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Anticancer and antibacterial activity for extract and isolation of natural alliospiroside A, from shallot (*Allium cepa* L. Aggregatum group)

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Abstract
In the present investigation, we isolated a steroidal saponin chemical, Cepa2, from the dry shallot roots, obtained from the Kingdom of Saudi Arabia. Using two-dimensional nuclear magnetic resonance (2D NMR) and comparison with data from the literature, the structure of the isolated Cepa2 was clarified. The cytotoxic activity of Cepa2 was then evaluated using a cell counter. Cells were allowed to settle for 24 hours before being treated with an individual concentration of, Cepa2 (3.125, 6.25, 12.5, 50,100ul). Treated cells were allowed to grow further for 48 hours. 20ul of Cell Titer 96® AQ ueous one solution Cell Proliferation Assay (Promega) were added at 37°C with a final concentration of 5 mg/ml following incubation and concentration point. For 2 hours, the 96-well plate was kept in the dark. Using a 96-well plate reader, the optical density of each treatment was calculated at an absorbance of 490 nm (Molecular Devices- SPECTRAmax- PLUS 384). The maximum inhibitory impact on the growth of the colon cancer cells was discovered at the concentration of 100ul while *in vitro* testing of the identified Cepa2 against colon cancer cell line. 48 hours after treatment, Cepa2's anticancer activity was highly effective, as evidenced by a decrease in cell viability. This is a brand-new study demonstrating the anticancer effects of the Cepa2 from the shallot plants, which is novel in terms of the view of drug safety and resistance. Additionally, the antimicrobial activities of the saponin extracts against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus have been reported.

Keywords: *Allium cepa* L, 2D NMR, steroidal saponins extract, anticancer activity, antibacterial activity

Introduction
The term "Therapeutic Plants" refers to a variety of plants used in herbal medicine, some of which have medicinal properties. These plants are regarded as rich sources of materials that can be used to create and produce medications. Some of these plants are walnut, green tea, ginger, and others. The active compounds in aspirin and toothpaste come primarily from other plants and their products. The perennial plant known as shallots, which is widely cultivated and consumed as food throughout the world, is also utilized as a medicinal herb. Plant bulbs have been well-known in traditional medicine to treat the symptoms of various infections. The onion powder is used to treat the initial stages of cough, mucous discharge and sore throat. *Allium ampeloprasum* consists of a range of wild and cultivated plants, in areas ranging from Iran to Portugal and North Africa. Leaves with large onions have long been cultivated by ancient Egyptians, Greeks and Romans. Leaves with large onions and small onions are now grown on all continents around the world. Leek is grown in many European countries, especially in Western Europe, in North America and Australia, rarely elsewhere in the world. *Allium* L. s a rich source of steroidal saponins, and according to the sapogenin it contains, it may be divided into furostanol, spirostanol, and cholestane saponins [1] through [2]. The pharmacological properties of steroidal saponins, such as their antifungal, cytotoxic, anti-inflammatory, antithrombotic, immunomodulatory, and hypocholesterolemic actions, have been covered in a number of papers [3, 4, 5]. The induction of apoptosis by saponins has been established in various investigations, including the prevention of cancer proliferation and migration [6, 7, 8]. Saponins are prospective anticancer drugs. Different human and animal cancer cell lines, including B16 melanoma, 4T1 breast carcinoma, pheochromocytomaPC12, hepatocellular carcinoma HepG2, and fibroblast 3T3-L1 cell lines, were all significantly harmed by steroidal saponins derived from various *Allium* specie. [9, 10, 11, 12]. Saponins isolated from different plants are considered directrix in a number of cancer cell lines. The induction of apoptosis by saponins has been described in various studies [7, 13, 14, 15].

Materials and methods
1. Extraction and isolation of the saponin compound from shallot
In a farm in the Al-Qassim region of the Kingdom of Saudi Arabia, shallot roots were harvested from their natural habitat and a pure saponin compound classified as Cepa2 was purified (refer with Figure.1: a, b). Fresh root–bulb basal stem of *Allium cepa* L. (80 g) was hand-cut and air-dried at room temperature (22 ºC), and the final dry weight (40 g) was gained and used in our study using n-hexane and 70% methanol. Methanol extract was dried in a rotary evaporator with a vacuum pump (v-700; BUCHI, Rotavapor R-3) at 50 ºC and then partitioned between BuOH and H2O (1:1). The BuOH layer was filtered and concentrated under vacuum, giving a saponins crude extract (1.69 g). [3]. In a nutshell, lipids and oils were extracted from dried roots using 100% n-hexane. Defatted roots were sonicated for 30 minutes before being further extracted with 80% methanol (Elmasonic Typ: s30 H made in Germany). The resulting extract was next dried at 50°C in a rotary evaporator (V-700; BUCHI, Rotavapor R-3), and then partitioned (1:1, v/v) between n-butanol and water. The BuOH phase was purified and concentrated to create crude extract of saponin. C300 silica gel column chromatography was used to

chromatograph an aliquot of the crude saponin that had been extracted (3 cm - 60 cm; Germany). Using a gradient solvent system of chloroform, the column was eluted ($CHCl_3$), $CHCl_3$: MeOH (9:1e1:9, v/v), MeOH and MeOH: H2O (9:1e7:4, v/v) to produce 8 fractions. Using silica gel plates for thin-layer chromatography (TLC), each fraction was rechromatographed (60 F254; Merck KgaA, Darmstadt, Germany). the creation of the chromatogram (60 F254; Merck KgaA, Darmstadt, Germany). The chromatogram was generated by $CHCl_3$: MeOH: H_2O (30:15:2.5, v/v/v). 8 mg of the pure Cepa2 chemical, which was produced via fraction 2, was then analyzed using a 2D NMR.

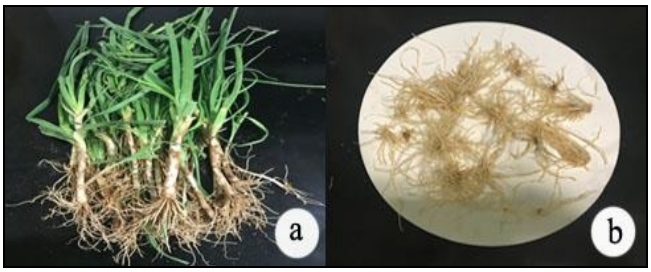

Fig 1: (a) *Allium cepa* L shallot (b) shallot roots *Allium cepaL*

2. Cell line and cell culture

(Central Laboratory of King Saud University, the girl's campus in Riyadh, Saudi Arabia) provided a colon cancer cell line (sw480), which was planted in 96-well plates at a density of 2x105 cells per well in 100 ml of optimized media. They utilized a cell counter to calculate the total number of cells employed across all of the trials using the trypan blue exclusion test (0.4%). Before treatment, cells were given a 24-hour period to settle.

3. Growth inhibition assay

With individual concentration of, i.e. (3.125, 6.25, 12.5,50 and100ul). Treated cells were allowed to grow further for 48 hours. 20ul of Cell Titer 96® AQ ueous one solution Cell Proliferation Assay (Promega) was added at 37°C with a final concentration of 5 mg/ml following the incubation period and concentration point. For 2 hours, the 96-well plate was kept in the dark. Using a 96-well plate reader, the optical density (O.D.) of each treatment was calculated at absorbance 490 nm (Molecular Devices- SPECTRA max-PLUS384), each experiment was performed in three replicates. Optical densities values were normalized according to the control (untreated cells).Therefore, cell viability values of untreated cells should be 100% while values of treated cells have below or above 100%.

4. Antibacterial activity

Agar well diffusion was used to test the crude saponin extract's antimicrobial properties [16]. The sterile petriplates were filled with around 20 mL of sterile nutritional agar (scharlau microbiology 01-140-Germany). Escherichia coli ATCC 25922, Staphlococcus aurous ATCC 25923 and Pseudomonas aeruginosa, ATCC 11778 (clinical isolate) were collected from the King Khalid University Hospital in Riyadh, Saudi Arabia, and were overnight cultured on triplicate plates. A sterile cork borer was used to gently puncture the solid nutritional agar medium in the petri dish plates to create a well (3wells in each plate). Finally, (100 L) were added to each well, and they were incubated at (37° C) for 24 hours. Following incubation, the diameter of the zone of inhibition was measured and quantified in millimeters (mm).

Result

Thesaponin crude extract was initially separated by column chromatography employing a gradient solvent method to isolate the steroidal saponin from shallot root plants, producing a partially purified fraction 2 of about 12 mg. 8 mg of pure saponin were produced after additional TLC purification of this fraction. Results showed that the structure of the isolated Cepa2 was identical to that of alliospiroside A, which was previously found in bulb onion (refer to Figure 2), and those available in published literature and the PubChem database (https://pubchem.ncbi.nlm.nih.gov) [17].

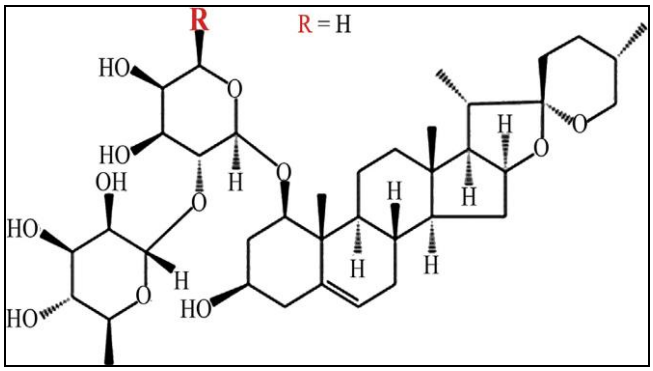

Fig 2: The chemical structure of Cepa2 isolated from shallot (*Allium cepa*L. Aggregatum group).

(Refer with: Table.1), fast-atom bombardment mass spectrometry and confirmed by the NMR data. Peaks in the range of (15.6–102.33) were visible in the Cepa2 aglycone's NMR spectra, (refer with: Table.2). We have been able to infer from the spectral properties the conformation of the carbohydrate rings, the arrangements of the glyosidic bonds, and the connection of the sugar chain to the aglycone. A Spirostanol saponin with two glycoside units is named alliospiroside A.

Table 1: Aglycone and sugar moieties of Cepa2/alliospiroside 13C NMR spectroscopic data from an isolated shallot

Position	Cepa2/alliospiroside A chemical shift (d) ppm
C1	98.5
C 2	46.65
C 3	55.2
C 4	23.6
C 5	102.33
C 6	23.11
C 7	23.54
C 8	70.33
C 9	98.56
C10	33.2
C11	15.60
C12	45.87
C13	39.18
C14	35.88
C 15	22.11
C 16	87.16
C17	43.71
C18	28.22

Table 2: Aglycone and sugar moieties of shallot-derived Cepa2/alliospiroside A were studied using 1H NMR spectroscopy

Protons	Cepa2/alliospiroside A chemical shift (d) ppm
CH3-18	1.4
H- 1	0.65
H- 3	2.5
H- 6	6.1
H-9	1.4
H -16	3.5
H-27	1.7

Antibacterial activity and cytotoxicity

The three different concentrations of Cepa2 showed a weak antibacterial activity against the types of bacteria used in this study (refer to figures. 3and 4). as such, the *in vitro* cytotoxicity effect of different concentrations of Cepa2 against colon cancer cell lines ($_{sw}$480) and cell viability values decreased when concentrations were increased compared to control (refer to figure. 5).

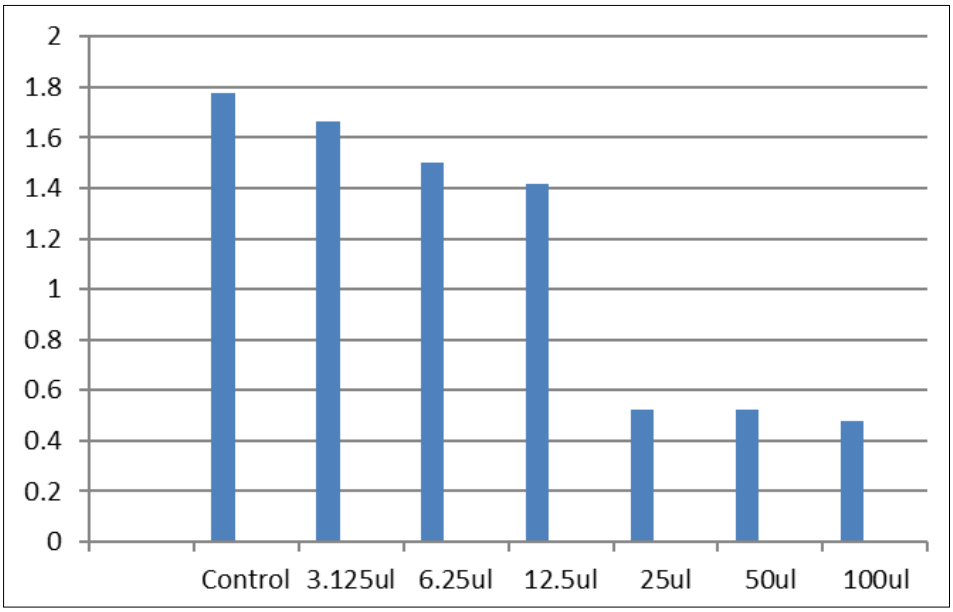

Fig 3: *In vitro* cytotoxic activity of different concentration (3.125,6.25,12.5,50, and 100ul) from Cepa2 against colone cancer cells for 48 h

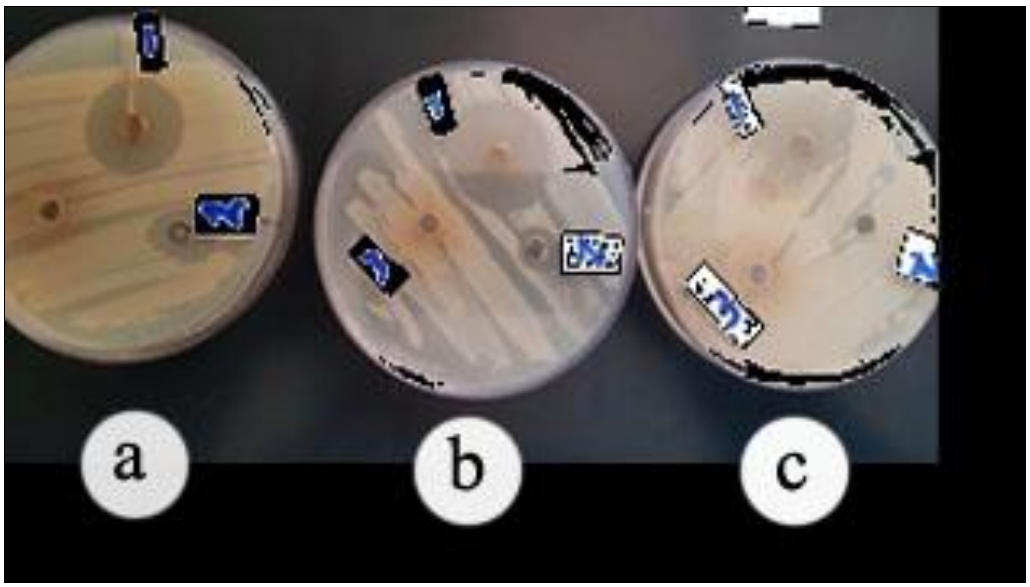

Fig 4: Antibacterial activity of three different concentrations of Cepa2against bacteria Types A) *Escherichia coli* (E. coli), B) *Staphylococcus aurous* and C) *Pseudomonas aeruginosa* for the number 1 indicates the higher concentration of Cepa2, number 2 indicates the average concentration and the number 3to the lowest concentrat

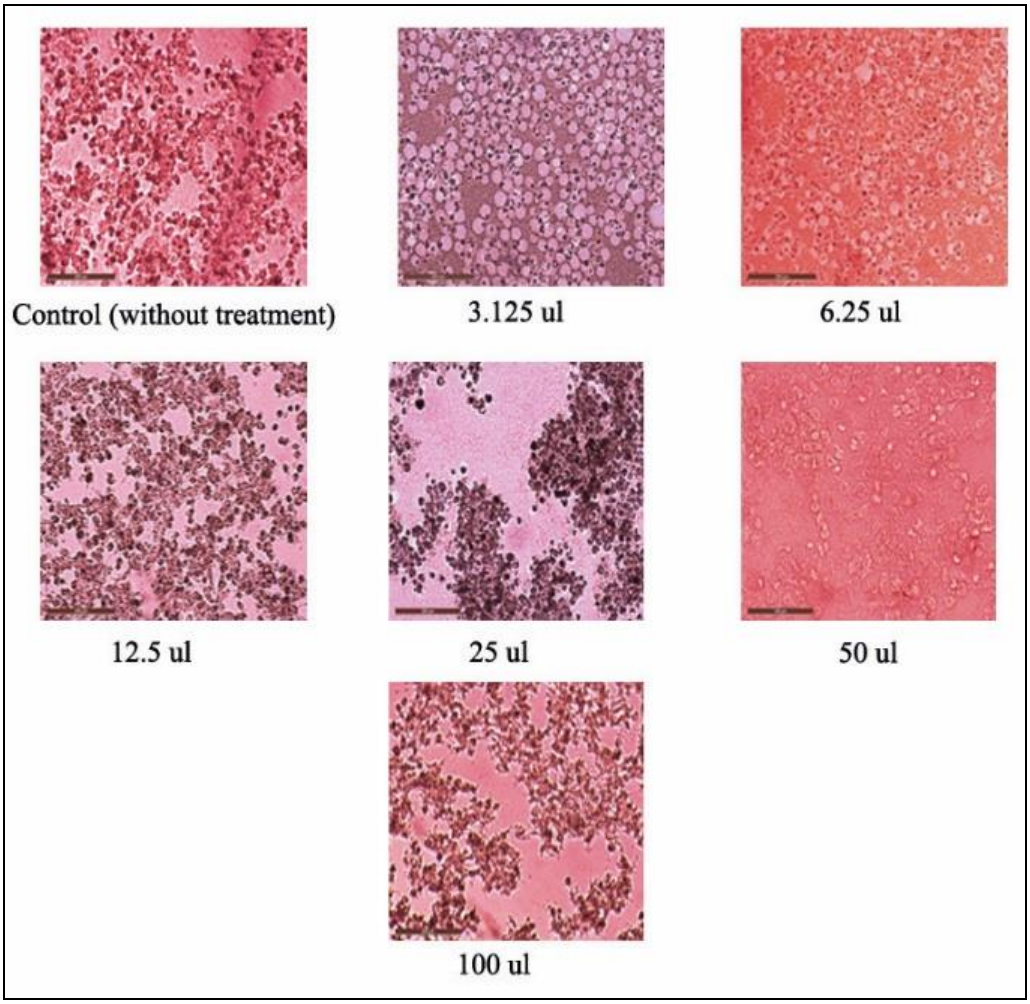

Fig 5: The morphological changes in colone cancer cells after being treated with different concentrations of Cepa2 (original magnification ×200

Discussion

A number of chemicals were obtained using chromatographic analysis of shallot roots that may be highly effective in combating pathogenic microbes. *Alliosiroside* compound resulted from the shallot roots, a compound that has antifungal activity as in the [18] but that this compound has anti-cancer properties still needs more specialized studies. The substance Cepa2 / alliospiroside A which was obtained also through chromatographic analysis has an effect on the Colone cancer cell line Modification factors of cytotoxic properties of the effect of saponins which change the structure of the polysaccharide chain. Shallots were found to be a source of antibodies linked to alcoholic scavengers with toxic capabilities against colorectal cancer cells in the current investigation. This is in line with research on [11]. Additionally, saponins have been said to have antibacterial properties [19]. However, only at low cell densities did three 5-spirostan-3-ol saponins after butanol extraction exhibit antibacterial efficacy against both prokaryotic and eukaryotic species. A number of chemicals were obtained using chromatographic analysis of shallot roots, which may be highly effective in combating pathogenic microbes. Alliosiroside compound resulted from the shallot roots, a compound that has antifungal activity as in the [18] but that this compound has anti-cancer properties still needs more specialized studies. The substance Cepa2 / alliospiroside A which was obtained also through chromatographic analysis has an effect on the Colone cancer cell line Modification factors of cytotoxic properties of the effect of saponins which change the structure of the polysaccharide chain. In the current study, shallots were identified as a source of antibodies associated with alcoholic scavengers with toxic properties against colone cancer cells. This is consistent with the study of [11]. Saponins have also been reported to have antimicrobial activity [19]. Three butanol-extractable 5-spirostan-3-ol saponins were shown to have antimicrobial activity on both prokaryotic and eukaryotic organisms, but only at low cell densities. The saponins did not inhibit microbial growth of dense populations. All four of the isolated saponins showed weak antibacterial activity. Results indicated that the tetraglycosidesaponins have stronger activity than the triglycosidesaponins. Hedyotis nudicaulis Wight and Arn (Rubiaceae) produced three new triterpenoidsaponins, Nudicaucins A, B, and C, as well as a known saponinguaiacin D, which were tested against Bacillus subtilis [20]. The four isolated saponins all had negligible antibacterial action. According to the findings, tetraglycosidesaponins have greater activity than triglycosidesaponins. Newly discovered jujubogeninsaponin from Colubrinaretusa L. (Rhamnaceae), jujubogenin 3-O--l-arabinofuranosyl-(1→2)-[3-O-(trans)-p-coumaroyl--d-glucopyranosyl-(1→3)] --l- arabinopyranoside, had antimycobacterial activity when tested against Mycobacterium intracellulare [19]. The jujubogen in saponin had antimycobacterial activity at a MIC of 10 g/ml. [21]

isolated three new furostanolsaponins along with seven known saponins from the seeds of *Capsicum annuum* L. var. acuminatum Fingerh. (Solanaceae). Gram-positive and Gram-negative bacteria were both subject to mild and no growth inhibition by the saponins (MIC >1000g/ml).

Conclusion

Our research has identified the natural chemical Cepa2/alliospiroside A and its efficient anti-cancer and anti-bacterial characteristics. The research focuses on a saponin natural chemical that plays a critical function in infection defense. This study is one of the most recent ones on the extraction of this natural component, as well as investigations on its use in different therapeutic contexts, such as as an antifungal.

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