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ORIGINAL ARTICLE

Biological activities and ecological aspects of *Limonium pruinosum* (L.) collected from Wadi Hof Eastern Desert, Egypt, as a promising attempt for potential medical applications

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Abstract
Very few researchers have focused on the biological efficacy of *Limonium* plants. In this concern, no investigations were commenced to delve into the in vitro and ex vivo biological actions of *Limonium pruinosum* in Egypt. Therefore, this work aims to assess for the first time the antimicrobial, antioxidant, and antitumor activities of *Limonium pruinosum* extract in addition to studying its ability to suppress the transcription of cell cycle–stimulating genes. *L. pruinosum* ethyl acetate extract exhibits considerable antibacterial and antibiofilm activity versus *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Results revealed that *L. pruinosum* exerts antioxidant effectiveness concerning DPPH, nitric oxide (NO), and hydroxyl radical (OH) scavenging ability with an IC_{50} (35.88 ± 2.2, 51.31 ± 1.06, and 65.87 ± 1.19 µg/mL) respectively. The results proved the effectiveness of *L. pruinosum* in closing wounds in gastric epithelial cells (GES-1) by (79.9343 ± 1.98%) compared with control (68.3637 ± 2.32%) in 48 h. Additionally, *L. pruinosum* had anticancer activity contrary to breast cancer MCF-7 and liver cancer HepG-2 cell lines with IC_{50} values of 96.73 ± 2.18 and 81.81 ± 0.99 µg/mL, respectively, while it had no cytotoxic activity against (Wi-38) normal cells. Also, *L. pruinosum* extract provoked considerable early- and late-apoptotic cell populations and was effective in inducing cell death of MCF-7. Our findings evoked that *L. pruinosum* has promising antibacterial, antioxidant, and wound healing activities and a good breast tumor suppressor arresting the cell cycle-stimulating genes, which may be an auspicious approach for the treatment of breast cancer.

Keywords Antibacterial · Antioxidant · Gene arrest · *Limonium pruinosum* · MCF-7 · Wi-38 · Wound healing

1 Introduction

The increasing incidence of multidrug-resistant species has become a critical challenge in the medication of a variety of microbial illnesses. As a result, employing plant extracts as sustainable antibacterial offers a desirable remedy for bacterial infection. Conventional plant-based substances are gaining significant attention in pharmacological aspects to cure untreatable disorders as they have no adverse effects, easy to recover in large amounts, and have a considerable degree of activity [1, 2]. So, it is critical to identify specific strategies for disease prevention [3, 4]. Resistance to antibiotics is among the most difficult clinical and global health issues; as a result, there has recently been a lot of interest in finding novel antimicrobial medications, specifically from natural sources. For the control and eradication of antibiotic-resistant pathogens, a variety of medicinal plants can be used as an alternative treatment [5]. Oxidative stress may be the root cause of serious disorders, including cancer, diabetes, chronic inflammation, and Alzheimer's disease [6]. This fact is brought on by either a reduction in an organism's innate antioxidant capacity or a rise in the concentration of reactive oxygen species (ROS), which destroys key components of cells, such as DNA and proteins, and it can also result in several illnesses [7]. In the food sector, synthetic antioxidants are frequently employed. They stop the creation of toxins

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or mutagenic substances that could be harmful to people's health. Because of these synthesized compounds' hazardous and cancer-causing characteristics, there has recently been some controversy about their unfavorable use [8]. As a result, it is crucial to use natural antioxidants in place of synthetic ones. Prospective sources of these natural antioxidants are plants. In reality, numerous antioxidants derived from plants have been scientifically demonstrated to be useful in protecting against tissue damage caused by free radicals. Through the manufacture of antioxidant cascades, higher plants have evolved several adaptive ways to decrease oxidative damage. Among them is halophyte *Limonium*. The genus *Limonium* has a worldwide distribution, but the Mediterranean region is the epicenter of its diversity [9–11]. The genus *Limonium*, also known as "Sea Lavender", or "Marsh-rosemary has a varying degree of colors from white to pink, produced on a branched panicle or corymb [12]. *Limonium pruinosum* (Family Plumbaginaceae) was native to Egypt, Palestine, Lebanon, Syria, Libya, and Tunisia (https://powo.science.kew.org/). In Egypt, *L. pruinosum* was very common in the Mediterranean coastal strip, the Isthmic desert, and Sinai [13]. According to [14], *L. pruinosum* is considered a medicinal plant. It is used in the treatment of many diseases such as fever, bleeding, and infections. Because it contains a high percentage of mineral salts and protein, it is used as animal feed, especially in arid regions [15] *L. pruinosum* can withstand salt and drought stress, as well as grow at high temperatures [16] *L. pruinosum* is very rich in polyphenols [15] and phenolic compounds [17]. *Limonium* possesses antibacterial, anti-inflammatory, and menstruation-regulating properties, and is being employed in the management of cervical cancer and abnormal uterine bleeding disorders in clinical practice [18]. *Limonium* species exhibit antioxidant, antibacterial, anti-inflammatory, antitumor, antimutagenic, hepatoprotective, anti-obesity, and enzyme-inhibitory actions, according to bioactivity investigations [19]. Aside from its therapeutic usefulness, the genus *Limonium* has ornamental value due to its stunning flowers, some species are consumed as foodstuffs [20]. Various literature surveys disclosed that *Limonium* species are rich in phenolics, terpenoids, and minerals [21]. Recently, phenolic compounds are in the spotlight because they show a critical part in stopping oxidation progressions and also have valuable and important features in improving health and preventing disorders such as cancer, inflammation, hypertension, and acute oxidative damage [22, 23]. One of the most prevalent cancers in women worldwide is breast cancer, and prevalence estimates have been rising quickly [24, 25]. The early diagnosis and advanced treatment of breast carcinoma have reduced patient mortality rates, but the disease's complicated mechanisms of growth and development still render it difficult to cure. Therefore, more research into the molecular pathways underlying carcinogenesis and breast cancer progression is still required. Human cancers, along with breast cancer, frequently exhibit cell cycle dysregulation, which is characterized by unchecked cell proliferation and cell cycle progression in cancer cells. This is one of the reasons why tumor cells can proliferate indefinitely and are resistant to existing remedies [26]. Hepatocellular carcinoma (HCC) is the fifth most prevalent malignancy worldwide and the third most common cause of cancer mortality [27, 28]. Relating wound healing medication, over 400 plant species have been recognized as potentially effective alternative medicine [29]. Numerous medicinal plants are utilized to treat a wide range of illnesses, microbial infections, and aging processes, according to ethnobotanical literature. For instance, various varieties of the *Limonium* genus have been shown to have hepatoprotective properties [30]. Unfortunately, very few researchers have focused on the biological efficacy of these plants [31]. This work aims to assess the vegetation and soil factors of the plant communities from Wadi Hof, Eastern Desert, Egypt, in which the *Limonium pruinosum* is present. No investigations were commenced to delve into the in vitro and ex vivo biological actions of *Limonium pruinosum* in Egypt. Thus, we assess for the first time the antimicrobial, antioxidant, and wound-healing activities of *Limonium pruinosum* extract in addition to studying its ability to suppress the transcription of cell cycle–stimulating genes, which may be a key molecular candidate for the therapy of breast cancer.

2 Materials and methods

2.1 Chemicals

All chemicals used in the present study were of analytical grade and were obtained from Sigma-Aldrich.

2.2 Collection of plant samples

The three plant communities in which *Limonium pruinosum* was found in Wadi Hof Eastern Desert, Egypt, were studied during the spring of 2020. *Limonium pruinosum* samples were collected from three stands of Wadi Hof terraces (Fig. 1). All wild plants in these plant communities were listed and identified according to [13] and [32–34]. Life forms and Phytogeographical species

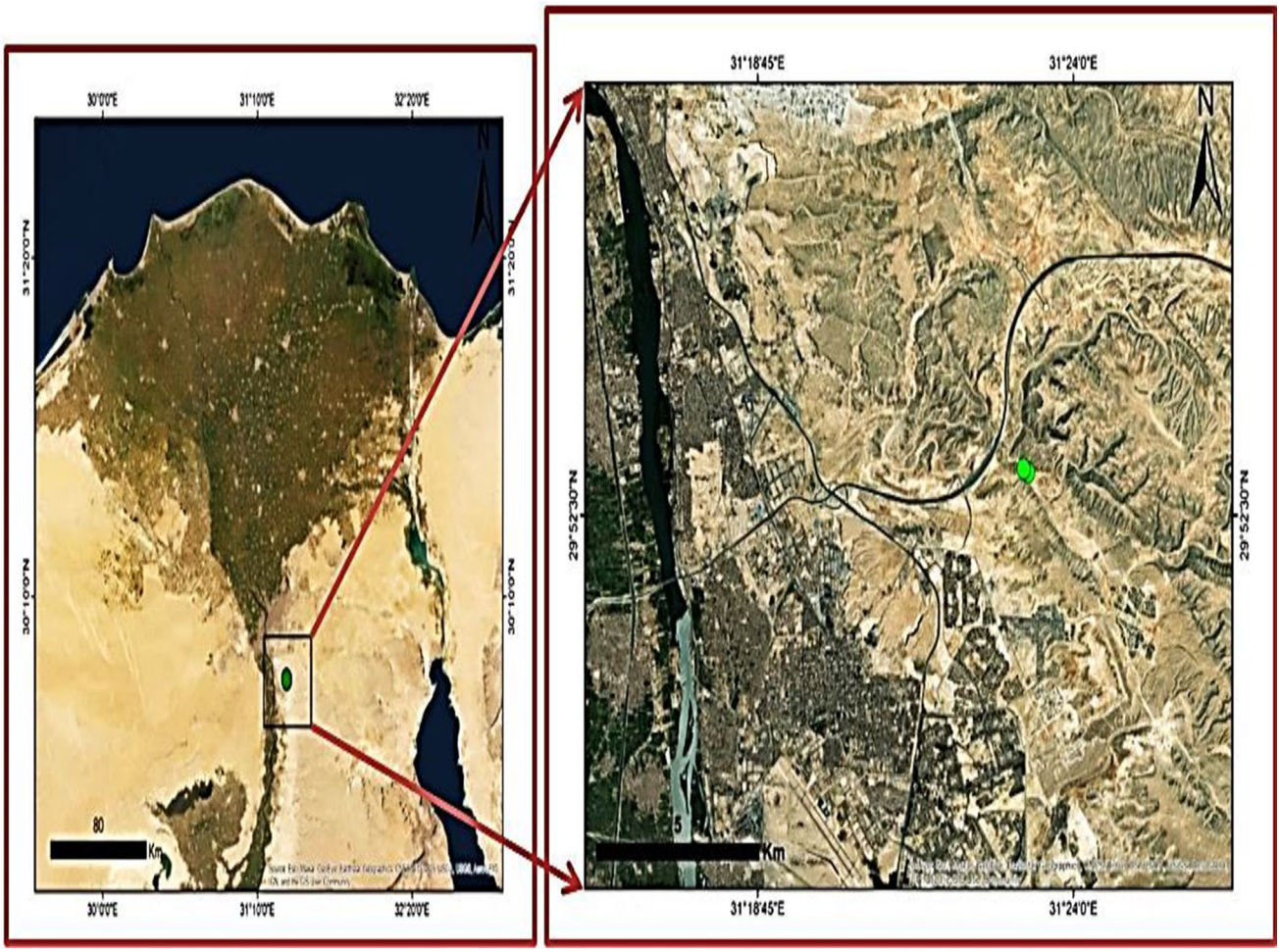


Fig. 1 A map illustrating the study zone and habitat of *Limonium pruinosum*

were identified after [35, 36], respectively. In each stand, five quadrates were studied, each quadrate with an area of 10*10 (100 m^2). The morphology of *Limonium pruinosum*; whole plant and flowers was illustrated in (Fig. 2). The importance value of all recorded species was calculated after [37] according to the following equation.

2.3 Importance value = relative density + relative abundance + relative frequency

All soil factors collected from each stand were determined according to [38]. The Sigma Plot 12.5 program was used to perform a descriptive statistical analysis of soil factors.

2.4 Preparation of plant extracts

The *Limonium pruinosum* arial part was washed appropriately with tap water, dried under the shade then ground into fine powder. Thirty grams of plant sample was dissolved separately in 300 mL of the selected solvents viz, (petroleum ether, hexane, butyl acetate, acetone, ethyl acetate, acetaldehyde, ethanol, methanol, diethyl ether, and water) maintaining a ratio of 1:10. All the plant preparations were incubated for 3 days until complete extraction. After that, the extracts were filtered out in previously weighed clean Petri plates which were then dried at 50 °C in an oven. After complete drying, the Petri plates were once more weighed to find out the expanse of solid extract secluded. The obtained extract was suspended in dimethyl sulfoxide (DMSO) in twice the amount of solid preparation and stored at -20 ˚C till further analysis.

2.5 Bacterial strains

Herein, antibiotic-resistant bacteria *(E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923*)* were graciously supplied by the Microbiology lab, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt. The test organisms were kept up on the appropriate agar slant and subcultured at regular times for 14 days.

Fig. 2 The morphology of *Limonium pruinosum*; whole plant and flowers. https://powo.science.kew.org/

2.6 Estimation of the antibacterial activity of *Limonium pruinosum* extracts

The antibacterial action of prepared extracts was assessed using the agar well diffusion method according to the method of [39] with minor modifications**.** Cell suspensions of 4×10^5 CFU/mL of *E. coli* and *staphylococcus aureus* were prepared. Next, bacterial suspensions were added to the surface of nutrient agar plates. *Limonium pruinosum* extracts (50 µg/mL) were applied to the cork porer (4 mm). An

antibiotic (10 µg/mL, Amikacin as a positive control) was employed to investigate the antibacterial activity. The experiment was run in triplicate and all plates got an overnight incubation at 37 °C. The zones of inhibition's (ZOI) diameter were measured for antimicrobial activity, and the mean results were estimated. The most active antibacterial extract was selected for further experiments throughout this work.

2.7 Quantitative determination of minimum inhibitory concentration of *L. pruinosum* extract

The test was done in accordance with CLSI standard M07-11 [40] with minor modifications. An isolated colony of *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were incubated separately in a nutrient broth medium at 30 °C for 18 h. A series of dilutions were then carried out to create a definitive inoculum of approximately 10^4 CFU/mL. Bacterial suspensions were applied to the surface of nutrient agar plates that had been amended with *L. pruinosum* extract at concentrations (6.25 to 100 µg·mL^{-1}). The experiment was run in triplicate and all plates got an overnight incubation at 37 °C. The minimum inhibitory concentrations (MICs) were identified as the least doses at which there was no discernible bacterial growth on the agar plates.

2.8 Studying the antibiofilm activity of *L. pruinosum* extract

According to the method of [41], with some modification, a qualitative assessment of biofilm development was made. The antibiofilm efficacy of *L. pruinosum* extract was determined against *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 and was assessed concerning the control one. Each tube was supplied with 4 mL of the nutrient broth, 250 µL of *L. pruinosum* extract, and 10 µL of tested bacteria (0.5 McFarland (1–2.5) X 10^8 CFU/ mL). Next, the bacteria were then incubated for 24 h at 37 °C. The nutrient broth in the treated and control tubes was removed and each was given a phosphate buffer saline (PBS; pH 7) treatment before being dried [42]. About (5.0 mL) of 3.0% sodium acetate was used to retain the adhering bacterial layers for 10 min, and then deionized water was used to rinse them. Biofilms were subsequently stained for 10 min with 0.1% Crystal Violet (CV), and the excess stain was then washed away with deionized water. Finally, the stain was dissolved in 2.0 mL of ethanol. A UV–Vis spectrophotometer (T60 U-UV–Vis-UK) was used to examine the bacterial biofilms (at 570 nm). The following equation was used to calculate the inhibition % [41].

$$\text{Inhibition}\% = (1)\text{ODc} - \text{Odt}/\text{ODc} \times 100$$

where, ODc: The absorbance of the control sample (without treatment), ODt: The absorbance of the treated samples.

2.9 DPPH scavenging of *L. pruinosum* extract

Limonium pruinosum antioxidant proficiency was estimated by DPPH scavenging test as depicted by [43, 44] including a few modifications. In summary, 100 µl of *Limonium pruinosum* extract was added to (0.004 percent in methanol) DPPH in separate tubes at concentrations of 6.25, 12.5, 25, 50, and 100 µg /mL. Following that, all of the tubes were placed in the dark at room temperature for 30 min, and ascorbic acid was used as a control sample. The decline in O.D. was detected at 517 nm using a Jenway spectrophotometer. The measurements were obtained as IC_{50} values, which required 50% DPPH radical scavenging inhibitory activity. The next equivalence was applied to calculate the inhibition ratio:

$$\%\text{ of Scavenging impact} = 1 - (\text{The absorbance of the test} / \text{and the absorbance of control}) \times 100$$

2.10 Hydroxyl radical scavenging test

The scavenging efficiency of the *L. pruinosum* extract concerning hydroxyl radical was assessed following the method of [39, 45]**.** Fifty microliters of plant extract and vitamin C at (6.25–100 µg/mL) concentrations were mixed with 9 mmol/L of each ($FeSO_4$, salicylic acid, and H_2O_2). The mixture was kept at 37 °C for 60 min before being spectrophotometrically measured at 510 nm using a Jenway spectrophotometer.

2.11 Nitric oxide radical scavenging test

The antioxidant efficiency of the *L. pruinosum* extract concerning nitric oxide was estimated subsequent to the procedure shown by [39, 46]**.** In a 96-well plate, 50 µl of the constant serial concentrations of *L. pruinosum* extract, positive control (vitamin C), and negative control (distilled H_2O) were combined with 50 µl of 10 mM sodium nitroprusside solution and incubated for 90 min. Finally, an equal amount of Griss reagent (1 percent sulphonyl amide, 0.1 percent naphthyl ethylenediamine in 2.5

percent H_3PO_4) was added to each well, and the nitrite level was instantly determined at 490 nm by Jenway spectrophotometer. Extracts' capacity to remove NO radicals was measured by the following formula:

$$\text{No scavenging activity } (\%) = (\text{AC} - \text{AE})/\text{AC}) \times 100$$

AC: The absorbance means of negative control, AE: The absorbance means of the test.

2.12 Quantification of total phenolic content of *L. pruinosum* extract

The total phenolics content of *L. pruinosum* extracts was established according to the method [47] using the Folin Ciocalteau reagent. The test was done in triplicates. The number of total phenolics was recorded as micrograms of gallic acid equivalents (µg GAE) per gram of extract.

2.13 Gas chromatography/mass spectrometry analysis of *L. pruinosum*

Gas chromatography in combination with mass spectrometry was used to figure out the chemical components of the *L. pruinosum* extract using a Shimadzu GC–MS-QP 2010 Plus equipped with an RTX-5 (60 mmL 9 0.25 mmL, D., 9 0.25 µm) capillary column in JNU, New Delhi, with helium as a carrier at 300 °C. The compounds of *L. pruinosum* were identified according to [48]**.** The (GC/MS) analysis was achieved at The Central Laboratories of Scientific Research and Biotechnological Applications City, New Borg El-Arab, Alexandria, Egypt.

2.14 The in vitro wound healing effect of *L. pruinosum* ethyl acetate extract using scratch assay method

The adherent cell surface is scratched with the tip of a pipette to create an artificially damaged area around the cells. Next, photographic images of cell migration were taken on time, from wound initiation to wound repair [49]**.** Herein, human gastric epithelial cells (GES-1) were obtained for this study from Cairo, Egypt's Cancer Institute. Cells were grown in 6 multi-well plates until confluent as illustrated by [50]**.** A linear scratch was created using a sterilized yellow pipette tip positioned at a 30-degree angle (to restrict the size of the scratch to a minimum) and imperiled to treatment with the extract of *L. pruinosum* for 48 h. Following that, imaging of mutually injured edges that could be seen using a 10 × objective was done. The data were shown as mean ± SE and variance with $p < 0.05$ being used to reveal statistical consequences. The aforementioned detailed test was done at Science Way Laboratory for scientific research and consultations, Cairo, Egypt.

2.15 Assessment of cytotoxicity of *L. pruinosum* extract against normal cell line

Human lung fibroblast (Wi-38) as a normal cell line was purchased from the American Type Culture Collection (Manassas, VA). The cells were cultured in 96-well tissue culture microtiter plates (Nunc-Denmark) and prepared in such a way using the MTT assay as indicated by [51]. After Adherent sheets of cells were created, the growth medium was removed carefully, and then *L. pruinosum* extract was applied to the cells. The plant extract was added to the cells at concentrations 390.62, 781.25, 1562.5, 3125, 6250, 12,500, and 25,000 µg / mL in volume 100 µl / well, and equal volumes of media were added to cells as control. Incubation of the plates took place for 24 h at 37 °C and 5% CO_2. After removing the medium, the plates were washed with phosphate-buffered saline (PBS), and the cells were again treated with 50 l/well of a solution containing 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-tetrazolium bromide (MTT) and incubated for 4 h. About 0.05 mL/well of DMSO solution was added. Through the use of an ELIZA reader Thermo Scientific™ Multiskan™ FC Filter-based Microplate Photometer, the absorbance of each well was measured at a wavelength of 560 nm. An inverted phase contrast microscope (Helmut Hund GmbH, Wetzlar, Germany) was used to investigate the morphological changes in cells. The viability percent was assessed by the following equation:

$$\text{Viability}\% = \text{MeanO.D.Treated}/\text{MeanO.D.Control} \times 100.$$

The IC_{50} was determined using GraphPad Instate software. The detailed test was done at Science Way Laboratory for scientific research and consultations, Cairo, Egypt.

2.16 Cytotoxicity test of *L. pruinosum* extract against malignant cell lines

Herein, MCF-7 and HepG2 cell lines were utilized to determine the tested *L. pruinosum* extract's anticancer effects, and their proliferation cytotoxic activity was evaluated using the MTT assay as indicated by [51]. MCF-7 cell line was obtained from Homo sapiens, humans, mammary gland, and breast, derived from the metastatic site, pleural effusion, and adenocarcinoma (ATCC: HTB-22). The HepG2 cell line was obtained from Homo sapiens, human, hepatocellular carcinoma liver tissue, (ATCC: HB-8065). The antitumor potential of the extract was investigated using a phase-contrast inverted microscope (Olympus, Japan). The anticancer activity of *L. pruinosum* extract was assumed by

appraising the IC_{50} value utilizing GraphPad Instat software version 3.1. The test was done at Science Way Laboratory for scientific research and consultations, Cairo, Egypt.

2.17 Cell cycle analysis

The *Limonium pruinosum* extract with pre-determined IC_{50} value was brought to MCF cells for 48 h. After being trypsinized, the cells were then repeatedly rinsed in phosphate-buffered saline, mixed in propidium iodide (PI) (500 L), and stained with RNase staining buffer for 15 min. Utilizing a Cytek® Northern Lights 2000 spectral flow cytometer and SpectroFloTM Software version 2.2.0.3, an examination of fluorescently stimulated cell sorting was performed according to [52, 53].

2.18 Real-time quantitative PCR

The Qiagen RNA extraction/BioRad cyber green PCR MMX kit was used. cDNA synthesis and PCR amplification are carried out in the same tube. The kit was optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity without compromising the fluorescent signal. The primers used were the following:

Bax: F 5'- TCAGGATGCGTCCACCAAGAAG-3',
Bax: R 5'- TGTGTCCACGGCGGCAATCATC-3'.
GAPDH: F 5'- GTCTCCTCTGACTTCAACAGCG-3'
GAPDH: R 5'- ACCACCCTGTTGCTGTAGCCAA-3'

The reader was Rotorgene RT- PCR system. This report was generated by Rotor-Gene 6000 Series Software 1.7 (Build 87).

2.19 Apoptosis analysis

After being exposed to *Limonium pruinosum* extract for 48 h, the MCF-7 cells were trypsinized and given two PBS washes. According to the manufacturer's instructions, Annexin V-FITC/PI analysis Kit was used to measure apoptosis. [53, 54].

2.20 Statistical analysis

All experiments on the antibacterial, antioxidant, and anticancer activity were done in triplicates. The data were stated as mean ± SD. The statistical analysis was achieved using the software SPSS version 15.

3 Results

3.1 Vegetation and soil analysis

A total of 22 plant species, belonging to 18 families, were listed in the studied stand. Asteraceae is the most common family (3 species). Both *Brassicaceae* and *Chenopodiaceae* have 2 species each, while 15 families (Apiaceae, Boraginaceae, Capparaceae, Caryophyllaceae, Ephedraceae, Geraniaceae, Menispermaceae, Nitrariaceae, Plumbaginaceae, Resedaceae, Rhamnaceae, Scrophulariaceae, Solanaceae, Tamaricaceae, and Zygophyllaceae) were denoted by one species (Fig. 3). The perennial life span is represented by 21 species, while the annual is represented by one species (*Erodium oxyrrhynchum*) (Table 1). Life forms included *chamaephyte* (13 species), phanerophyte (5 species), hemicryptophyte

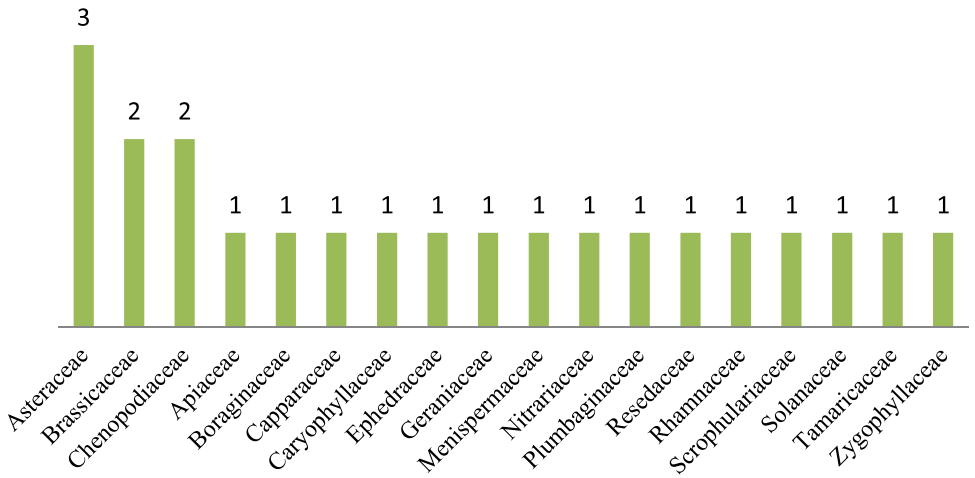


Fig. 3 Number of species in plant families

Table 1 Floristic composition and vegetation analysis of the studied stands in Wadi Hof

Species	Family	Life span	Life form	Floristic category	IVI
Deverra tortuosa (Desf.) DC	Apiaceae	Pe	Ch	SA-SI	46.50
Achillea fragrantissima (Forssk.) Sch. Bip	Asteraceae	Pe	Ch	SA-SI + IR-TR	25.50
Launea nudicalus (L.) Hook. f		Pe	Hem	SA-SI	5.80
Echinops spinosus L		Per	Hem	ME + SA-SI	23.59
Trichodesma africanum (L.) R.Br	Boraginaceae	Pe	Ch	SA-SI + S-Z	20.72
Farsetia aegyptia Turra	Brassicaceae	Pe	Ch	SA-SI + S-Z	22.04
Zilla spinosa (L.) Prant		Pe	Ch	SA-SI	20.35
Capparis spinosa L	Capparaceae	Pe	Ch	ME	15.94
Gymnocarpos decandrus Forssk	Caryophyllaceae	Per	Ch	SA-SI	11.31
Haloxylon salicornicum (Moq.) Bunge	Chenopodiaceae	Pe	Ch	SA-SI	13.66
Atriplex halimus L		Pe	Ph	ME + SA-SI	11.31
Ephedra alata Decne	Ephedraceae	Pe	Ch	SA-SI	5.80
Erodium oxyrrhynchum M. Bieb	Geraniaceae	An	Th	IR-TR	8.96
Cocculus pendulus (J.R. & G. Forst.) Diels	Menispermaceae	Pe	Ch	S-Z	8.96
Nitraria retusa (Forssk.) Asch	Nitrariaceae	Pe	Ph	SA-SI	9.47
Limonium pruinosum (L.) Chaz	Plumbaginaceae	Pe	Hem	SA-SI	5.80
Ochradenus baccatus Delile	Resedaceae	Pe	Ph	SA-SI	8.96
Ziziphus spina–christi (L.) Desf	Rhamnaceae	Pe	Ph	ME	5.80
Scrophularia deserti Delile	Scrophulariaceae	Pe	Ch	SA-SI	8.96
Lycium shawii Roem. & Schult	Solanaceae	Per	Ph	SA-SI + S-Z	5.80
Tamarix aphylla (L.) H. Karst	Tamaricaceae	Pe	Ch	SA-SI + IR-TR + S-Z	8.96
Zygophyllum coccineum L	Zygophyllaceae	Pe	Ch	SA-SI + S-Z	5.80

Pe, perennial; *An*, annual; *Ch*, chamaephyte; *Hem*, hemicryptophyte; *Ph*, phanerophyte; *Th*, therophyte; *SA-SI*, Saharo–Sindian; *S-Z*, Sudano–Zambesian; *ME*, Mediterranean; *IR-TR*, Irano–Turanian

(3 species), and therophyte (single species) (Table 1 and Fig. 4a). Concerning listed species floristic categories, Saharo–Sindian region has 10 species, Saharo–Sindian–Sudano–Zambesian have 4 species, Mediterranean and Mediterranean–Saharo–Sindian have 2 species each, Irano–Turanian, Irano–Turanian–Saharo–Sindian, Irano–Turanian–Saharo–Sindian–Sudano–Zambesian and Sudano–Zambesian have one species each (Fig. 4b).

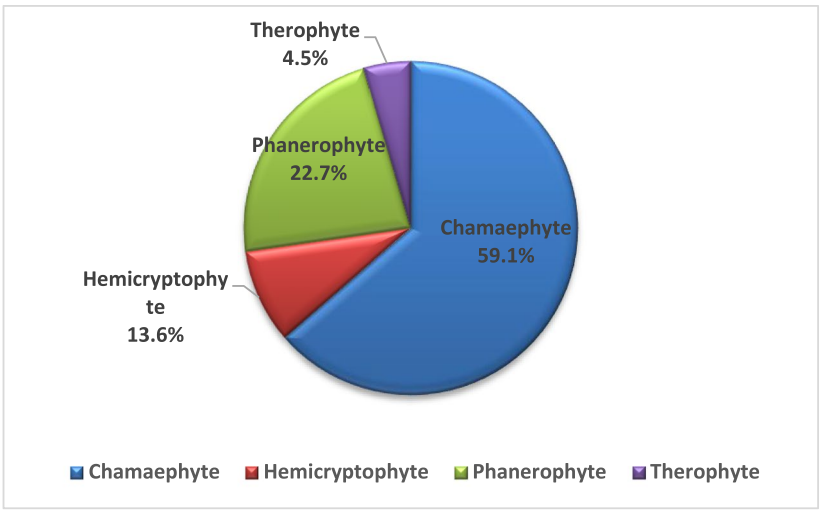
The importance value index (IVI) of the species range was between 5.8 (*Ephedra alata*) and 46.5 (*Deverra tortuosa*) (Table 1). Results of the descriptive analysis (mean, standard deviation, range, maximum, minimum, and median) of soil factors are summarized in Table 2.

3.2 Determination of the antibacterial activity of *L. pruinosum* ethyl acetate extract

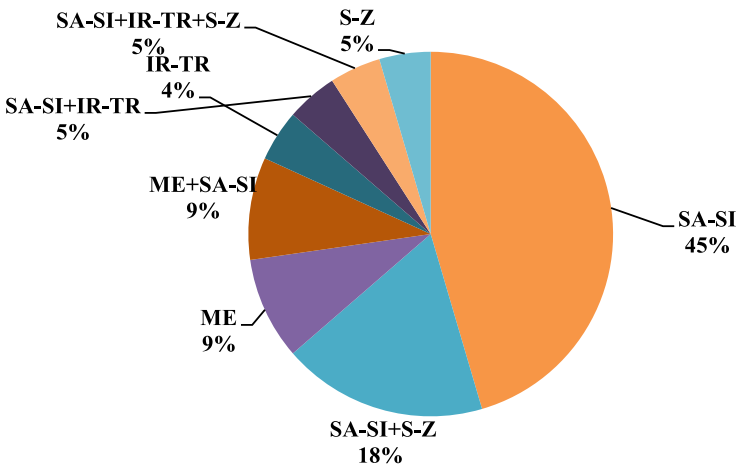
In the current work, the antibacterial activities of *L. pruinosum* using ten different solvents were determined against chosen antibiotic-resistant pathogens related to 10 µg/mL Amikacin as a positive control. The highest antibacterial activity was exerted by ethyl acetate extract. So, based on these outcomes, the ethyl acetate extract was chosen and used for further investigations. As indicated in (Fig. 5) (ethyl acetate *L. pruinosum* extract (50 µg/mL) displayed antibacterial action versus *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 with a zone of inhibition (ZOI) of about 20 and 23 mm, compared to the inhibition zone of positive control (27 and 24), respectively. The MIC assessment of *L. pruinosum* ethyl acetate extract toward *E. coli* was 25 µg/mL while that toward *S. aureus* was 12.5 µg/mL, (Fig. 6). *L. pruinosum* ethyl acetate extract has dual behavior acting as bacteriostatic in its lower concentration and behaving as bactericidal in its higher concentration.

3.3 Antibiofilm activity of *Limonium pruinosum* ethyl acetate extract

Regarding the antibiofilm activity of *L. pruinosum* extract, the data presented in Table 3 illustrate the inhibition % of



(a): The percentage of plant species' life forms.



(b): Percentage of plant species floristic categories.

Fig. 4 (**a**) The percentage of plant species' life forms. (**b**) Percentage of plant species floristic categories

the biofilm formation. The highest repression was recorded as 81.05% for *E. coli* and 75.14% for *S. aureus*.

3.4 Quantification of total phenolic content of *L. pruinosum* ethyl acetate extract

Quantification of the total phenolic content (TPC) of *L. pruinosum* extract using the folic-Ciocalteau method evoked the presence of a respectable amount of total phenolics (210 ± 0.11 µg gallic acid equivalent/mg).

3.5 Estimation of antioxidant potency of *L. pruinosum* ethyl acetate extract

In this investigation, the antioxidant potency of *L. pruinosum* ethyl acetate extract was assessed by DPPH, nitric oxide, and hydroxyl radical scavenging activity. The results confirmed the high antioxidant activities of *L. pruinosum* extract. The IC_{50} value for the DPPH method of *L. pruinosum* was found to be 35.88 ± 2.2 µg/mL, while for nitric oxide scavenging was 51.31 ± 1.06 µg/mL, and for hydroxyl radical scavenging activity 65.87 ± 1.19 µg/mL, respectively compared to vitamin C (Fig. 7). According to these findings, the ethyl acetate extract demonstrated antioxidant defense against peroxyl radicals.

Table 2 Descriptive statistics for soil factors of the studied stands in Wadi Hof

Soil factor	Mean ± standard deviation	Range (maximum—minimum)	Median
pH	6.5 ± 0.1	0.2 (6.6–6.4)	6.5
Electrical conductivity (deciSiemens per meter)	1.833 ± 0.0577	0.1 (1.9–1.8)	1.8
Total soluble salts	1173.333 ± 36.95	64 (1216–1152)	1152
(Part per million)			
Cations (milliequivalents per liter)			
Ca^{++}	7.8 ± 0.1	0.2 (7.9–7.7)	7.8
Mg^{++}	3.4 ± 0.1	0.2 (3.5–3.3)	3.4
Na^{+}	6.1 ± 0.1	0.2 (6.2–6)	6.1
K^{+}	1.2 ± 0.1	0.2 (1.3–1.1)	1.2
Anions (milliequivalents per liter)			
CO_3^{-}	0 ± 0	0 (0–0)	0
HCO_3^{--}	3.467 ± 0.0577	0.1(3.5–3.4)	3.5
Cl^{-}	8.1 ± 0.1	0.2 (8.2–8)	8.1
SO_4^{--}	5.533 ± 2.888	5.1 (7.3–2.2)	7.1

All values were expressed as mean ± SEM ($n = 3$)

3.6 The in vitro wound healing effect of *L. pruinosum* ethyl acetate extract using scratch assay method

In the present work, human gastric epithelial cells (GES-1) were treated with *L. pruinosum* ethyl acetate extract to measure the cellular recovery and, consequently, the extract's ability to close wounds at distinct intervals. According to the findings in Fig. 8, the cell-free zones were almost entirely closed by 79.9343 ± 1.98% compared with the untreated control 68.3637 ± 2.32% from day 0 (wound induction day) to 48 h. Additionally, wound termination in GES-1 cells handled with *L. pruinosum* extract as compared to control is shown in (Fig. 9A) whereas the

Fig. 5 Antimicrobial activity of *L. pruinosum* ethyl acetate extract (50 µg/mL) against (**A**) *Escherichia coli* ATCC 25922, 1: -Ve Control, 2: + Ve control, 3: *L. pruinosum* extract (**B**) *Staphylococcus aureus* ATCC 25923, 1: -Ve control, 2: + Ve control, 3: *L. pruinosum* ethyl acetate extract

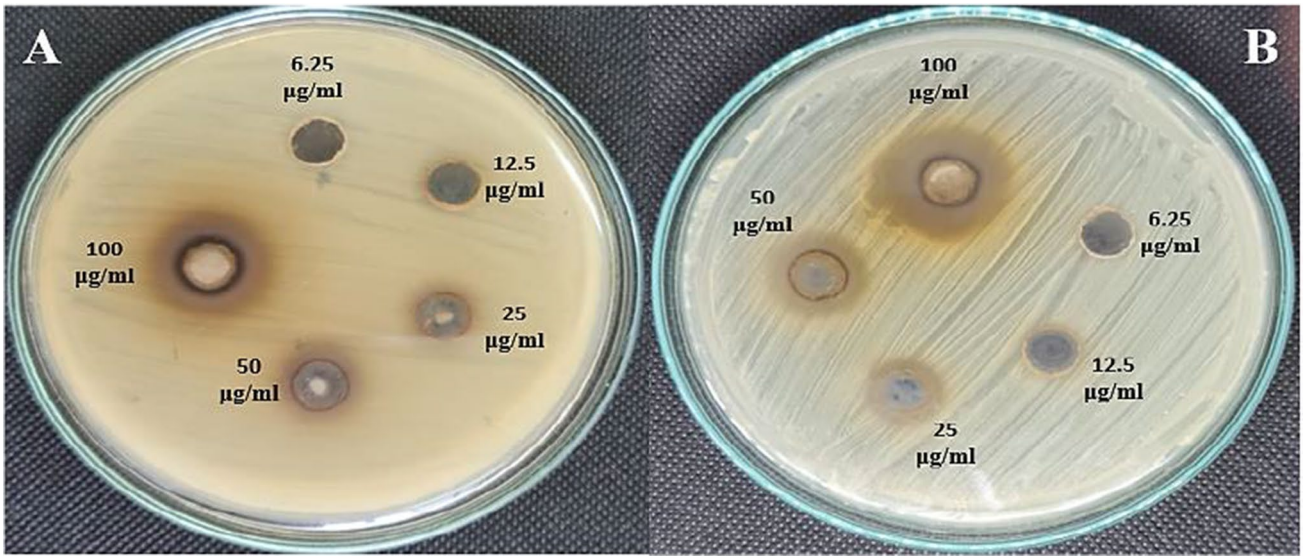


Fig. 6 The minimum inhibitory concentration of *Limonium pruinosum* ethyl acetate extract against (**A**) *Escherichia coli* ATCC 25922 and (**B**) *Staphylococcus aureus* ATCC 25923

Table 3 Antibiofilm activity of *L. pruinosum* ethyl acetate extract

Tested bacteria	O.D. of control at 570 nm	O.D. of treated samples with *Limonium pruinosum* extract at 570 nm	Biofilm inhibition (%)
Escherichia coli ATCC 25922	1.251 ± 0.007	0.237 ± 0.00	81.05
Staphylococcus aureus ATCC 25923	0.700 ± 0.003	0.174 ± 0.005	75.14

All values were expressed as mean ± SEM ($n = 3$)

rate of cell migration is depicted in (Fig. 9B). The in vitro wound healing assay suggested that *L. pruinosum* extract exhibited faster-wound healing activity than the untreated control.

3.7 Characterization of GC/MS analysis

The active compounds in the *L. pruinosum* ethyl acetate extract were detected by GC–MS analysis. The records listed in (Table 4) point to the occurrence of 15 metabolites in the extract.

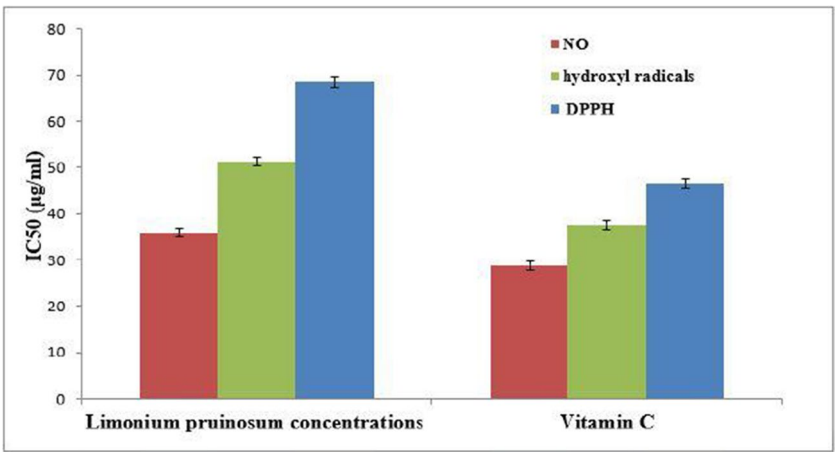


Fig. 7 Scavenging activity of *Limonium pruinosum* ethyl acetate extract against DPPH free radicals, NO radicals, and hydroxyl radicals

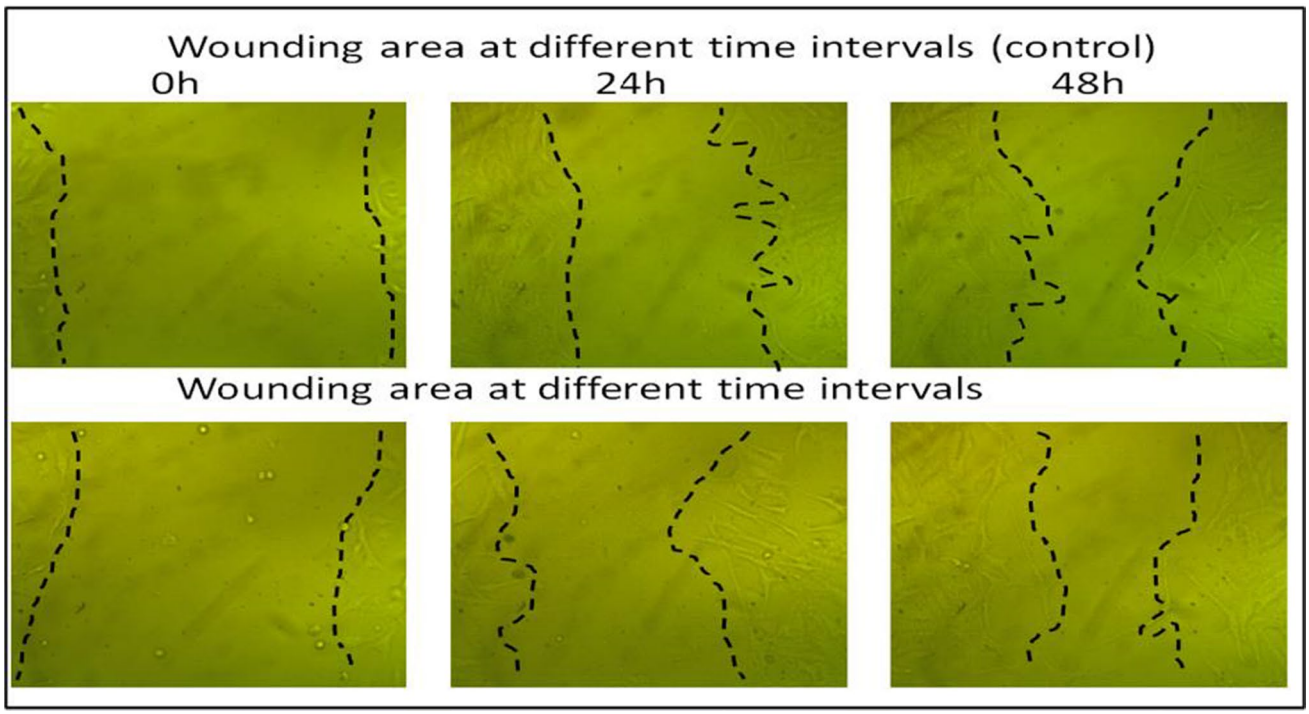


Fig. 8 The wound healing effect of *L. pruinosum* ethyl acetate extract compared to control from day 0 (wound induction day) to 48 h in GES-1 cells

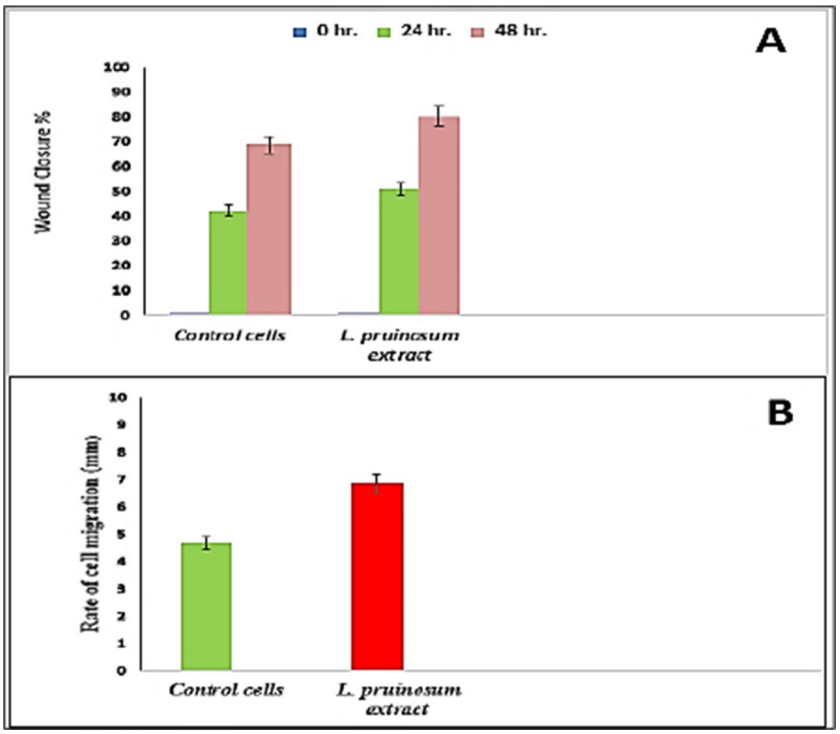


Fig. 9 (**A**) Estimation of relative wound closing in GES-1cells reconciled with *L. pruinosum* ethyl acetate extract compared to control. (**B**) Rate of cell migration. The whole statistics were conveyed as mean ± SEM and interpreted as significantly different at $p \leq 0.05$*

Table 4 Bioactive compounds of *L. pruinosum* ethyl acetate extract identified through GC–MS analysis

ID	Name	R. time	Mol. formula	mol. wt	Reported bioactivity and usage
1	Formamide, N, N-dimethyl-	4.76	C_3H_7NO	(73)	Antimicrobial activity [94] used in the manufacture of high-quality polyurethane and polyamide coatings commonly used as a solvent in the electronics industry, in pesticides, in industrial paint-stripping applications, and as a reaction and crystallizing solvent in the pharmaceutical industry [85]
2	Benzene, (1-butylheptyl)-	32.7	$C_{17}H_{28}$	(223)	Antimicrobial activity [95]
3	Benzene, (1-pentylhexyl)-	35.35	$C_{16}H_{26}$	(218)	Antimicrobial activity [95]
4	Benzene, (1-butylnonyl)-	35.485	$C_{18}H_{30}$	(246)	Antimicrobial activity [95]
5	Trimethyl (4-(1,1,3,3, -tetramethyl butyl) phenoxy) silane	23.44	$C_{17}H_{30}OSi$	(278)	Antibacterial activity [96]
6	1,2,4-Triazole-3-amine	37.13	$C_8H_{13}N_7$	(207)	Anticancer activity [97]
7	2-Octadecyl-propane-1,3-diol	17.55	$C_{21}H_{44}O_2$	(328)	Antibacterial activity [98]
8	Decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene	17.12	$C_{16}H_{30}$	(222)	Anticancer, antioxidant activity [99]
9	Eicosane	34.17	C20H42	(282.5)	Antibacterial and antifungal activity [79]
10	Hexadecane	38.89	C16H34	(282.5)	Antibacterial and antifungal activity [79, 80]
11	Cyclohexane, 1,1'-(2-tridecyl-1,3-propanediyl) bis	29.112	$C_{28}H_{54}$	(390)	Anticancer activity, [84]
12	Tetrapentacontane, 1,54-dibromo	29.985	$C_{54}H_{108}Br_2$	(914)	Antioxidant, antitumor, antiviral, hypolipidemic [100]
13	1,2-Benzisothiazol-3-amine	35.10	$C_{13}H_{20}N_2SSi$	(264)	Antidiabetic, [101]
14	1-Docosene	31.00	$C_{22}H_{44}$	308	Antibacterial [81] antioxidant, anti-inflammatory, and anti-cancer activities [102]
15	Tetradecanoic acid	18.23	C14H28O2	228.4	Antimicrobial [82]

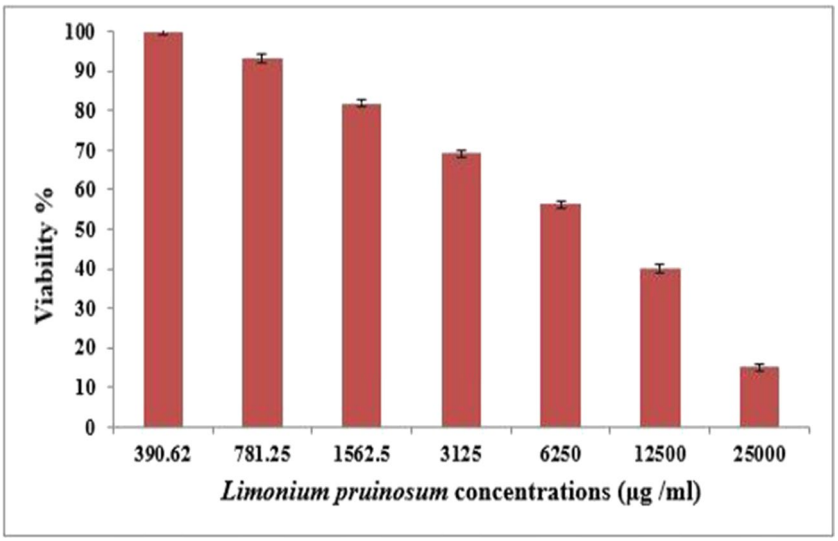


Fig. 10 Cell viability % of normal human lung fibroblast (Wi-38) cells after exposure to different concentrations of *L. pruinosum* ethyl acetate extract

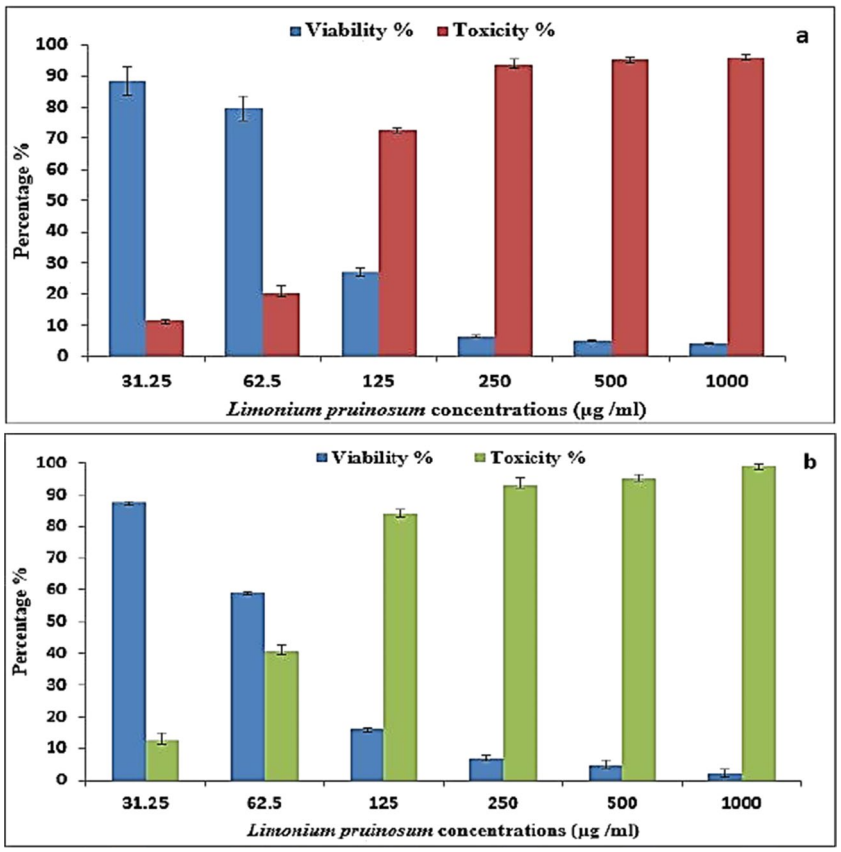


Fig. 11 (**a**) Appreciation of *L. pruinosum* ethyl acetate extract ($IC_{50} = 96.73 \pm 2.18$ µg/mL) produced cell viability/cell death in MCF-7 cell lines. (**b**) Appreciation of *L. pruinosum* ethyl acetate extract ($IC_{50} = 81.81 \pm 0.99$ µg/mL) produced cell viability/cell death on HepG2 cell line

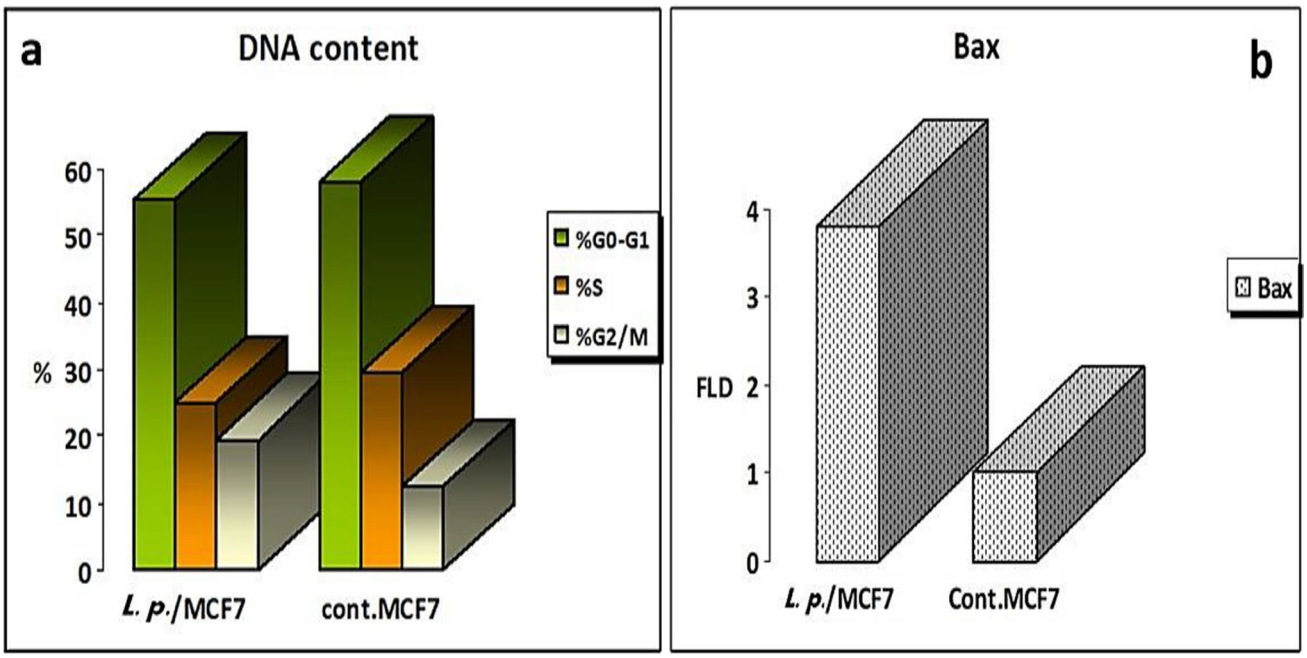
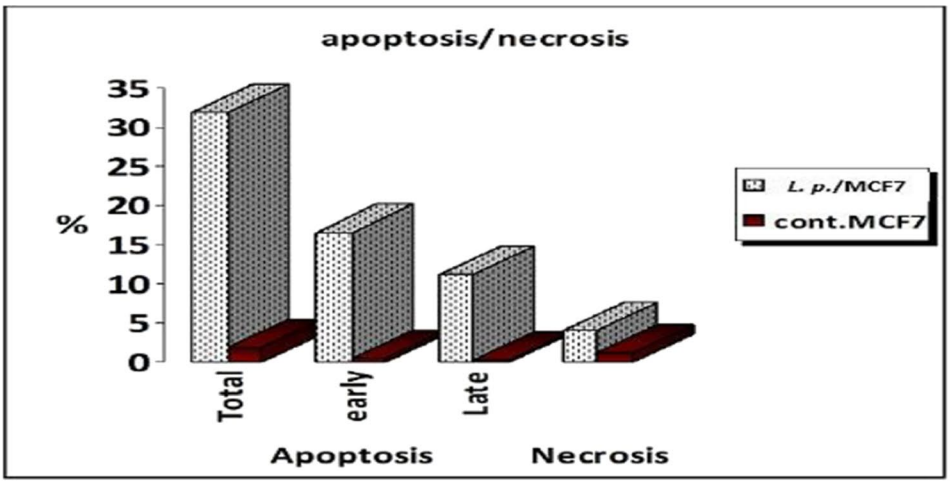
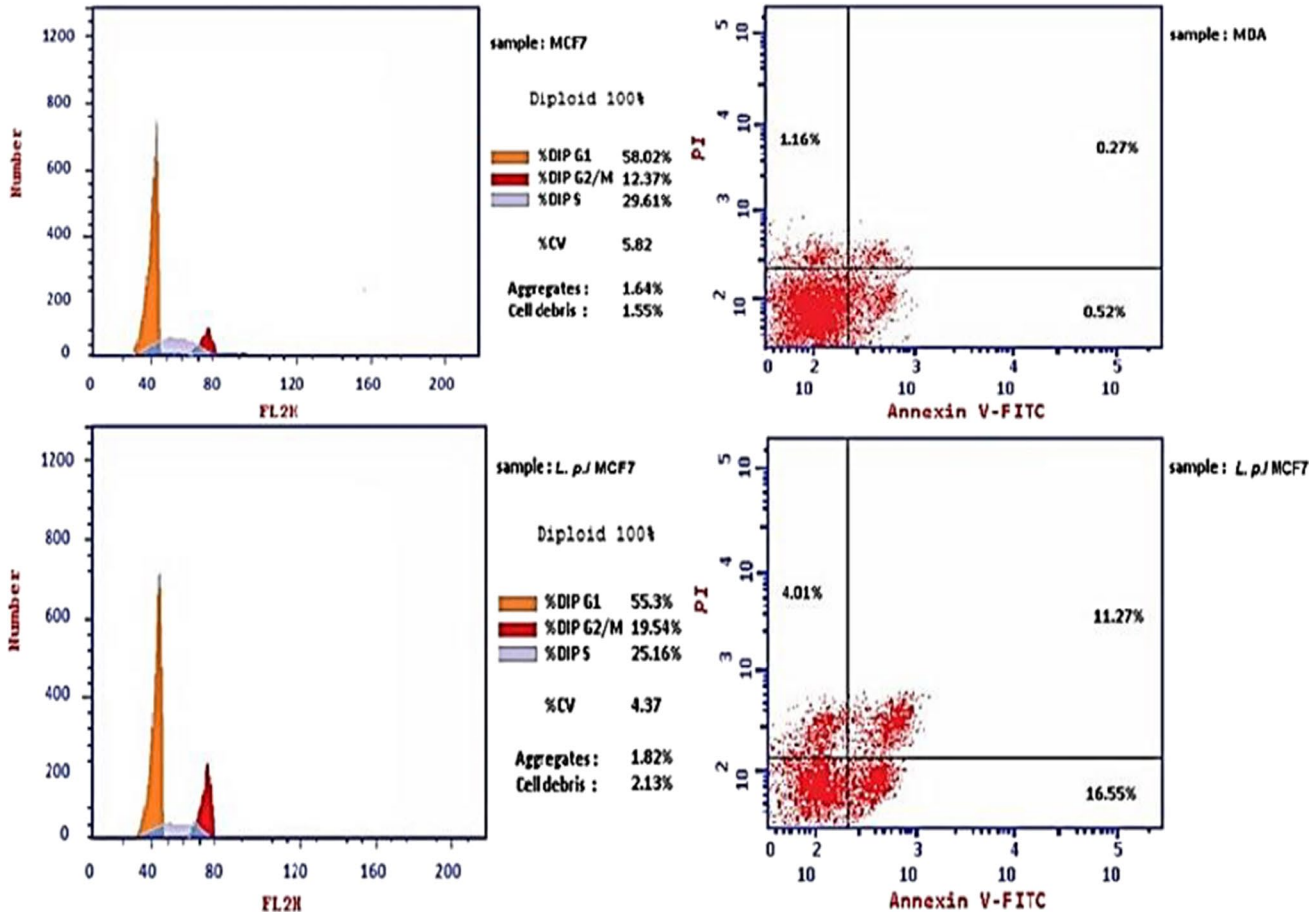


Fig. 12 (**a**, **b**) Cell cycle distribution of MCF-7 after treatment with *L. pruinosum* (*L. p.*) ethyl acetate extract compared to control cell line using flow cytometer



(a): The percentages of total apoptosis/necrosis in *L. pruinosum* (*L. p.*) ethyl acetate extract-treated human MCF-7 cell line.



(b): The apoptotic impact in MCF-7 cancerous cells after treatment with *L. pruinosum* (*L. p.*) ethyl acetate extract compared to control cell line by annexin V−FITC/PI staining using flow cytometer

Fig. 13 (**a**) The percentages of total apoptosis/necrosis in *L. pruinosum* (*L. p.*) ethyl acetate extract-treated human MCF-7 cell line. (**b**) The apoptotic impact in MCF-7 cancerous cells after treatment with *L. pruinosum* (*L. p.*) ethyl acetate extract compared to control cell line by annexin V – FITC/PI staining using flow cytometer

3.8 Estimation of cytotoxicity of *L. pruinosum* ethyl acetate extract on normal human lung fibroblast (Wi-38) cell

The cytotoxic potential of *L. pruinosum* ethyl acetate extract was assessed antagonistically to the Wi-38 normal cell line to check the extract's safety against normal cells. The results showed that *L. pruinosum* extract has no substantial harmful impacts on the Wi-38 normal cell line and the cells have a normal morphological appearance. Moreover, the high concentrations (12,500 and 25,000 µg /mL) produced minimal cell death as the cells shrink, appeared irregular in shape, and be likely to detach from the substratum (Fig. 10).

3.9 Cytotoxicity test of *L. pruinosum* ethyl acetate extract against malignant cell lines

To ascertain the cytotoxic effect of *L. pruinosum* ethyl acetate extract against cancerous cells, the viability/cytotoxicity (MTT) assay was carried out as opposed to breast cancer (MCF-7) and liver cancer (HepG-2) cell lines. The results evoked that *L. pruinosum* exerts effective cytotoxic impacts against MCF-7 and HepG-2 cell lines in a dose-dependent manner. When the concentrations of *L. pruinosum* extract develop from 31.25 to 1000 µg/mL, the cell viability is reduced. The utmost cytotoxic effect of *L. pruinosum* was achieved at 1000 µg/mL which realized the highest inhibition percentage (98.9 and 95.8%) toward (MCF-7 and HepG-2) cell lines respectively, (Fig. 11a, b). Moreover, the results indicated a 50% decrease in cell inhibition (IC_{50}) equal to 96.73 ± 2.18 and 81.81 ± 0.99 µg/mL for (MCF-7) and (HepG-2) cell lines, respectively.

3.10 Cell cycle analysis

The *L. pruinosum* ethyl acetate extract was explored for cell cycle distribution to determine the intracellular mode of action. The MCF-7 cancerous cells and control were treated with pre-calculated IC_{50} for 48 h stained with propidium iodide and observed for cell cycle distribution by flow cytometry. As illustrated in Fig. 12 (a and b), *L. pruinosum* ethyl acetate extract decreased the cell division in the G1 phase from 58.02 to 55.3%, compared to the control cells. Also, the S phase decreased from 29.61 to 25.16%, whereas the *L. pruinosum* ethyl acetate extract performs a protruding increase in cell distribution in the G2 phase to 19.54%.

3.11 Apoptosis studies

The results illustrated in Fig. 13 (a and b) indicated that *L. pruinosum* ethyl acetate extract provoked considerable early- and late-apoptotic cell populations, boosting them by 16.55 and 11.27, respectively. The results revealed that *L. pruinosum* extract was effective in inducing cell death of MCF-7 and display 31.83 total apoptosis and 4.01 necrosis. Moreover, *L. pruinosum* extract was found to be morphologically cytotoxic to the cancer cells which showed morphological alterations involving loss of membrane integrity, membrane blebbing, and decreased cell size.

4 Discussion

Asteraceae was the most common family in the Mediterranean Flora of North Africa [55]. This is consistent with many studies that were carried out on Wadi Hagul [56–59], as well as Wadi Asyouti and Wadi Habib [60] which are also in the Eastern Egyptian desert.

There is, unfortunately, not much research concerning the antibacterial properties of *Limonium* species in the publications. In the specific case of the *Limonium* genus, antimicrobial investigations have been conducted for other species, such as *L. brasiliense* [61], *L. socotranum* (Vierh.) Radcl.-Sm. [62], and *L. echioides* [63], yet not for *L. pruinosum*, thereby making it difficult to make substantial efficacy comparisons. Moreover, [64] revealed the antibacterial potential of *L. globuliferum*, *L. effusum*, and *L. lilacinum* extracts. In another study conducted by [62], it was informed that the methanol extract of *L. socotranum* displayed better antibacterial activity toward *Micrococcus luteus S. aureus* and *P. aeruginosa.* Additionally, [61] revealed that the ethyl-acetate fraction of *L. brasiliense* extracts effectively inhibited the growth of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium.*

Moreover, the obtained results indicated the ability of *L. pruinosum* extract to suppress the biofilm formation by *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (Table 3). As stated by [65], a biofilm is a collection of microbial cells that have become permanently attached to a surface (they cannot be removed by mild rinsing) and are contained within a polysaccharide-based matrix. Exopolysaccharide-producing pathogenic bacteria have been found to produce biofilms [42]**.** *E. coli* and *Staphylococcus spp*. have been categorized as strong biofilm formers and have been shown in numerous investigations to have a very potent capacity to establish bacterial adhesion [66]. It has been established that biofilms are a prevalent cause of infections, and over 80% of persistent bacterial infections worldwide were linked to biofilms [67]**.** Antibacterial drugs were employed to penetrate the polysaccharide layers and diminish the bacterial biofilm, which therefore made it easier to suppress the bacterial cells.

The quantification of TPC of the *L. pruinosum* extract under study evoked the presence of a respectable amount of

total phenolics (210 ± 0.11 µg gallic acid equivalent/mg). It was greater than that informed for *L. sinuatum* (23–34 mg GAE/g DW) [22, 68], while lesser than that published for *L. algarvense* (228 mg GAE/g DW) by [69, 70]**.** One of the major plant bioactive chemical groups associated with antioxidant action is the phenolic family. Phenolic compounds were originally considered phytochemicals that promote health [71]. These chemicals also have numerous health benefits due to their high antioxidant power [72]. Several factors, including abiotic and biotic stress, retirement, and extraction procedure, have a significant effect on the quantities of these compounds [73]. A recent investigation by [74–76] revealed that polyphenols have a variety of functions, such as immunomodulatory, antibacterial, wound repair, and antioxidant. Various literature surveys disclosed that *Limonium* species are rich in phenolics, terpenoids, and minerals [21, 77]**.** Recently, phenolic compounds are in the spotlight because they show a critical part in stopping oxidation progressions and also have valuable and important features in improving health and preventing disorders such as cancer, inflammation, hypertension, and acute oxidative damage [22, 23]**.** Concerning the antioxidant activity of *L. pruinosum* extract, our conclusions were in deal with the investigation performed by [78] which implied that the extracts of *L. densiflorum* display the ability to drastically diminish the generation of ROS and so safeguard cells against oxidative damage. In the food sector, synthetic antioxidants are frequently employed. They stop the creation of toxins or mutagenic substances that could be harmful to people's health. Because of these synthesized compounds' hazardous and cancer-causing characteristics, there has recently been some controversy about their unfavorable use [8]**.** As a result, it is crucial to use natural antioxidants in place of synthetic ones. Aside from its therapeutic usefulness, the genus *Limonium* has ornamental value due to its stunning flowers, some species are consumed as foodstuffs, and some species are utilized as an antioxidant in cosmetics [20]**.** On the other hand, *L. pruinosum* extract executes cellular recovery of (GES-1) and, consequently, wound curing at distinct intervals.

Our findings were consistent with those obtained by [79–81] who disclosed that Hexadecane and Docosene have antibacterial activity. Also, our results concurred with those of [82, 83] who found that tetradecanoic acid has been shown to have antibacterial impacts versus *Listeria monocytogenes* and *C. albicans*. On the other hand, it was reported by [79, 80] that Eicosane is efficient against *S. aureus*, *Salmonella typhi,* and *Escherichia coli*. Interestingly, Cyclohexane, 1,1'-(2-tridecyl-1,3-propanediyl) bis-, and Decahydro-8a-ethyl-1,1,4a, 6-tetramethylnaphthalene have shown to possess antioxidant and Anticancer activities as described by [84]**.** Also**,** N, N-dimethylformamide is primarily employed as a polar solvent in the production of polyacrylonitrile fibers, and trends in its production are analogous to those of the polyacrylic fiber industry. It is also employed in the production of premium polyurethane and polyamide coatings, such as those for leather or synthetic leather textiles [85]. Lately, *L. pruinosum* and *L. tunetanum* were confirmed as rich sources of bioactive compounds [71].

Relating to the cytotoxic efficacy, the results showed that *L. pruinosum* extract has no substantial harmful impacts on the Wi-38 normal cell line while exerting effective cytotoxic impacts against cancerous MCF-7 and HepG-2 cell lines in a dose-dependent manner. Because most expensive chemotherapy agents have devastating side effects and non-specific cytotoxicity against healthy body cells, which can result in a variety of detrimental outcomes, so, there is a growing need for new anti-cancer treatments to give novel biological control ways [44, 86]**.** As stated by [87]. The superlative chemotherapy treatment ought to be more specialized and capable of distinguishing healthy cells from malignant cells. Several studies conducted by [2, 44] informed that the bulk of anticancer medications still lacks pharmacological specificity, killing both cancer cells and healthy cells at the same time. To find new chemotherapeutic medications with high potency and specificity, amazing efforts are put forth. This study revealed the specificity of *L. pruinosum* extract and indicated that this plant extract was less toxic against normal cell lines compared to the malignant cell lines. These vital results provide a new approach to the cytotoxic potential of MCF-7 and HepG-2 cell lines with high specificity. Despite the widespread usage of the genus *Limonium* in folk medicine, very few researchers have concentrated on the biological features of the diverse species that comprise this genus. *L. pruinosum* anticancer action has not been reported anywhere in previous publications. We investigated this species' potential anticancer properties because of its significant anti-inflammatory and antioxidant properties as well as its abundance of phenolic chemicals. It has been demonstrated that natural substances found in plants can stop the growth of malignant cells and altering the signaling pathways that lead to cellular death [88]**.** Regarding the anticancer potential of the genus *Limonium* [78]*,* recorded that *Limonium densiflorum* extract evoked the strongest anticancer activity against human lung carcinoma (A-549) and colon adenocarcinoma (A-549) (DLD-1). *L. pruinosum* extract interestingly demonstrated full selectivity between human cancer cell lines and normal human cells.

According to [89], cell cycle arrest is a phase in cell growth where the cell is not consistently engaged in the procedures related to replication and division. It has been demonstrated that inhibiting high-dose cytarabine (IHDAC) has antiproliferative effects by causing cell-cycle arrest in G1 through the upregulation of cyclin-dependent kinase CDK inhibition or the downregulation of cyclins and CDKs [90]. Moreover, as described early by [91, 92]**,** most plant-based anticancer drugs that are prescribed to fight cancer

restrict the proliferation of cancer cells by controlling the cell cycle, especially G2/M accumulation. Also, according to [93], *Limonia acidissima* fruit extract had a G2/M cell cycle arrest-mediated inhibitory impact on MDA-MB435 breast cancer cells. As a result, it may react with tubulin similarly to other plant-derived chemotherapies. The cell type and culture circumstances may, however, affect this plant's antitumor effect. The results of the present work evoked that *L. pruinosum* extract provoked considerable early- and late-apoptotic cell populations in MCF-7 cancerous cells. Apoptosis is a form of cellular death that has an essential task in controlling cell proliferation [44, 86]. The findings suggest that the *L. pruinosum* extract under study was able to induce cell cycle arrest. However, more research is required to determine the molecular pathways underlying this plant's anticancer properties. They, therefore, seem to be promising candidates for more study and therapeutic treatment.

5 Conclusion

In conclusion, it is clear from these findings that *L. pruinosum* exhibited higher biological activity both in vitro and ex vivo. *L. pruinosum* exerts noticeable antioxidant effectiveness concerning DPPH, NO, and hydroxyl radicals. Additionally, this study revealed the specificity of *L. pruinosum* extract and indicated that this plant extract was less toxic against normal cell lines (Wi-38) compared to the malignant cell lines with high specificity. Our findings demonstrated that *L. pruinosum* is a potent breast tumor suppressor causing suppression of the cell cycle and induction of apoptosis and showed superior tumor cell regression efficacy which might be a potential molecular target for breast cancer treatment. This study also shed light on the effectiveness of *L. pruinosum* in curing wounds in gastric epithelial cells (GES-1) by ($79.9343 \pm 1.98\%$) compared with control ($68.3637 \pm 2.32\%$) in 48 h. Gas chromatography-mass spectrometry (GC–MS) analysis revealed the presence of several effective metabolites, the majority of which are responsible for the extract's antibacterial and immunomodulatory impacts. Overall, *L. pruinosum* has several beneficial properties and may be put forward as a potential new additive in pharmaceutical and food products.

Author contribution Conceptualization; M.H.S, A.Y.M, and R.B., Methodology; A.Y.M, G.E.D, R.B, M.H.S, O.G.R, and E.A. Data Analysis; M H.S, A.Y.M, G.E.D, O.G.R and R.B. Figures and tables preparation; A.Y.M, G.E.D, E.A, R.B, and M.H.S. Writing original draft preparation; A.Y.M, and R.B. Writing review and editing A.Y.M, G.E.D, R.B. All authors have read and agreed to the published version of the manuscript.

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Data availability This investigation offers all the data collected or estimated throughout this effort.

Declarations

Ethical approval There are no experiments on persons or animals in this study.

Informed consent Not applicable.

Conflict of interest The authors declare no competing interests.

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