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Original article

Phytochemical profiling of *Salsola tetragona* Delile by LC-HR/MS and investigation of the antioxidant, anti-inflammatory, cytotoxic, antibacterial and anti-SARS-CoV-2 activities

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ABSTRACT

This study aimed to investigate the phytochemical composition and biological activity of *Salsola tetragona* Delile. (Amaranthaceae), a medicinal plant. The study evaluated the antioxidant potential of the crude extract and five fractions of *S. tetragona* using DPPH•, ABTS•+, CUPRAC, and metal chelating assays. The anti-inflammatory activity was determined using a protein denaturation assay, and the antibacterial activity was determined by the Minimum inhibitory concentrations (MICs) for the growth of *Escherichia coli* and *Staphylococcus aureus* strains. The MTT test and an in vitro scratch assay evaluated the effects on cell viability and cell migration. The potential anti-SARS-CoV-2 activity was assessed by analyzing the effects on the interaction between ACE2 and Spike protein. The bioactive compounds present in the plant were identified using LC-HR/MS analysis. The crude hydromethanolic extract (STM) and five fractions of *S. tetragona*, n-hexane (STH), dichloromethane (STD), ethyl acetate (STE), n-butanol (STB), and aqueous (STW) showed significant antioxidant activity in four different tests. In the anti-inflammatory assay, the ethyl acetate fraction exhibited significantly higher activity than Aspirin® (IC_{50} = 13 ± 5 µg/mL). The crude extract and its fractions showed positive antibacterial activity with similar MICs. In the cytotoxicity assay against the breast cancer cell line MCF7, the dichloromethane fractions (STD) were very effective and demonstrated superiority over the other fractions (IC_{50} = 98 µg/mL). Moreover, the potential of the extract and fractions as anti-SARS-CoV-2, the ethyl acetate, and dichloromethane fractions demonstrated important activity in this test. LC-HR/MS analysis identified 16 different phenolic compounds, Eleven of which had not been previously reported in the genus *Salsola*. The results suggest that the extracts of *S. tetragona* have the potential to become new sources for developing plant-based therapies for managing a range of diseases.

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1. Introduction

The *Salsola* L. genus, also known as Russian thistle or Saltwort, is a halophyte plant that belongs to the Amaranthaceae family. It is a large genus consisting of semi-dwarf to dwarf shrubs and woody tree species. The name of the genus comes from the Latin word

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"salsus," which means "salty," referring to the plant's salt-tolerant nature. Salsola is a cosmopolitan group of plants that are distributed and naturalized worldwide. Over 64 species have been reported, which are widespread in arid and semi-arid regions of Central Asia, the Middle East, Africa, and Europe (Hanif et al., 2018; Murshid et al., 2022).

Salsola species have a variety of features that make them a potential forage species in semi-arid to dry settings along sea beaches. These features include extensive seed production and resistance to extreme climatic conditions, including high temperature and extended drought conditions. These plants typically grow on flat, generally dry and/or slightly saline soils, with some species occurring in salt marshes (ElNaggar et al., 2022).

The *Salsola* genus is rich in various classes of Phytoconstituents, such as flavonoids, phenolic compounds, nitrogenous compounds, saponins, triterpenes, sterols, volatile constituents, lignans, coumarins, and cardiac glycosides. Moreover, it shows different biological activities, including Alzheimer, antihypertensive, antioxidant, antidepressant, anti-analgesic, anti-inflammatory, antiviral, antibacterial, anticancer, cardioprotective, and hepatoprotective activities. The genus *Salsola* is frequently overlooked, and few people are aware of its significance. The majority of studies focus on pollen morphology and species identification, while little research has looked at its phytochemical makeup or biological effects(Ahmad et al., 2008; Tundis et al., 2008; Munir et al., 2014).

Salsola tetragona Delile is native to the deserts of Algeria, Tunisia, Morocco, Libya, and Mauritania (Ozenda, 1977). In Algeria, this species is relatively common in the Northern and Western Sahara sectors. The plant grows in stony and loamy saline desert pastures, sebkhas, and slightly saline steppes in desert regions; this specie is well-adapted to survive in harsh desert environments and has been previously used in traditional medicine in these regions (Chehma, 2006). The leaves and aerial parts of *S. tetragona* have a significant role in conventional medicine. In southeastern Algeria, it treats indigestion, constipation, abdominal and gastric pain, hypertension, kidney disease, and diabetes (Lakhdari et al., 2016). The powder or decoction of the leaves of *S. tetragona* is used to alleviate gastrointestinal pains, gastric pains, intestinal worms, microbial infections, cancer, and arrhythmia (Daoud et al., 2016; Ghourri et al., 2012). In Morocco, *S. tetragona* is used to treat diabetes, and the parts used for this purpose include the plant's leaves, roots, bark, and fruit. These remedies are often administered in powder form or decoction (Ghourri et al., 2013).

Considering the potential of this genus, it is important to reveal the phytochemical characters of *S. tetragona*. The purpose of this work is to quantify the phenolic contents and identify the main phenolic compounds present in the crude extract of the aerial part using LC-HR/MS analysis. The unexplored biological activity of the extract, together with its fractions will be evaluated for antioxidant, anti-inflammatory, and anti-cancer potentials.

2. Materials and methods

2.1. Plant material collection

The aerial parts of *S. tetragona* Delile, Amaranthaceae, were collected in May 2020 from the El-Magrane region (El-Oued, southeast Algeria 33°48′39″ N 6°55′19.5″ E). This plant was identified by Dr. Youcef Halis (Director of the Technical Research Center on Arid Regions, Algeria). A voucher specimen (LOST.St07/09) is kept in the herbarium of the Faculty of Life and Natural Sciences, El-Oued University.The plant was washed well and parched at room temperature for 20 days, in conditions away from moisture, light, dust, and dirt, with adequate ventilation. After drying, it was crushed, and the powder was stored in a closed glass container.

2.2. Preparation of the extract and fractionation of plant material

The aerial part of *S. tetragona* (200 g) was macerated in a hydroalcoholic solution of 600 mL Methanol / Water (80: 20: v / v) for 24 h at room temperature. This operation was repeated three times with renewal solvent every 24 h. At a later stage, the hydroalcoholic extract has been filtered and concentrated in Rotary Evaporator at a temperature equal to 40 °C in order to obtain the crude dried extract (10%).

The crude extract was dissolved in distilled water, filtered, and subjected to fractionation using different solvents of increasing polarity, starting with n-hexane (STH) (600 mL × 3), dichloromethane (STD) (600 mL × 3), ethyl acetate (STE) (600 mL × 3) and then n-butanol (STB) (600 mL × 3). All fractions including the aqueous phase were dried by rotavapor at 38–40 °C. The five organic fractions were concentrated to provide dryness to extracts; n-hexane fraction (1.6%) dichloromethane fraction (%1.1) ethyl acetate fraction (0.9%) and butanol fraction (1.2%) and the remaining aqueous fraction (%4.9) and they were stored at 4 °C in the dark, in a refrigerator until use.

2.3. Phytochemical studies

Phytochemical screening was conducted according to reported methods (Matos, 1997). a quantitative analysis of *S. tetragona*, where then detection of phenols, alkaloids, coumarins, mucilage, saponins, tannins, sterols, terpenes, and oils.

2.4. Determination of total phenolics

The total phenolic content (TPC) of the extract from *S. tetragona* and its fractions was determined through the utilization Folin-Ciocalteau method (Li et al., 2007). A 0.2 mL of the sample was combined with 1 mL of 10% Folin-Ciocalteau reagent and allowed to incubate for 5 min. Subsequently, 0.8 mL of a 7.5% Na_2CO_3 solution was introduced into the mixture and left to incubate for a span of 40 min in the obscurity of ambient temperature. The measurement of the absorbance of the mixture was done at 765 nm, and the quantification of total phenolic content was determined in terms of the comparable measure of gallic acid, expressed in micrograms per milligram of extract, employing the equation $y = 0.006x + 0.0007$, where $R^2 = 0.99$.

2.5. Determination of total flavonoid

According to the reported method (Mbaebie et al., 2012), equal volumes of sample and aluminum chloride solution (0.2 %) were mixed. The mixtures were subjected to an hour-long incubation at ambient temperature after thorough agitation. Subsequently, the absorbance was measured at 415 nm, and the quantification of total flavonoid content (TFC) was expressed in micrograms of quercetin equivalents per milligram of dry extract, utilizing the equation $y = 0.005x + 0.0402$, where $R^2 = 0.99$.

2.6. Determination of tannins content

The reported method (Kokoska et al., 2008; Muthukrishnan et al., 2018) were employed to measure the total condensed tannins. In coated test tubes, 0.5 mL of the extracts were combined with 1.5 mL of strong HCl and 3 mL of vanillin reagent (4%, w/v in methanol). The tubes were incubated at 20 °C followed by reading absorption at 500 nm absorption was observed. The TCT amount was expressed in µg of catechin (C) equivalents per mil-

ligram of dry extract. Based on a regression equation of catechin's calibration curve (y = 0.0036x + 0.0249, R^2 = 0.996).

2.7. LC-HR/MS analysis

LC-HR/MS, which stands for liquid chromatography-high resolution mass spectrometry, was used to analyze the phenolic compounds of the hydromethanolic extracts of *S. tetragona*. LC–MS analysis of the 65 phenolic standards was conducted with a Thermo Orbitrap Q-Exactive. A C18 column (150 × 3 mm × 5 μ) was used, maintained at 30 °C. The mobile phases used were water with 1.0% (v/v) formic acid (A) and methanol with 1.0% (v/v) formic acid (B), with a gradient of 50% B (initial–1.0 min), 100% B (1.0–3.0 min), 100% B (3.0–6.0 min), 50% B (6.0–7.0 min), 100% B (7.0–15.0 min), and 10% B (15.1–18.0 min). The flow rate was 0.35 mL/min, and an injection volume of 1 μL was used. MS detection was conducted with a positive and negative ion ESI–MS. The optimized analytical parameters were as follows: Mass Scan Cycles: 100–900 *m*/*z*, Sheath gas flow rate: 45, Aux gas flow rate: 10, Spray voltage (kV): 3.80, Capillary temp. (°C): 320, Aux gas heater temp (°C): 320, S-lens RF level: 50.0.

2.8. In vitro antioxidant activity

2.8.1. DPPH free radical scavenging assay

The putative radical-scavenging activity of *S. tetragona* extract and fractions was evaluated against $DPPH^{\bullet}$ free radicals using the method described by (Jafri et al., 2017). Equal amounts of samples and 0.1 mM of $DPPH^{\bullet}$ solution were blended and placed in the dark for 30 min at ambient temperature. The absorbance of the solutions was determined at a wavelength of 517 nm, α-tocopherol and BHA were used as standards in this assay, and the radical scavenging potential of $DPPH^{\bullet}$ is determined as IC_{50} values calculated according to the below equation:

$$InhibitionPercentage = [1 - (Abs/Abc)] \times 100 \quad (1)$$

Ab_S: absorbance of the sample or standard solution.
Ab_C: absorbance of control solution.

2.8.2. ABTS cation radical scavenging assay

The test was conducted as described earlier (Re et al., 1999), 4 mL of ABTS (7 mM) solution were mixed with 4 mL 2.45 mM $K_2S_2O_8$ solution and stored for 16 h at room temperature. Prior to testing the ABTS solution was concentration with ethanol till the absorbance value at 734 nm came out to be 0.703 ± 0.025. Volumes of 950 μL of $ABTS^{\bullet+}$ solutions were mixed with 50 μL different concentrations of plant samples. Ethanol was used as negative control, while α-tocopherol and BHA were used as standard. The graph of the percentage scavenging effect with sample concentration was used to obtain the IC_{50} values.

$$ABTS^{\cdot+} scavengingpower(\%) = [(Ab_0 - Ab_1)/Ab_0] \times 100 \quad (2)$$

Ab_0: absorbance of $ABTS^{\bullet+}$ solution.
Ab_1: absorbance of $ABTS^{\bullet+}$ standard or extract.

2.8.3. Cupric reducing antioxidant activity (CUPRAC)

The procedure of Apak et al (Apak et al., 2004) was used to determine the antioxidant capacity for reducing cupric ions. The absorbance of the plant extracts was measured and compared with the absorbance of the antioxidant standards BHA and α-tocopherol.

2.8.4. Metal chelating assay

With some modifications, the Ferrin method (Decker and Welch, 1990) was used to measure the extract and fractions ability to chelate and inhibit free Fe^{2+} in the solution. Volumes of 80 μL of different samples concentrations were added to 40 μL (0.2 mM) of ferrous chloride ($FeCl_2$). The reaction began by the addion of 80 μL (0.5 mM) of Ferene iron reagent. At ambient temperature, the mixture was stirred hard for 10 min. The absorbance was measured at 593 nm after the mixture had reached equilibrium. Results were expressed as IC_{50} (μg/mL) using EDTA as the antioxidant standard and calculated by using equation 03:

$$Metalchelatingactivity(\%) = [(Ab_c - Ab_s)/Ab_c] \times 100 \quad (3)$$

Ab_c: control solution absorption.
Ab_s: chelator with a (sample or standard) absorption.

2.9. In vitro anti-inflammatory capacities

The method of Gheraissa et al (Gheraissa et al., 2022) was used with few modifications to explore the anti-inflammatory potential of *S. tetragona* samples *in-vitro*. Human albumin (5%), in a volume of 1 mL, was mixed with 1 mL of different samples concentrations and 20 mL of 1 N HCl. Tubes were incubated at 27 °C for 15 min, then heated in a water bath for 10 min at 70 °C. The absorbance was measured at 660 nm spectrophotometrically after cooling to ambient temperature. Aspirin® was used as a standard. Results were expressed as IC_{50} values calculated as follows equation 04:

$$Percentage\ \ inhibition\ (\%) = [(Ab_c - Ab_s)/\ Ab_c] \times 100 \quad (4)$$

Ab_c: control solution absorption.
Ab_s: sample or standard absorption.

2.10. Antibacterial activity

Escherichia coli (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213) used in the study were obtained from Pharmaceutical Microbiology Department, Bezmialem Vakıf University. A sterile 96-well microplate was used in the antibacterial susceptibility test based on the microdilution method (Matuschek et al., 2022). From Muller Broth 50 μL were added to each well of the microplate. Vancomycin and ciprofloxacin were used as control. Serial dilution of the *S. tetragona* curd extract and its various fractions was performed starting from a concentration of 1000 μg/mL (the dilution concentrations to be obtained are 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8 and 3.9 μg/mL) respectively. A stock solution was prepared by adding Muller broth from the prepared bacterial strains to 1 × 10^8 (CFU/mL) cells per milliliter. 10 μL of the prepared bacterial stock solution was added to all wells and the microplates were incubated at 37℃ for 24 h in a CO_2 environment. At the end of the incubation, the sensitivity expressed as MIC values of the tested extract and fractions were compared with that of vancomycin and ciprofloxacin antibiotics detected manually by the unaided eye.

2.11. Cytotoxic activity

Human healthy skin fibroblasts CCD-1079Sk (CRL-2097™) and human breast cancer cells MCF7 (HTB-22™) were acquired from American Type Culture Collection (ATCC, VA, USA). In addition to 10% fetal bovine serum and 1% penicillin–streptomycin (Gibco, USA), the cancer cell lines were cultured in DMEM-F12 medium. The process of sub-culturing was carried out at intervals of 2–3 days. Before treatment, a total of 10^4 cells/well were seeded and incubated for 24 h in a 96-well. The cells were treated with *S. tetragona* extracts (treatmenti concentrationsi: 0–250 μg/mL or 0–260 μ/mL, according to the solubility) for 24 h, and their cytotoxic activities were analyzed by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay (Mosmann, 1983). The negative control/vehicle control was cell culture medium containing same amount of vehicle (0.1% DMSO). After 24 h treatment, a volume of 20 μL MTT solution (5 mg/mL in PBS) was introduced into each well,

followed by a period of incubation in darkness for 3 h at a temperature of 37 °C, in an environment characterized by 5% CO_2 and 92% humidity. The solution was then carefully removed and the resultant formazan crystals were dissolved within 100 µL of DMSO. The absorbance was subsequently recorded at a wavelength of 590 nm. The determination of cell viability was accomplished through the division of the absorbance values obtained from the experimental groups by those originating from the solvent control cells. IC_{50} values were calculated using the GraphPad Prism program, and the concentration-dependent % of cell viability data was calculated according to the vehicle control samples.

2.12. Cell migration assay

STD and STE extract treatments with concentrations lower than the IC_{50} values were chosen according to the cell viability assay (100 µg/mL and 50 µg/mL for STD; 150 µg/mL and 75 µg/mL for STE). The CytoSelect™ 24-well Wound Healing Assay Kit (Cell Biolabs Inc, USA) was used for evaluating the migration rate of the MCF7 cells. The inserts were placed inside every well of a 24-well plate using sterile forceps, all aligned in the same direction. 500 µL of medium containing $2x10^5$ cells was added to the gaps of the inserts. After 24 h incubation, the inserts were removed, and the images of each well were taken before treatment. After taking the images, STD and STE extract treatments were done (100 µg/mL and 50 µg/mL for STD; 150 µg/mL and 75 µg/mL for STE). After 24 h treatment the images of the same area of the wounds were taken. For calculating the wound healing percentage, the program ImageJ (National Institutes of Health, USA) was used, and a comparison was made between the treatment groups with the vehicle control group. The calculation of the wound healing percentage was done according to the kit manufacturer's protocol.

2.13. Anti-SARS-CoV-2 activity

For evaluating the anti-SARS-CoV-2 activity, the protocol of the SARS-CoV-2 Spike-ACE2 Interaction Inhibitor Screening Assay Kit (Cayman Chemical, USA) was used. A recombinant rabbit Fc-tagged SARS-CoV-2 spike S1 RBD that binds to a plate precoated with a mouse anti-rabbit antibody is used and a recombinant His-tagged ACE2 protein binds to the spike RBD. This complex is detected with an HRP-conjugated anti-His antibody and the absorbance is read at 450 nm. A control is used for competition of the SARS-CoV-2 spike RBD-ACE2 interaction. STD and STE concentrations (0–50 µg-mL) were decided according to the cytotoxicity assay. The activity percentage was calculated according to the given formula:

% Activity = (corrected inhibitor activity) / (corrected 100% initial activity) × 100 (Equation 05).

2.14. Statistical analysis

Quantitative estimates and the antioxidant, anti-inflammatory, antiviral, cytotoxic, and cell migration activities were carried out in triplicates. The results obtained have been included as mean ± SD (standard deviation). One-way ANOVA and the Tukey test were used to assess statistical differences or by the statistical test of student's (*t*-test) to evaluate means differences, and P values < 0.05 were considered significant.

3. Results

3.1. Phytochemical studies

The standard references of phytochemical screening were used to identify the preliminary phytochemicals in *S. tetragona* hydromethanolic extract by color reaction with various reagents (Gul et al., 2017). Table 1 displays the results of analyses for the presence or absence of phytoconstituents. The hydromethanolic extract reacted positively with the reagents identifying the presence of alkaloids, coumarins, mucilage, phenols, saponins, sterols, tannins, and terpenes, with the absence of oils.

3.2. Phenolic profile of S. tetragona crude extract by LC-MS

The hydromethanolic extract of *S. tetragona* was analyzed using LC/HR-MS to identify and estimate phenolic and flavonoid compounds. The analysis revealed the existence of 16 phenolic compounds in *S. tetragona* (See Table 2), 11 of which were detected for the first time in the genus *Salsola*. The dominant compound in the extract was salicylic acid (274 mg/kg extract), followed by ascorbic acid (159.60 mg/kg extract), naringin (33.60 mg/kg extract), gypsogenic acid (16.50 mg/kg extract), dihydrocaffeic acid (15.25 mg/kg extract), and 3′-O-methyl quercetin (13.50 mg/kg extract).

3.3. Total phenolic content (TPC)

The total phenolic content of the *S. tetragona* crude extract and the five fractions was expressed as gallic acid equivalents and is presented in Table 3. The results showed that the total phenolic content of the fractions ranged from 15.65 to 46.55 µg GAE/mg. The crude extract had the highest concentration of polyphenols among the fractions (46.55 ± 0.10 µg GAE/mg), followed by the ethyl acetate fraction (39.76 ± 0.25 µg GAE/mg). The aqueous fraction (15.65 ± 0.17 µg GAE/mg) had the lowest amounts of total phenolic compounds among the five fractions.

Table 1
Preliminary phytochemical screening of *S. tetragona* hydromethanolic extract.

Compounds	Observation
Alkaloids	(+)
Coumarins	(+)
Mucilage	(+)
Phenols	(+)
Tannins	(+)
Saponins	(+)
Sterols and terpenes	(+)
Oils	(-)

The presence of phytochemical compounds is indicated by the plus sign (+), while their absence is indicated by the minus sign (-).

Table 2
Phenolic compounds of the hydromethanolic extract of *S. tetragona* by LC/HR-MS.

Phenolic standards	(mg analyte/ kg extract)	U%
Ascorbic acid	159.60	3.94
Chlorogenic acid	4.30	3.58
(+)-*trans* taxifolin	0.25	3.35
Naringin	33.60	4.20
Hyperoside	4.40	3.46
Ellagic acid	3.60	4.20
Quercitrin	7.10	3.78
Quercetin	0.50	2.95
Salicylic acid	274.00	1.89
Naringenin	3.30	4.20
3′-*O*-Methyl quercetin	13.50	3.58
Caffeic asit phenethyl ester	0.10	3.13
Gypsogenic acid	16.50	3.34
Pyrogallol	6.80	3.74
Dihydrocaffeic acid	15.25	0.86
Chrysoeriol	1.30	2.08

3.4. Total flavonoid content (TFC)

The total flavonoid content of the hydromethanolic extract and its fractions of *S. tetragona* was expressed as quercetin (Q) equivalents (μg/mg) and are presented in Table 3. The ethyl acetate fraction contained the highest amount of flavonoids (15.77 ± 0.09 μg QE/mg), followed by the crude extract (13.17 ± 0.11 μg QE/mg).

3.5. Total condensed tannins content (TCT)

The quantitative content of condensed tannins in the *S. tetragona* extract and its fractions were estimated using catechin (C) and expressed in microgram equivalents per milligram of the extract (Table 3). The n-butanol fraction had the highest concentration of condensed tannins (13.61 ± 0.16 μg CE/mg), followed by the crude extract (11.33 ± 0.43 μg CE/mg). The remaining fractions had similar concentrations of condensed tannins.

Table 3
Values of total polyphenol, flavonoid, and condensed tannins of *S. tetragona* crude extract and its fractions.

Samples	TPC (μg GAE/mg ED)	TFC (μg QE/mg ED)	TCT (μg CE/mg ED)
CE	46.55 ± 0.10[a]	13.17 ± 0.11[b]	11.33 ± 0.43[b]
STH	26.41 ± 0.18[d]	11.17 ± 0.23[c]	3.97 ± 0.41[c]
STD	35.65 ± 0.22[c]	11.57 ± 0.41[c]	3.48 ± 0.22[c]
STE	39.76 ± 0.25[b]	15.77 ± 0.09[a]	3.87 ± 0.08[c]
STB	25.62 ± 0.14[d]	13.24 ± 0.05[b]	13.61 ± 0.16[a]
STW	15.65 ± 0.17[e]	8.04 ± 0.01[d]	2.52 ± 0.14[d]

The values are given as mean ± SD (n = 3). [a-e]Means withi distinct letters in each columni differ substantially ($p < 0.05$). Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

3.6. In vitro antioxidant activity

Table 4 illustrates the antioxidant power of *S. tetragona* extract and its fractions. The results were significant and were expressed by IC_{50} values, where the dichloromethane fraction expressed the highest antioxidant activity in the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ tests, with IC_{50} = 62.54 ± 0.48 μg/mL, IC_{50} = 19.11 ± 0.70 μg/mL respectively, the ethyl acetate fraction was superior in the CUPRAC test, with an IC_{50} of 116.74 ± 2.39 μg/mL, while the hexane fraction was the best in the metal chelating activity, with an IC_{50} = 95.13 ± 0.6 9 μg/mL. The results were compared to α-tocopherol, BHA and EDTA for the metal chelation test. The antioxidant activity with higher IC_{50} values is less potent.

3.7. In vitro anti-inflammatory capacities

The anti-inflammatory activity of *S. tetragona* was evaluated by the albumin denaturation assay at different concentrations. The results are given in terms of IC_{50} (listed in Table 5), which is the concentration that protects 50% of the albumin against denaturation. According to the obtained results, it was found that the crude extract and its fractions had a very high anti-inflammatory activity, where both the ethyl acetate and the aqueous fractions exceeded the positive control Aspirin®, with an IC_{50} value of 13 ± 5 μg/mL, 40 ± 7 μg/mL respectively.

3.8. Antibacterial activity

The activity of the *S. tetragona* extracts as an antibacterial agent against two bacterial strains was investigated using Muller Hinton agar as the experimental medium The results (Table 6) showed that the extracts had a moderate effect on stopping the growth of the tested bacteria. The minimum inhibitory concentrations (MICs) of the curd extract of *S. tetragona* and its fraction were similar against the two types of bacteria studied, except the value contra *Escherichia coli* ATCC-25922, was 125 μg/mL in the crude extract.

Table 4
In vitro antioxidant activity of the different fractions of *S. tetragona*.

		DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)	CUPRAC $A_{0.5}$ (μg/mL)	Metal Chelating IC_{50} (μg/mL)
Samples	CE	478.30 ± 0.66[f]	64.37 ± 0.61 [g]	306.87 ± 5.01[e]	405.79 ± 4.05[c]
	STH	815.12 ± 8.71 [g]	36.58 ± 0.89[f]	479.63 ± 7.42[f]	95.13 ± 0.69[b]
	STD	62.54 ± 0.48[c]	19.11 ± 0.70[b]	203.84 ± 1.35[d]	4624.27 ± 4,10[f]
	STE	79.02 ± 0.83[d]	21.53 ± 0.54[c]	116.74 ± 2.39[b]	3250.19 ± 4.40[e]
	STB	258.39 ± 2.54[e]	31.80 ± 0.42[e]	133.51 ± 2.88[c]	1945.27 ± 4.33[d]
	STW	1449.50 ± 10.02 [h]	104.91 ± 9.23 [h]	1007.75 ± 2.47[g]	3250.19 ± 4.40[e]
Standards	BHA	28.59 ± 0.06[a]	7.23 ± 0.01[a]	24.49 ± 0.19[a]	–
	α-tocopherol	36.35 ± 0.24[b]	27.70 ± 0.28[d]	134.53 ± 0.19[c]	–
	EDTA	–	–	–	26.85 ± 1.50[a]

The values are given as mean ± SD (n = 3). [a-h]Means withi distinct letters in each columni differ substantially ($p < 0.05$). Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction; BHA, Butylated hydroxyanisole.

Table 5
IC_{50} values of albumin denaturation assay of hydromethanolic extract of *S. tetragona* and its fractions.

Samples	CE	STH	STD	STE	STB	STW	Aspirin®
IC_{50} (μg/mL)	636 ± 10[e]	1189 ± 100[f]	1500 ± 150 [g]	13 ± 5[a]	120 ± 20[d]	40 ± 7[b]	94 ± 12[c]

The valuesi arei given asi mean ± SD (n = 3). [a-g]Means withi distinct letters ini each rowi differi substantially ($p < 0.05$). Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

Table 6
S. tetragona extracts antibacterial activity (MICs).

Bacterial strains	MIC(μg/mL)						
	CE	STH	STD	STE	STB	STW	Ampicillin
E.coli ATCC 25922	125	250	250	250	250	250	7.8
S.aureus ATCC 29213	250	250	250	250	250	250	3.9

Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

3.9. Cytotoxic activity

According to the MTT test results (Fig. 1 and Fig. 2), STD and STE were the most active against the cell viability of CCD-1079Sk and MCF7 cell lines, showing cytotoxicity in a concentration-dependent manner. Additionally, the extracts were more potent against the normal cell line when compared to the cancer cell line. STH and STM showed slight inhibitor effects, while STB and STW were not cytotoxic at the studied concentration range. The IC_{50} values can be found in Table 7.

3.10. Cell migration assay

After 24 h treatment with STD, cell migration rate of the MCF7 cells was decreased after treatment with 25 μg/mL and 50 μg/mL significantly (pi < 0.05) compared to the vehicle control group. STE also inhibited cell migration after treatment with 75 μg/mL and 150 μg/mL concentrations, although not significant (Fig. 3).

3.11. Anti-SARS-CoV-2 activity

The interaction percentage of ACE2 and spike protein was decreased significantly ($p < 0.05$) after STD exposure in the highest studied concentrations of STD (25 μg/mL and 50 μg/mL), while STE also had inhibitory activity in all concentrations, although insignificant. The results are given in Fig. 4.

4. Discussion

The current study is the first to identify and characterize the phytochemical constituents of *S. tetragona* and evaluate their biological activity, including their potential as an antioxidant, anti-inflammatory, cytotoxic, and anti-viral.

Polyphenols, flavonoids, and tannins are important secondary metabolites that have been shown to possess various biological activities, such as antioxidant, anti-inflammatory, and anticancer properties (Murshid et al., 2022). The presence and quantity of these compounds in plants can be influenced by several factors, such as genetic makeup, environmental conditions, and plant age (Boulaaba et al., 2019).

By comparing the polyphenols and flavonoids content of the crude extract of *Salsola tetragona*, which is estimated at 46.55 ± 0.10 mg GAE/g ED and 13.17 ± 0.11 mg QE/g ED, respectively, with the content of polyphenols and flavonoids in the plant species belonging to the same genus, it was found that the plant contains an average amount. The plant excels in the quantitative content of polyphenols and flavonoids over the species *Salsola imbricata*, *Salsola kali*, and *Salsola tomentosa*, where the quantitative content of polyphenols was estimated at 2.60 mg GAE/g ED; 6.97 ± 0.1 mg QE/g ED; 31.73 ± 0.09 mg GAE/g ED and flavonoids estimated at 0.571 mg QE/100 g ED; 13.6 ± 0.2 mg GAE/g ED; 4.56 ± 0.2 mg QE/g ED, respectively (Aniss et al., 2014; Mohammadi et al., 2016; Shehab and Abu-Gharbieh, 2014). However, *Salsola cyclophylla* and *Salsola vermiculata* outperformed *S. tetragona*. The quantitative content of polyphenols and flavonoids was as follows (126.6 ± 0.81 mg GAE/g ED; 20.5 ± 1.02 mg RE/g ED) and (135.2 mg GAE/g ED; 18.2 mg QE/g ED). Respectively (Al-Omar et al., 2020; Amin et al., 2022).

Through the results of the LC / HR-MS analysis, it was found that there are different phenolic compounds, five of which were present in *Salsola kali*, *Salsola grandis*, and *Salsola cyclophylla*, namely hyperoside, quercitrin, naringenin, quercetin, and salicylic acid. Quercetin was also found in *Salsola collina* Pall, *Salsola imbricata*, *Salsola nvermiculata*, *Salsola tetrandra*, and *Salsola grandis* (ElNaggar et al., 2022). Additionally, eleven phenolic compounds were detected for the first time in the genus *Salsola*, including ascorbic acid, (+)-trans taxifolin, naringin, ellagic acid, 3′-O-methyl quercetin, caffeic acid phenethyl ester (CAPE), gypsogenic acid, pyrogallol, dihydrocaffeic acid, and chrysoeriol.

The major phenolic compounds of the crude extract of *S. tetragona* were identified using LC-HR/MS analysis as salicylic acid (274.00 mg/kg extract), ascorbic acid (159.60 mg/kg extract), and naringin (33.60 mg/kg extract). The results demonstrated a significant relationship between the quantity and quality of these phenolic compounds and their biological activity. This suggests that the effectiveness of the crude extract and its fractions in the studied assays may be influenced by the presence and concentration of these compounds (Chemsa et al., 2018). However, it is important to note that the biological activity of these compounds is not solely dependent on their polyphenolic nature but rather may also be influenced by their specific structures, functional groups, and potential synergistic effects among compounds (Ahmed et al., 2012).

According to the results of the DPPH scavenging test, the crude extract showed weak activity when compared to the standard compounds BHA and α-tocopherol. The IC_{50} value of the crude extract was 478.30 ± 0.66 μg/mL, which was better than the activity value achieved by the hydroethanolic extract of *S. cyclophylla* according to the study conducted by Mohammed et al. (2021), which recorded a scavenging ability with an IC_{50} value of 1350 ± 0.16 μg/mL. While it was lower than the value of DPPH anti-free radical activity achieved by the methanolic extract of *S. villosa* IC_{50} = 290.7 μg/mL according to the study conducted by (Amin et al., 2022). However, some fractins of *S. tetragona* extract dichloromethane (STD) and ethyl acetate (STE) recorded significant antioxidant activity with IC_{50} values of 62.54 ± 0.48 μg/mL and 79.02 ± 0.83 μg/mL, respectively. And by comparing the results of ABTS•+ free radical scavenging, it was found that the extracts of *S. tetragona* plant possess strong activity, as the crude extract recorded CE (EC_{50} = 64.37 ± 0.61 μg /mL), outperforming the activity recorded by the plant *Salsola imbricata* (EC_{50} = 137.7 ± 1.2 mg/mL and *Salsola kali* (EC_{50} = 457.66 μg/mL)(Ajaib and Shah, 2023). For the metal Chelating assay, the reductive activity of the crude extract *S. tetragona* (IC_{50} = 405.79 ± 4.05 μg/mL). Better than *Salsola nitraria* (IC_{50} = 4450 ± 0.00 μg/mL) and *Salsola halophila* (IC_{50} = 2 840 ± 0.00 μg/mL) both studied by (Şirin and Aslım, 2018).

The capacity of DPPH free radical scavenging by the crude extract and fractions related to the presence of antioxidant molecules such as ascorbic acid, quercetin, chlorogenic acid, naringin, ellagic acid, and others. These compounds can neutralize the free

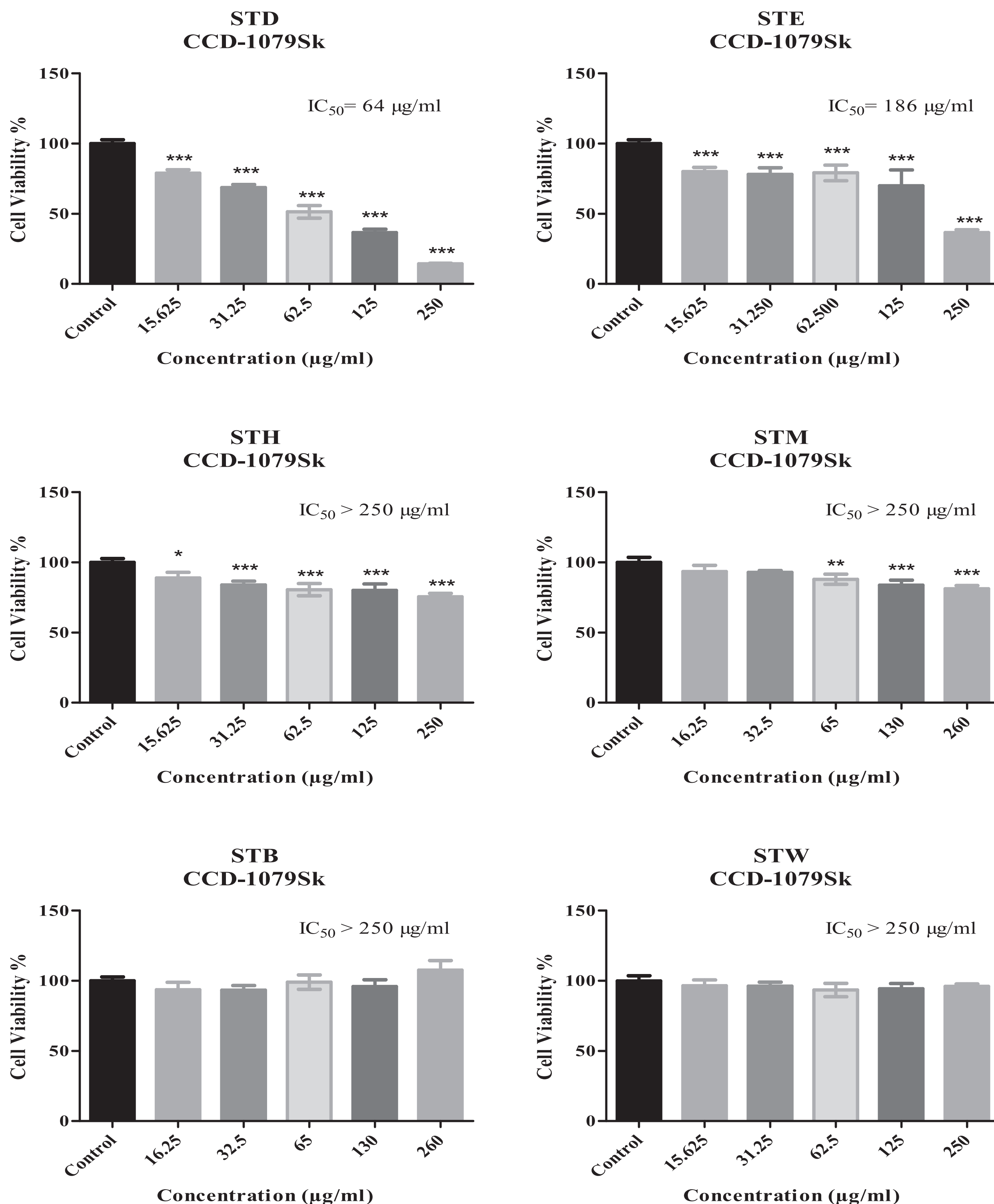

Fig. 1. MTT assay results affect STD, STE, STH, STM, STB, and STW on CCD-1079Sk cell viability following 240 h treatment. Treatment concentrations: 0–250 µg/mL, 0–260 µg/mL. Data are expressed as mean = SD, *p < 0.050, **p < 0.010, ***p < 0.0010 versus the control group. Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona n*-butanol fraction; STW, *S. tetragona* remaining aqueous fraction; MTT, 3-(4,5-Dimethylthiazoli-2-yl)-2,5-Diphenyltetrazoliumi Bromide.

radical by giving hydrogen (Oluwole et al., 2022). The radical's reactivity capacity is attributed to the type and amount of these phenolic compounds and their ability to scavenge DPPH•, an important indicator of the extract's ability to prevent the formation of ABTS•+. This appeared when the extract's power was very high in removing the ABTS•+ radical (Gulcin et al., 2004). So, at higher concentrations, the plant extracts may help treat pathological damage caused by free radicals. The ability of metal ions to form chelates is a vital antioxidant mechanism as it reduces the concentration of transition metals, which are known to accelerate lipid peroxidation (Prior et al., 2005). Chelating agents can disrupt ferrozine-Fe^{+2} complexes, resulting in a decrease in the red color of the complex.

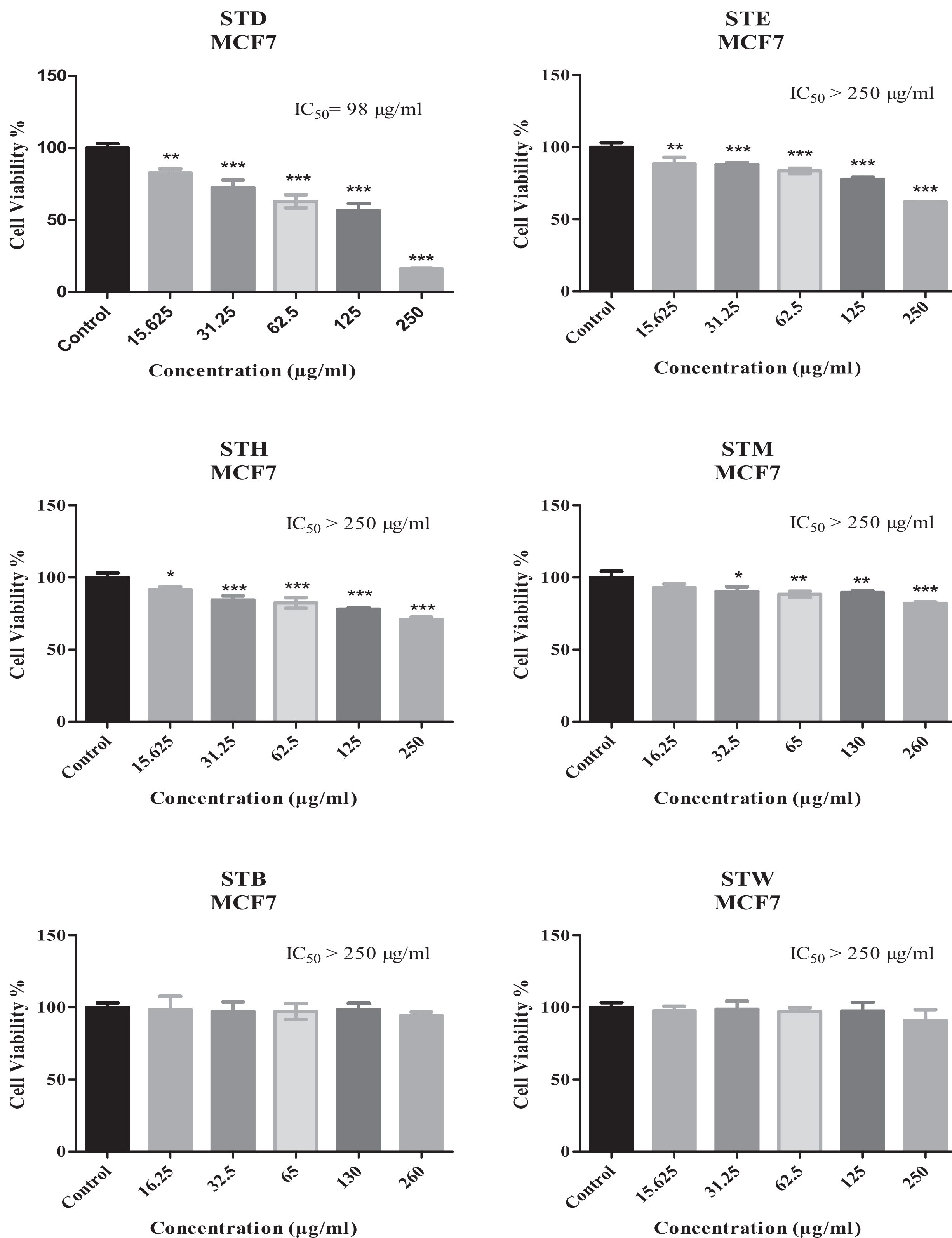

Fig. 2. MTT assay results affect STD, STE, STH, STM, STB, and STW on MCF7 celli viability following 240 h treatment. Treatment concentrations: 0–250 µg/mL, 0–260 µg/mL. Data are expressed as mean = SD, *p < 0.050, **p < 0.010, ***p < 0.0010 versus the control group. MTTi: (3-(4,5-Dimethylthiazoli-2-yl)-2,5-Diphenyltetrazoliumi Bromide). Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

The *n*-hexane fraction of *S. tetragona* (STH) exhibited high metal chelating activity, which varied among the other fractions. The CUPRAC assay is based on the reduction of an oxidant through electron transfer, which can be observed as a change in color (Lekouaghet et al., 2020). In this particular test, the extracts of *S. tetragona* showed significant antioxidant action.

Table 7
IC_{50} values of *S. tetragona* extracts estimated in the MTT cytotoxicity assay.

Cell Lines	STB	STD	STE	STH	STM	STW
MCF7	> 250 μg/mL	**98 μg/mL**	> 250 μg/mL	> 250 μg/mL	> 250 μg/mL	> 250 μg/mL
CCD-1079Sk	> 250 μg/mL	**64 μg/mL**	**186 μg/mL**	> 250 μg/mL	> 250 μg/mL	> 250 μg/mL

Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

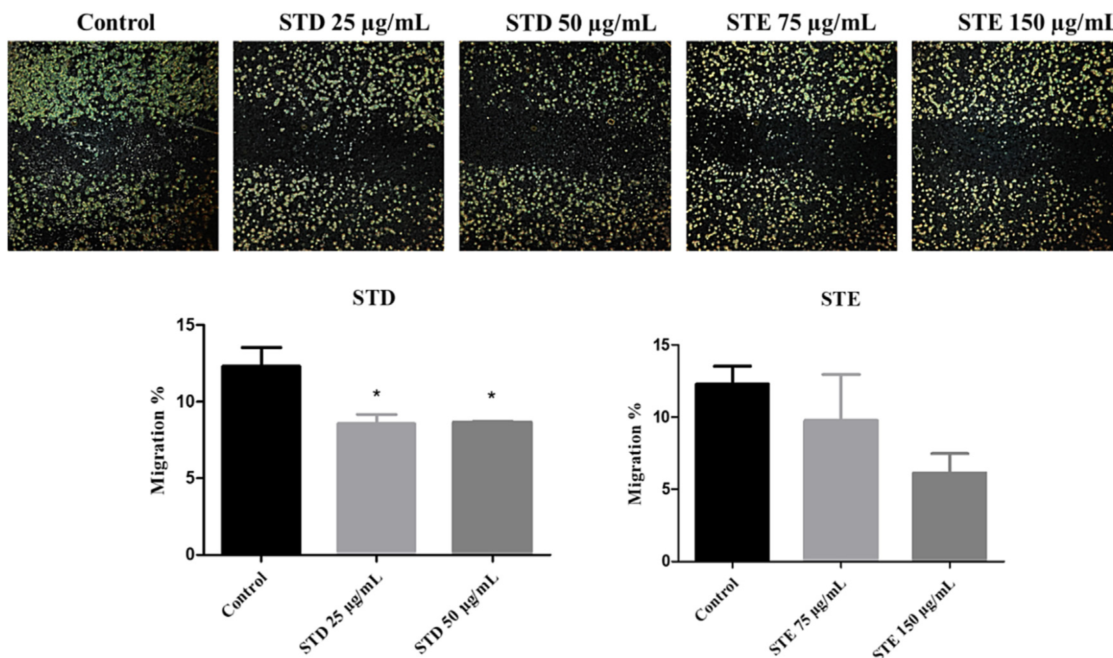

Fig. 3. Effects of STD and STE on cell migration of MCF7 cells. Treatment concentrations for STD: 250 μg/mL and 500 μg/mL. Treatment concentrations for STE: 75 μg/mL and 150 μg/mL. Data are expressed as mean = SD, *p < 0.05 versus the control group. Abbreviations: STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction.

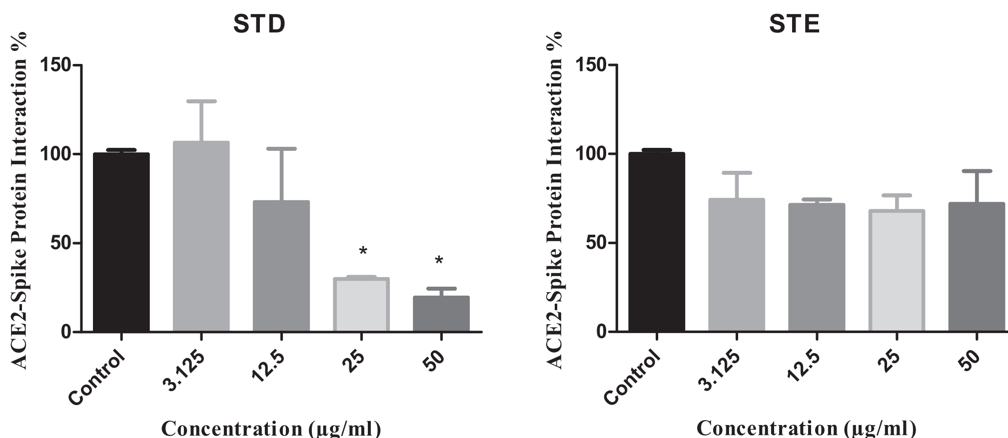

Fig. 4. Effects of STD and STE on ACE2-Spike Protein Interaction. Treatment concentrations: 0–50 μg/mL. Data are expressed as mean = SD, *p < 0.05 versus the control group. Abbreviations**:** STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction.

Protein denaturation is the process whereby proteins undergo structural unraveling of their secondary and tertiary conformations as a consequence of external perturbation, such as heat, acidity, basicity, concentrated inorganic salt, or organic solvent (Anoop and Bindu, 2015). This process is a major contributor to inflammation, as it occurs in various inflammatory diseases (Osman et al., 2016). Protein denaturation is caused by the disruption of electrostatic, hydrophobic, disulfide, and hydrogen bonds that maintain the three-dimensional structure of proteins (Chirisa and Mukanganyama, 2016). Some phenolic compounds, such as flavonoids and phenolic acids, have been shown to bind to plasma proteins and protect these bonds from being broken (Kurlbaum and Hogger, 2011). This may explain the potent anti-inflammatory activity of various fractions and the crude hydromethanolic extract

of *S. tetragona*, which contains bioflavonoids such as chlorogenic acid, quercetin, and naringenin (Chen and Wu, 2014; Oluwole et al., 2022).

For the antibacterial activity of *S. tetragona* fractions were evaluated against *Staphylococcus aureus* and *Escherichia coli*, two bacteria commonly responsible for superficial and systemic infections. The results demonstrated that the fractions exhibited substantial efficacy in suppressing the proliferation of both gram-positive and gram-negative bacteria, demonstrating comparable inhibitory potential. The antibacterial activity of the crude extract and of the fractions of *S. tetragona*, which was very close and most of its MICs values were equal to 250 µg/mL, was much better than that of its close relatives of the same genus such as *Salsola vermiculata*, which recorded an MIC value of 85 ± 2.2 mg/mL in *Escherichia coli* and did not show efficacy with *Staphylococcus aureus* or *S. cyclophylla*, whose MICs value for *Escherichia coli* and *Staphylococcus aureus* was 79 ± 5.1 mg/mL and 75 ± 3.5 mg/mL, respectively (Mohammed et al., 2019). The main reason for the antibacterial activity recorded in *S. tetragona* was attributed to the presence of secondary metabolites belonging to polyphenols (Bouarab-Chibane et al., 2019) and flavonoids (naringenin and quercetin) in these fractions, which can damage the plasma membranes of the tested bacteria (Oluwole et al.., 2022).

The cytotoxicity of *S. tetragona* extracts against human skin cells, and human breast cancer cells show the potential possibility of especially dichloromethane and ethyl acetate fractions (STD and STE) for further anticancer drug development investigation along with the anti-migration effects of the extracts. Although the extracts were more potent against the normal skin cells when compared to the cancer cells, further experiments should be done in other cell lines to conclude their precise cytotoxic effects. Since the total phenolic content (TPC) of STD and STE was observed to be higher compared to the other fractions, this may be the underlying reason for their higher potency. Phenolic compounds are known for their therapeutic effects on serious diseases like cancer. The different fractions are not observed to be toxic to the cells, which suggests they could be further analyzed for various therapeutic areas besides cancer.

Steps for entry of SARS-CoV-2 into host cells include binding of glycoprotein (S) to its receptor, angiotensin-converting enzyme 2 (ACE2), and subsequent membrane fusion. Thus, one strategy to prevent virus entry is to find compounds that can bind to the S protein, preventing membrane adhesion, the interaction between the SARS-CoV-2 protein and angiotensin-converting enzyme 2 (ACE2) (Heleno et al., 2023; Praharaj et al., 2021). From the results shown in Fig. 4, it was found that STD and STE extracts had an inhibitory effect on ACE2-Spike Protein Interaction, where STD extract had better activity than STE. STD was 20% less effective in inhibition tests at a concentration of 50 µg/mL. Also, referring to previous studies, several plant-based compounds have been explored for their potential antiviral activity against SARS-CoV-2 (Islam et al., 2021). Plant secondary metabolites, especially flavonoids, alkaloids, and phenolic acids, showed antiviral activity against several samples of the Coronaviridae family (Campos et al., 2023). It has also been reported through a pre-docking study that 17 of the 18 chemical components of the oils inhibit the binding of ACE-2 to a virus-protein, and these 17 compounds account for 99.4% of all essential oils (Ankri & Mirelman, 1999). In an in-silico study (Arokiyaraj et al., 2020), the researchers suggested that the polyphenols found in Geranii herba may have interactions beyond the active sites of the SARS-CoV-2 protein. These polyphenols can interact with cell surface receptor-regulatory protein 78 (GRP78) to regulate cell signaling, triggering endoplasmic reticulum (ER) stress and other processes. Moreover, it may directly or indirectly interact with Cys/its pair of major proteases, thus inhibiting viral replication. As a result, polyphenols can disrupt the pathogenesis of viral disease at different stages (de Oliveira et al., 2022). DNA-binding alkaloids, such as berberine and tetrandrine, have also been found to inhibit viral replication in cells. Flavonoid glucosides, such as isoquercetin and isoorientin, have been identified in Livistona decipiens extracts as potential inhibitors of SARS-CoV-2 replication. Other studies have investigated the antiviral activities of plant secondary metabolites, including flavonoids, phenols, tannins, terpenoids, proanthocyanidins, lignins, thiosulfonates, steroids, and polysaccharides, against SARS-CoV-2 and other viruses (Aloufi et al., 2022; Behl et al., 2021; El Gizawy et al., 2021; Selim et al., 2022). These results indicate that plant secondary metabolites have the potential to be developed into natural remedies against COVID-19. The results presented in this study provide evidence to suggest that some compounds in *S. tetragona* extracts, particularly chalcone and flavones, have the potential to impair binding between the viral spike protein and its ACE2 receptor. This binding inhibition is a critical mechanism involved in the entry and replication of SARS-CoV-2 viruses. However, further research is needed to evaluate their efficacy and safety in vivo.

5. Conclusion

The results of this study suggest that the fractions of crude hydromethanolic extract of *S. tetragona* have significant in vitro antioxidant, anti-inflammatory, antibacterial, cytotoxic, and anti-SARS-CoV-2 effects. The qualitative and quantitative composition of phenolic, flavonoid, and other chemicals in the fractions varied. The LC/HR-MS analysis of the hydromethanolic extract of *S. tetragona* revealed the presence of several phenolic and flavonoid compounds. Based on these results, the dichloromethane (STD) and ethyl acetate fractions (STE) of the *S. tetragona* can be considered a source of bioactive substances. Further phytochemical analysis of the extract is needed to identify the specific compounds responsible for these biological and pharmacological effects, and to evaluate the in vivo effects of these compounds on the immune system and disease prevention.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.101731.

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