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Original Article

Phytochemical Screening, Antioxidant Potential, and α-Glucosidase Inhibition of *Causonis trifolia* Leaf Extracts: A Solvent-Based Comparative Study

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ABSTRACT

Introduction: Medicinal plants are used in diabetes mellitus due to their proven minimal adverse effects in humans. *Causonis trifolia* leaves have been used as traditional medicine for various treatments. **Objective:** The aim of this research was to investigate the phytochemicals, the total flavonoid and phenolic content, and *in-vitro* antioxidant and antidiabetic activities of the extracts of *C. trifolia* leaves. **Method:** The leaves of *C. trifolia* were sequentially extracted with maceration in hexane, ethyl acetate, ethanol, and 50% ethanol. Then, the *C. trifolia* leaf extracts were assessed for antioxidant activity by the DPPH and ABTS radical scavenging and FRAP assay. Total phenolic and flavonoid contents were determined by the Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. Antidiabetic activity was evaluated by determination of α-glucosidase inhibitory effect. **Results:** Preliminary phytochemical analysis of ethanol and 50% ethanol extracts of *C. trifolia* leaves showed positive results for alkaloids, coumarin, tannins, saponins, cardiac glycosides, terpenoids, and steroids. The 50% ethanol extract had the best antioxidant activities of the three antioxidant assays ($p < 0.05$). The results revealed higher phenolic and flavonoid contents in 50% ethanol extract than in the other extracts ($p < 0.05$). Moreover, the 50% ethanol extract exhibited the best potential as α-glucosidase inhibitors of all the extracts and acarbose ($p < 0.05$). **Conclusions:** The results conclude that the *C. trifolia* leaves with 50% ethanol as the solvent possessed the potential to extract the highest levels of phytochemical content and have potential antioxidant and α-glucosidase inhibitory activities for diabetic therapy.

Key words: *Causonis trifolia*, Antioxidant, α-Glucosidase, Antidiabetic.

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INTRODUCTION

Diabetes mellitus is a complex metabolic disorder characterized by continued elevated blood glucose levels due to anomalies in either insulin secretion or insulin action or both.[1] The World Health Organization (WHO) estimated that there were 1.6 million deaths caused by diabetes in 2021 with 47% of all diabetes-related deaths occurring before the age of 70 years. Moreover, the number of people living with diabetes rose from 200 million in 1990 to 830 million in 2022.[2]

α-Glucosidase inhibitors, such as acarbose, miglitol, and voglibose, are among the therapeutic agents used in the management of type 2 diabetes mellitus.[3] Approved by the Food and Drug Administration (FDA) for treating adults with type 2 diabetes mellitus, acarbose is a complex oligosaccharide that functions as a competitive and reversible inhibitor of pancreatic α-amylase and intestinal α-glucosidase enzymes. These enzymes are responsible for breaking down complex carbohydrates, including oligosaccharides, trisaccharides, and disaccharides (e.g., sucrose and maltose), into monosaccharides (e.g., glucose and fructose) in the brush border of the small intestine. By inhibiting these enzymes, acarbose delays the absorption of monosaccharides, reducing postprandial blood glucose spikes. The delayed breakdown and absorption of carbohydrates lead to a slower transport of glucose across the intestinal mucosa into blood circulation. However, acarbose therapy is associated with certain gastrointestinal side effects, including flatulence, diarrhea, and abdominal discomfort.[4] Traditional medicine from plant extracts has been used for a long time and plays an important role as an alternative medication option. Medicinal plants are rich sources of the secondary metabolites used in various treatments, including diabetes mellitus, and their use is proven to have minimal adverse effects in humans.[5] One of these herbal medicines with high potential is *Causonis trifolia* (L.) Mabb. & J.Wen.

Causonis trifolia (L.) Mabb. & J.Wen (vitaceae), commonly known as "fox grape" (synonym: *Cayratia trifolia* (L.) Domin), is a perennial climber that is native to Australia and several countries in Asia, including Thailand. The leaves of *C. trifolia* are traditionally used externally for nose ulcers, muscle pain, abscess, fever and asthma as well as being used as an expectorant, carminative, and blood purifier.[6,7] *C. trifoli*a leaves have been scientifically tested and proven to have several biological activities, such as anti-ulcer[8], antimitotic[9], antioxidant[10,11,12], and antibacterial activities.[13] The study conducted by Yusuf *et al.* investigated the antidiabetic potential of an ethanolic stem extract against a streptozotocin-induced mice model.[14] However, the activity of its leaf extract as an antidiabetic has not been evaluated. Therefore, this study was conducted to investigate the phytochemicals, total flavonoid and polyphenol content, and *in-vitro* antioxidant and antidiabetic potential of sequentially extracted polar extracts of *C. trifolia* leaves to provide the scientific evidence for the folkloric/traditional use of this plant.

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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), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), α-glucosidase from *Saccharomyces cerevisiae*, *p*-nitrophenyl-a-D-glucopyranoside (*p*-NPG), and acarbose were obtained from Sigma-Aldrich (Germany). All other reagents which were used in this study were analytical grade.

Plant materials

The Causonis trifolia leaves were collected in Nakorn Sri Thammarat Province, Thailand in June to July 2022 and identified by the Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation, Thailand. The fresh leaves of *C. trifolia* were washed under running tap water, and then dried at 50 °C for 72 h. The resulting dried leaves were ground into coarse powder using a mechanical grinder in preparation for their use in experiments.

Preparation of the plant extracts

Three hundred grams of the coarse powder were sequentially extracted with maceration in four solvents, namely hexane, ethyl acetate, ethanol and 50% ethanol, with an increasing order of polarity. The coarse powder underwent this extraction process three times with each solvent at room temperature over three days. Each solvent extract was pooled and then filtered through a Whatman No.1 paper filter. Then, the filtrate was concentrated at a temperature not exceeding 50 °C using a rotary evaporator, or freeze-dried at -80 °C for 72 h. All extracts were stored in a dark container at 4 °C after being weighed for further analysis. The extraction yield (%) was calculated as follows:

$$\text{Extraction yeild (\%)} = \frac{\textit{weight of the extract after solvent exporation and freeze drying}}{\text{dry weight of the sample}} \times 100$$

Preliminary analysis of phytochemicals

The preliminary screening for the presence of phytochemicals, including alkaloids, anthraquinones, cardiac glycosides, coumarin, saponins, steroids, tannins, and terpenoids in different solvent extracts of *C. trifolia* leaves was conducted by the modified methods described earlier.[15,16] The results of the phytochemical screening were stated as "+" or "–" denoting the presence or absence of the phytochemicals, respectively.

Determination of total phenolic content

The total phenolic content (TPC) in the different solvent extracts of *C. trifolia* leaves was determined using the Folin-Ciocalteu method adapted from a previous study with some modifications.[17] A total of 25 µL of the diluted samples from each extract was added to a 96-well microplate, followed by the addition of 100 µL of 1:4 diluted Folin-Ciocalteu reagent. The mixture was gently shaken and left for 5 min at room temperature, and then 75 µL of sodium carbonate solution (100 g/L) was added. The mixture was thoroughly shaken and incubated for 2 h at room temperature. Subsequently, the absorbance was measured at a wavelength of 765 nm using a microplate reader (SPECTRO Star Nano, BMG LabTech). Gallic acid dilutions (25-200 ug/mL) were used to construct a calibration curve. Total phenolic contents were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). All measurements were calculated from the value obtained from assays performed in triplicate.

Determination of total flavonoid content

The total flavonoid content (TFC) in different solvent extracts of *C. trifolia* leaves was quantified using the aluminum chloride colorimetric method, modified from Sari *et al.*[18] A total of 50 µL of the diluted samples from each extract and 100 µL of ethanol were mixed in 96-well microplate. Subsequently, 50 µL of 10% aluminum chloride ($AlCl_3$) was added to each well. The mixture was gently shaken and kept in incubation for 3 min at room temperature. After the incubation period, 20 µL of 1 M sodium acetate was added, followed by the addition of 60 µL of ethanol. The mixture was then incubated in the dark for 40 min at room temperature, and the absorbance was recorded at 430 nm using a microplate reader (SPECTRO Star Nano, BMG LabTech). All determination was carried out in triplicate. Various concentrations of quercetin (12.5-200 ug/mL) were used to construct a calibration curve, and quantification was presented as milligrams of quercetin equivalent per gram of extract (mg QE/ g extract).

DPPH radical scavenging activity

The DPPH assay was slightly adapted from a previously described method.[19] A total of 50 µL of varying sample concentrations from each extract (50-1000 ug/mL, dissolved in 5% DMSO) was combined with 150 µL of a 200 µM DPPH in a 96-well microplate. Subsequently, the microplate was incubated in the dark for 30 min at room temperature, and absorbance was measured at 517 nm using a microplate reader (SPECTRO Star Nano, BMG LabTech). Then, 5% DMSO was used as a substitute for the crude extract in the control and was tested in the blank sample. Ascorbic acid was served as a standard at concentrations ranging from 1 to 20 ug/mL. The experiment was carried out in triplicate. The inhibition percentages of the DPPH scavenging activity of the samples were calculated according to the following equation:

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

Where $A_{control}$ = the absorbance in the absence of the sample, and A_{sample} = the absorbance in the presence of the sample.

The results were plotted as the percentage of scavenging activity against the sample concentration, and IC_{50} (the concentration at which 50% of the DPPH radical was scavenged) was subsequently determined.

ABTS radical cation scavenging activity

The ABTS assay was conducted according to a method modified from previous research.[20,21] Solutions of ABTS and potassium persulfate ($K_2S_2O_8$) were prepared at concentrations of 7 mM and 2.42 mM, respectively, and these were then combined at a ratio of 1:1 (v/v) before being incubated for 12-16 h in the dark to make ABTS reaction solution. The ABTS reaction solution was then diluted with distilled water to obtain absorbance of 0.8 ± 0.1 units at 743 nm. The concentrations of the extracts were as previously described for the DPPH assay, and the ascorbic acid was used as a standard within a concentration range of 40 to 120 ug/mL. A total of 25 µL of the different concentrations of each extract was added to a volume of 180 µL of ABTS working solution in a 96-well microplate and then incubated at room temperature in the dark for 30 min. The absorbance was determined at 743 nm using a microplate (SPECTRO Star Nano, BMG LabTech). The experiments were conducted in triplicate. The percentage of inhibition was calculated in the same way as for the DPPH, and the average concentration responsible for 50% inhibition (IC_{50}) was determined.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined using the method described by Xiao *et al.*[22] with slight modification. The FRAP reagent was made with a

mixture of 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ solution, and acetate buffer (300 mM, pH 3.6) at the ratio of 1:1:10. A total of 20 µL of the diluted samples from each extract was mixed with 180 µL FRAP reagent and then incubated in the dark at 37 °C for 15 mins. The absorbance was measured at 593 nm using a microplate reader (SPECTRO Star Nano, BMG LabTech). All determinations were performed in triplicate. The calibration curve was prepared using ferrous sulphate ($FeSO_4.7H_2O$) solution in concentrations of 0.5 to 20 mM/mL. The results were calculated from the standard curve prepared from ferrous sulphate solution and were expressed as mM $FeSO_4$/mg extract.

In-vitro antidiabetic activity

The antidiabetic activity was determined by a method of inhibitory activity of α-glucosidase using the modified method previously reported.[23] The concentration of plant extracts ranged from 31.25 to 1000 µg/mL. A total of 50 µL of the diluted samples were added into a 96-well microplate and mixed with 50 µL of 0.075 U/ mL of α-glucosidase enzyme in 0.1 M sodium phosphate buffer (pH 6.8). The sample mixtures were left at 37°C for 10 min. 40 µL of 1 mM *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) was then added as a substrate and the mixtures were further incubated at 37 °C for 5 min. After the incubation period, the reaction was stopped with the addition of 40 µL of 0.2 M sodium carbonate (Na_2CO_3) solution. The absorbance was measured by a microplate reader (SPECTRO Star Nano, BMG LabTech) at a wavelength of 405 nm. Acarbose was used as a positive control under the same conditions as the assay. The results of the inhibition of enzymatic activity were expressed as the average of the 50% inhibitory concentration (IC_{50}). The percentage inhibition was calculated using the formula:

$$\alpha - \text{glucosidase activity inhibition (\%)} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Where $A_{control}$ = reaction control absorbance - reaction control blank absorbance, A_{sample} = sample absorbance - sample blank absorbance.

Statistical analysis

All experiments were conducted in triplicate and the results expressed as mean and standard deviation (SD). One-way analysis of variance (ANOVA) and post-hoc Tukey tests were used in the analysis, with data analyzed using GraphPad Prism 10.4.0. Pearson's correlation coefficient was calculated using Microsoft Excel 2021. The level of significance was at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction

Crude extracts from *Causonis trifolia* leaves were prepared using solvents of increasing order of polarity, such as hexane, ethyl acetate ethanol, and 50% ethanol. The percentage yield (% yield) was calculated using the dry weights of the crude extracts (Table 1). The highest value of the % yield of the extracts of *C. trifolia* leaves was obtained through ethanol solvent extraction which was 6.19% and the lowest yield was from ethyl acetate at 1.44%.

A quantitative assessment of total phenolic and flavonoid content was performed. The results revealed that the total phenolic content of *C. trifolia* extracts in different solvents was in the range of 6.58 ± 0.87 to 133.07 ± 1.98 mg GAE/g extract. The 50% ethanol extract significantly demonstrated the highest total phenolic content (133.07 ± 1.98 mg GAE/g extract) compared to ethanol, ethyl acetate, and hexane extracts ($p < 0.05$). The total flavonoid content of *C. trifolia* extracts

Table 1: Percentage of extraction yield from different solvents of *C. trifolia*.

Solvents	Extraction yield (%)
Hexane	2.43
Ethyl acetate	1.44
Ethanol	6.19
50%ethanol	3.81

from different solvents was in the range of 23.27 ± 1.39 to 42.07 ± 2.00 mg QE/g extract. The 50% ethanol extract significantly presented the highest flavonoid content (42.07 ± 2.00 mg QE/g extract) when compared to other solvents ($p < 0.05$) (Figure 1).

The effect of solvent polarity is one of the most critical extraction parameters that influences the extraction efficiency of phytochemicals.[24] The most successful solvent for extracting the chemical compounds in *C. trifolia* leaves was 50% ethanol, while nonpolar solvents were less effective (Figure 1). From the current study, it has been shown that 50% ethanol of *C. trifolia* leaves was the most effective in dissolving polar and polar intermediate molecules containing COO or OH groups, such as phenolics and flavonoids.

DPPH and ABTS radical scavenging activity

DPPH and ABTS radical scavenging assays are widely used to determine the radical scavenging ability of various samples. DPPH radical scavenging is an accepted method to evaluate the ability of plant extracts to donate hydrogen atoms. DPPH, a stable free radical with an unpaired electron, reacts with hydrogen-donating compounds, leading to its reduction. This process causes a color change from purple to yellow, and the decrease in absorbance indicates the plant extract's antioxidant capacity.[25] The ABTS free radical scavenging assay can evaluate both hydrogen-donating and chain-breaking antioxidants. Antioxidants in the extract neutralize $ABTS^+$ free radicals, which are generated by the ABTS reacting with potassium persulfate. This method is commonly employed for the preliminarily assessment of the radical scavenging properties of antioxidant compounds or plant extracts.[26]

All extracts from different solvents were assessed for their DPPH and ABTS radical scavenging activities and presented as IC_{50} (Figure 2). The different solvents of *C. trifolia* leaf extract showed antioxidant potential from their IC_{50} values. IC_{50}, known as the half maximal inhibitory concentration, is a key parameter used to assess the antioxidant activity of a substance. It refers to the concentration of plant extract needed to inhibit or neutralize 50% of free radical activity. A lower IC_{50} value signifies stronger antioxidant potential, while a higher value indicates weaker activity.[27] According to the results, established in DPPH and ABTS scavenging activities, the 50% ethanol extract significantly possessed the highest free-radical scavenging activity with IC_{50} values of 137.29 ± 1.81 µg/mL and 107.07 ± 2.74 µg/mL, respectively ($p < 0.05$), while the hexane extract significantly exhibited the lowest activity among the extracts, with IC_{50} values of 5269.70 ± 578.64 µg/mL and 1969.47 ± 23.44 µg/mL, respectively ($p < 0.05$). Whereas the IC_{50} values of ascorbic acid by DPPH and ABTS assays were 12.34 ± 0.48 and 70.51 ± 11.92 µg/mL, respectively. The results in the current study indicate that solvents with high polarity demonstrated high radical scavenging activity, which might be due to the presence of phenolic and flavonoid contents (Figure 1). It was further found that the radical scavenging effects of the extracts were directly proportional to the phenolic content present in the extracts. Additionally, these results correspond to previous studies in which the best antioxidant capacity was observed in polar solvents, particularly in methanol and organic solvent-water mixtures,[28,29,30] which was associated with the presence of phenolic compounds that were highly soluble in polar solvents.[31]

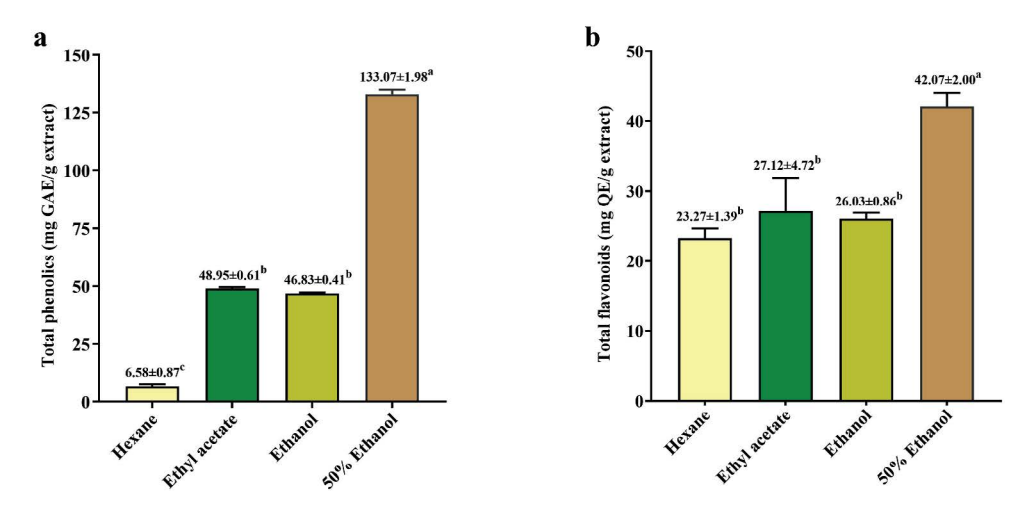

Figure 1: Total phenolic and flavonoid contents of *C. trifolia* extracts in different solvents (a) total phenolics (mg GAE/g extract) and (b) total flavonoids (mg QE/g extract). The results are expressed as mean ± SD (n = 3) and evaluated using one-way ANOVA together with post-hoc Tukey tests. Different letters represent significant differences compared among extracts (p < 0.05). GAE: Gallic acid equivalent; QE: Quercetin equivalent.

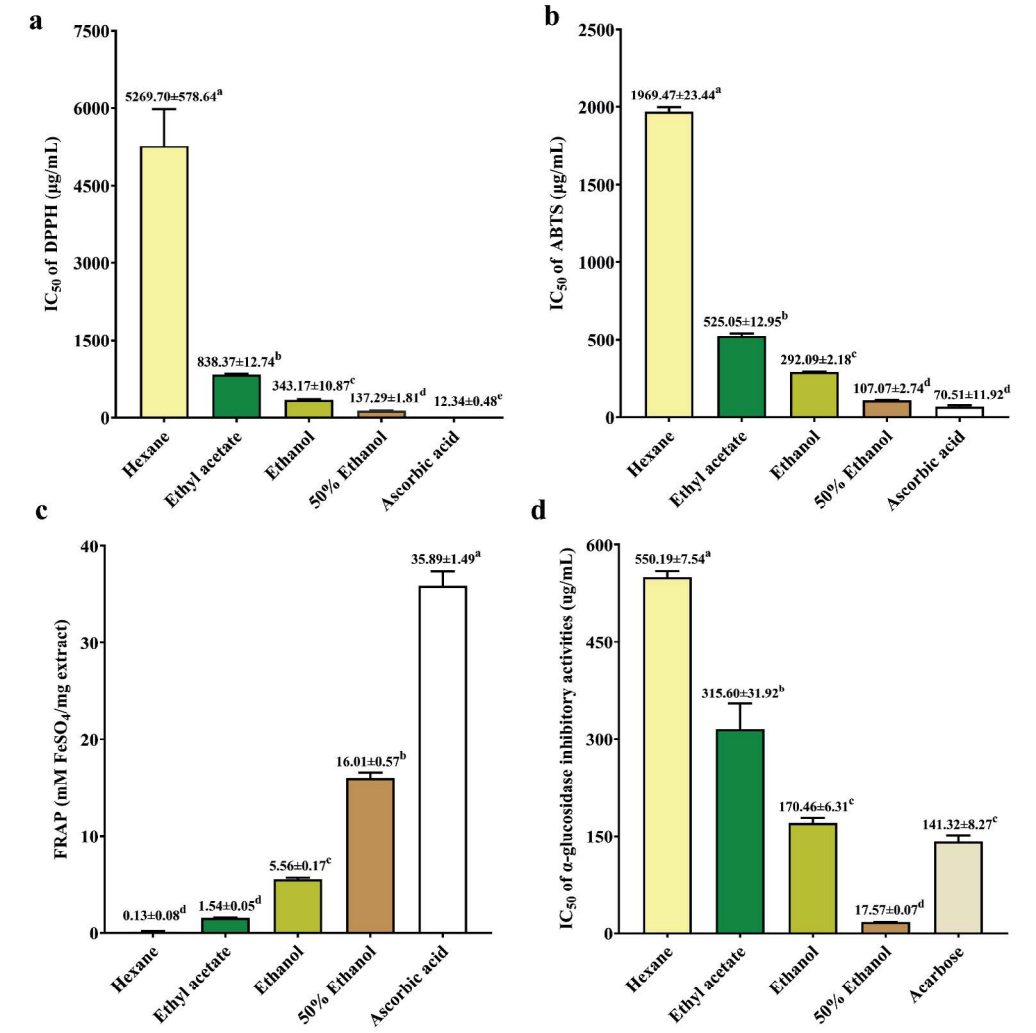

Figure 2: Antioxidant and α-glucosidase inhibitory activities of *C. trifolia* leaf extracts in different solvents (a) IC_{50} of DPPH (ug/mL), (b) IC_{50} of ABTS (ug/mL), (c) FRAP (mM $FeSO_4$/mg extract), and (d) IC_{50} of α-glucosidase inhibitory activities (ug/mL). The results are expressed as mean ± SD (n = 3) and evaluated using one-way ANOVA together with post-hoc Tukey tests. Different letters represent significant differences compared among extracts (p < 0.05).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was used to measure the antioxidant potential in a sample based on the ability to reduce the complexity of ferric-tripyridyl triazine (Fe^{3+}-TPTZ) and change it to blue colored Fe^{2+}-TPTZ in acidic conditions. The reaction was observed by determining the change of absorbance at 593 nm. The reducing properties of compounds are attributed to their ability to donate hydrogen atoms, which effectively break free radical chain reactions.[32]

The results of the FRAP assay displayed the same trend as the DPPH and ABTS assays (Figure 2). The current results showed the 50% ethanol extract (16.01 ± 0.57 mM $FeSO_4$/mg extract) exhibited the significantly highest reducing power compared to the other extracts ($p < 0.05$). This result from the FRAP assay, indicating a positive correlation between reducing potential and phenolic content in the extracts (Figure 1), corresponded to previous research.[33,34] In the same way as from the DPPH and ABTS assays, the polar solvent extraction obtained high phenolic content, which could be attributed to better antioxidant activity.[28,30]

In-vitro α-glucosidase inhibitory activity

α-Glucosidase inhibitory activity is one of the known methods used to assess antidiabetic activity. α-Glucosidase is the enzyme which is responsible for breaking down disaccharides and complex carbohydrates into glucose. Thus, inhibition of this enzyme leads to delaying the absorption of glucose in the gastrointestinal tract and prevents an increase in postprandial glucose levels.[35]

The α-glucosidase inhibitory activity, presented as IC_{50} values, of all *C. trifolia* leaf extracts is represented in Figure 2. The different solvents of *C. trifolia* leaf extract showed inhibitory activity of α-glucosidase at IC_{50} values ranging from 17.57 ± 0.07 to 550.19 ± 7.54 ug/mL. The 50% ethanol extract exhibited the significantly highest α-glucosidase inhibitory activity at IC_{50} of 17.57 ± 0.07 ug/mL ($p < 0.05$), followed by ethanol, ethyl acetate, and hexane extracts with IC_{50} values of 170.46 ± 6.31, 315.60 ± 31.92, and 550.19 ± 7.54 ug/mL, respectively. Notably, the inhibitory activity of 50% ethanol extract on α-glucosidase was found to be significantly higher than that of acarbose with an IC_{50} value of 141.32 ± 8.27 ug/mL ($p < 0.05$), which implies it has potent antidiabetic property. The current study indicated that the 50% ethanol extract showed the highest α-glucosidase inhibitory activity out of all the extracts, possibly due to the extract containing higher amounts of TPC and TFC, which could inhibit α-glucosidase activity.[36,37] The findings of this study are also consistent with those of earlier studies in showing that the extracts derived from plants are strong α-glucosidase inhibitors.[35]

Preliminary phytochemical screening

The results of the phytochemical screening analysis are presented in Table 2. Various tests of phytochemical screening were performed, revealing the presence of different bioactive compounds in the leaf extracts of *C. trifolia*, namely alkaloids, anthraquinones, cardiac glycosides, coumarin, saponins, tannins, terpenoids, and steroids. Anthraquinones were absent from the leaf extracts of *C. trifolia*. Hexane extract was found only in the steroids, and ethyl acetate extract was presented in coumarin, saponins, and steroids. The ethanol and 50% ethanol extracts contained a wide range of bioactive components, including alkaloids, cardiac glycosides, coumarin, saponins, steroids, tannins, and terpenoids. This result corresponds with the earlier report that the *C. trifolia* leaf extract contained various phytochemical compounds, such as alkaloids, phenolic compounds, terpenoids, steroids, and saponins.[6]

Table 2: Phytochemical constituents of *C. trifolia* leaf extracts in different solvents.

Phytochemicals	*C. trifolia* leaf extracts			
	Hexane extracts	Ethyl acetate extracts	Ethanol extracts	50% ethanol extracts
Alkaloids	-	-	+	+
Anthraquinones	-	-	-	-
Cardiac glycoside	-	-	+	+
Coumarin	-	+	+	+
Saponins	-	+	+	+
Steroids	+	+	+	+
Tannins	-	-	+	+
Terpenoids	-	-	+	+

+, present; -, absent

Table 3: Correlation coefficient between total phenolic content (TPC) and total flavonoid content (TFC) with antioxidant and α-glucosidase inhibitory activities of *C. trifolia* leaf extracts in different solvent.

	TPC	TFC	DPPH	ABTS	FRAP	α-glucosidase
TPC	1					
TFC	0.982	1				
DPPH	-0.716	-0.569	1			
ABTS	-0.767	-0.630	0.996	1		
FRAP	0.960	0.959	-0.614	-0.679	1	
α-Glucosidase	-0.903	-0.814	0.900	0.934	-0.888	1

Correlation between TPC and TFC with antioxidant and α-glucosidase inhibitory activities of *C. trifolia* leaf extracts in different solvents

This study investigated the correlation between TPC and TFC with antioxidant activities (DPPH, ABTS, and FRAP) and α-glucosidase inhibitory activity of *C. trifolia* leaf extracts in different solvents (Table 3). The TPC positively correlated with TFC (R = 0.982) but negatively correlated with IC_{50} values of DPPH, ABTS, and α-glucosidase inhibitory activities of R = 0.716, 0.767, and 0.903, respectively. Comparable to TPC, TFC also negatively correlated with IC_{50} values of DPPH, ABTS, and α-glucosidase inhibitory activity of R = 0.569, 0.630, and 0.814, respectively. On the other hand, there was a notably positive correlation between FRAP assay and TPC and TFC at R = 0.960 and 0.959, respectively. These results suggest the high levels of TPC and TFC in *C. trifolia* leaf extracts were correlated with well free radical scavenging and anti-α-glucosidase activities. Several studies have reported on a positive relationship with antioxidant components, such as polyphenols, and α-glucosidase inhibition.[37,38,39]

CONCLUSION

From the findings of this study, it is concluded that various solvent extracts of *C. trifolia* leaves have potential total phenolic and flavonoid contents as well as antioxidant and antidiabetic activities. The results demonstrated that *C. trifolia* leaf extract, using 50% ethanol, exhibited the highest total phenolic and flavonoid contents, DPPH and ABTS radical scavenging activities, ferric reducing antioxidant power, and inhibitory activity of α-glucosidase, possibly due to the extract containing high amounts of total phenolic and flavonoid contents. Further studies are needed to isolate and identify individual bioactive components from the crude extracts of *C. trifolia* leaves based on the polarity of the extraction solvents.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this manuscript.

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