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ORIGINAL ARTICLE

In vitro Assessment of Antioxidant, Antimicrobial, Cytotoxic, Anti-inflammatory, and Antidiabetic Activities of *Campanula retrorsa* Crude Extracts

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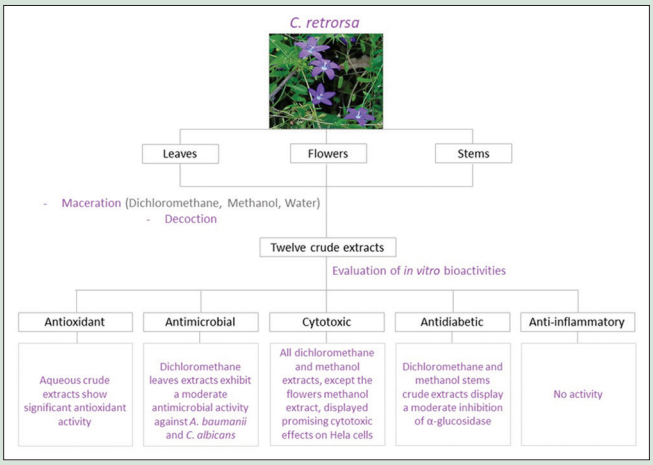
ABSTRACT

Background: *Campanula retrorsa* belongs to the Campanulaceae family and the *Campanula* genus. The species of this latter are traditionally known for their antiallergic, spasmolytic, antiphlogistic, antioxidant, and antiviral properties. Few studies were realized on the bioactivity of *Calicotome villosa* crude extracts. **Objective:** The objective was to investigate, for the first time, the antioxidant, antimicrobial, cytotoxic, antidiabetic, and anti-inflammatory potentials of dichloromethane, methanol, and aqueous crude extracts of *C. retrorsa* leaves, flowers, and stems. **Materials and Methods:** The antioxidant activity of different crude extracts was determined using the free radical, 1, 1-diphenyl-2-picrylhydrazyl. Antimicrobial activity against three Gram-positive (*Staphylococcus aureus, Bacillus cereus,* and *Enterococcus faecium*), two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumanii*) bacterial strains and a fungal strain (*Candida albicans*) was evaluated in a plate-hole diffusion assay. Cytotoxic activity of *C. retrorsa* dichloromethane and methanol crude extracts on Hela cells was realized using the Trypan blue dye exclusion technique. Antidiabetic activity against α-glucosidase and anti-inflammatory activity against bee venom phospholipase A2 (PLA2) were assessed using a colorimetric test. **Results:** Leaf and flower aqueous extracts possessed very high radical scavenging activity = 90%. The dichloromethane extracts of leaves and flowers were the most active against *A. baumanii* and *C. albicans*. After 48 h of treatment, all extracts except the methanol flower extract showed an inhibition of 90% of cell growth at 2 mg/mL. Dichloromethane and methanol stem crude extracts showed a modest inhibition of α-glucosidase. While no inhibition of the PLA2 was displayed by any of the tested extracts. **Conclusion:** Obtained results give an overall view on *C. retrorsa* crude extracts' bioactivities.

Key words: Antidiabetic activity, bioactivity, *Campanula retrorsa*, crude extracts, cytotoxicity

SUMMARY

- The results show that all *Campanula retrorsa* aqueous crude extracts have significant antioxidant activity
- *C. retrorsa* dichloromethane leaf extracts exhibit a moderate antimicrobial activity against *Acinetobacter baumanii* and *Candida albicans*
- All dichloromethane and methanol extracts, except the flowers methanol extract, displayed promising cytotoxic effects on Hela cells
- Dichloromethane and methanol extracts show no anti-inflammatory activity
- Dichloromethane and methanol stems crude extracts display a moderate inhibition of the enzyme α-glucosidase at 2 mg/mL
- *C. retrorsa* species could be used as a possible new source of natural cytotoxic agents.



Abbreviations Used: PLA2: Phospholipase A2; DPPH: 2, 2-Diphenyl-1-picrylhydrazy; CFU: Colony-forming unit; DMSO: Dimethyl sulfoxide; IZ: Inhibition zone; DMEM: Dulbecco Modified Eagle Medium; NaCl: Sodium Chloride; NaOH: Sodium Hydroxide; U: Enzyme Unit; CMUL: Microbiological Collection of the Lebanese University; ATCC: American Type Culture Collection; EC: Enzyme Commission number; CH_2Cl_2: Dichloromethane; CH_3OH: Methanol; H_2O: Water.

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INTRODUCTION

The Mediterranean climate in Lebanon favors the growth of a large number of plant species. The Lebanese flora has 2607 species, distributed in 783 genus. Many are used in the traditional medicine and a relatively small number of these species are studied.

The genus *Campanula* belongs to the Campanulaceae family and includes 300 species. All the species are herbaceous. The name refers to bell-shaped, blue flowers of the majority of the species. Most of *Campanula* species grows in Asia, the Black Sea, and Mediterranean

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regions. According to the study of Tohme 18 *Campanula* species grow in Lebanon.[1-4]

The species of *Campanula* genus have been used in traditional medicine for treatment of laryngitis, constipation, warts, tonsillitis, and bronchitis.[5] Furthermore, they possess antiallergic, spasmolytic, antiphlogistic, antioxidant, and antiviral properties.[6-9] Fresh roots have been chewed for lung and heart problems treatment; their infusions have been used for ear inflammations. The plant decoction has been used in the treatment of sore eyes.[10]

Flavonoids, flavones, and anthocyanins such as luteolin, quercetin, kaempferol, luteolin -7- rutinoside, luteolin 3',7- diglucoside, luteolin 7- glucoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-D galactoside, kaempferol 3-*O*-β-D glucopyranoside, kaempferol 3-*O*-β-D galactopyranoside, violdephin, bisdeacylplatyconin, monodeacylcampanin, and campanin have been isolated from *Campanula* genus.[11-13]

Campanula retrorsa is a green soft plant that can grow between 20 and 100 cm. The stems are very angular and scabrous as well as leaves margins. Lower leaves are obovate, slightly crenate, and briefly petiolate; upper ones are sessile, lanceolate, and acute. The capsules are glabrous and conic. It is surmounted by the lobes of the crescents calyx. The blue-violet flowers bloom between April and May on humid lands in the Mediterranean area.[4]

As part of a systematic research study on the constituents and biological activities of Lebanese natural plants, we here examine the antioxidant, antibacterial, and antifungal activities of the dichloromethane, methanol, and aqueous extracts of *C. retrorsa* leaves, flowers, and stems. We also report cytotoxic activity on Hela cell lines, anti-inflammatory activity against phospholipase A2 (PLA2) isolated from bee venom and anti-diabetic activity against α-glucosidase of *C. retrorsa* leaves, flowers, and stems dichloromethane and methanol extracts.

No study has been reported yet on this species.

MATERIALS AND METHODS

Materials and reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) (97%), Vitamin C (99%), α-glucosidase from *Saccharomyces cerevisiae* (10.8 U/mg), *p*-nitrophenyl α-d-glucopyranoside (>99%), acarbose (>95%), and PLA2 from *Apis mellifera* (600–2400 U/mg) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Manoalide (>98%) was purchased from Abcam (Cambridge, MA, USA). All other chemical reagents that are used in the research were purchased from reliable commercial sources.

Plant material

The whole plants of *C. retrorsa* were collected in Ardeh, North of Lebanon, in May 2015 and identified by Professors Georges and Henriette Tohme (Professors in natural substances).

The plant material was dried in the shade at room temperature for 2 weeks and stored in a dry place.

Extraction and isolation

The air-dried parts (leaves, flowers, and stems) of *C. retrorsa* were macerated for 48 h at room temperature by successively dichloromethane, methanol, and water.

Decoctions were prepared by boiling dried plant material, broken into small pieces, in distilled water for 10 min.

Upon extraction, the mixtures were filtered and evaporated. The extracts were stored at 4°C in dark until use.

Antioxidant assay

The antioxidant activity of *C. retrorsa* different extracts was determined by evaluation of the scavenging effect of these samples on the stable radical DPPH, according to the method described in the literature.[14,15] Different concentrations of each sample were prepared in the solvent of extraction (0.3, 0.5, and 1.0 mg/mL). Briefly, 1.0 mL aliquot of each concentration of test sample was added to 1.0 mL of 0.16 mM DPPH methanolic solution. The mixture was vortexed and left at room temperature for 30 min in the dark, then absorbance was read at 517 nm at $t = 0$ and $t = 30$ min. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{(A_{\text{control}})} \times 100$$

Where $A_{control}$ is the absorbance of the control (DPPH solution without sample), and the A_{sample} is the absorbance of the test sample (DPPH solution with test sample) at 517 nm. Triplicate measurements were carried out. Vitamin C was used as positive control.

Antimicrobial assay

The inhibition zones were determined by whole diffusion by using a cell suspension of about 1.5×10^6 colony-forming unit/mL obtained from a McFarland turbidity standard N 0.5. The suspension was standardized by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120-01 spectrophotometer). A hole of 6-mm diameter was then made on the plate (8-mm thick). 50 µL of the different extracts solubilized in dimethyl sulfoxide (DMSO) (for the dichloromethane and methanol extracts) or water (for the aqueous extracts) of different concentrations (1.0, 2.0, and 10.0 mg/mL) was added to each well in the specific position. The negative control (DMSO) and the positive control (antibiotic or antifungal depending on the test) were added in two different wells. The inoculated plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the zone of growth inhibition (IZ) around the hole. The assay was repeated twice, and the results recorded as mean IZ were expressed as signs: (−) for not active samples, (+) for samples with 3 mm < IZ <4 mm, and (++) for samples with IZ >5 mm.

Tests were performed against six bacterial reference strains: Gram-positive bacteria: *Staphylococcus aureus, Bacillus cereus*, and *Enterococcus faecium,* Gram-negative bacteria: *Escherichia coli, Pseudomonas aeroginosa, Acinetobacter baumanii*, and a yeast: *Candida albicans.* The microorganisms were obtained from the Health and Environment Microbiology laboratory at Azm Center for Research on Biotechnology Sciences and its applications, Tripoli, Lebanon.

Cytotoxic assay

The cytotoxic activity of *C. retrorsa* dichloromethane and methanol extracts on Hela cells was performed using the Trypan blue dye exclusion technique described by Alhage *et al.*[16] Hela cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics. A 100 µL aliquot cell suspension containing 10^4 cells was seeded in each well of a 96-well plate and incubated in a humidified atmosphere at 37°C for 24 h. The medium was removed after 24 h, and the cells were then treated with 100 µL of the extracts diluted with DMEM at 2 and 5 mg/mL. The 96 well-plates were further incubated for 24 and 48 h. The Trypan blue dye exclusion test was then performed by adding one part of a 0.4% solution of Trypan blue to one part of cell suspension. The cells were exposed to Trypan blue for 3 min at room temperature and the percentage of stained cells was determined using a Malassez counting chamber. Cells growth control was performed using the DMEM alone or with DMSO instead of plant

extract. The DMSO concentration in the dilutions and in the solvent control wells was not higher than 1%. All experiments were performed in duplicate. The percentage of proliferation rate was then calculated with respect to not treated cells (100%) using the following formula:

$$\% \text{ of proliferation} = 100 \times \frac{\frac{\text{Living cells}}{\text{Dead cells}} \text{ of treated sample}}{\frac{\text{Living cells}}{\text{Dead cells}} \text{ of untreated control}}$$

α-glucosidase inhibition assay

The α-glucosidase enzyme inhibitory assay was performed in a 96-well plate as described by Kumar *et al.* with slight modifications.[17] Briefly 15 µL of crude extract solution at different concentrations, in 0.1 M phosphate buffer (pH – 6.8), was mixed with 15 µL of Baker's yeast α-glucosidase solution containing 0.5 U/mL in 0.1 M phosphate buffer (pH – 6.8). The mixed solution was incubated at 37°C for 10 min to allow inhibition of the enzyme by the tested extracts or pure compounds. After incubation, 15 µL of the substrate, *p*-nitrophenyl α-D-Glucopyranoside, 0.5 mM solution in 0.1 M phosphate buffer (pH-6.8), was added to the above mixture and incubated at 37°C for 30 min. Then, 100 µL of 0.2 M sodium carbonate solution was added to stop the reaction. The absorbance of the solution containing *p*-nitrophenol was measured at 405 nm using a microplate reader. A stock solution of α-glucosidase with 1 mg/mL (1 mg = 7 U) concentration was prepared in 0.1 M phosphate buffer (pH – 6.8) and diluted to 0.5 U/mL at the time of assay. The extract and fractions were dissolved in DMSO and diluted to desired concentrations with 0.1 M phosphate buffer. The final DMSO concentration was maintained below 2% (v/v), which was found not to have any effect on the enzyme activities. Different concentrations were tested of each crude extract (2, 1, 0.5, 0.4, 0.3, 0.2, and 0.1 mg/mL). The uninhibited enzyme was taken as control. Appropriate blank was used for all the extracts. Acarbose was used as the reference inhibitor of the enzyme. The assay was performed in triplicate. The α-glucosidase inhibition percentage was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Phospholipase A2 inhibition assay

The inhibitory effect of *C. retrorsa* dichloromethane and methanol extracts was performed. PLA2 (from *Apis mellifera* venom, Sigma-Aldrich) activity was determined colorimetrically in 96-well plates according to the method reported by de Araújo and Radvanyi with slight modifications.[18] Different concentrations of each sample were prepared in DMSO (1, 2, 5, and 10 mg/mL). The enzymatic solution was prepared in distilled water (1 mg/mL). Substrate solution contained L-α- phosphatidylcholine (3.5 mM), NaCl (100 mM), calcium chloride (10 mM), Triton X-100 (7 mM), and phenol red (0.055 mM). The pH is adjusted to 7.6 with 40 mM NaOH solution in a final volume of 100 mL. Briefly, 10 µL of different concentrations of each extract was incubated with 1 µL of the enzymatic solution for 1 h at 37°C. Then, 200 µL of the substrate solution was added. The mixture was incubated for 5 min at 37°C. Colorimetric measurements were made at time 0 and after 5 min and read at 558 nm on a microplate reader. The decrease in the absorbance A_{558} was proportional to pH change as due to the release of fatty acids in the assay conditions. The inhibition percentage was calculated by comparison with a control experiment (absence of extract) using the following equation:

$$\% \text{ Inhibition} = \left(1 - \frac{\left(A_{(P0)} - A_{(P5)}\right)}{\left(A_{(T0)} - A_{(T5)}\right)}\right) \times 100$$

Where $A_{(P0)}$ is the absorbance of the product at $t = 0$ min, $A_{(P5)}$ is the absorbance of the product at $t = 5$ min, $A_{(T0)}$ is the absorbance of the control experiment (DMSO without test sample) at $t = 0$ min and $A_{(T5)}$ is the absorbance of the control experiment (DMSO without test sample) at $t = 5$ min at 558 nm. Triplicate measurements were carried out. Manoalide was used as positive control.

RESULTS AND DISCUSSION

C. retrorsa parts were harvested and then dried at room temperature under shade. The dried leaves, flowers, and stems were macerated with dichloromethane, methanol, and water for 48 h, and decocted with water for 10 min. The obtained mixtures were then filtered and evaporated to dryness under reduced pressure. The antioxidant and antimicrobial activities of all extracts and the cytotoxic activity on Hela cells, anti-inflammatory activity, and anti-diabetic activity of dichloromethane and methanol extracts were then investigated. For the nomenclature, CR refers to the first initials of the plant species, followed by the first initial of the plant part (L for leaves, S for stems, and F for flowers). This letter is followed by the type of extraction (D for decoction and M for maceration). For the latter, it is mentioned the solvent used for the extraction (CH_2Cl_2, CH_3OH, or H_2O). The details of the extraction and nomenclature of each extract are summarized in Table 1.

Antioxidant activity

Free radicals due to physical stress, chemicals, foods, and environmental pollutants may lead to many disorders such as atherosclerosis, arthritis, and many other diseases. Antioxidants by their scavenging capacity are useful for the management of those disorders.[19] The large discovery of plant secondary metabolites with an antioxidant potential during the last few decades made medicinal plants researches more valuable.[20,21] The antioxidant compounds isolated from the different parts of plants

Table 1: Details of procedures used for extraction of *Campanula retrorsa*

Extract	Extraction material	Solvent	Method	Temperature (°C)	Time
CR-L-M-DCM	Dry leaves	Dichloromethane	Maceration	25	48 h
CR-L-M-MeOH		Methanol			
CR-L-M-H_2O		Water			
CR-L-D-H_2O		Water	Decoction	100	10 min
CR-F-M-DCM	Dry flowers	Dichloromethane	Maceration	25	48 h
CR-F-M-MeOH		Methanol			
CR-F-M-H_2O		Water			
CR-F-D-H_2O		Water	Decoction	100	10 min
CR-S-M-DCM	Dry stems	Dichloromethane	Maceration	25	48 h
CR-S-M-MeOH		Methanol			
CR-S-M-H_2O		Water			
CR-S-D-H_2O		Water	Decoction	100	10 min

play a major role in reducing the incidence of chronic diseases caused by radicals.[22]

DPPH has been widely used to investigate the free radical scavenging activity of antioxidant compounds.[23] It is a free radical, stable at room temperature which produces a violet solution in methanol. The reduction of DPPH is monitored by the decrease in its absorbance at 517 nm. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant, the absorption disappears.[24]

The dichloromethane, methanol, and aqueous extracts of *C. retrorsa* have been tested for their antioxidant activity using DPPH. Vitamin C was used as reference standard. The concentrations tested were 0.3, 0.5, and 1 mg/mL. The solution of DPPH alone in methanol was considered as the negative control with zero inhibition.

The results presented in Figure 1 show a difference in the antioxidant activity between samples.

The extracts prepared by hot water extraction were more active than the extracts prepared at room temperature. These results are in agreement with previous works carried out with other plants, in which the decocted extracts have shown the best antioxidant activity between other macerated ones. It seems that hot water extraction increases the quantity of antioxidant compounds without affecting their activity. Furthermore, it could be contributed to the hydrolysis of glycoside bonds by the heat from phenolic compounds, resulting an increase of phenolic hydroxyl groups and antioxidant activity.[21,25]

The more potent activity was for the decocted leaves extract. It was comparatively relative with the radical scavenging activity (RSA) of Vitamin C standard. Decocted extracts have shown the best in comparison with other samples, in which 0.3 mg/mL of the leaves decocted extract inhibits about 94% of the DPPH solution after 30 min. This result is similar to the inhibition shown by the Vitamin C standard.

All aqueous extracts have shown a good antioxidant activity. These results are correlated with other studies where higher antioxidant activity was obtained in polar plant extracts.[26,27] This can be due to polyphenols, flavones, and flavonoids known to have antioxidant activity.[28,29]

All the dichloromethane crude extracts showed a weak antioxidant activity (RSA ~50%). This may be explained by the lack of hydrogen-donating capacity of compounds extracted. Previous studies demonstrated that the interaction between an antioxidant and the radical DPPH depends on its structural conformation. The number of DPPH molecules that are reduced seems to be correlated with the number of available hydroxyl groups.[24,30] In summary, the results showed that the polarity of the extracts strongly influences the antioxidant activity.

Methanol crude extracts of different parts of *C. retrorsa* have shown a moderate antioxidant activity. This is in agreement with a previous study where the methanol extract of aerial parts of this species showed a scavenging activity about seven times lower than the standard.[9]

To our knowledge, no studies on DPPH scavenging activity of *Campanula retrorsa* extracts been previously reported.

This scavenging activity may be due to flavonoids and anthocyanin previously isolated from different species of *Campanula* genus and reported for their antioxidant activity such as kaempferol and rhamnetin.[31,32]

Antimicrobial assay

Natural substances are usually evaluated for their antimicrobial activity by the agar-diffusion techniques for they are inexpensive and easy methods for determining antibacterial activity against pathogenic bacterial and fungal strains.[33,34]

In this study, the hole-plated diffusion method was used to serve as a screening procedure for *C. retrorsa* crude extracts against *S. aureus*, *B. cereus, E. faecium, E. coli*, *P. aeroginosa, Acinetobacter baumanii* and *C. albicans*. Dichloromethane and methanol extracts were dissolved in DMSO, while aqueous ones were dissolved in water.

The diameters of the inhibition zones obtained with *C. retrorsa* active crude extracts are presented in Table 2.

Screening of the antimicrobial activity of the extracts revealed a moderate activity by the dichloromethane extracts against *A. baumanii*. Previous

Table 2: Antimicrobial effects of *Campanula retrorsa* active crude extracts

Code	Strains			
	Gram-Bacteria		Yeast	
	Acinetobacter baumannii		*Candida albicans*	
	CMUL 271		ATCC 10,231	
Concentration (mg/mL)	2	10	2	10
CR-L-M-DCM	+	++	~	~
CR-L-M-MeOH	-	+	-	+
CR-F-M-DCM	-	-	-	~
CR-F-M-MeOH	-	+	-	~
CR-S-M-MeOH	~	+	-	-

CMUL: Microbiological Collection of the Lebanese University; ATCC: American Type Culture Collection; -: No antimicrobial activity, i.z. of sample 1 mm; ~: Slight antimicrobial activity, i.z. of sample 1-2 mm; +: Moderate antimicrobial activity, i.z. of sample 3-4 mm; ++: Clear antimicrobial activity, i.z. of sample 5-10 mm

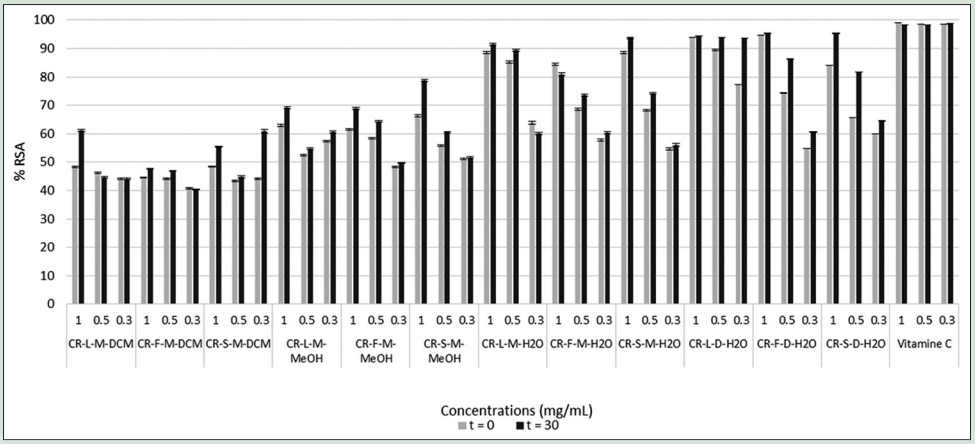

Figure 1: DPPH radical scavenging activity of the extracts and Vitamin C. All values are expressed as mean of triplicate ± standard division

studies on plant extracts have shown that plant extracts are more active against Gram-positive bacteria than against Gram-negative bacteria.[35,36] While in this study, the dichloromethane crude extracts of *C. retrorsa* and especially the leaves dichloromethane extract have inhibited the growth of the Gram-negative strain *A. baumanii*.

In addition, *C. albicans* was slightly inhibited by the dichloromethane and methanol crude extracts of *C. retrorsa* leaves and flowers at 10 mg/mL.

On the other hand, methanol and aqueous crude extracts of *C. retrorsa* failed to inhibit the growth of the bacterial or fungal isolates tested in the present study at any of the employed concentration. This may be due to the fact that most compounds that have been identified active against antimicrobial strains are not water soluble.[34,37]

The results of this present study are in strong agreement with what has been reported earlier on antimicrobial activity of aqueous crude extracts of some of *Campanula* species such as *Campanula americana* L., *Campanula rapunculoides* L., and *Campanula rotundifolia* L.[38]

However, this is the first study to demonstrate that dichloromethane extracts of *C. retrorsa* contain antimicrobial substances with antibacterial and anticandidal effects. Further studies can be subjected on the leaves dichloromethane extract to isolate the therapeutic antimicrobial compounds.

Cytotoxic assay

Dye exclusion is a simple and rapid technique for measuring cell viability. In order to evaluate the cytotoxic effects of *C. retrorsa* dichloromethane and methanol extracts, cell viability experiments were performed on Hela cell line. Cells were incubated 24 h and 48 h with the extracts previously dissolved in DMEM, at final concentrations of 5 and 2 mg/mL. Figure 2 shows the cytotoxic effects of dichloromethane and methanol crude extracts on Hela cells. Cell survival analyses indicated that *C. retrorsa* extracts caused growth inhibition of Hela cells in time-dependent manner. All extracts showed a better inhibition of Hela cells at 48 h than the inhibition at 24 h. While, an inhibition of cell growth was remarked in a dose-dependent manner only in the flower methanol extract, in which cell viabilities decreased significantly from 92% at 2 mg/mL to 27% at 5 mg/mL after 24 h of treatment. Leaf and stem extracts showed the most cytotoxic activity at 2 mg/mL with 90% of cell growth inhibition. This remarkable inhibition may be due to a large variety of polyphenols previously reported in other species of *Campanula* genus such as chlorogenic acid, kaempferol, quercetin, isoquercitrin, and astragalin.[39-42] Those are well known for their anticancer potency.[43-50] From the above, we can see that *C. retrorsa* could be a promoting plant for further studies investigating cytotoxic compounds.

α-Glucosidase inhibition assay

According to many *in vivo* studies, inhibition of α-glucosidase is believed to be one of the most effective approaches for diabetes care.[51-53] α-glucosidase (EC 3.2.1.20) catalyzes the final step of the digestive process of carbohydrates acting upon 1,4-α bonds and giving as a result glucose.[54] Synthetic α-glucosidase inhibitors may cause several side effects such as meteorism, abdominal distention, cholestasis, hypoglycemia, and flatulence.[55] Hence, it is essential to find new effective natural compounds having no or fewer side effects to regulate the blood glucose level. The dichloromethane and methanol extracts of *C. retrorsa* have been evaluated for their activity against α-glucosidase for the first time. Acarbose was used as reference standard. The concentrations tested were 2, 1, 0.5, 0.4, 0.3, 0.2, and 0.1 mg/mL. The mixture of the enzyme and the substrate alone was considered as the negative control with zero inhibition. The results showed that most of the extracts were not capable of inhibiting the activity of α-glucosidase. Dichloromethane and methanol stems crude extracts showed, respectively, a 20% and 13% inhibition of the enzyme α-glucosidase at 2 mg/mL. The inhibition rate of these two extracts can be considered a good sign for the anti-diabetic activity because, as crude extracts, they are constituted of a large number of secondary metabolites. The active compound/compounds may be present in low quantities in the mixture, so further purification of these two extracts is required to be able to characterize the active molecules.

To our knowledge, this is the first study to evaluate the anti-diabetic effect of *C. retrorsa* crude extracts.

Phospholipase A2 inhibition assay

PLA2 is an enzyme that catalyzes the hydrolysis of esters of phospholipids to produce free fatty acids and lysophospholipids.[56] The released fatty acids initiate the biosynthesis of eicosanoids, such as prostaglandins, thromboxanes, and leukotrienes, which are known to mediate inflammation.[57] Thus, PLA2 inhibitors have been targeted as potential therapeutic agents in the treatment of inflammation. Many of these inhibitors have been isolated from plants crude extracts such as Manoalide and Halenaquinone.[56,58] The dichloromethane and methanol extracts of C. retrorsa have been evaluated for activity against bee venom PLA2. Manoalide was used as reference standard. The concentrations tested were 10, 5, 2, and 1 mg/mL. The mixture of DMSO alone with the enzyme and the substrate was considered as the negative control with zero inhibition. The results showed that all the tested extracts were inactive, and no inhibition of PLA2 was detected at any of the tested concentrations. This may be due to the fact that compounds with interesting activities are often present in very low amounts in plant crude extracts.[59]

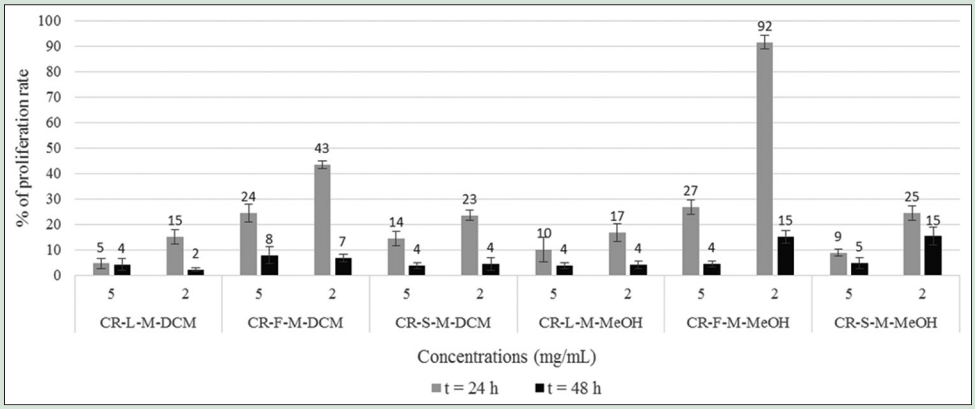

Figure 2: Cytotoxic activity on Hela cells of the extracts. All values are expressed as mean of duplicate ± standard division

Nevertheless, this is the first study to evaluate the anti-inflammatory potency of *C. retrorsa* crude extracts.

CONCLUSION

The data of our present study showed that all aqueous crude extracts of *C. retrorsa* have significant antioxidant activity. Decocted leaves extract have the best DPPH scavenging activity at all the tested concentration. The results clearly show that *C. retrorsa* extracts are a significant natural source of antioxidants.

Among all the crude extracts, only the dichloromethane leaves extracts of *C. retrorsa* have shown a moderate antimicrobial activity against *A. baumanii* and *C. albicans*. All dichloromethane and methanol extracts, except the flowers methanol extract, displayed promising cytotoxic effects on Hela cells after 48 h of treatment at 2 mg/mL.

Dichloromethane and methanol have exhibited no anti-inflammatory activity. While the dichloromethane and methanol stems crude extracts showed a moderate inhibition of the enzyme α-glucosidase at 2 mg/mL.

Further studies focused on the elucidation and characterization of active compounds of the promising extracts are required.

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Conflicts of interest

There are no conflicts of interest.

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