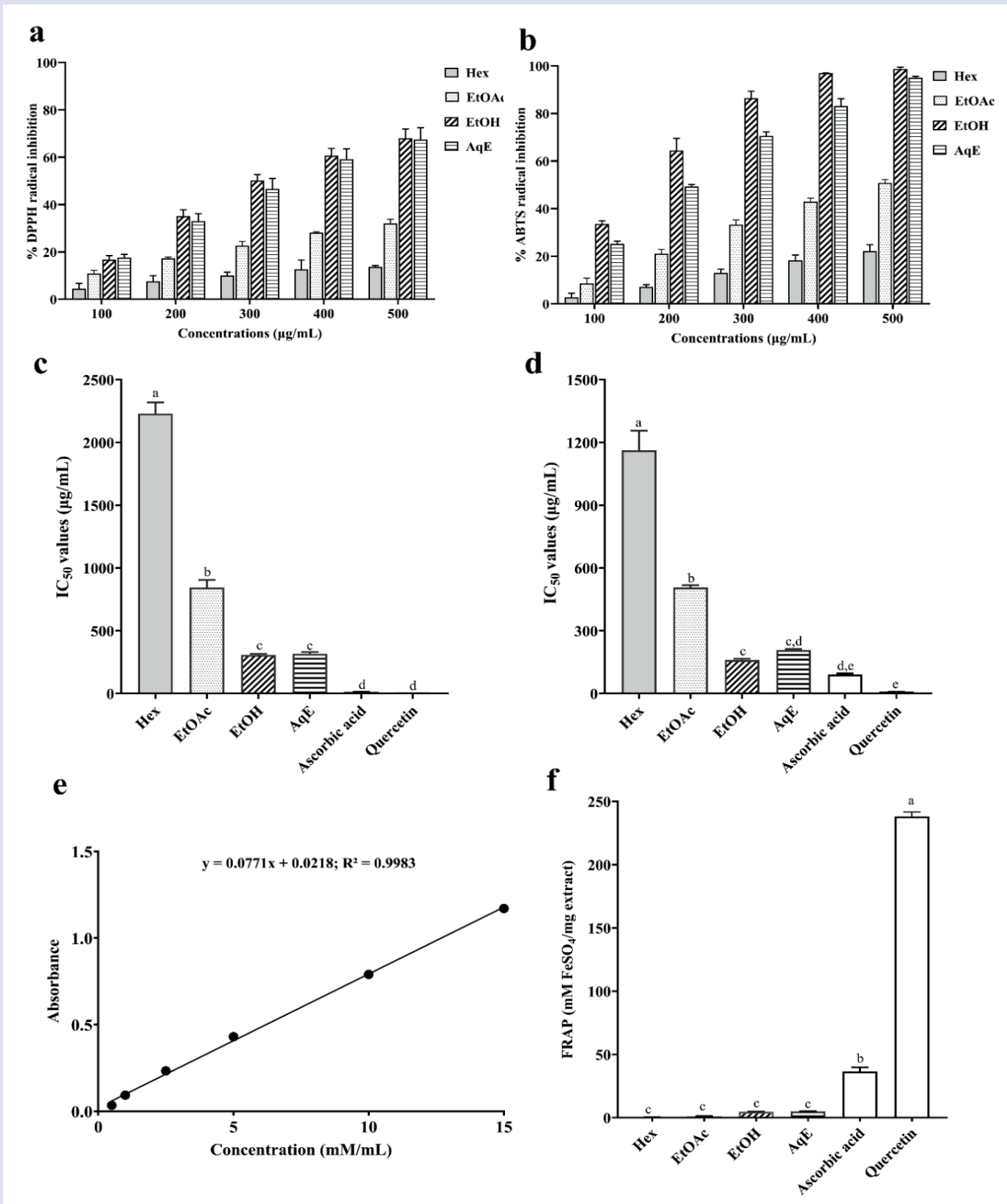

Figure 3. Antioxidant activities in different solvent extracts of *C. hastata* leaves. (a) percentage of DPPH radical inhibition by different solvent extracts of *C. hastata* leaves; (b) percentage of ABTS radical inhibition by different solvent extracts of *C. hastata* leaves; (c) IC_{50} values for scavenging DPPH; (d) IC_{50} values for scavenging ABTS; (e) calibration curve of $FeSO_4$; and (f) FRAP values. The data are presented as mean ± SD based on three independent replicates ($n = 3$). Statistically significant differences among the extracts were indicated by different superscript letters ($p < 0.05$). Extract abbreviations: Hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE).

Table 3. Antibacterial activity of different solvent extracts of *C. hastata* leaves against gram-positive and gram-negative pathogens.

Samples	(mg/mL)	Diameter of zone of inhibition (mm)				
		S. aureus	MRSA	*B. cereus*	*E. coli*	*S. typhimurium*
Hex	25	12.03 ± 0.06^{d,e}	11.56 ± 0.74^{b}	14.40 ± 0.88^{b,c}	NI	NI
	50	13.41 ± 0.73^{c,d}	12.99 ± 1.21^{a,b}	14.57 ± 0.40^{b,c}	NI	NI
	100	14.81 ± 0.77^{b,c}	12.63 ± 1.24^{a,b}	14.86 ± 0.52^{b,c}	NI	NI
EtOAc	25	NI	NI	13.63 ± 1.77^{b,d}	NI	NI
	50	NI	NI	14.39 ± 0.27^{b,c}	NI	NI
	100	16·00 ± 0.70^{b}	NI	15.23 ± 0.88^{b}	NI	NI
EtOH	25	NI	NI	10.87 ± 0.31^{d}	NI	NI
	50	NI	NI	12.01 ± 1.19^{c,d}	NI	NI
	100	NI	NI	12.91 ± 1.44^{b,d}	NI	NI
AqE	25	10.77 ± 0.35^{e}	NI	12.08 ± 0.52^{c,d}	NI	NI
	50	12.11 ± 0.14^{d,e}	11.27 ± 0.35^{b}	13.93 ± 1.39^{b,d}	NI	NI
	100	15.43 ± 0.46^{b}	14.76 ± 0.58^{a}	15.66 ± 1.04^{b}	NI	NI
Gentamicin 10 µg/mL		20.51 ± 0.20^{a}	NI	24.64 ± 0.27^{a}	16.87 ± 0.33	25.27 ± 0.23

The data are presented as mean ± SD based on three independent replicates (n = 3). Statistically significant differences among the extracts were indicated by different superscript letters in the same column ($p < 0.05$). NI = No inhibition zone. Extract abbreviations: Hexane (Hex), ethyl acetate (EtOAc), 95% ethanol (EtOH), and 50% ethanol (AqE).

specific antibacterial activity against *B. cereus*, with increasing effectiveness from 13.63 ± 1.77 mm at 25 mg/mL to 15.23 ± 0.88 mm at 100 mg/mL. Additionally, an inhibition zone of 16·00 ± 0.70 mm was observed against *S. aureus* at 100 mg/mL. The EtOH extract followed a similar pattern to the EtOAc extract, displaying inhibitory activity solely against *B. cereus*, with the zones of inhibition ranging from 10.87 ± 0.31 mm (25 mg/mL) to 12.91 ± 1.44 mm (100 mg/mL). The AqE extract at 100 mg/mL showed significant inhibition against *S. aureus*, MRSA, and *B. cereus*, with zones of inhibition of 15.43 ± 0.46 mm, 14.76 ± 0.58 mm, and 15.66 ± 1.04 mm, respectively. As a positive control, gentamicin (10 µg/mL) exhibited antibacterial activity, with the zones of inhibition against *S. aureus* (20.51 ± 0.20 mm), *B. cereus* (24.64 ± 0.27 mm), *S. typhimurium* (25.27 ± 0.23 mm), and *E. coli* (16.87 ± 0.33 mm), affirming its broad-spectrum efficacy.

Previous studies on other *Cissus* species, such as *Cissus quadrangularis* and *Cissus rotundifolia*, have reported significant antibacterial activity against both Gram-positive and Gram-negative bacteria[36,37]. However, our study found that none of the *C. hastata* leaf extracts exhibited antibacterial activity against the tested Gram-negative strains *(E. coli* and *S. typhimurium*) at different concentrations (25-100 mg/mL). The structural differences in the cell walls of Gram-positive and Gram-negative bacteria influence their susceptibility to phytochemicals. Gram-negative bacteria possess a more complex cell wall that includes an outer lipopolysaccharide layer, which often acts as a barrier to limit the entry of various substances. In contrast, Gram-positive bacteria have a simpler structure consisting mainly of a thick peptidoglycan layer, which allows for greater permeability. As a result, phytochemicals are generally more effective against Gram-positive bacteria due to their easier access to the cell structure[38].

Phytochemical analysis suggests that the antibacterial effects may be attributed to bioactive constituents such as alkaloids, polyphenols, and flavonoids[39]. Particularly, polyphenolic compounds have been demonstrated to have antibacterial action through various mechanisms that are closely associated with their structural characteristics, chemical composition, and lipophilicity. For instance, polyphenols may interact directly with bacterial membranes, causing structural damage and leading to the leakage of intracellular contents. This disruptive effect is largely facilitated by the hydroxyl groups within polyphenol structures, which form hydrogen bonds with membrane components[40]. Thus, the comprehensive study of *C. hastata* leaf extracts has identified a range of bioactive compounds with notable antibacterial properties.

CONCLUSION

The current study highlights the significant antioxidant and antibacterial potential of different solvent extracts of *Cissus hastata* leaves, particularly those extracts using ethanol-based solvents. The findings of this study provide a foundational basis for the potential development of this indigenous plant as a therapeutic agent. Its notable antioxidant and antibacterial activities indicate a promising role in alleviating various diseases, including the development of treatments for infectious diseases. Markedly, this study contributes scientific evidence supporting the medicinal properties of *C. hastata*, thereby advancing its potential application in evidence-based therapeutics. Further studies are needed to isolate and identify individual bioactive components from the crude extracts of *C. hastata* leaves based on the polarity of the extraction solvents.

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