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Exploring *in vitro* Biological Activities of *Calligonum Comosum*: Antibacterial and Anticancer Therapeutics

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Abstract: The study investigated the phytochemical composition, antimicrobial activity, and anticancer potential of *Calligonum comosum*, a desert shrub found in Egypt's coastal regions. Phytochemical analysis revealed the presence of saponins, tannins, phenols, flavonoids, and alkaloids in *C. comosum* extracts. The methanolic extracts showed antibacterial activity against several bacterial strains like *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus cereus* with zones of inhibition ranging from 15-22 mm. The extracts exhibited dose-dependent cytotoxic effects against the Hepatocellular carcinoma (HePG-2) cell line, with 67.63% inhibition at 100 µg/mL concentration. The IC_{50} value against HePG-2 cells was 58.05 µg/mL. Normal WI-38 cells were less affected, with only 16.67% inhibition at 100 µg/mL, suggesting selective cytotoxicity towards cancer cells. In summary, this study highlights the therapeutic potential of the desert plant *C. comosum* as a source of antibacterial and anticancer agents, providing a basis for further research into its bioactive constituents.

keywords: *Calligonum comosum*; Coastal Desert; Antimicrobial, Phytochemical, Antitomer.

1.Introduction

Scientists have extensively investigated the utilization of natural products in drug development, and these naturally derived substances are commonly employed in cancer research. Plants have a crucial role in the field of medicine since they provide herbal compounds that may be used to cure diseases. These compounds can be used either on their own or in combination with other compounds [1,2].

Traditional medicinal systems around the world have long made use of compounds produced from plants to treat a wide range of illnesses [3]. Research into the features, antioxidant, antibacterial, and anticancer capabilities of functional components extracted from plants is an ongoing effort by scientists. Kondhare and Lade [4] note that finding modern scientific evidence to support the traditional therapy approach is another critical topic that requires extensive examination.

Approximately 50% of the medications now used in healthcare are derived from natural sources, highlighting the substantial role that natural products play in advancing the pharmaceutical business. In order to encourage the use of plant materials as possible sources of antibacterial activities, it is crucial to thoroughly evaluate their composition and biological activity before using them [4,6]. The emergence of antibiotic-resistant microorganisms has spurred the development of novel antibacterial treatments. The increase in the occurrence of multiple drug-resistant bacteria may be linked to the careless use or insufficient control of antibiotics, resulting in a reduced effectiveness of some antibiotics against certain microorganisms [7,8].

Calligonum comosum (Arta plant, family Polygonaceae) is a small shrub with woody and rigid branches, usually 4 to 6 feet high but occasionally may reach even 10 feet in height with a girth of 1 to 2 feet [9]. It commonly grows on dry sandy soils and on sand dunes. It is very hardy and being capable of growing under adverse conditions of soil and moisture. It produces root suckers and is easily propagated by cutting and layering [10].

Previous investigation of genus *Calligonum* revealed that several plants of this genus are used as antidiabetic, antibacterial, anticancer and antirheumatic. Kamil *et al.* [11] studied the pharmacognostic and phytochemical standardization of *C. comosum*. Heneidy and Bidak [12] reported that *C. cosmosum* has medicinal value, used as fuel and used as forage.

The aim of this research was to analyze the botanical specimen *Calligonum comosum*, obtained from the Mediterranean coastal desert, in order to determine its chemical composition and assess its antibacterial and anticancer characteristics.

2. Materials and Methods

2.1. Plant material

A group of healthy *Calligonum comosum* specimens were collected in May 2023 from sand formations in the northern Nile Delta region of Egypt (30°7'21.73"N, 31°21'44.68"E). References such as Tackholm [13] and Boulos [9] were used extensively during the process of plant identification. After being manually cleaned, the specimen was rinsed three times with distilled water to eliminate any remaining pollutants and particle matter. After that, the sample was left to air-dry in a shaded area for a few days at room temperature (25 ± 3 °C) until full drying had occurred. The dried specimen was then ground into a fine powder by pulverization. The next step was to carefully place the specimens into paper bags and store them at room temperature, away from direct light, until the analysis was ready.

2.2. Extraction

Conventional solvent extraction is used to extract the therapeutic components of plants [14]. Extraction refers to the procedure of separating water-soluble constituents of plants, while retaining the insoluble cellular residues. A total of 50 grams of dried plant parts were immersed in a 75% methanol solution at room temperature for a duration of three days [14]. The cell walls are delicately disintegrated and shattered to release water-soluble phytochemicals. Once three days have elapsed, the solution that has been filtered is now prepared for use. Traditional techniques depend on the transfer of heat by convection and conduction, as well as the selection of solvents for extracting samples [15]. Once the extracts underwent filtration, evaporation, and dissolution in DMSO, they were suitable for use.

2.3. Phytochemical constituents

The methods used in this investigation to evaluate the levels of tannin, saponin, flavonoid, alkaloid, and total phenol concentrations were the ones outlined by Sadasiveam and Manieckam [16], Harborne [17], Bohim and Kocipai-Abyazian [18], and Obadonei and Ochuiko [19].

2.4. Antibacterial activity

The antibacterial activity of the investigated substances was measured using a modified Kirby-Bauer disc diffusion technique. To conduct the antibacterial assay, sterilized filter paper discs with a diameter of five milliliters were immersed in plant extracts overnight and loaded over nutrient agar medium inoculated with pathogenic microorganisms [20]. A filter disc impregnated with 10 µl of solvent (DMSO) served as the negative control. The Petri plates were incubated at 37°C for 24 hours, and the inhibitory zone diameter (mm) was determined.

The different extracts of three selected xerophytes were tested against four-Gram negative bacteria: *Klebsiella pneumoniae* (ATCC10031), *Pseudomonas aeruginosa* (BAP19128), *Escherichia coli* (ATCC10536) and *Salmonella typhi* (ATCC25566) and three-Gram positive bacteria; *Bacillus subtilis* (ATCC6538), *Bacillus cereus* (AZR58453), and *Staphylococcus aureus* (ATCC6312).

2.5. Cytotoxic activity

The cytotoxic activity of *Calligonum comosum* extract was assessed using an MTT colorimetric test developed by Terblanche *et al*. [21]. The cell strains were cultured in RPMI-1640 media containing 10% fetal bovine serum. Antibiotics, penicillin (100 units/mL) and streptomycin (100 µg/mL), were added at 37°C in a 5% CO_2 incubator. Cell lines were sown in a 96-well plate at a density of 1.0×10^4 cells/well and incubated at 37°C for 48 hours with 5% CO_2. Following incubation, cells were treated with varying doses of the tested materials and incubated for another 24 hours.

After 24 hours of medication treatment, MTT solution (5 mg/mL, 20 µL) was added and incubated for an additional 4 hours. DMSO (100 µL) was applied to each well to dissolve the violet formazan. The colorimetric examination was done, with absorbance values obtained at 570 nm using a plate reader (EXL 800, New York, NY, USA). The IC_{50} values were determined using nonlinear regression (sigmoid type) and examined using Origin 8.0® software (Origin Lab. Corporation, https://www.originlab.com/ (accessed on March 19, 2021). Equation was used to compute inhibition percentage as well as relative cell viability in percent.

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

3. Results and Discussion

3.1. Phytochemical analysis

Phytochemistry, a field concerned with the chemical composition of plants and their many constituents, is widely acknowledged as an early subdivision of organic chemistry. The characterization and discovery of plant-derived compounds with medicinal properties have considerable importance [22]. The detailed evaluation of the analytical results pertaining to *Calligonum comosum* revealed the unique attributes of the investigated plant, along with the diversified assortment of phytoconstituents that exhibited differences across different plant samples.

The examination revealed that the plant under investigation demonstrated a substantial presence of saponins, tannins, phenols, flavonoids, and alkaloids. The medicinal effects of many chemical classes, such as alkaloids, saponins, tannins, anthraquinones, and flavonoids, have been acknowledged for their efficacy against numerous diseases. Thus, these substances have historically been utilized for the management of many medical conditions, as evidenced by the investigations undertaken by Hassan *et al*. [23] and Usman and Osuji [24].

Table 1. Active organic compounds (mg g^{-1} dry wt.) of *Calligonum comosum* collected from the coastal desert.

Plant sample	Active organic compounds				
	Alkaloids	Flavonoids	Phenols	Saponins	Tannins
Calligonum comosum	16.32±0.54	20.41±0.75	25.08±1.02	31.26±1.15	40.84±2.09

3.3. Antimicrobial activity

The methanol extracts of the chosen plant showed significant activity against seven distinct pathogenic bacterial strains at a concentration of 30 µg/mL, as indicated in Table 2, compared to the control. The zone of inhibition (ZOI) created by the extract from the shoots of *Calligonum comosum* in a culture medium that was infected with *Escherichia coli* was measured to be 22.24 mm. This result was determined to be significant when compared to a previous research conducted by Kumar *et al*. [25]. The culture of *Bacillus cereus* exhibited a notable zone of inhibition (ZOI) when treated with an extract derived from *Calligonum comosum* shoot, measuring 18.71 mm in diameter. The findings we obtained showed comparable activity (15.32 mm) to those published by Kumar *et al.* [25]. On the other hand, when tested against *Klebsiella pneumoniae* and *Staphylococcus aureus*, *Calligonum comosum* produced results that were not positive.

Due to the fact that the shoot extract is likely to have unique phytochemical effects, the results need to be viewed as significant. The extracts of different parts (roots, stems, buds, flowers and seeds) of *C. comosum* revealed that the presence of flavonoids, alkaloids, proteins, tannins, steroids, phenol, carbohydrates and terpenoids [26].

Table 2. Antibacterial activities represented by the inhibition zone diameter (mm) of the MeOH extract of *Calligonum comosum* and standard antibiotics.

Tested organisms	*C. comosum* extract(30 µg/mL)	Standard antibiotic		
		Ampicillin	Cefotaxime	Tetracycline
Escherichia coli	22.24	25.45	14.98	25.09
Klebsiella pneumoniae	0	14.67	14.23	24.87
Pseudomonas aeruginosa	15.01	13.07	12.03	13.66
Salmonella typhi	15.83	0	15.22	16.43
Bacillus cereus	18.71	23.34	27.8	25.66
Bacillus subtilis	16.37	0	22.95	0
Staphylococcus aureus	0	13.23	16.18	22.68

3.3. Cytotoxic activity

A cytotoxicity assay was performed for *Calligonum comosum* shoot extract using the MTT assay in the HePG-2 cancer cell line, while WI-38 cells were used as a control (Figure 1). The tests included seven different doses of plant extract (1.56, 3.125, 6.25, 12.50, 25, 50, and 100 µg mL^{-1}) that were generated by a serial dilution process (Figure 1). The findings indicate that the methanolic extracts of *Calligonum comosum* had dose-dependent cytotoxic activity, similar to that of doxorubicin, which was used as a reference standard. The extracts of the *Calligonum comosum* sample exhibited inhibitory activities of 67.63% and 16.67% against HePG-2 and normal cell (WI-38) respectively, at a concentration of 100 µg mL^{-1}. Nevertheless, the sample with the lowest dosage (1.56 µg mL^{-1}) has the least cytotoxic activity among all the samples (Figure 1). Previous investigation of genus *Calligonum* revealed that several plants of this genus are used as antidiabetic, antibacterial, anticancer and antirheumatic [11,27].

Our results suggest that *Calligonum comosum* shoot extracts are more cytotoxic to cancer cells than normal cells. Next, IC_{50} value of the highest potential extract (*Calligonum comosum*) were observed to be 58.05 and >100 µg/mL against HePG-2 and normal cell (WI-38), respectively. The *in vivo* anticancer properties of numerous medicinal plants have been evaluated using diverse animal models. While plant-based chemicals have shown lower toxicity compared to typical synthetic compounds, there is increasing evidence about the adverse consequences of uncontrolled use of these plants for treating various ailments. The issue is in the lack of adequate evidence pertaining to the quality, safety, and effectiveness of herbal medications [28].

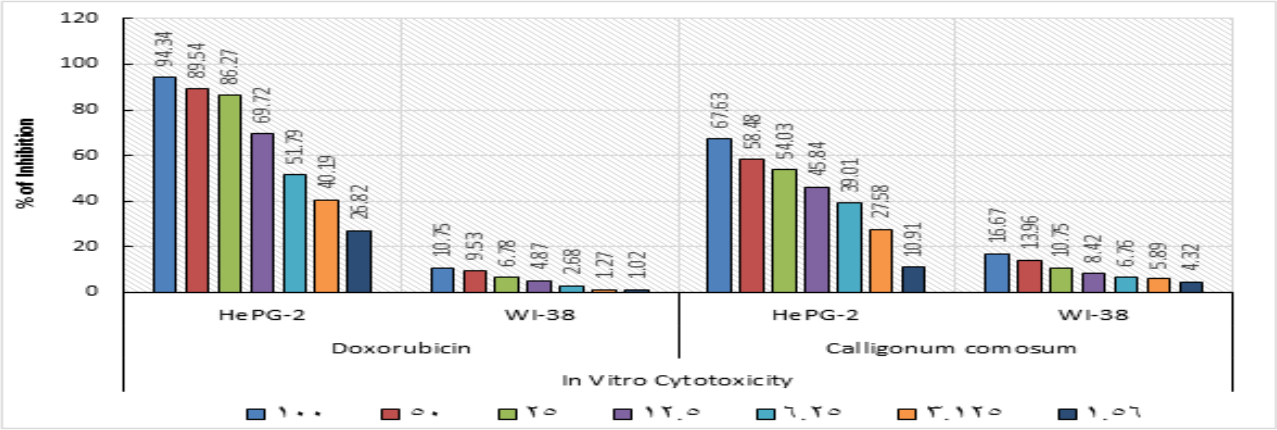

Figure 1. The inhibition percentage of the prepared plant sample against the tumor and normal cells at different concentrations, and doxorubicin as standard. Hepatocellular carcinoma (HePG-2), and normal cell (WI-38).

4. Conclusion

Based on the collective findings of this study, we can deduce that the phytoconstituents present in the extract of *Calligonum comosum* shoots have the ability to function as agents that may combat cancer and microbial infections. Hence, the primary objectives of this research will include the separation and characterization of the chemical compounds found in the extract of *Calligonum comosum*, as well as the investigation of their molecular targets in cancer cells. These aims may be further explored in future studies.

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