Trends Phytochem. Res. 8(2) 2024 131-140

Trends in Phytochemical Research (TPR)

Journal Homepage: https://sanad.iau.ir/journal/tpr

Original Research Article

Exploring the biological potential of *Cruciata taurica* (Pall. ex Willd.) Ehrend.: Evaluating antibacterial, antioxidant, antidiabetic, and cytotoxic properties

Shiva Khalil-Moghaddam[1]*, Roya Moghimi[2], Thomas Efferth[3], And Sajedeh Mousavian[4]

[1]Department of Biology, Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University, Tehran, Iran
[2]Department of Organic Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran
[3]Department of Pharmaceutical Biology, Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University Mainz, Germany
[4]Departmant of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

ABSTRACT

This study focused on the biological properties of *Cruciata taurica*, a plant found in the Irano-Turanian region, which has not been extensively studied in terms of its phytochemical and biological activities. The methanolic extract of the plant was obtained and its cytotoxic, antibacterial, antidiabetic, and antioxidant properties were investigated. The antibacterial activity of the fractions was evaluated using broth microdilution method, while the antioxidant properties were assessed through the DPPH assay and the cytotoxic properties were determined using the RRA assay. This study revealed that the residual aqueous fraction exhibited the highest extraction yield and the most potent antioxidant activity. The EtOAc fraction showed strong antibacterial activity against certain bacteria, while the *n*-hexane fraction exhibited greater antidiabetic effects. Additionally, the EtOAc fraction was found to be the most cytotoxic. Overall, the study suggested that *C. taurica* has potential therapeutic applications due to its various biological properties.

ARTICLE HISTORY

Received: 06 February 2024
Revised: 29 May 2024
Accepted: 17 June 2024
ePublished: 28 June 2024

KEYWORDS

Bioactivity
Cancer
Cruciata taurica (Pall. ex Willd.) Ehrend.
Cytotoxic
Ethnopharmacology
Multidrug resistance
Plant extract

1. Introduction

The Rubiaceae family, also known as the Madder family, Bedstraw family, or coffee family, is named after the Madder genus *Rubia*. Currently, the Rubiaceae family comprises approximately 637 genera and over 13,000 species, making it one of the six largest angiosperm families in terms of both genus and species diversity (Wooster et al., 2008; Avoseh et al., 2020; Nyobe et al., 2020; Wonkam et al., 2022). It shares this distinction with the Asteraceae, Orchidaceae, Fabaceae, Poaceae, and Euphorbiaceae families (Bremer and Eriksson, 2009). Rubiaceae species are predominantly found in warmer and tropical regions across the globe. This family exhibits a wide range of growth forms, with shrubs being the most common, although its members can also be trees, lianas, or herbs. While the majority of species are woody, less than 20% of the genera are herbaceous (Karou et al., 2011). The *Cruciata* genus includes only nine species (Tava et al., 2020). *Cruciata* genus has central hermaphrodite and lateral male flowers (Huysmans et al., 2003). The species within the *Cruciata* genus have diverse applications in traditional medicine. For instance, *C. laevipes* has been traditionally recognized for its wound-healing properties, has historically been used for both external and internal purposes (De Rosa et al., 2003). The internal uses of this herbal species include treatment of stomach and bowel obstructions, appetite stimulation, rheumatism, and dropsy (Tava et al., 2020). *C. taurica* is distributed in Crimea, East Greece, Southwest Asia, and Iran (De Rosa et al., 2003). This plant is a small shrub with yellow flowers. It is a perennial, semi-woody, herbaceous plant with primary roots and sometimes adventitious roots. The stems are typically upright from the base, with erect to ascending branches that are quadrangular, and often have sparse or no trichomes. The leaves are ovate to almost circular, approximately 30 mm long and 15

* Corresponding author: Shiva Khalil-Moghaddam
Tel: +989127752895; Fax: +989127752895
E-mail address: shiva.moghaddam@yahoo.com, **doi: 10.71596/tpr.2024.1126086**

mm wide, and tend towards yellow or purplish colors. After flowering, the leaves are retrorsed. The secondary leaves are ovate to almost round, about 15 mm long and 12 mm wide, and may have trichomes that are roughened. The flower has a golden yellow to yellowish cup that is ovate to oblong and sharp-beaked. The fruit has a nut-like, usually solitary, globose structure without trichomes or sometimes with appressed trichomes (Ehrendorfer et al, 2005). The midpoint of diversification and the source of this species is the Irano-Turanian area (Ehrendorfer and Schönbeck-Temesy, 1982; Salim and Necattin, 2018). Phytochemical studies have shown that *C. taurica* contains coumarins, *e.g.*, umbelliferon and scopoletin (Borisov, 1974), flavonoids, *e.g.*, hyperoside, quercetin, and iridoid glycosides (Ergun et al., 1984; De Rosa et al., 2003). *C. taurica* has been used in traditional medicine as a mixed juice of crushed material with fresh milk (Salim and Necattin, 2018).

Herbal products have different biological activities including anti-inflammatory (Karakas et al., 2017; Lopez-Corona et al., 2022; Yeshi et al., 2022), antibacterial (Singh, 2022; Eruygur et al., 2023), anticancer (Ng et al., 2022), antioxidant (Ghagane et al., 2017; Alam et al., 2022a; Sbieh et al., 2022), cardioprotective (Tomou et al., 2023), neuroprotective (Zieneldien et al., 2022), antidiabetic (Alam et al., 2022b), antiparasitic (Ranasinghe et al., 2023), and antiviral properties (Saifulazmi et al., 2022). Diabetes mellitus is characterized by impaired and prolonged insulin activity in target tissues, as well as disruptions that affect the metabolism of carbohydrates, fats, and proteins (Choudhury et al., 2018). One of the most important remedies for diabetes is controlling postprandial hyperglycemia (Méril-Mamert et al., 2022). Low and stable blood glucose levels can be achieved by delaying glucose absorption through inhibiting enzymes such as α-amylase and α-glucosidase, which break down carbohydrates in the digestive system (Khadayat et al., 2020). There are numerous commercial products available worldwide that are designed to manage diabetes and are derived from medicinal plants (Ansari et al., 2022). Natural products with antioxidant properties neutralize free radicals and safeguard cells against damage and degradation (Zhang et al., 2023). These herbal products exhibit notable bioactivities and can help inhibit the progression of diabetes mellitus, cancer, and heart disease. Cancer is a major global cause of mortality (Tohma et al., 2019; Behdarvand et al., 2020; Goli et al., 2021). Recently, there has been increased focus on natural bioactive compounds from medicinal plants that exhibit anticancer activity (Abd Wahab et al., 2020; Khan et al., 2022; Yuan et al., 2022). To the best of our knowledge, there are only few studies on the biological activity of *C. taurica* (De Rosa et al., 2002, 2003). Furthermore, there is a lack of research on the liquid-liquid extraction of *C. taurica*. This study represents the first investigation of the cytotoxic effects of *C. taurica* extracts on leukemia cells. In this study, we report on the methanolic extract of the aerial parts of the plant, obtained through maceration and liquid-liquid extraction. The plant was fractionated into different fractions (MeOH, EtOAc, *n*-hexane, and residual aqueous fractions), and their biological activities were investigated, including antioxidant, antidiabetic, antibacterial, and cytotoxic activities.

2. Experimental

2.1. Plant collection

The aerial parts of the *C. taurica* plant were collected from Iran, specifically from the Kurdistan Province, Marivan City, and Miraji Mountain, in May 2021 (Fig. 1). The plant was collected and identified by Dr. Nastaran Jalilian. A voucher number (RANK-8420) was deposited at the herbarium of the Agricultural and Natural Resources Research and Education Center in Kermanshah, Iran. The specimen was obtained from an area characterized by inceptisol soil composition, which consists of minimally developed soils interspersed with rock fragments. The region's climate is Mediterranean and cold. The plant collection was conducted under the authority of the official national plant protection organization.

2.2. Chemicals and reagents

The following compounds were used: DPPH (Merck Millipore), α-amylase (Merck-Millipore), Starch (Merck Millipore), acarbose (Sigma-Aldrich, Taufkirchen, Germany), 3,5-dinitrosalicylic acid (Sigma-Aldrich), resazurin reagent (Sigma-Aldrich), RPMI 1640 culture medium (Gibco BRL, Eggenstein, Germany), fetal bovine serum (FBS; Gibco), penicillin and streptomycin sulfate (Biochrom, Germany). All solvents were purchased from Merck Millipore. All chemicals used in this study were of 99% purity or higher. An Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) was used for fluorescence measurement. Also, UV absorbance was conducted using a spectrophotometer (Jasco V-750, Japan).

2.3. Plant extract

The maceration method was used for the preparation of the plant extracts. The cleaned aerial parts of the plant were dried at room temperature and then powdered. Subsequently, 200 g of *C. taurica* powder was extracted with methanol (MeOH) at room temperature for 48 h under shaking conditions (150 rpm), at plant material-to-solvent ratio of 1:10 (*w/v*%). The plant material was removed from the solvents by filtration through Whatman paper, and the filtrate was concentrated using a rotary evaporator. The MeOH extract was suspended in distilled water, and further extraction was performed using EtOAc and *n*-hexane solvents (1:1 *v/v*%) by the liquid-liquid extraction method (Mazzola et al., 2008).

2.4. Yield of extraction

The extraction yield was represented as the dry matter content of the extracts and was calculated using the following equation (Eqn. 1). For this purpose, MeOH, aqueous, EtOAc, and *n*-hexane fractions were dried using a freeze dryer at -60 °C. The residual aqueous solution was obtained from the remaining liquid-liquid extraction process.

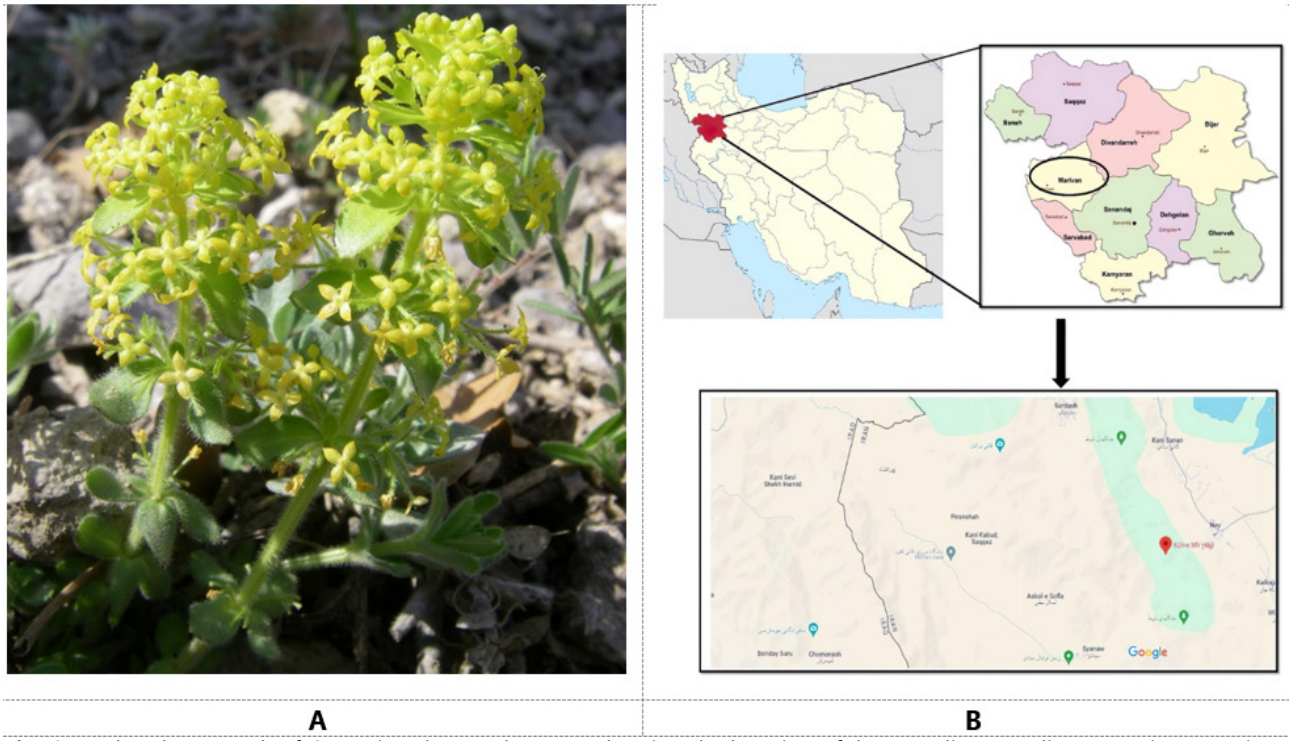

Fig. 1. A: The photograph of *C. taurica* plant and **B:** Map showing the location of the sampling (Kurdistan Province, Marivan City, Miraji Mountain, Iran).

Extraction yield = $(W_1/W_2) \times 100$ (Eqn. 1)

Where W_1 is the dry weight of the MeOH extract or fractions, and W_2 is the weight of the dried aerial parts of *C. taurica*.

2.5. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH free radical scavenging assay, as recently described with slight modifications (Khalil-Moghaddam et al., 2021) was used to determine the antioxidant activity of various plant fractions. In summary, diluted DMSO solutions of the samples were prepared at concentrations ranging from 0.009 to 10 mg/mL. A solution of DPPH (80 µg/mL) was then prepared in MeOH, and 150 µL of this solution was added to the different concentrations of the plant fractions. Sample blanks were also prepared by substituting MeOH for the DPPH solution. The plates were then incubated at 25 °C for 30 minutes, followed by measurement of the absorbance of each well at 517 nm using a microplate reader. As a positive control, butylated hydroxytoluene (BHT) was used.
The percent inhibition was calculated as follows (Eqn. 2):

Inhibition (%) = $(A_{Blank}\text{-}A_{Sample}/A_{Blank}) \times 100$ (Eqn. 2)

The terms A_{Blank} and A_{Sample} respectively account for the corresponding absorbance of the control reaction (DPPH solution), and the plant extraction combined with DPPH, both at 517 nm. Furthermore, the solution concentration that led to 50% inhibition (IC_{50}) was determined by plotting the inhibition percentage against each plant extraction concentration.

2.6. Antibacterial activity

2.6.1. Microbial strain

Four bacterial standard strains were evaluated in this study, *i.e., Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11788, and *Escherichia coli* ATCC 25922.

2.6.2. Determination of the minimum inhibitory concentration (MIC)

MICs were assigned using a broth microdilution assay according to CLSI method (CLSI, 2006) in a 96-well microtiter plate. Different fractions of the *C. taurica* extract were dissolved in DMSO to produce an initial concentration of 36 mg/mL. Subsequently, aliquots of the samples were consecutively diluted in a 96-well plate containing Mueller-Hinton Broth (MHB) medium to yield a concentration range of 0.017-36 mg/mL. A standardized bacterial suspension (100 µL, 10^6 CFU/mL) was inoculated in each well and incubated overnight at 37 °C. The lowest concentration that showed no visible growth was considered the minimum inhibitory concentration. The antibacterial activity of DMSO was also analyzed, and no antibacterial activity was observed at the examined concentrations.

2.7. α-Amylase inhibitory activity

The α-amylase inhibition assay was conducted using the 3,5-dinitrosalicylic acid (DNSA) method with certain

modifications (Wickramaratne et al., 2016). Acarbose was employed as the positive control. To prepare the DNSA reagent, DNSA (1.0 g) and sodium potassium tartaric acid (30 g) were dissolved in 20 mL of NaOH (2.0 N). The sample was then diluted to 100 mL using distilled water. The plant extract was dissolved in a minimal amount of dimethyl sulfoxide (DMSO: 50 mg/mL). In the initial step, two concentrations (3 and 300 µg/mL) of various fractions were evaluated to determine the most effective fraction with significant antidiabetic activity. Subsequently, concentration ranges of 0.3, 3, 30, and 300 µg/mL were analyzed for the best fraction (*n*-hexane), while concentrations of 0.3, 0.75, 1.5, 3.75, and 7.5 were assessed for acarbose. Additionally, the IC_{50} values for both the *n*-hexane fraction and acarbose were calculated during this stage. To initiate the assay, 200 µL of α-amylase solution (1.0 mg/mL) was added to 200 µL of plant extract and incubated for 30 min at 37 °C. Subsequently, 200 µL of an aqueous starch solution (1.0% *w/v* in buffer solution) was added to each sample and incubated at 37 °C for 3 min. The absorbance of each tube was measured at 540 nm using a UV-Visible spectrophotometer.

2.8. Cytotoxic effect

2.8.1. Cell lines and culture

Human CCRF-CEM acute lymphoblastic leukemia cells and their multidrug resistant subline CEM/ADR5000 Axel Sauerbrey (Department of Pediatrics, University of Jena, Jena, Germany) were used for cytotoxicity assays. The generation of CEM/ADR5000 cells was described by selection with doxorubicin and the characterization of their multidrug resistance phenotype (Efferth et al., 2008). The cell lines were maintained in RPMI 1640 culture medium supplemented with fetal bovine serum (FBS, 10.0%), (penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL). The resistance phenotype of CEM/ADR5000 was maintained by treating the cells every other week with 5000 ng/mL doxorubicin. The cells were incubated at 37 °C in a CO_2 incubator (5.0% CO_2).

2.8.2. Resazurin reduction assay (RRA)

The investigation into the cytotoxicity of the plant fractions was conducted by employing the RRA assay, with certain modifications (Youmbi et al., 2023). Following appropriate growth for experimental purposes, the cell lines underwent a series of procedures, including washing, trypsinization, and resuspension in a fresh medium. At the initial stage, the cells were cultured in 96-well plates, with or without varying concentrations of the plant fractions (10 and 100 µg/mL), for a duration of 72 hours. Subsequently, the most potent fraction exhibiting the most effective antiproliferative activity was chosen for IC_{50} determination. To this end, ten concentrations of the optimal fraction (ranging from 0.01 to 1000 µg/mL) were prepared and tested against two different cell lines and the IC_{50} representing the concentration at which 50% of the cells were killed was subsequently determined. The fluorescence emitted during the experiment was measured using an Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany). The excitation and emission wavelengths were 544 nm and 590 nm, respectively.

2.9. Statistical analysis

All experiments were performed in triplicate with each of the six parallel measurements. The results are presented as means ± standard deviation (SD). Statistical analysis was performed employing Graph Pad Prism software version 9.2.0. The comparison between the three groups was accomplished by the use of one-way analysis of variance (ANOVA).

3. Results and Discussion

3.1. Extraction yield and antioxidant activity

The results of the extraction yield and DPPH assays for the MeOH, EtOAc, *n*-hexane, and residual aqueous fractions are presented in Table 1. As can be seen in this table, the lowest and highest extraction yield was obtained for the EtOAc (0.2%) and the residual aqueous (2.2%) fractions, respectively. The DPPH assay showed that the residual aqueous fraction had the lowest IC_{50} (10.0 ± 2.6 µg/mL). A comparison of the antioxidant activity of different plant fractions with BHT revealed that *C. taurica* extracts had potent antioxidant activity, and the IC_{50} of different fractions was comparable to that of the synthetic standard antioxidant compound (BHT). As far as we know, there are no comprehensive studies on the antioxidant activity of the *Cruciata* genus in the literature. In one study, some compounds isolated from *Cruciata articulata* did not exhibit antioxidant or antibacterial activity (Liu et al., 2021). The antioxidant properties of the Rubiaceae family have been discussed in a recently published review, which highlights that the family contains active biological compounds with potent antioxidant activity, capable of preventing oxidative stress (González-Castelazo et al., 2023). The antioxidant activity of *C. cruciata* may be attributed to the presence of flavonoids such as hyperoside, quercetin, and rutin (De Rosa et al., 2003). Flavonoids with antioxidant properties can effectively modulate essential stages of cell growth and differentiation, thereby influencing the regulation of overall plant development and formation of individual organs (Agati et al., 2012).

3.2. Antibacterial activity

The antibacterial activities of the plant fractions were investigated against two Gram-negative bacteria (*E. coli* and *P. aerosinosa*) and two Gram-positive bacteria (*B. cereus* and *S. aureus*). Among the plant fractions screened, the EtOAc fraction showed the most significant inhibitory effect against *E. coli* and *B. cereus*, with an MIC value of 0.1 mg/mL (Table 2). Moreover, the *n*-hexane fraction had more excellent antibacterial activity against *P. aeruginosa* than the other fractions (MIC: 2.2 mg/mL). On the other hand, the residual aqueous fraction showed the weakest antibacterial activity among all fractions against *E. coli* (MIC: 18 mg/mL). Several studies have reported MIC values of less

Table 1
Yield of plant extraction and antioxidant activity of different *C. taurica* fractions.

Samples	Yield of extraction (%)	DPPH assay IC_{50} (µg/mL)
MeOH	1.8	150.0 ± 12.0
EtOAc	0.2	20.0 ± 2.0
n-Hexane	1.2	1010.0 ± 81.0
Water	2.2	10.0 ± 2.6
BHT	-	10.0 ± 2.0

Table 2
MIC values of *C. taurica* fractions (mg/mL).

Samples	*S. aureus* ATCC 25923	*P. aeruginosa* ATCC27853	*B. cereus* ATCC 11778	*E. coli* ATCC 25922
MeOH	4.5	4.5	0.6	1.1
EtOAc	1.1	4.5	0.1	0.1
n-Hexane	1.1	2.2	0.3	0.3
Water	4.5	4.5	4.5	18
Chloramphenicol	2.0[a]	64	1	8

[a] MIC value of Chloramphenicol was represented as µg/mL.

than 0.5 mg/mL, indicating the potent and significant antibacterial activity of plant extracts (Sartoratto et al., 2004; Mogana et al., 2020). As a result, the *n*-hexane and EtOAc fractions exhibited strong antibacterial activity against *B. cereus* and *E. coli*.
C. cruciata is a rich source of aromatic monoterpenoid glycosides. These glycoside compounds can be extracted using methanol and exhibit significant antimicrobial activity (De Rosa et al., 2003). Therefore, the antibacterial activity observed in different fractions of *C. cruciata*'s methanolic extract can be attributed to the presence of these compounds. A previous research has examined the antibacterial activity of many plants belonging to the Rubiaceae family (Chassagne et al., 2021). The aqueous and ethanol extracts of *Mtracarpus villosus* leaves, for instance, were shown to have antibacterial activity in a research. In accordance with this study, the results indicated that the MICs against *S. aureus*, *B. subtilis*, *S. faecalis*, and *E. coli* ranged from 0.1 to 8.0 mg/mL (Irobi and Daramola, 1994). In another study, the ethanolic extracts from the leaves of *M. scabrum* were tested for their potential antibacterial efficacy against different pathogens including *E. coli* and *S. aureus*. In this connection, the greatest results were obtained with the hydroalcoholic extract with an MIC of 50 mg/mL (Ouadja et al., 2018). A previous study reported the antibacterial and antifungal activities of selected plants (75 crude extracts) from the Rubiaceae family. Specifically, 85% of *n*-hexane extracts from these plants demonstrated significant antibacterial activity. Our results are consistent with the previous study, which found that the *n*-hexane extract of plants belonging to the Rubiaceae family exhibited significant antibacterial activity (Niño et al., 2012).

3.3. α-Amylase inhibitory assay

The α-amylase inhibitory activity of the different plant fractions was plotted as a function of the extract concentration (Fig. 2A), and the IC_{50} values were subsequently calculated (Fig. 2B). As seen, the results for the two concentrations of each fraction were significantly different ($p < 0.05$). In addition, significant differences were found between the IC_{50} values of the sample and the standard ($p < 0.001$). Evaluation of the inhibitory activity of the plant fractions against α-amylase at two concentrations (3 and 300 µg/mL) revealed that the *n*-hexane fraction was the most potent. The IC_{50} of the *n*-hexane fraction was determined to be 183.1 ± 8.6 µg/mL. In comparison, the standard positive control, acarbose, exhibited an IC_{50} of 3.8 ± 0.1 µg/mL. Our findings also showed that all plant fractions had relatively weak antidiabetic activity compared to the standard compound, acarbose. Several studies have previously evaluated the α-amylase inhibitory activities of plant extracts (Borhan et al., 2013; Namjoyan et al., 2015; Wickramaratne et al., 2016; Sai et al., 2019; Abdollahzadeh et al., 2021; Abd Elkader et al., 2022; Kumar et al., 2022). The α-amylase inhibitory activity of different extracts of loquat (*Eriobotrya japonica*) leaves

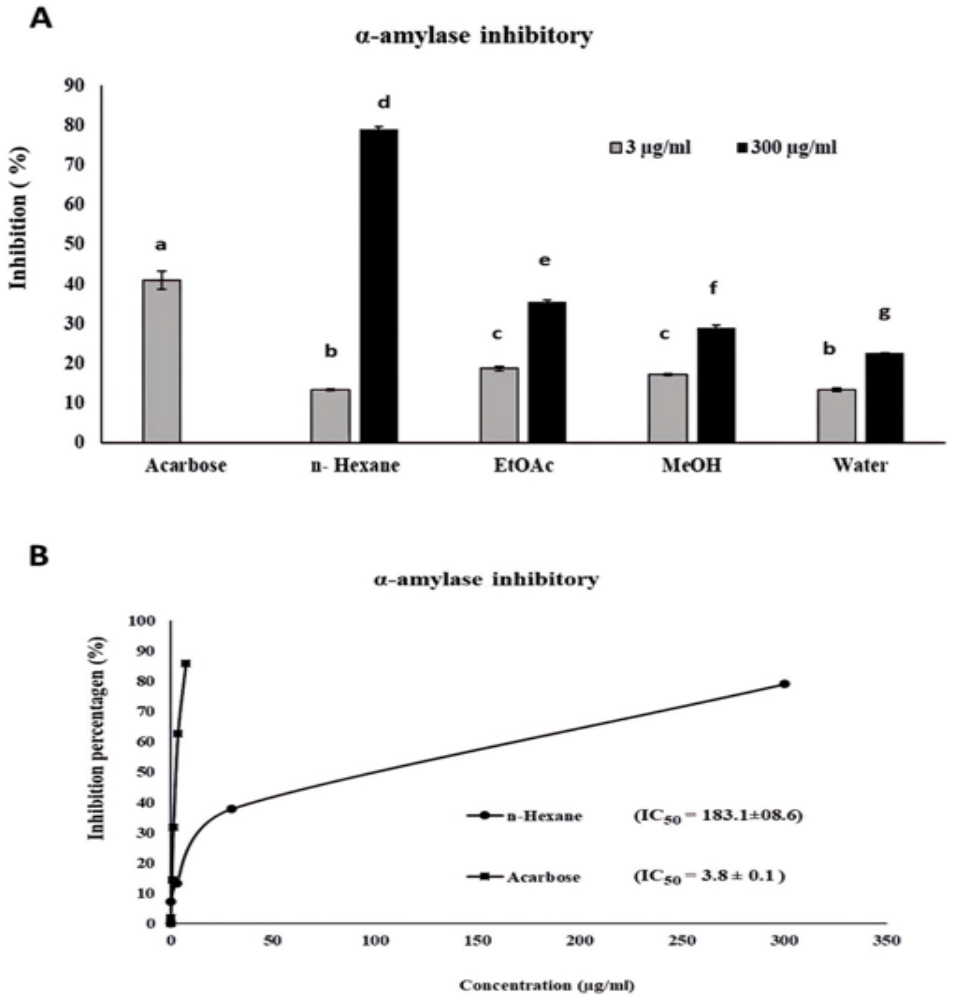

Fig. 2. Enzyme inhibition (%) (**A**) For different fractions of *C. taurica* at two concentrations (3 and 300 µg/mL), (**B**) As a function of optimum fraction (*n*-hexane) extract concentration for determination of IC_{50} *(*Note: Different letters in the same pattern represent significant difference ($p < 0.05$).

has been the subject of a recently published report (Mogole et al., 2020). In another study, the α-amylase inhibitory activity of nine traditional plant extracts from Southeastern Nigeria was investigated. The results indicated that eight of the plant extracts exhibited moderate α-amylase inhibitory activity (Oyedemi et al., 2017). The α-amylase enzyme facilitates the process of breaking down starch through a double substitution mechanism, which involves the formation and subsequent breakdown of a covalent β-glycosyl enzyme intermediate within its active site. Inhibiting the α-amylase with plant-derived compounds appears to be a promising approach for treating diabetes. Coumarins and iridoid glycoside are a potent source for antidiabetic effect of *C. cruciate* extracts (Chen et al., 2016; Li et al., 2017). Also, flavonoids had high inhibitory capacity against α-amylase. The proposed inhibitory capacity of flavonoids action mechanism correlates inhibition potency of these compounds with the number of hydroxyl groups in the B ring of the flavonoid (Shah et al., 2018). Typically, flavonoids exhibit desirable structural characteristics as enzyme inhibitors, which encompass adaptable backbones, a hydrophobic composition, and multiple accessible hydrogen bond (H-bond) donors and acceptors (Rasouli et al., 2017). Our findings are consistent with previously published studies, which reported moderate to weak antidiabetic effects of plant extracts. We hypothesized that the results might be improved if the α-amylase inhibitory activity of pure active compounds from these plants was investigated.

3.4. Cytotoxic activity

The plant extracts were analyzed at concentrations of 10 and 100 µg/mL while using doxorubicin as a positive control drug on human CEM-CCRF lymphoblastic leukemia cells (drug-sensitive) (Fig. 3A) and their multidrug-resistant sub-line CEM/ADR5000 (multidrug-resistant) (Fig. 3B). Among the plant fractions tested, the EtOAc fraction demonstrated cytotoxic activity on cell proliferation of both cell lines at a concentration

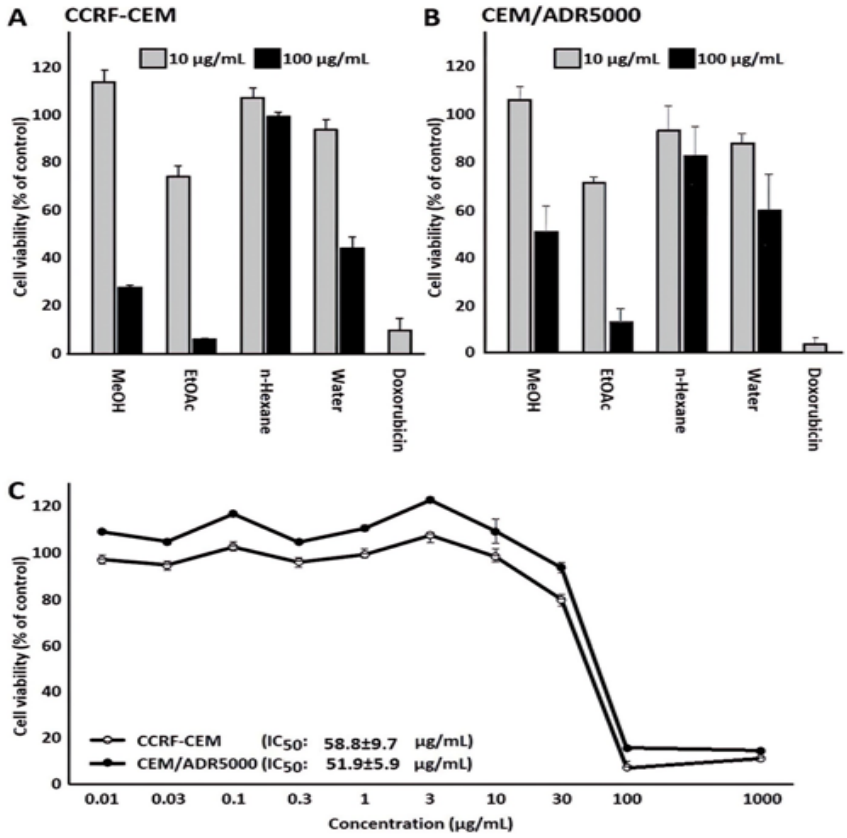

Fig. 3. Growth inhibition of *C. taurica* plant fractions. Effect of fixed concentrations (10 and 100 µg/mL) towards: **A**: Drug-sensitive CCRF-CEM, **B:** Multidrug-resistant CEM/ADR5000 and **C:** Effect of a dose range from 0.01 to 1000 µg/mL EtOAc fraction to both CEM-CCRF and CEM/ADR5000 cells.

of 100 µg/mL. In contrast, the other fractions showed no apparent cytotoxic effects at the concentrations used. Consequently, the EtOAc fraction was selected as the optimal fraction, exhibiting the best cytotoxic activity. Further investigation of the EtOAc fraction at various concentrations (0.01-1000 µg/mL) led to the determination of the IC_{50} value (Fig. 3C). The IC_{50} values obtained were 58.8 ± 9.7 and 51.9 ± 5.9 µg/mL for CCRF-CEM and CEM/ADR5000 cells, respectively. Although the IC_{50} values indicate relatively weak activity of the extract, there was no cross-resistance of CEM/ADR5000 cells to the *C. taurica* extract. The presence of flavonoids is a crucial factor in the cytotoxic properties of the *C. taurica* extract (Sak, 2014). This is noteworthy since CEM/ADR5000 cells exhibited high resistance to doxorubicin and many other clinically established anticancer drugs (Efferth et al., 2008). Multidrug resistance is a frequent cause of failure of chemotherapy, which leads to the death of many cancer patients (Volm and Efferth, 2015). Therefore, the cytotoxic potency of plant extracts and natural products that can inhibit multidrug-resistant cells with similar efficacy to drug-sensitive tumor cells may have the potential to overcome drug resistance.

Numerous studies have focused on the anticancer activities of plant extracts and isolated phytochemicals (Molnár et al., 2010; Kumar and Jaitak, 2019; Luo et al., 2019). In one of the recently published reports, multidrug-resistant. However, many inhibited sensitive and multidrug-resistant cells with similar efficacies (Atanasov et al., 2021). This may open a new horizon to treat multidrug-resistant tumors with plant extracts and natural products.

4. Concluding remarks

In this study, the methanol (MeOH) extract of *C. taurica* was obtained. Subsequently, several fractions were made from the initial extract, including residual aqueous, *n*-hexane, and EtOAc fractions. To gain a better understanding of the plant's biological activity, some complimentary tests were conducted to assess its antibacterial, antioxidant, anticancer, and antidiabetic properties. The results confirmed that the residual aqueous fraction had remarkable antioxidant activity. The EtOAc fraction was potent against *E. coli* and *B. cereus*, with a minimum inhibitory concentration (MIC) of 0.1 mg/mL. The *n*-hexane and EtOAc fractions exhibited the best antidiabetic and anticancer effects, respectively. Different plant extracts can be a valuable natural resource for isolating active ingredients with potent biological activity. The investigations presented here demonstrate that *C. taurica* could be a rich source of natural bioactive compounds with antibacterial, anticancer, antidiabetic, and antioxidant properties.

The key limitation of this work was the assessment of the biological activity of the crude extracts. Therefore, further investigation is recommended to confirm the potential of the extracts as phytotherapeutic agents. This includes: i) Isolating and characterizing the main active compounds from the extracts. ii) Exploring the biological activities and mechanisms of action of the isolated compounds. By addressing these aspects, the full potential of *C. taurica* as a source of natural bioactive compounds can be more thoroughly explored and validated.

Conflict of interest

The authors declare that there is no conflict of interest.

Author contribution

Shiva Khalil-Moghaddam: Investigation and methodology, Project administration, Supervision; Roya moghimi: Conceptualization, writing-review & editing; Thomas Efferthc: Investigation and methodology; Sajedeh Mousavian: Investigation and methodology.

Acknowledgments

Not applicable.

List of Abbreviations

BHT: Butylated hydroxytoluene; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **DNSA:** 3, 5-Dinitrosalicylic Acid; **FBS:** Fetal Bovine Serum; **IC_{50}:** Inhibitory Concentration (50%); **MHB:** Mueller-Hinton Broth; **MIC:** Minimum Inhibitory Concentration; **RRA:** Resazurin Reduction Assay; **UV:** Ultraviolet.

References

Abd Elkader, A.M., Labib, S., Taha, T.F., Althobaiti, F., Aldhahrani, A., Salem, H.M., Saad, A., Ibrahim, F.M., 2022. Phytogenic compounds from avocado (*Persea americana* L.) extracts; antioxidant activity, amylase inhibitory activity, therapeutic potential of type 2 diabetes. Saudi J. Biol. Sci. 29, 1428-1433.

Abd Wahab, N.Z., Azizul, A., Ibrahim, N., 2020. Phytochemistry, cytotoxicity and antiviral activity of *Catharanthus roseus*. Iran. J. Microbiol. 12, 460.

Abdollahzadeh, E., Nematollahi, A., Hosseini, H., 2021. Composition of antimicrobial edible films and methods for assessing their antimicrobial activity: A review. Trends Food Sci. Technol. 110, 291-303.

Agati, G., Azzarello, E., Pollastri, S., Tattini, M., 2012. Flavonoids as antioxidants in plants: Location and functional significance. Plant Sci. 196, 67-76.

Alam, M.W., Najeeb, J., Naeem, S., Usman, S.M., Nahvi, I., Alismail, F., Abuzir, A., Farhan, M., Nawaz, A., 2022a. Electrochemical methodologies for investigating the antioxidant potential of plant and fruit extracts: A review. Antioxidants 11, 1205.

Alam, S., Sarker, M.M.R., Sultana, T.N., Chowdhury, M.N.R., Rashid, M.A., Chaity, N.I., Zhao, C., Xiao, J., Hafez, E.E., Khan, S.A., 2022b. Antidiabetic phytochemicals from medicinal plants: Prospective candidates for new drug discovery and development. Front. Endocrinol. (Lausanne) 13.

Ansari, P., Akther, S., Hannan, J.M.A., Seidel, V., Nujat, N.J., Abdel-Wahab, Y.H.A., 2022. Pharmacologically active phytomolecules isolated from traditional antidiabetic plants and their therapeutic role for the management of diabetes mellitus. Molecules 27, 4278.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Orhan, I.E., Banach, M., Rollinger, J.M., Barreca, D., Weckwerth, W., Bauer, R., Bayer, E.A., Majeed, M., Bishayee, A., Bochkov, V., Bonn, G.K., Braidy, N., Bucar, F., Cifuentes, A., D'Onofrio, G., Bodkin, M., Diederich, M., Dinkova-Kostova, A.T., Efferth, T., El Bairi, K., Arkells, N., Fan, T.P., Fiebich, B.L., Freissmuth, M., Georgiev, M.I., Gibbons, S., Godfrey, K.M., Gruber, C.W., Heer, J., Huber, L.A., Ibanez, E., Kijjoa, A., Kiss, A.K., Lu, A., Macias, F.A., Miller, M.J.S., Mocan, A., Müller, R., Nicoletti, F., Perry, G., Pittalà, V., Rastrelli, L., Ristow, M., Russo, G.L., Silva, A.S., Schuster, D., Sheridan, H., Skalicka-Woźniak, K., Skaltsounis, L., Sobarzo-Sánchez, E., Bredt, D.S., Stuppner, H., Sureda, A., Tzvetkov, N.T., Vacca, R.A., Aggarwal, B.B., Battino, M., Giampieri, F., Wink, M., Wolfender, J.L., Xiao, J., Yeung, A.W.K., Lizard, G., Popp, M.A., Heinrich, M., Berindan-Neagoe, I., Stadler, M., Daglia, M., Verpoorte, R., Supuran, C.T., 2021. Natural products in drug discovery: Advances and opportunities. Nat. Rev. Drug Discov. 20, 200-216.

Avoseh, N.O., Lawal, O.A., Ogunwande, I.A., Ascrizzi, R., Flamini, G., Amoo, E., 2020. *In vivo* anti-inflammatory and anti-nociceptive activities, and chemical constituents of essential oil from the leaf of *Gardenia jasminoides* J. Ellis (Rubiaceae). Trends Phytochem. Res. 4, 203-212.

Behdarvand, N., Bikhof Torbati, M., Shaabanzadeh, M., 2020. Tamoxifen-loaded PLA/DPPE-PEG lipid-polymeric nanocapsules for inhibiting the growth of estrogen-positive human breast cancer cells through cell cycle arrest. J. Nanoparticle Res. 22, 262.

Borhan, M.Z., Norhidayah, A., Ahmad, R., Rusop, M., Abdullah, S., 2013. Production of herbal nanopowders: Effect of milling time. Adv. Mater. Res. 686, 3-7.

Borisov, M.I., 1974. Coumarins of the genera *Asperula* and *Galium*. Chem. Nat. Compd. 10, 78.

Bremer, B., Eriksson, T., 2009. Time tree of Rubiaceae: Phylogeny and dating the family, subfamilies, and tribes. Int. J. Plant Sci. 170, 766-793.

Chassagne, F., Samarakoon, T., Porras, G., Lyles, J.T., Dettweiler, M., Marquez, L., Salam, A.M., Shabih, S., Farrokhi, D.R., Quave, C.L., 2021. A systematic review of plants with antibacterial activities: A taxonomic and phylogenetic perspective. Front. Pharmacol. 11, 2069.

Chen, Y., Wu, Y., Gan, X., Liu, K., Lv, X., Shen, H., Dai, G., Xu, H., 2016. Iridoid glycoside from *Cornus officinalis* ameliorated diabetes mellitus-induced testicular damage in male rats: Involvement of suppression of the AGEs/RAGE/p38 MAPK signaling pathway. J. Ethnopharmacol. 194, 850-860.

Choudhury, H., Pandey, M., Hua, C.K., Mun, C.S., Jing, J.K., Kong, L., Ern, L.Y., Ashraf, N.A., Kit, S.W., Yee, T.S., Pichika, M.R., Gorain, B., Kesharwani, P., 2018. An update on natural compounds in the remedy of diabetes mellitus: A systematic review. J. Tradit. Complement.

Med. 8(3), 361-376.
CLSI, W., 2006. Clinical and Laboratory Standards Institute Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approv. Stand. M7-A7, CLSI, Seventh Ed, PA, USA.
De Rosa, S., Mitova, M., Handjieva, N., Çalış, İ., 2002. Coumarin glucosides from *Cruciata taurica*. Phytochemistry 59, 447-450.
De Rosa, S., Mitova, M., Handjieva, N., Ersoz, T., Calis, I., 2003. Aromatic monoterpenoid glycosides from *Cruciata taurica*. Nat. Prod. Res. 17, 109-113.
Efferth, T., Konkimalla, V.B., Wang, Y.-F., Sauerbrey, A., Meinhardt, S., Zintl, F., Mattern, J., Volm, M., 2008. Prediction of broad spectrum resistance of tumors towards anticancer drugs. Clin. Cancer Res. 14, 2405-2412.
Ehrendorfer, F., Schönbeck-Temesy, E., 1982. Flora of Turkey and the East Aegean Islands.
Ergun, F., Küsmenoglu, Ş., Şener, B., 1984. High-performance liquid chromatographic determination of iridoids in *Cruciata Taurica*. J. Liq. Chromatogr. 7, 1685-1689.
Ehrendorfer, F., Schonbeck-Temesy, E., Puff, C., Rechinger, W., 2005. Rubiaceae. In: Rechinger, K H (ed.) Flora Iranica, No. 176-Graz, p. 443.
Eruygur, N., Buyukyildirim, T., Tetik Rama, S., Ayaz, F., Tekin, M., Tuzcu, M., Akcakavak, G., Abdullah Yilmaz, M., 2023. Phytochemical profiling and biological activity of *Achillea sintenisii* Hub.-Mor. Chem. Biodivers. 20, e202201258.
Ghagane, S.C., Puranik, S.I., Kumbar, V.M., Nerli, R.B., Jalalpure, S.S., Hiremath, M.B., Neelagund, S., Aladakatti, R., 2017. *In vitro* antioxidant and anticancer activity of *Leea indica* leaf extracts on human prostate cancer cell lines. Integr. Med. Res. 6, 79-87.
Goli, P.P., Torbati, M.B., Parivar, K., Khiavi, A.A., Yousefi, M., 2021. Preparation and evaluation of gemcitabin and cisplatin-entrapped Folate-PEGylated liposomes as targeting co-drug delivery system in cancer therapy. J. Drug Deliv. Sci. Technol. 65, 102756.
González-Castelazo, F., Soria-Jasso, L.E., Torre-Villalvazo, I., Cariño-Cortés, R., Muñoz-Pérez, V.M., Ortiz, M.I., Fernández-Martínez, E., 2023. Plants of the Rubiaceae family with effect on metabolic syndrome: Constituents, pharmacology, and molecular targets. Plants 12, 3583.
Huysmans, S., Dessein, S., Smets, E., Robbrecht, E., 2003. Pollen morphology of NW European representatives confirms monophyly of Rubieae (Rubiaceae). Rev. Palaeobot. Palynol. 127, 219-240.
Irobi, O.N., Daramola, S.O., 1994. Bactericidal properties of crude extracts of *Mitracarpus villosus*. J. Ethnopharmacol. 42, 39-43.
Karakas, F.P., Turker, A.U., Karakas, A., Mshvildadze, V., Pichette, A., Legault, J., 2017. *In vitro* cytotoxic, antibacterial, anti-inflammatory and antioxidant activities and phenolic content in wild-grown flowers of common daisy—A medicinal plant. J. Herb. Med. 8, 31-39.
Karou, S.D., Tchacondo, T., Ilboudo, D.P., Simpore, J., 2011. Sub-Saharan Rubiaceae: A review of their traditional uses, phytochemistry and biological activities. Pak. J. Biol. Sci. 14, 149-169.
Khadayat, K., Marasini, B.P., Gautam, H., Ghaju, S., Parajuli, N., 2020. Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. Clin. Phytosci. 6, 1-8.
Khalil-Moghaddam, S., Aliahmadi, A., Jalilian, N., Tabad, M.A., 2021. The study of antioxidant and cellular toxicity effects of methanol, ethyl acetate, aqueous and *n*-hexane extracts of *symphytum kurdicum* plant. J. Chem. Heal. Risks 11, 105-111.
Khan, M.I., Bouyahya, A., Hachlafi, N.E.L., Menyiy, N. El, Akram, M., Sultana, S., Zengin, G., Ponomareva, L., Shariati, M.A., Ojo, O.A., 2022. Anticancer properties of medicinal plants and their bioactive compounds against breast cancer: A review on recent investigations. Environ. Sci. Pollut. Res. 29, 24411-24444.
Kumar, A., Aswal, S., Chauhan, A., Semwal, R.B., Singh, R., Andola, H.C., Joshi, S.K., Semwal, D.K., 2022. Antidiabetic effect of aqueous-ethanol extract from the aerial parts of *Artemisia roxburghiana*. Nat. Prod. Res. 36, 1300-1305.
Kumar, A., Jaitak, V., 2019. Natural products as multidrug resistance modulators in cancer. Eur. J. Med. Chem. 176, 268-291.
Li, H., Yao, Y., Li, L., 2017. Coumarins as potential antidiabetic agents. J. Pharm. Pharmacol. 69, 1253-1264.
Liu, X., Wang, Y., Alizade, V., Khutsishvili, M., Atha, D., Borris, R.P., Clark, B.R., 2021. Cruciasides C-G, monoterpenoid glycosides from *Cruciata articulata*. Phytochemistry 189, 112821.
Lopez-Corona, A.V., Valencia-Espinosa, I., González-Sánchez, F.A., Sánchez-López, A.L., Garcia-Amezquita, L.E., Garcia-Varela, R., 2022. Antioxidant, anti-inflammatory and cytotoxic activity of phenolic compound family extracted from raspberries (*Rubus idaeus*): A general review. Antioxidants 11, 1192.
Luo, H., Vong, C.T., Chen, H., Gao, Y., Lyu, P., Qiu, L., Zhao, M., Liu, Q., Cheng, Z., Zou, J., 2019. Naturally occurring anti-cancer compounds: Shining from Chinese herbal medicine. Chin. Med. 14, 48.
Mazzola, P.G., Lopes, A.M., Hasmann, F.A., Jozala, A.F., Penna, T.C. V, Magalhaes, P.O., Rangel-Yagui, C.O., Pessoa Jr, A., 2008. Liquid-liquid extraction of biomolecules: An overview and update of the main techniques. J. Chem. Technol. Biotechnol. Int. Res. Process. Environ. Clean Technol. 83, 143-157.
Méril-Mamert, V., Ponce-Mora, A., Sylvestre, M., Lawrence, G., Bejarano, E., Cebrián-Torrejón, G., 2022. Antidiabetic potential of plants from the Caribbean basin. Plants 11, 1360.
Mogana, R., Adhikari, A., Tzar, M.N., Ramliza, R., Wiart, C., 2020. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. BMC Complement. Med. Ther. 20, 55.
Mogole, L., Omwoyo, W., Mtunzi, F., 2020. Phytochemical screening, anti-oxidant activity and α-amylase inhibition study using different extracts of loquat (*Eriobotrya japonica*) leaves. Heliyon 6(8), e04736.
Molnár, J., Engi, H., Hohmann, J., Molnár, P., Deli, J., Wesolowska, O., Michalak, K., Wang, Q., 2010. Reversal of multidrug resistance by natural substances from plants. Curr. Top. Med. Chem. 10, 1757-1768.
Namjoyan, F., Dastjerdi, Z.M., Azemi, M.E., 2015. Alpha amylase inhibition activity of some plants extract of

Teucrium species. Eur. J. Biol. Sci. 7, 26-31.
Ng, C.X., Affendi, M.M., Chong, P.P., Lee, S.H., 2022. The potential of plant-derived extracts and compounds to augment anticancer effects of chemotherapeutic drugs. Nutr. Cancer 74, 3058-3076.
Niño, J., Mosquera, O.M., Correa, Y.M., 2012. Antibacterial and antifungal activities of crude plant extracts from Colombian biodiversity. Rev. Biol. Trop. 60, 1535-1542.
Nyobe, J.C.N., Bikélé, D.M., Fodouop, M.B., Mpondo, E.M., Ndom, J.C., 2020. A new pyrrolidinyl-piperazine alkaloid derivative from *Oxyanthus speciosus* DC.(Rubiaceae). Trends Phytochem. Res. 4, 109-116.
Ouadja, B., Anani, K., Djeri, B., Ameyapoh, Y.O., Karou, D.S., 2018. Evaluation of the phytochemical composition, antimicrobial and anti-radical activities of *Mitracarpus scaber* (Rubiaceae). J. Med. plants Res. 12, 493-499.
Oyedemi, S.O., Oyedemi, B.O., Ijeh, I.I., Ohanyerem, P.E., Coopoosamy, R.M., Aiyegoro, O.A., 2017. Alpha-amylase inhibition and antioxidative capacity of some antidiabetic plants used by the traditional healers in Southeastern Nigeria. Sci. World J. 2017, 3592491.
Ranasinghe, S., Armson, A., Lymbery, A.J., Zahedi, A., Ash, A., 2023. Medicinal plants as a source of antiparasitics: An overview of experimental studies. Pathog. Glob. Health 1-19.
Rasouli, H., Hosseini-Ghazvini, S.M.-B., Adibi, H., Khodarahmi, R., 2017. Differential α-amylase/α-glucosidase inhibitory activities of plant-derived phenolic compounds: A virtual screening perspective for the treatment of obesity and diabetes. Food Funct. 8, 1942-1954.
Sai, K., Thapa, R., Devkota, H.P., Joshi, K.R., 2019. Phytochemical screening, free radical scavenging and α-amylase inhibitory activities of selected medicinal plants from Western Nepal. Medicines 6, 70.
Saifulazmi, N.F., Rohani, E.R., Harun, S., Bunawan, H., Hamezah, H.S., Nor Muhammad, N.A., Azizan, K.A., Ahmed, Q.U., Fakurazi, S., Mediani, A., 2022. A review with updated perspectives on the antiviral potentials of traditional medicinal plants and their prospects in antiviral therapy. Life 12, 1287.
Sak, K., 2014. Cytotoxicity of dietary flavonoids on different human cancer types. Pharmacogn. Rev. 8, 122.
Salim, M., Necattin, T., 2018. A survey on wild plants with ethnobotanical use in the Bahçe and Hasanbeyli districts of Osmaniye, Turkey. GSC Biol. Pharm. Sci. 05, 28-35.
Sartoratto, A., Machado, A.L.M., Delarmelina, C., Figueira, G.M., Duarte, M.C.T., Rehder, V.L.G., 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian J. Microbiol. 35, 275-280.
Sbieh, R., Al-Lahham, S., Jaradat, N., 2022. Antioxidant, antimicrobial and cytotoxic properties of four different extracts derived from the aerial parts of *Chiliadenus iphinoides*. Eur. J. Integr. Med. 54, 102149.
Shah, S.B., Sartaj, L., Ali, F., Shah, S.I.A., Khan, M.T., 2018. Plant extracts are the potential inhibitors of α-amylase: A review. MOJ Bioequiv. Availab. 5, 270-273.
Singh, A.K., 2022. A review on plant extract-based route for synthesis of cobalt nanoparticles: Photocatalytic, electrochemical sensing and antibacterial applications. Curr. Res. Green Sustain. Chem. 100270.
Tava, A., Biazzi, E., Ronga, D., Avato, P., 2020. Identification of the volatile components of *Galium verum* L. and *Cruciata leavipes* Opiz from the Western Italian Alps. Molecules 25.
Tohma, H., Altay, A., Köksal, E., Gören, A.C., Gülçin, İ., 2019. Measurement of anticancer, antidiabetic and anticholinergic properties of sumac (*Rhus coriaria*): Analysis of its phenolic compounds by LC-MS/MS. J. Food Meas. Charact. 13, 1607-1619.
Tomou, E.-M., Papakyriakopoulou, P., Skaltsa, H., Valsami, G., Kadoglou, N.P.E., 2023. Bio-actives from natural products with potential cardioprotective properties: Isolation, identification, and pharmacological actions of apigenin, quercetin, and silibinin. Molecules 28, 2387.
Volm, M., Efferth, T., 2015. Role of P-glycoprotein for Resistance of Tumors to Anticancer Drugs: From bench to Bedside. Resistance to Targeted ABC Transporters in Cancer. pp. 1-26.
Wickramaratne, M.N., Punchihewa, J.C., Wickramaratne, D.B.M., 2016. *In-vitro* alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*. BMC Complement. Altern. Med. 16, 1-5.
Wooster, T.J., Golding, M., Sanguansri, P., 2008. Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir 24, 12758-12765.
Wonkam, A.K.N., Njanpa, C.A.N., Ateba, J.E.T., Fongang, Y.S.F., Awantu, A.F., Bankeu, J.J.K., Chouna, J.R., Boyom, F.F., Sewald, N., Lenta, B.N., 2022. Constituents of the ripe fruits of *Nauclea latifolia* Sm. (Rubiaceae) and their antileishmanial activities. Trends Phytochem. Res. 6(4), 292-300.
Yeshi, K., Turpin, G., Jamtsho, T., Wangchuk, P., 2022. Indigenous uses, phytochemical analysis, and anti-inflammatory properties of Australian tropical medicinal plants. Molecules 27, 3849.
Youmbi, L.M., Makong, Y.S.D., Mbaveng, A.T., Tankeo, S.B., Fotso, G.W., Ndjakou, B.L., Wansi, J.D., Beng, V.P., Sewald, N., Ngadjui, B.T., 2023. Cytotoxicity of the methanol extracts and compounds of *Brucea antidysenterica* (Simaroubaceae) towards multifactorial drug-resistant human cancer cell lines. BMC Complement. Med. Ther. 23, 48.
Yuan, M., Zhang, G., Bai, W., Han, X., Li, C., Bian, S., 2022. The role of bioactive compounds in natural products extracted from plants in cancer treatment and their mechanisms related to anticancer effects. Oxid. Med. Cell. Longev. 2022.
Zhang, J., Chu, W.-C., Li, L.-Z., 2023. Isolation and structure elucidation of antioxidant compounds from stem and root barks of *Daphne giraldii*. J. Asian Nat. Prod. Res. 1-10.
Zieneldien, T., Kim, J., Cao, C., 2022. The multifaceted role of neuroprotective plants in Alzheimer's Disease treatment. Geriatrics 7, 24.