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Original Article

In vitro investigations of chemical composition, antibacterial, antioxidant, antidiabetic, and anti-inflammatory activities of *Chromolaena odorata* flower extracts

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Abstract

The purpose of this study was to conduct preliminary investigations into the chemical composition and biological characteristics of *Chromolaena odorata* flower extracts. The results revealed that *C. odorata* flowers include alkaloids, flavonoids, glycosides, phenols, tannins, triterpenoids, and saponins. The antibacterial activity of the extracts was assessed using the antibacterial ring diameter, minimum inhibitory concentration, and minimum bactericidal concentration. The extracts were more efficient against Gram-positive bacteria than Gram-negative bacteria, with minimum bactericidal concentrations ranging from 125 to 1000 µg/mL. *C. odorata* flower extracts had significant antioxidant and anti-inflammatory activity, with IC_{50} values ranging from 10.44±0.46 to 152.81±8.63 µg/mL. Ethyl acetate fraction extracted from *C. odorata* flowers showed the ability to more effectively inhibit α-amylase (IC_{50} = 109.24±1.12 µg/mL) and α-glucosidase (IC_{50} = 53.87±0.42 µg/mL) than the other remaining fractions. These findings indicate that *C. odorata* flowers might be used as a natural source of antibacterial, antioxidant, antidiabetic, and anti-inflammatory compounds.

Keywords: *C. odorata* flowers, antibacterial, antioxidant, anti-inflammatory, anti-diabetes

1. Introduction

Diabetes is one of the world's most common illnesses, with a high mortality rate. Inhibiting enzymes responsible for glucose metabolism, such as α-amylase and α-glucosidase, is vital for treating diabetes and preventing its complications. Inhibiting the enzymes α-amylase and α-glucosidase slows carbohydrate metabolism, lowering glucose absorption from the small intestine and postprandial blood glucose levels. To properly monitor and cure diabetes mellitus, inhibiting α-amylase and α-glucosidase is necessary (Haguet *et al*., 2023). Diabetes mellitus is primarily connected with oxidative stress and inflammation caused by the overproduction of reactive oxygen and nitrogen species, as well as alterations in antioxidant status. The inflammatory process encourages the formation of free radicals, causing

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tissue damage in diabetic complications (Nedosugova *et al*., 2022). Research into the screening of compounds with antioxidant and anti-inflammatory activities is critical for controlling diabetes and its complications. Furthermore, diabetic patients frequently experience limb amputations and are readily contaminated with disease-causing micro organisms. Plants are also thought to contain compounds that have antibacterial properties. Plants have antibacterial properties due to the secondary metabolites (Vaou, Stavropoulou, Voidarou, Tsigalou, & Bezirtzoglou, 2021). There is a rising interest in plant-based products because of its anti-diabetic, antioxidant, anti-inflammatory, and antibiotic properties. Plants have the ability to heal a variety of ailments effectively, safely, and affordably.

C. odorata (CO) is a fragrant weed native to Central and South America. Its rapid spread has caused risks and obstructions to agriculture, animal husbandry, firefighting, and the natural environment. CO is used to treat a wide range of illnesses and ailments in sub-Saharan Africa, including diabetes, inflammation, wounds, and fever (Olawale, Olofinsan, & Iwaloye, 2022). Currently, research on the biological activity and chemical composition of CO is mostly undertaken on the stems, leaves, roots, aboveground parts, or complete plant, assessing these for wound healing, anti-parasitic, anti-infection, anti-cancer and antipyretic effects (Olawale, Olofinsan, & Iwaloye, 2022).

There has been little investigation into the chemical composition and biological activities of extracts from CO flowers, especially as regards those CO flowers growing in Can Tho, Vietnam. Isosakuranetin (5,7-dihydroxy-4'-methoxy flavanone), persicogenin (5,3'-dihydroxy-7,4'-dimethoxy flavanone), 5,6,7,4'-tetramethoxyflavanone, 4'-hydroxy-5,6,7-trimethoxyflavanone, chalcone, 2'-hydroxy-4,4',5',6'-tetra methoxychalcone, 4,2'-dihydroxy-4',5',6'-trimethoxychal cone, acacetin (5,7-dihydroxy-4'-methoxyflavone), and luteolin (5,7,3',4'-tetrahydroxyflavone) were found in research on the chemical composition of CO flowers in Thailand. These very potent chemicals are members of the flavonoid group. According to (Bunyapraphatsara & Chokechaijaroenporn, 2000), CO flowers are frequently used as a tonic, fever reducer, and heart tonic in Thailand. Additionally, flowers of CO are frequently used in Vietnamese traditional medicine treatments for hemostasis, diabetes, coccidiosis, acne, and diarrhea. These are only traditional usage, though, and they require experimental validation. Consequently, our study will contribute to the establishment of a strong scientific basis for the chemical makeup and potential biological uses of CO flowers in Vietnam.

2. Materials and Methods

2.1 Materials

CO were collected at Can Tho, Vietnam's Phong Dien district, on March 3rd, 2023. Figure 1 shows some photos of CO. Dr. Nguyen Thi Kim Hue (Deputy Dean of Biology, College of Natural Sciences, Can Tho University) defines CO, which is stored in the Clinical Biochemistry Laboratory (Room C11.105), Department of Biochemistry, Faculty of Medicine and Pharmacy, Tra Vinh University, under the storage code CT_Cod202303010010. Following processing,

Figure 1. Some pictures of *Chromolaena odorata*

the research team received 1,750 g of CO flowers (fresh flowers). The flowers of CO were dried at 50±2ºC and ground to obtain 618 g of powder. Then, the powder was passed through a tray screen to obtain medicinal powder particles of 60 mesh size, and the moisture level was determined using the YOKE DSH-10A moisture analysis balance (YOKE, China). Medicinal powder with a moisture content of 7.10±0.21% was then preserved in a PE plastic bag, placed in a glass and plastic box, and stored at 4ºC.

2.2 Preparation of ethanol extract and fractional extracts

The 500 g medicinal powder sample was steeped in 5,000 mL of 99.5% ethanol at room temperature for 24 hours. Following that, the soaking solution was collected, filtered through filter paper, and the solvent evaporated to yield an ethanol extract (25.47 grams). The ethanol extract (10 g) was then liquid-liquid extracted with increasing polarity solvents to get fractions: n-hexane (1.36 g), dichloromethane (2.59 g), and ethyl acetate (3.88 g). Heidolph, Germany supplied a rotary vacuum evaporator that was used to evaporate ethanol extract and fraction extracts. The extracts were kept in glass vials at 4°C and utilized in future studies.

2.3 Phytochemical analysis

The study team carried out qualitative chemical groupings of alkaloids, flavonoids, triterpenoids, steroids, tannins, saponins, and glycosides as reported by (Anh *et al*., 2021).

2.4 Quantification of polyphenols, flavonoids, alkaloids, and tannins

The tannins, alkaloids, flavonoids, and total polyphenols were determined using the methods described by

(Anh *et al*., 2021; Bhat, Sridhar, & Yokotani, 2007; Shamsa, Monsef, Ghamooshi, & Verdianrizi, 2008). The total concentration of tannins, alkaloids, flavonoids, and polyphenols was calculated equivalently as mg of catechin (CE), atropine (AE), quercetin (QE), or gallic acid (GAE) per 1 g of extract, using the standard curve equations for CE ($y=0.003x+0.0113$; $R^2=0.9986$), AE ($y=0.0011x+0.0148$; $R^2=0.9943$), QE ($y=0.0064x-0.0004$; $R^2=0.9999$) or GAE ($y=0.0164x-0.019$; $R^2=0.9988$).

2.5 Investigation of the in vitro antioxidant activity

The technique for testing neutralizing 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, reducing power (RP), ferric reducing-antioxidant power (FRAP), and total antioxidant capacity (TAC) as described by (Anh *et al*., 2021) was employed to determine the antioxidant properties of CO floral extracts. Ascorbic acid was employed as a positive control, while ethanol served as a negative control.

2.6 Investigation of the in vitro antidiabetic activity

CO flower extracts were investigated for antidiabetic efficacy in vitro using the α-amylase and α-glucosidase enzyme inhibition techniques reported by (Anh *et al*., 2021). Acarbose was utilized as a positive control, with dimethyl sulfoxide serving as the negative control.

2.7 Investigation of the in vitro anti-inflammatory activity

CO flower extract products were tested for anti-inflammatory efficacy in vitro utilizing procedures described by (Alisi & Onyeze, 2008; Modak, Paul, Sarkar, Thakur, & Bhattacharjee, 2021). These approaches included preventing BSA denaturation, preserving red blood cells, and inhibiting nitric oxide ($NO^{\bullet}$). Diclofenac and ascorbic acid were used as positive controls, with dimethyl sulfoxide serving as a negative control.

2.8 Investigation of the antibacterial activity

Bacterial strains (Table 1) are stored at the Department of Biology, College of Natural Sciences, Can Tho University. Bacterial strains were cultivated and evaluated for antibacterial activity using Luria-Bertani Broth and Agar medium from HiMedia, India. The agar well diffusion method was used to determine the diameter of the antibacterial ring; the broth dilution method was performed in a 96-well plate with the color indicator resazurin to determine the minimum inhibitory concentration (MIC); and the drop plate method of counting viable bacteria was used to determine the minimum bactericidal concentration (MBC), as expressed by (Ngan, Moon, Kim, Shibamoto, & Anh, 2012), to indicate the antibacterial activity. As a positive control, TW25 Pharmaceutical Joint Stock Company, Vietnam's commercial antibiotic tetracycline was utilized. Dimethyl sulfoxide (10%) was employed as a negative control and to dissolve the test material.

2.9 Statistical analysis

The *in vitro* antioxidant, anti-inflammatory, and anti-diabetic properties of CO flower extract were compared to a standard (ascorbic acid/diclofenac/acarbose) at a 50% inhibitory concentration (IC_{50}). The IC_{50} value was calculated as explained by (Anh *et al*., 2021).

The mean data are reported as Mean±Standard Deviation (SD) and analyzed using one-way ANOVA (Tukey's studentized range) in Minitab 16 for Windows. Differences were judged significant at $p < 0.05$. Each test was conducted three times.

3. Results and Discussion

3.1 Preliminary results of the chemical composition

According to studies, CO flower extracts include alkaloids, flavonoids, triterpenoids, steroids, tannins, and glycosides. As an example, saponins were found in the ethyl acetate fraction (Table 2). The study focused on polyphenols, flavonoids, alkaloids, and tannins since these are the key chemical classes with substantial pharmacological effects. The results in Table 3 indicate that the ethyl acetate fraction contained the most tannin, alkaloids, flavonoids, and polyphenols. The n-hexane extract had less polyphenols, flavonoids, alkaloids, and tannins than the other extracts. Thus, solvent polarity can influence the composition and concentration of plant-level metabolites. Aromatic rings, glycosyl forms, and chain linkages can all affect polyphenols, flavonoids, alkaloids, and tannins. The more hydroxyl groups in the molecule and the higher the polarity of polyphenols, flavonoids, alkaloids and tannins, the easier these dissolve in

Table 1. Minimum inhibitory concentration and minimum bactericidal concentration

Bacterial strain	Minimum inhibitory concentration (MIC, µg⁄mL)			Minimum bactericidal concentration (MBC, µg⁄mL)		
	EE	EaF	TC	EE	EaF	TC
Salmonella typhi ATCC ® 13311TM	500	250	25	1000	500	50
Vibrio parahaemolyticus ATCC ® 17802TM	250	250	25	500	500	50
Pseudomonas aeruginosa ATCC 27855	250	250	25	500	500	50
Escherichia coli ATCC ® 25922TM	250	250	25	500	500	50
Listeria innocua ATCC 33090	125	62.5	12.5	250	125	25
Staphilococcus aureus ATCC 6538	125	62.5	12.5	250	125	25

Note: EE is ethanol extract; EaF is ethyl acetate fraction; TC is tetracyline

Table 2. Qualitative results of compound groups in extracts

Compound group	Test	EE	HF	DF	EaF
Alkaloids	Dragendroff's test	+	+	+	+
	Wagner's test	+	+	+	+
	Mayer's test	+	+	+	+
Flavonoids	Amyl alcohol	+	+	+	+
	Shinoda test	-	-	-	-
Triterpenoids	Rosenthaler test	+	+	+	+
Steroids	Salkowski's test	+	+	+	+
Tannins	Lead acetate	+	+	+	+
	Vanilin-Hydrocloride	+	+	+	+
	$FeCl_3$	+	+	+	+
	Zinc-Hydrochloride	+	+	+	+
Saponins	Shake in distilled water	-	-	-	+
Glycosides	Borntrager's	+	+	+	+
	Fehling test	+	+	+	+

Note: EE is ethanol extract; HF is n-hexane fraction; DF is dichloromethane fraction; EaF is ethyl acetate fraction. Besides, (+) means the presence of a substance group; (-) means there is no presence of the compound group.

Table 3. Analysis of correlation coefficients of factors on polyphenol, flavonoid, alkaloid and tannin content

Value	EE	HF	DF	EaF
TPC (mg GAE/g extract)	337.40^{b}±7,01	130.16^{d}±2.68	245.33^{c}±4.49	466.67^{a}±2.75
TFC (mg QE/g extract)	147.32^{b}±3.71	98.98^{d}±3.73	118.39^{c}±3.52	221.25^{a}±4.74
TAC (mg AE/g extract)	136.59^{b}±2.27	74.47^{d}±6.46	83.94^{c}±3.99	166.52^{a}±2.86
TTC (mg CE/g extract)	64.88^{b}±1.10	32.10^{d}±2.10	41.40^{c}±2.29	83.63^{a}±3.25

Note*:* values followed by the same letter in the same column are not significantly different at the 5*%* level. EE is ethanol extract; HF is n-hexane fraction; DF is dichloromethane fraction; EaF is ethyl acetate fraction; TPC is total polyphenol content; TFC is total flavonoid content; TAC is total alkaloid content; TTC is total tannin content.

highly polar solvents (Hayat *et al*., 2020). In a study by (Solihah, Munawwaroh, & Rasyid, 2020), an ethanol extract from CO leaves had a flavonoid concentration of 126.459±0.163 QE mg/g extract, which was lower than in the ethanol extract and ethyl acetate fraction extract isolated from CO flowers by 0.003 and 1.75 times, respectively. CO flowers have a wide variety of secondary metabolites with great biological activity.

3.2 *In vitro* antioxidant activity

Figure 2 displays the antioxidant activity of CO flower extracts as measured by five different antioxidant techniques. Table 4 shows the IC50 values established by the study team for CO floral extracts. The ethyl acetate fraction has the greatest antioxidant capacity, whereas the n-hexane fraction has the lowest. This conclusion is completely compatible with the analysis of polyphenols, flavonoids, alkaloids, and tannins in the extracts. These families of chemicals contain redox potential, which allows them to function as reducing agents, providing hydrogen and deactivating free oxygen radicals. (Mazumder, Tolaema, Chaikhemarat, & Rawdkuen, 2023). The chemicals 2*S*,5''*R*-eupodoratin A, 2*S*,5''*S*-eupodoratin A, drahebephin A, and drahebephin B were isolated from CO flowers and reported by (Yang *et al*., 2023). These substances have shown efficacy in scavenging $ABTS^{\bullet+}$ free radicals.

(Molyneux, 2004) classified the ethyl acetate fractional extract from CO flower as having extremely significant antioxidant activity, with IC_{50} values ranging from 10.44±0.46 to 30.52±0.46 µg/mL in all 5 examination methodologies.

3.3 *In vitro* antidiabetic activity

The IC_{50} value was determined by the study based on the inhibitory efficiency displayed in Figure 3 (Table 4). The most efficient fraction for inhibiting α-amylase and α-glucosidase is ethyl acetate extract (IC_{50}, α-amylase= 109.24±1.12 µg/mL; IC_{50}, α-glucosidase=53.87±0.42 µg/mL). Numerous studies have shown that plant extracts can reduce the activity of α-amylase and α-glucosidase depending on the secondary metabolite level. The above-mentioned compounds' hydroxyl groups, both in number and location, block the enzymes α-amylase and α-glucosidase. These groups contains hydroxyl groups that can create hydrogen linkages with the -OH group in the functional amino acid active side chain of the enzyme. This inhibits the activity of α-amylase, and the carbohydrates prevent the hydrolysis of carbohydrates (Li *et al*., 2018). The ability of chemicals isolated from CO flowers to inhibit the enzymes α-amylase and α-glucosidase has not yet been shown, but according to a study by (Lekshmi *et al*., 2015), kaempferide, an acacetin molecule, is present in CO leaves. Besides, (Lam *et al*., 2024; Proença *et al*., 2019) have described these chemicals and shown that they can block the enzymes α-amylase and α-glucosidase. The extract's ability to inhibit the enzymes α-amylase and α-glucosidase may be associated with the presence of these chemicals.

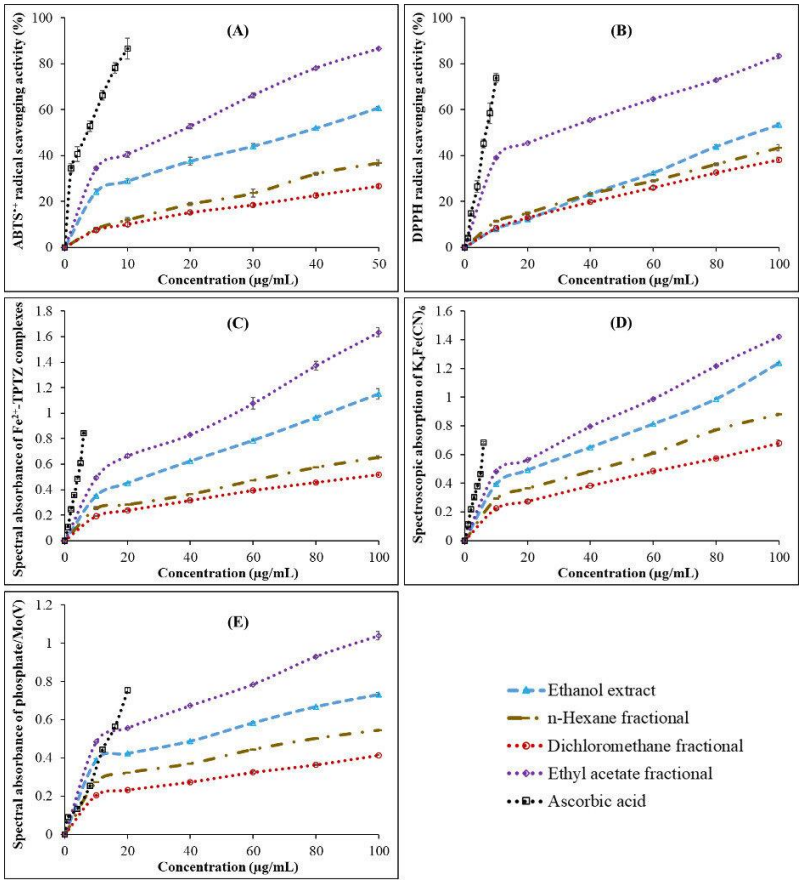

Figure 2. Neutralization or reduction performance of oxidizing agents of CO flower extracts
Note: (A), (B), (C), (D) and (E) refer to the ABTS$^{\bullet+}$, DPPH, FRAP, RP and TAC methods respectively.

Table 4. IC_{50} values (µg/mL) obtained from *in vitro* antioxidant, antidiabetic and anti-inflammatory methods

Method	The IC_{50} (µg/mL) values of extracts in different testing methods				
	EE	HF	DF	EaF	SS
ABTS$^{\bullet+}$	36.77^{c}±0.86	103.22^{a}±5.11	69.67^{b}±1.36	17.56^{d}±0.68	6.91^{e}±0.06
DPPH	93.25^{c}±0.67	134.00^{a}±3.18	118.77^{b}±3.20	30.52^{d}±0.46	6.85^{e}±0.32
FRAP	26.33^{d}±0.13	92.78^{a}±1.97	66.22^{b}±0.40	10.44^{c}±0.46	3.90^{e}±0.05
RP	22.58^{c}±0.25	64.24^{a}±0.96	41.35^{b}±0.56	12.68^{d}±0.23	4.84^{e}±0.06
TAC	39.48^{c}±0.43	137.20^{a}±0.42	81.93^{b}±0.34	11.92^{d}±0.71	13.71^{e}±0.21
α-Amylase	148.05^{c}±1.19	321.18^{a}±9.85	205.70^{b}±3.36	109.24^{d}±1.12	5.50±0.34
α-Glucosidase	90.74^{c}±0.97	255.51^{a}±3.57	179.73^{b}±8.50	53.87^{d}±0.42	5.04^{e}±0.03
BSA	22.73^{c}±0.33	80.96^{a}±9.30	45.40^{b}±2.22	15.95^{d}±1.81	6.30^{e}±0.15
RBCs	16.61^{c}±0.14	28.52^{a}±0.40	22.08^{b}±0.08	13.91^{d}±0.19	7.21^{e}±0.08
NO$^{\bullet}$	99.40^{c}±0.65	152.81^{a}±8.63	139.90^{b}±4.31	63.92^{d}±2.08	66.50^{e}±1.08

Note: values followed by the same letter in the same method are not statistically different ($p>0.05$); EE is ethanol extract; HF is n-hexane fraction; DF is dichloromethane fraction; EaF is ethyl acetate fraction; SS is standard substance; ascorbic acid was used as a standard for ABTS$^{\bullet+}$, DPPH, FRAP, RP, TAC and NO$^{\bullet+}$ methods. Acarbose is used as a standard for α-amylase and α-glucosidase methods. Diclofenac is used as a standard for BSA and RBCs methods.

3.4 *In vitro* anti-inflammatory activity

BSA denatures and discloses antigens associated with type 3 hypersensitivity responses in conditions including lupus erythematosus, rheumatoid arthritis, and glomerulonephritis when it is subjected to high temperatures. Because of this, lowering BSA denaturation is a mechanism by which plants can treat inflammatory diseases. At doses ranging from 3.26±0.22% at 0.78125 µg/mL to 71.46±6.52% at 25 µg/mL, CO flower extracts prevented BSA denaturation

(Figure 4A). The most effective inhibitor of BSA denaturation is the ethyl acetate fraction (IC_{50}=15.95±1.81 µg/mL), as Table 4 shows.

Because RBCs are essential to human health, safeguarding them can prevent illness and promote wellness. Since lysosomal membranes and erythrocytes are similar, the anti-inflammatory action of plant materials is assessed by inhibiting hypotension-induced erythrocyte lysis. At dosages ranging from 0.78125 to 25 µg/mL, CO flower extracts successfully shielded RBCs from heat-induced hemolysis (Figure 4B). With an IC_{50} value of 13.91±0.19 µg/mL, among CO flower extracts the strongest capacity to preserve RBCs was in the ethyl acetate fraction (Table 4).

The free radical nitric oxide, which is generated in large amounts in a range of inflammatory illnesses, can have detrimental effects on tissues near the site of inflammation despite its physiological role as a vasodilator and protein modulator. Hence, one of the most important strategies for reducing inflammation is the ability to inhibit the production of free radicals from nitric oxide. At doses of 10 to 100 µg/mL, Figure 4C demonstrates that extracts from CO flowers inhibit the generation of nitric oxide free radicals with the efficiency ranging from 11.78±0.33 to 65.89±1.57%. The most efficient fraction for inhibiting the production of nitric oxide free radicals is the ethyl acetate fraction (IC_{50}=63.92±2.08 µg/mL), as Table 4 demonstrates.

Researchers have shown that CO leaves contain dihydrokaempferide and acacetin (Lekshmi *et al*., 2015). Studies have demonstrated the anti-inflammatory effects of acacetin and dihydrokaempferide (Codo *et al*., 2022; Singh, Gupta, Meena, & Luqman, 2020). The anti-inflammatory qualities of CO flowers could be attributed to these chemicals.

3.5 Antibacterial activity

Diabetes causes reduced T cell response, neutrophil function, and humoral immune disorders. Therefore, diabetes increases the likelihood of bacterial infections. Respiratory

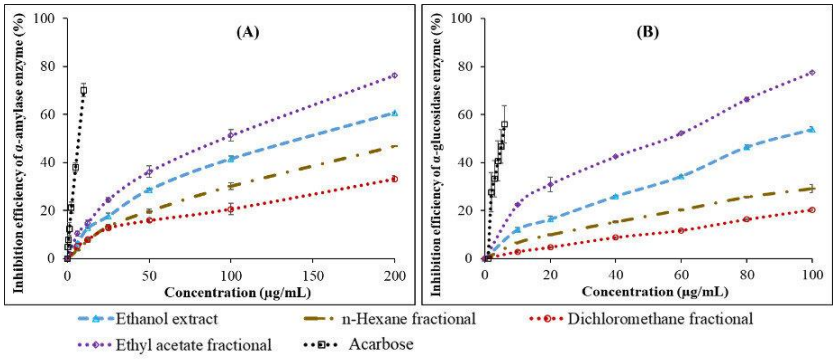

Figure 3. Inhibition efficiencies of CO flower extracts against enzymes α-amylase and α-glucosidase.
Note: (A), (B) are respectively the inhibition efficiency of α-amylase and α-glucosidase enzymes.

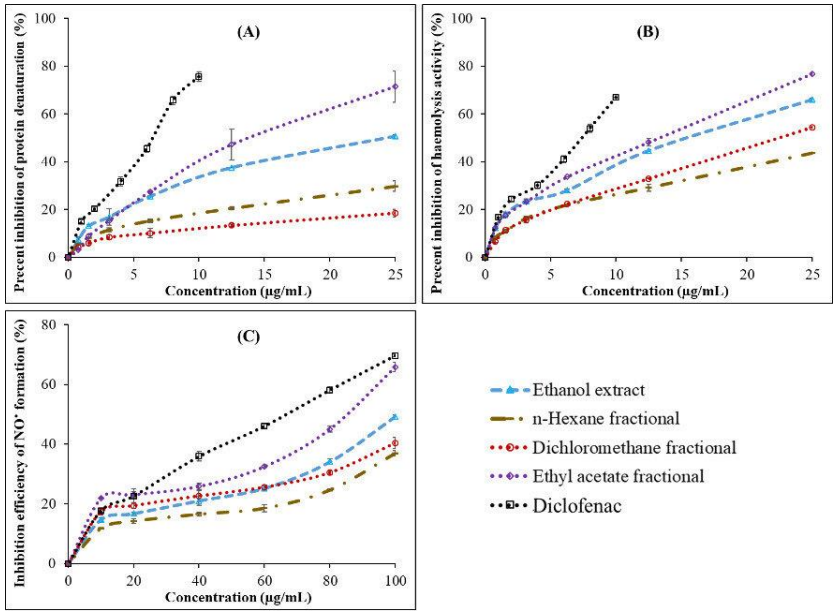

Figure 4. The *in vitro* anti-inflammatory performance of CO flower extracts
Note: (A), (B), and (C) are respectively the inhibition efficiency of BSA denaturation, protection efficiency of RBCs, and inhibition efficiency of nitric oxide formation.

tract infections, skin and soft tissue infections, and gastrointestinal and genitourinary tract infections all occur more frequently in people with diabetes (Juliana, Janine, & Cresio, 2012). The research team investigated the antibacterial activity of all four extracts. However, only ethanol extract and ethyl acetate fraction extract showed antibacterial activity in the investigated concentration range. Therefore, the research team only presented the antibacterial results of ethanol extract and ethyl acetate fraction extract. Figure 5 shows that antibacterial rings with diameters ranging from 0 to 22.83±1.04 mm were generated by the ethanol extract and ethyl acetate fraction. As shown in Figure 6, the MIC was determined by utilizing the resazurin reagent's color reaction. Compared to Gram-negative pathogens, the ethanol extract and ethyl acetate fraction exhibit greater efficacy against Gram-positive bacterial strains. The explanation might be that Gram-negative bacteria have an outer membrane covered with lipopolysaccharide and a cell wall composed of a thin layer of peptidoglycan, while Gram-positive bacteria have a cell wall composed mostly of peptidoglycan.

Plant extracts contain antibacterial components such as flavonoids, tannins, alkaloids, and polyphenols that interact with enzymes and proteins in bacterial cell membranes, causing proton flow to the outside of the cell and cell death. They can also disrupt bacterial amino acid biosynthesis enzymes. The investigated plant extracts, on the other hand, have an inhibitory effect because of their hydrophobic properties, which allow them to react with proteins found in bacterial cell membranes and mitochondrial membranes, altering the structure so that bacterial membrane permeabilitychanges (Shamsudin *et al*., 2022). In flowers, CO has been shown to contain 2S,5''R-eupodoratin A (1), 2S,5''S-eupodoratin A, drahebephin A, drahebephin B (Yang *et al*., 2023). These compounds showed inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

4. Conclusions

The chemical composition and biological activity of CO flowers were determined during this preliminary investigation. CO flowers' biological activities are rationally linked to their secondary metabolite concentrations. Ethyl acetate fractional extraction of CO flowers had the most polyphenols, flavonoids, alkaloids, and tannins, suggesting higher biological activity than of the other extracts. As a consequence, CO flowers may be able to replace synthetic drugs in the treatment of free radical-related disorders, inflammation, and infections, as well as diabetes.

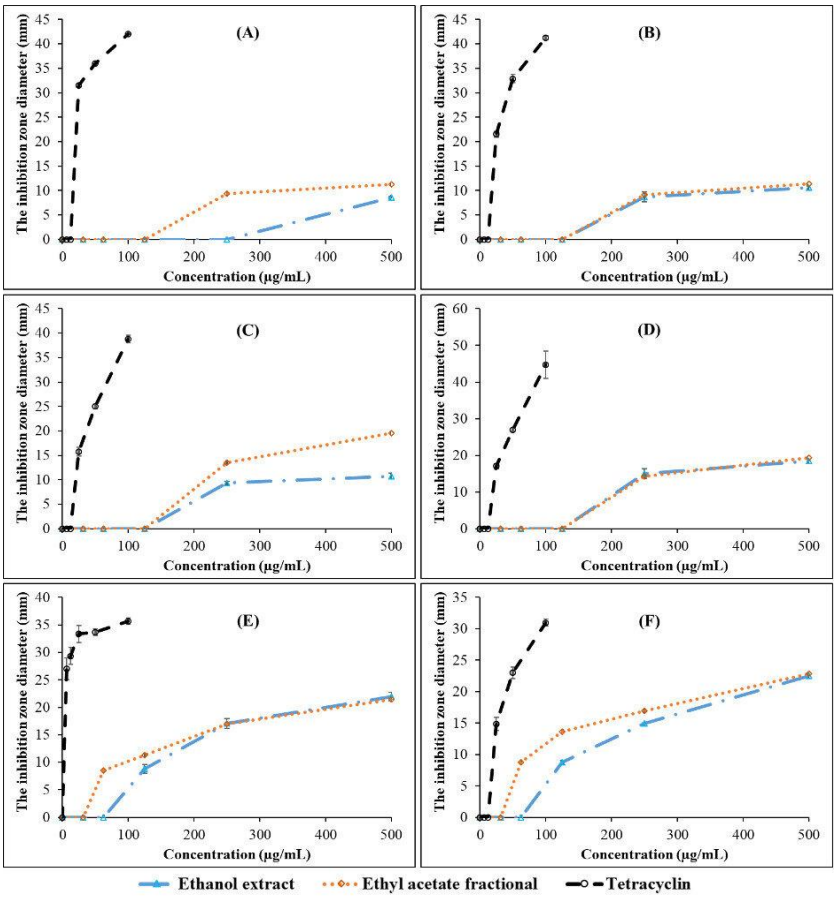

Figure 5. Inhibition zone diameters of CO flower extracts against bacterial strains.
Note: (A), (B), (C), (D), (E), (F) are the diameter of the antibacterial ring against *Salmonella typhi, Vibrio parahaemolyticus, Pseudomonas aeruginosa, Escherichia coli, Listeria innocua and Staphilococcus aureus*, respectively.

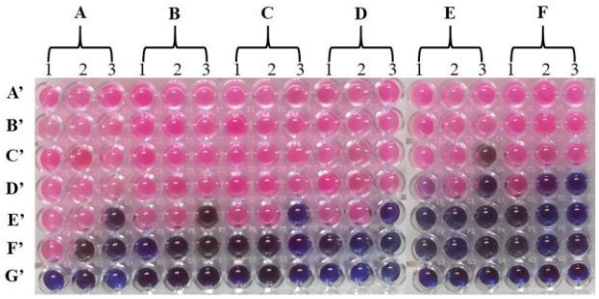

Figure 6. The color of resazurin under the influence of bacterial strains and test samples

Notes: (A) Salmonella typhi; (B) Vibrio parahaemolyticus; (C) Pseudomonas aeruginosa; (D) Escherichia coli; (E) Listeria innocua; (F) Staphilococcus aureus. (1) ethanol extract; (2) ethyl acetate fraction; (3) tetracycline. (A') is bacteria; (B') is bacteria + dimethyl sulfoxide (solvent to dissolve the test sample); (C'), (D'), (E'), (F') and (G') are bacteria + sample concentrations of 31.25, 62.5, 125, 250, 500 µg⁄mL, respectively. Tetracycline alone was tested at concentration ranges of 6.25, 12.5, 25, 40, 50, and 100 µg⁄mL.

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