http://www.herbmedpharmacol.com doi: 10.34172/jhp.2024.48140

Journal of Herbmed Pharmacology

Antioxidant, photoprotective, cytotoxic, and antimicrobial activities of *Albuca amoena* Batt.

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 14 July 2023
Accepted: 25 September 2023

Keywords:
Albuca amoena
Antimicrobial activity
Antioxidant activity
Cytotoxic activity
Photoprotective activity

ABSTRACT

Introduction: *Albuca amoena* Batt. is an endemic plant from Morocco used for treating melanoma or leishmaniasis. The aim of this study is to test the antioxidant, photoprotective, cytotoxic, and antimicrobial properties of this plant.
Methods: The extracts of the aerial and bulbous parts were obtained by hydroalcoholic maceration of the plant. Their antioxidant potentials were evaluated *in vitro* using four methods of 2,2-diphenyl-1picrylhydrazil (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP) assay, and total antioxidant capacity assay (TAC). The photoprotective properties were evaluated through the study of UVA and UVB absorbance and antityrosinase activity. The cytotoxic activity was determined against two breast cancer cell lines (MCF-7 and MDA-MB-231) and one human colorectal cancer cell line (HT-29) using the 3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) method. In addition, the antimicrobial activity was tested on six pathogens: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Candida albicans* by using the disc diffusion method.
Results: The results showed that the UVA and UVB absorptive proprieties of the extracts were similar to those used as positive controls: Methyl salicylate and zinc oxide. Moreover, the antioxidant and antityrosinase properties of the studied extracts were found to be weakly active. The extracts had strong cytotoxicity activities, especially against HT-29 cell line. However, the extracts did not show any antibacterial activity.
Conclusion: According to the results of this study, the extracts can be used as a sunscreen to avoid dermal anomalies and as a new source of cytotoxic bioactive compound.

Implication for health policy/practice/research/medical education:
Our findings reveal that *Albuca amoena* extracts have a remarkable cytotoxic activity and a beneficial effect on the skin. By carrying out other phytochemical tests, this plant might be used as a source of molecules for drug development.

Please cite this paper as: Zakhour R, El-Guourrami O, Bouothmany K, El Ftouh S, Benkhouili FZ, Benzeid H, et al. Antioxidant, photoprotective, cytotoxic, and antimicrobial activities of *Albuca amoena* Batt. J Herbmed Pharmacol. 2024;13(1):101-110. doi: 10.34172/jhp.2024.48140.

Introduction

Medicinal plants contain an immense reservoir of active principles that are responsible of pharmacological activities, which provide unlimited possibilities for the discovery of new drugs from nature. Human was and remains linked to their use to treat and relieve various health problems (1,2). Based on the World Health Organization (WHO) calculations, 80% of world population utilizes plants as remedies for curing several diseases (3).

Because of its geographical position, Morocco is

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characterized by an important medicinal flora that represents a very attractive track for the valorization of the therapeutic activities of these plants (4). Many species have been used by Moroccan people, mostly in rural regions, for centuries in folk medicine for medicinal purposes using traditional practices for preparations; most of them have been registered in the Moroccan pharmacopoeia (5). *Albuca amoena* Batt., called Ouliad in Morocco, is a fast-growing herbaceous annual plant from the Asparagaceae family. This medicinal plant is found on sandy and stony desert pastures of northwestern Sahara in Algeria and the adjacent regions of Morocco. Traditionally, *A. amoena* is known among the native population by its use at the dermal level for the treatment of melanoma or leishmaniasis, and at the sedative level against pain or excitement (6–9). There is no report in the literature about the biological activity of this species. However, various species of the genus *Albuca* have been found to have antioxidant, antidiabetic, antibacterial, cytotoxicity, and anti-inflammatory activities (10–13).

Oxidative stress exerts a significant influence on the initiation and progression of numerous disorders, for example, cancer, diabetes, aging, autoimmune disorders, and even cardiovascular and neurodegenerative diseases (14). Plant-derived antioxidant compounds are considered effective in suppressing or inhibiting cellular damage caused by free radicals (15). Polyphenols are the major secondary metabolites of plants that have an aromatic ring with at least one hydroxyl group. They function as agents for reducing, scavenging of singlet and triplet oxygens, and providers of hydrogen (16).

Nowadays, the development of antibiotic resistance is due to the excessive use of antimicrobial agents in clinical practices, where bacteria develop this strategy to promote resistance to antimicrobial agents (17). Several studies have been conducted on natural antimicrobials of plant origin with the aim of discovering new and natural bioactive compounds with antimicrobial characteristics for drug development (18).

Ultraviolet (UV) radiation, particularly UVA and UVB rays, reach the earth and can penetrate the skin, resulting in various unhealthy effects (19). The primary impact of these radiations is the generation of reactive oxygen species (20). Skin protection against these external aggressions remain a priority, and only chemical protection, meaning the use of sunscreens, remains effective, because their chemical composition is based on the use of compounds capable of absorbing radiation (21).

Tyrosinase is an enzyme that plays an essential role in melanogenesis (22). It is involved in a speed-limiting step in melanin production, catalyzing the oxidation of L-tyrosine and L-DOPA (L-3,4-dihydroxyphenylalanine) to dopaquinone in melanocyte melanosomes (23). Melanin is responsible for pigmentation of the eyes, skin, and hair, and plays an important role in protecting the skin from UV damage (24). However, it has been asserted that hyperpigmentation anomalies are implicated in melasma and melanoma, and are also responsible for significant and adverse medical incidents, particularly in the elderly (25).

In the context of the valorization of an endemic plant, this work proposed the testing of various biological properties (antioxidant, photoprotective, cytotoxic, and antimicrobial) of the hydroalcoholic extracts obtained from the aerial and bulbous parts of *A. amoena*.

Materials and Methods

Plant materials and extraction process

The hydroalcoholic extracts from aerial part (AP) and bulbous part (BP) of *A. amoena* used in this study were the same as those prepared in our previous work (26). The specimen of the plant was gathered in March 2019 from Boudnib, Morocco. A vouchers specimen was placed in the herbarium unit of the Scientific Institute of Rabat, Morocco, under the reference code RAB108289. The extracts were prepared using cold maceration (during 24 h) in a mixture solution of ethanol and distilled water (1V: 1V).

Phytochemical tests

Determination of total polyphenols content

The content of total polyphenols was conducted by the Folin-Ciocalteu method (27). The results were presented using a standard curve based on gallic acid. Then, the total polyphenol content was expressed as milligram of gallic acid per gram of extract (mg GAE/g extract).

Determination of total flavonoids content

To determine the flavonoids present in the hydroalcoholic extracts of *A. amoena*, the aluminum chloride colorimetric method was used (28). A quercetin calibration curve was used to present the results in milligram equivalent of quercetin per gram of the extract (mg EQ/g extract).

Determination of total flavonols content

The detection of flavonol content in the extracts was determined according to the method described by Yermakov et al (29). The expression of the flavonol content was in milligram equivalent of quercetin per gram of any extract (mg EQ/g extract) from the calibration curve of quercetin, established under the same operating conditions.

Determination of total condensed tannins content

To detect the condensed tannin content, the method described by Karray-Bouraoui et al. (30) was used. A catechin standard was also prepared under the same conditions to present the results in equivalent milligrams of catechin per milligram of the extract (mg EC/mg extract).

Antioxidant activity

DPPH free radical scavenging assay

According to Şahin et al method (31), *A. amoena* extracts were tested for their antiradical activity. The results were expressed as an inhibition concentration of 50% of DPPH (IC_{50}). Indeed, quercetin was used as a positive control for comparison. The calculation of percentage of inhibition was determined using the following formula:

$$\text{Percentage of inhibition (\%)} = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}} \times 100$$

ABTS radical scavenging assay

The effects of the extracts on stabilizing 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a source of free radicals were evaluated according to the method described by Tuberoso et al (32). The ascorbic acid was used as positive control and the percentage of inhibition was calculated in the same way as for DPPH.

Ferric reducing antioxidant power assay

The ability of the extracts to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) was determined according to the method described by El Jemli et al (33). The standard employed in this assay was catechin.

Total antioxidant capacity assay

The total antioxidant capacity (TAC) of *A. amoena* extracts was determined according to the method adjusted from Ohikhena et al (34). The TAC was expressed in equivalent milligrams of acid ascorbic per milligram of the extract (mg EAA/mg extract).

Photoprotection activity

UV absorption assay

For this test, the absorbance of *A. amoena* extracts at a concentration of 5 mg/mL was read at two wavelengths, which exhibited UVA (365 nm) and UVB (300 nm). Methyl salicylate (100 µg/mL) and zinc oxide (5%) were used as positive controls (35).

Tyrosinase activity assay

The determination of the anti-tyrosinase activity of *A. amoena* extracts was carried out according to a previous work (36). Kojic acid served as a positive control, and the calculation of percentage of inhibition was carried out in following manner:

$$\text{Percentage of inhibition (\%)} = \frac{(\text{Control} - \text{Blanc}) - (\text{Sample} - \text{Blanc sample})}{(\text{Control} - \text{Blanc})} \times 100$$

Cytotoxic activity

Cell culture

In order to appreciate the cytotoxic effects of the extracts, three cancerous cell lines were employed in this study. Two breast cancer cell lines (MCF-7 and MDA-MB-231) and a colon cancer cell line (HT-29) were kindly obtained by Prof. Hamid Morjani from UFR of pharmacy, Reims, France. The culture of the cell lines was made in DMEM media with 10% Gibco BRL fetal serum, 1% penicillin-streptomycin, and 1% L-glutamine at 37 °C in an atmosphere of 5% CO_2 and 95% air.

Cell viability: MTT assay

The evaluation of cancer cell growth inhibition was carried out based on the MTT colorimetric assay (37). The cell cultures were distributed in 96-well microplates at a density of 8000 cells per well. After 24 hours, different concentrations of the extracts, ranging from 3125 to 100 µg/mL, were added to the cells and the whole was re-incubated for 72 hours at 37 °C. In the end, 10 µL of MTT (5 mg/mL) was added and the plates were incubated for 4 h. In these experiments, the negative control consisted of a medium without the extract, while Mitomycin C served as the positive controls. Afterwards, the optical density of each well was read using Wallac Victor X3 multiplate reader, at a wavelength of 570 nm. All *in vitro* experiments were performed in triplicates for each tested substance as well as the control. The cytotoxic effect or the percentage of cell lysis was measured in relation to the viability of untreated cells, according to the following formula:

$$\text{Cytotoxicity (\%)} = \frac{\text{OD Control} - \text{OD Essai}}{\text{OD Control}} \times 100$$

Where OD is optical density.

Antimicrobial activity

The antimicrobial activities of the hydroalcoholic extracts of AP and BP were evaluated against six pathogens, including Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 3354, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, and *Proteus mirabilis* ATCC 21100), Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213), and one yeast (*Candida albicans* ATCC 90028) available in the Research Laboratory of the Ibn Sina Hospital in Rabat, Morocco.

Preparation of the inoculum

Each species was inoculated beforehand on nutrient agar to obtain a 24 h culture. Then, 4 to 5 well-isolated bacterial colonies were suspended in 0.9% NaCl physiological water. Finally, this suspension was adjusted to the McFarland 0.25 standard using a turbidimeter.

Diffusion method in agar medium: Sensitivity test

The well technique consisted of distributing Muller-Hinton medium for bacteria and Sabouraud simple medium for yeast in Petri dishes. After solidification, the plates were flooded with the pathogenic strain. Identical

volume wells of 5 mm in diameter were made using a sterile Pasteur pipette. Each well received 100 μL of the extract (50, 100, or 200 mg/mL). After 30 minutes of diffusion at laboratory temperature, the dishes were incubated for 24 hours at 37 °C. Tetracycline and Ceftazidime served as positive controls, while distilled water was employed as a negative control. The presence or absence of an inhibition zone was observed (38).

Statistical analysis

We employed the "GraphPad Prism 8" software for conducting statistical analysis. The outcomes were reported as the mean value along with the standard deviation (SD), based on three replicates. The data underwent scrutiny through one-way analysis of variance (ANOVA), followed by a post hoc assessment, with statistical significance set at a P value < 0.05."

Results

Phytochemical test

The total polyphenols and total condensed tannins contents were determined using the calibration curves of gallic acid and catechin, respectively. While, total flavonoids and total flavonols were determined using the calibration curve of quercetin. The results of total polyphenols, flavonoids, flavonols, and condensed tannins contents of *A. amoena* extracts were summarized in Table 1. Generally, it can be noted that the two hydroalcoholic extracts of AP and BP contained a source of phenolic compounds. Indeed, the total phenolic contents in AP and BP were found to be 6.40 ± 0.06 and 1.34 ± 0.07 mg EAG/g extract, respectively. In addition, the concentration of flavonoids of *A. amoena* extracts ranged between 8.21 ± 0.03 and 2.80 ± 0.16 mg EQ/g extract. However, the flavonols contents were absent in both extracts. The AP and BP hydroalcoholic extracts also contained condensed tannins with 5.80 ± 0.10 and 2.83 ± 0.11 mg EC/g extract, respectively.

Antioxidant activity

The antioxidant activity of the two extracts of *A. amoena* was assisted *in vitro* using four complementary tests: DPPH, ABTS, FRAP, and TAC assays. As summarized in Table 2, the hydroalcoholic extract from the aerial part of *A. amoena* showed the highest antioxidant capacity compared to the hydroalcoholic extract from the bulbous part in the four tests. The AP extract presented free radical neutralization capacities, with IC_{50} of 0.250 ± 0.009 and 0.380 ± 0.008 mg/mL for DPPH and ABTS, respectively. However, the BP extract was inactive against DPPH and ABTS. The results of reducing power and the TAC were 0.270 ± 0.030 mg/mL and 0.094 ± 0.003 mg AAE/g extract for AP extract and 1.520 ± 0.010 mg/mL and 0.010 ± 0.001 mg AAE/g extract for BP extract.

Photoprotection activity

UV absorption assay

The absorbance values of UVA and UVB of AP and BP extracts are reported in Table 3. Zinc oxide (5%) was considered as a positive control for UVA absorption and Methyl salicylate (100 μg/mL) for UVB absorption. At a concentration of 5 mg/mL, the absorbance of UVB by AP extract was comparable to Methyl salicylate (100 μg/mL), which absorbed 2.19 ± 0.01 and 2.23 ± 0.02, respectively. In addition, there was a slight difference between the

Table 1. Total polyphenols, flavonoids, flavonols, and condensed tannins contents in hydroalcoholic extracts of *Albuca amoena*

Compounds	Aerial part	Bulbous part
Total polyphenols content (mg EAG/g extract)	6.40 ± 0.06[a]	1.34 ± 0.07[b]
Total flavonoids content (mg EQ/g extract)	8.21 ± 0.03[a]	2.80 ± 0.16[b]
Total flavonols content (mg EQ/g extract)	Nd	Nd
Total condensed tannins content (mg CE/g extract)	5.80 ± 0.10[a]	2.83 ± 0.11[b]

Values are means ± standard deviation of three replicates. Values on the same line with different superscript letters indicate significant differences (P value < 0.05).
Nd: not detected; GAE: gallic acid equivalent; QE: quercetin equivalent; CE: catechin equivalent.

Table 2. The antioxidant activity of hydroalcoholic extracts of *Albuca amoena*

Tests	Aerial part	Bulbous part	Quercetin	Catechin	Ascorbic acid
DPPH IC_{50} (mg/mL)	0.250 ± 0.009[a]	Inactive	0.005 ± 0.001[b]	-	-
ABTS IC_{50} (mg/mL)	0.380 ± 0.008[a]	Inactive	-	-	0.002 ± 0.001[b]
FRAP IC_{50} (mg/mL)	0.270 ± 0.030[a]	1.520 ± 0.010[b]	-	0.013 ± 0.001[c]	-
TAC (mg AAE/g extract)	0.094 ± 0.003[a]	0.010 ± 0.001[b]	-	-	-

Values are means ± standard deviation of three replicates. Values on the same line with different superscript letters indicate significant differences (P value < 0.05).
AAE: Acid ascorbic equivalent; ABTS: 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-Diphenyl-1picrylhydrazil; FRAP: Ferric reducing antioxidant power assay; IC_{50}: The concentration to exert 50% of the maximal inhibition; TAC: Total antioxidant capacity.

Table 3. The absorbance values of UVA and UVB of hydroalcoholic extracts of *Albuca amoena*

Product	UVA	UVB
Aerial part (5 mg/mL)	1.97 ± 0.01[a]	2.19 ± 0.01[a]
Bulbous part (5 mg/mL)	0.52 ± 0.03[b]	1.59 ± 0.03[b]
Methyl salicylate (100 µg/mL)	0.03 ± 0.01[c]	2.23 ± 0.02[a]
Zinc oxide (5%)	2.33 ± 0.04[a]	3.25 ± 0.02[c]

Values are means ± standard deviation of three replicates. Values on the same column with different superscript letters indicate significant differences (*P* value < 0.05).

absorbances of Zinc oxide (5%) and AP extract. The latter absorbed 1.97 ± 0.01 of UVA compared to 2.33 ± 0.04 of the control absorbance.

All UVA and UVB absorbances by BP extract were lower than those of AP extract. Both of them had tendency to absorb UVB more than UVA.

Tyrosinase activity assay

As summarized in Table 4, the results of this test showed that to inhibit 50% of the tyrosinase effect, concentrations of 1.69 ± 0.05 mg/mL and 1.67 ± 0.04 mg/mL of AP and BP extracts, respectively, were required. The extracts showed poor results when compared with the kojic acid result (positive control).

Cytotoxicity activity

In order to evaluate the cytotoxic potential of *A. amoena*, we confronted our extracts to three tumor cell lines, two breast cancer cell lines (MCF-7 and MDA-MB-231) and one colorectal cancer cell line (HT-29).

First, the observation of the morphology of cells optical microscope allowed us to notice the effect of the extracts. Indeed, the treated cells lost their anchoring properties and detached by adopting a round morphology, compared to the untreated cells, which retained their anchoring properties and remained attached to each other.

Treatment of MCF-7 and MDA-MB-231 human breast cancer cell lines, and HT-29 human colon cancer cells for 72 hours with increasing concentrations of the extracts ranging from 100 to 3.125 µg/mL caused remarkable morphological changes (Figure 1). For HT-29 cells, considerable morphological changes were observed at all concentrations tested, even at low concentrations (3.125 µg/mL) (Figure 1C and D). The cytotoxic effects of AP and BP extracts on the proliferation of the MCF-7, MDA-MB-231, and HT-29 cell lines were evaluated by MTT assay. The calculation of the IC_{50} values was performed using a dose-response curve and the values are presented in Table 5.

Using cell viability indices, the MTT test revealed that both extracts reduced cell viability in all cancerous cells tested in a dose-dependent manner after 72 hours of treatment (studied range 3.125 to 100 µg/mL) with a

Table 4. The anti-tyrosinase activities of hydroalcoholic extracts of *Albuca amoena*

Product	IC_{50} (mg/mL)
Aerial part	1.69 ± 0.0500[a]
Bulbous part	1.67 ± 0.0400[a]
Kojic acid	0.01 ± 0.0007[b]

Values are means ± standard deviation of three replicates. Values on the same column with different superscript letters indicate significant differences (*P* value < 0.05). IC_{50}: The concentration to exert 50% of the maximal inhibition.

Table 5. The cytotoxic effects of hydroalcoholic extracts of *Albuca amoena* on various cell lines

Product	IC_{50} (µg/mL)		
	HT-29	MCF-7	MDA-MB-231
Aerial part	18.55 ± 2.15[a]	12.92 ± 2.30[a]	75.69 ± 9.40[a]
Bulbous part	12.39 ± 2.37[b]	4.012 ± 0.12[b]	7.94 ± 1.86[b]
Mitomycin	-	1.220 ± 0.30[c]	6.60 ± 1.44[b]

Values are means ± standard deviation of three replicates. Values on the same column with different superscript letters indicate significant differences (*P* value < 0.05). IC_{50}: The concentration to exert 50% of the maximal inhibition.

pronounced effect of extracts on HT-29 cells after 72 post incubation. The responses of MCF-7, MDA-MB-231, and HT-29 cancer cells to increasing concentrations of both extracts are shown in Figures 2 and 3.

Results showed that the MCF-7 cells were more sensitive to BP and AP extracts recording IC_{50} of 4.012 ± 0.12 and 12.92 ± 2.30 µg/mL, respectively; the IC_{50} corresponding to BP and AP extracts on MDA-MB-231 cells were 7.94 ± 1.86 and 75.69 ± 9.40 µg/mL, respectively.

The viability of colorectal tumor cells (HT-29) was found to be very low after 48 and 72 hours of incubation; however, IC_{50} was determined after only 24 hours of incubation (Figure 1C and D), which indicated that the extracts had a strong cytotoxic effect on the HT-29 cell line.

Antimicrobial activity

The results tabulated in Table 6 show that the hydroalcoholic extracts of *A. amoena* were not as active as positive controls (tetracycline and ceftazidime); they did not inhibit bacterial growth, therefore they did not form an inhibitory zone.

Discussion

In this study, the antioxidant activities of *A. amoena* extracts were assessed using four tests frequently used for the valorization of plant extracts namely DPPH, ABTS, FRAP, and TAC. The first two methods (DPPH and ABTS) rely on the ability of the product to inhibit free radicals by giving up protons, while the two others (FRAP and TAC) require electrons (39,40). The AP extract was active in all four methods, while the BP extract was inactive in DPPH

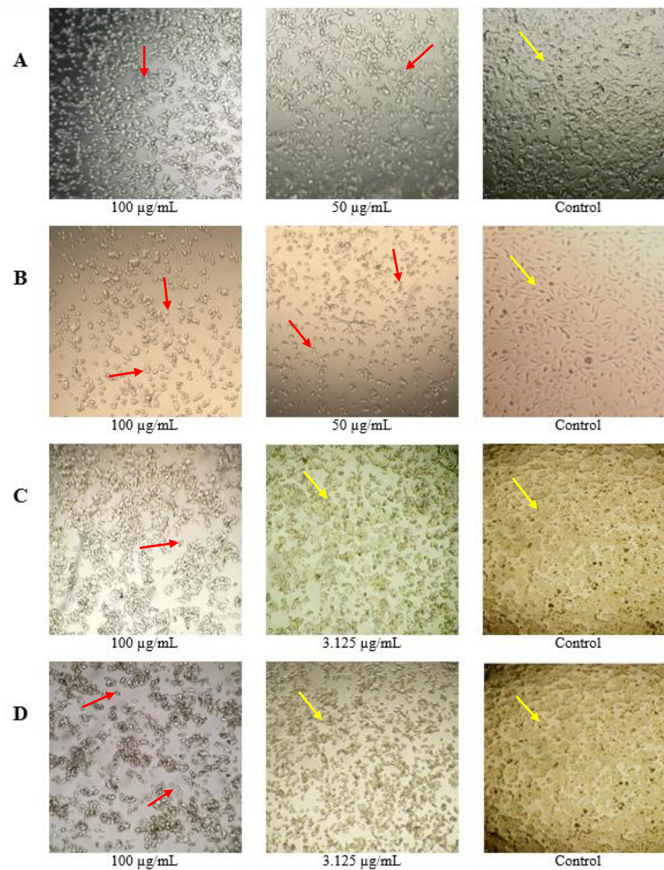

Figure 1. Microscopic observation of the cytotoxic effect of bulbous part extract on MCF-7 cell line (A), MDA-MB-231 cell line (B), HT-29 cell line 24 h post incubation (C), and HT-29 cell line 72 h post incubation (D). Red arrows: Cancer cell detached as well as the loss of anchor properties. Yellow arrows: Cancer cells attached to neighbors with preservation of anchor properties.

and ABTS. According to the results, we can deduce that both extracts have antioxidant activities with a different order of action: AP tends to scavenge free radicals by giving up electrons or protons, while BP only gives up electrons. One of the chemical groups present in plants is the polyphenols, which have a great biological activities, especially antioxidant activity (41,42). As show in our results, AP extract is more concentered of polyphenols and has more antioxidant activity than BP extract.

Exposure to sunlight causes an oxidative stress and a stimulation of tyrosinase for L-Dopa to oxidize and turn into melanin. However, a high exposure to sunlight induces a deregulation of melanogenesis and causes pigmentary anomalies and even cancers. To prevent this

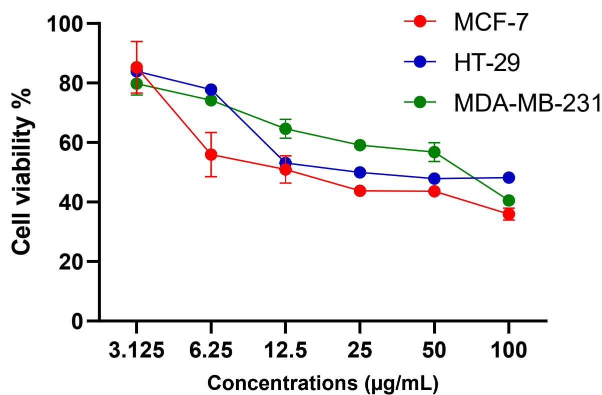

Figure 2. The dose-response curves for cell viability assay following the treatment with aerial part extract.

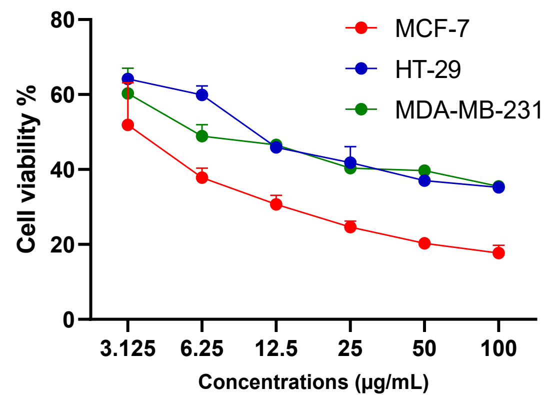

Figure 3. The dose-response curve for cell viability assay following the treatment with bulbous part extract.

Table 6. Antimicrobial susceptibility of hydroalcoholic extracts of *Albuca amoena* at different concentrations

Microorganisms	Diameters of inhibition zones (mm)								
	Aerial part (mg/mL)			Bulbous part (mg/mL)			Distilled water	Tetracycline	Ceftazidime
	50	100	200	50	100	200			
Staphylococcus aureus	0	0	0	0	0	0	0	20.66 ± 0.57	Nt
Pseudomonas aeruginosa	0	0	0	0	0	0	0	Nt	15 ± 0.15
Escherichia coli	0	0	0	0	0	0	0	24 ± 0.10	20.37 ± 0.41
Klebsiella pneumoniae	0	0	0	0	0	0	0	21 ± 0.87	Nt
Proteus mirabilis	0	0	0	0	0	0	0	Nt	Nt
Candida albicans	0	0	0	0	0	0	0	Nt	Nt

Values are means ± standard deviation of three replicates.
Nt: not tested.

disequilibrium, the photoprotectors intervene, which are either in the form of physical or chemical barriers (43). Our work indicate that AP and BP absorb the UVA and UVB radiations like a sunscreen. However, the extracts have a low antityrosinase activity compared with Kojic acid. In the other hand, *A. amoena* extracts have tendency to protect the skin against sunlight by a physical and not chemical barrier. Some natural compounds are as active as sunscreen agents, such as some flavonoids (44), terpenoids (45), amino acids (46), lipids (47), glycosides (48), and polyphenols (49). Other works show that *A. amoena* is composed of those phytochemical compounds (26,50), and in this work the polyphenols have been detected and dosed explaining the photoprotective activity of AP and BP extracts.

The results indicated that the BP extract of *A. amoena* was more potent as a cytotoxic bioagent than the AP extract, which explains the use of the bulbs by the indigenous population (9). The cytotoxic effects of the extracts should be due to colchicine and polysaccharides present in this plant (51). The mechanism by which colchicine causes cell death or apoptosis involves the interruption of the mitotic cell cycle. This occurs when it binds to the surface of two tubulin heterodimers next to the exchangeable GTP-binding site that can be exchanged, leading to the depolymerization of microtubule (52).

Colchicine also exerts an effect on gastric cancer (AGS and NCI-N87) by inducing caspase-3-mediated mitochondrial apoptosis (53), on hypopharyngeal cancer (FaDu and SNU1041) by inhibiting the phosphorylated FAK/SRC complex and paxillin (54), on breast cancer (MCF-7) by inhibiting the MMP-2 expression (55), and on colon cancer (HT-29) by decreasing the AKT phosphorylation (56).

Our experimental study demonstrated that *A. amoena* extracts could destroy colorectal tumor cells strongly, like a polysaccharide. The polysaccharides are a part of the chemical composition of plants; they are excellent antioxidant and anti-inflammatory compounds. Further, they provide colon-protective effects that are summarized in the maintenance of homeostasis as well as the alteration of colon cancer cells. The polysaccharides have an effect on colon cancer cells via different signaling pathways (PI3K/AKT signaling, NF-κB signaling and MAPK signaling), via mitochondrial apoptotic pathway, TP53 apoptotic pathway, gut microflora and the disruption of cell cycle (57). Some polysaccharides extracted from *Albuca bracteata*, a species of the same genus as *A. amoena*, have been reported to have a synergistic effect with 5-fluorouracil against colorectal cancer (58,59).

Traditional Moroccan medicine offers a diversity of medicinal plants for antitumor use; two plants of the Asparagaceae family are used in this sense *Asparagus officinalis* L. and *Drimia maritima* (L.) Stearn in the form of a decoction or an infusion, orally (60,61). The steroidal saponin (25S)-5β-spirostan-3β-ol-3-O-β-Dglucopyranosyl-(1→2)-β-Dglucopyranoside isolated from *A. officinalis* has proven to be effective against several tumor cell lines, including human esophageal carcinoma (Eca-109), human gastric carcinoma (MGC-803), human lung adenocarcinoma (LTEP-a-2), nasopharyngeal (KB), and lymphocytic leukemia (L1210) (62). Some studies have proven the antiproliferative ability of *D. maritime*, given its chemical composition rich in cardiotonic heteroside, especially bufadienolide, against human lymphoma (U-937GTB) (63), hepatocellular cancer (HepG-2), colon cancer (HCT-116), cervical cancer (HeLa), and breast cancer (MCF-7) (64).

Some spices of *Albuca* genus like *Albuca bracteata*, and *Albuca setosa*, are known for their antioxidant and antibacterial activities (12,13). According to our results, the extracts of *A. amoena* did not have the same antibacterial activity. Therefore, this plant is not promising in terms of antimicrobial activity, at least for the six isolates tested in our experimental conditions.

Conclusion

In conclusion, the extracts of *A. amoena,* particularly AP extracts, revealed their richness in phenolic compounds. Our findings brought novel insights to the existing

literature regarding the cytotoxicity activity of this plant. Moreover, the extracts displayed high photoprotective activity. However, the antioxidant and antityrosinase properties of the extracts studied were found to be of low activity. The extracts also did not show any antibacterial activity. Hence, additional research is needed to delve into the primary compounds found in *A. amoena* and elucidate their mechanisms of actions.

Authors' contributions

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Conflict of interests

Authors state no conflict of interest to declare.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been observed by the authors.

Funding/Support

None to declare.

References

1. Al-Adhroey AH, Nor ZM, Al-Mekhlafi HM, Mahmud R. Ethnobotanical study on some Malaysian anti-malarial plants: a community based survey. J Ethnopharmacol. 2010;132(1):362-4. doi: 10.1016/j.jep.2010.08.006.
2. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001;109(Suppl 1):69-75. doi: 10.1289/ehp.01109s169.
3. World Health Organization (WHO). Diet, Nutrition and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation (WHO Technical Report Series 916). Geneva: WHO; 2003.
4. Jamaleddine M, El Oualidi J, Taleb MS, El Alaoui-Faris FE, Benzine A. Les plantes aromatiques et médicinales au Maroc: statut, endémisme, chorologie et bioclimat. Médecine thérapeutique. 2019;25(3):185-91. doi: 10.1684/met.2019.0776.
5. Fennane M, Rejdali M. Aromatic and medicinal plants of Morocco: richness, diversity and threats. Bulletin de l'Institut Scientifique (Rabat). 2016(38):27-42.
6. Azizi N, Amirouche R, Amirouche N. Cytotaxonomic diversity of some medicinal species of Hyacinthaceae from Algeria. Pharmacogn Commun. 2016;6(1):34-8. doi: 10.5530/pc.2016.1.6.
7. Bellakhdar J. La Pharmacopée Traditionnelle Marocaine. Paris: Ibis Press; 1997. p. 753.
8. El Hadj MDO, Hadj-Mahammed M, Zabeirou H, Chehma A. Importance of spontaneous medicinal plants in the traditional pharmacopoeia of the Ouargla region (northern Sahara-Eastern Algeria). Sci Technol C Biotechnol. 2003(20):73-8.
9. Ajjoun M, Kharchoufa L, Alami Merrouni I, Elachouri M. Moroccan medicinal plants traditionally used for the treatment of skin diseases: from ethnobotany to clinical trials. J Ethnopharmacol. 2022;297:115532. doi: 10.1016/j.jep.2022.115532.
10. Odeyemi S, Afolayan A, Bradley G. In vitro anti-inflammatory and free radical scavenging activities of crude saponins extracted from *Albuca bracteata* Jacq. bulb. Afr J Tradit Complement Altern Med. 2015;12(4):34-40. doi: 10.4314/ajtcam.v12i4.6.
11. Ndebia EJ, Umapathy E, Nkeh-Chungag BN, Iputo JE. Anti-inflammatory properties of *Albuca setosa* and its possible mechanism of action. J Med Plants Res. 2011;5(18):4658-64. doi: 10.5897/jmpr.9000103.
12. Odeyemi S, Afolayan A, Bradley G. Phytochemical analysis and anti-oxidant activities of *Albuca bracteata* Jacq. and *Albuca setosa* Jacq bulb extracts used for the management of diabetes in the Eastern Cape, South Africa. Asian Pac J Trop Biomed. 2017;7(6):577-84. doi: 10.1016/j.apjtb.2017.05.013.
13. Odeyemi SW, Afolayan AJ. Characterization and cytotoxicity evaluation of biologically synthesized silver nanoparticles from *Albuca setosa* aqueous bulb extract. Int J Nanosci. 2019;18(2):1850023. doi: 10.1142/s0219581x18500230.
14. Mssillou I, Agour A, Slighoua M, Tourabi M, Nouioura G, Lyoussi B, et al. Phytochemical characterization, antioxidant activity, and in vitro investigation of antimicrobial potential of *Dittrichia viscosa* L. leaf extracts against nosocomial infections. Acta Ecol Sin. 2022;42(6):661-9. doi: 10.1016/j.chnaes.2021.09.021.
15. Akinmoladun AC, Obuotor EM, Farombi EO. Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. J Med Food. 2010;13(2):444-51. doi: 10.1089/jmf.2008.0292.
16. Lü JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. J Cell Mol Med. 2010;14(4):840-60. doi: 10.1111/j.1582-4934.2009.00897.x.
17. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277-83.
18. Falah F, Shirani K, Vasiee A, Tabatabaee Yazdi F, Alizadeh Behbahani B. In vitro screening of phytochemicals, antioxidant, antimicrobial, and cytotoxic activity of *Echinops setifer* extract. Biocatal Agric Biotechnol. 2021;35:102102.

doi: 10.1016/j.bcab.2021.102102.

19. Afaq F, Mukhtar H. Botanical antioxidants in the prevention of photocarcinogenesis and photoaging. Exp Dermatol. 2006;15(9):678-84. doi: 10.1111/j.1600-0625.2006.00466.x.
20. de Oliveira-Júnior RG, Ferraz CAA, Souza GR, Guimarães AL, de Oliveira AP, de Lima-Saraiva SRG, et al. Phytochemical analysis and evaluation of antioxidant and photoprotective activities of extracts from flowers of *Bromelia laciniosa* (Bromeliaceae). Biotechnol Biotechnol Equip. 2017;31(3):600-5. doi: 10.1080/13102818.2017.1288073.
21. Costa SC, Detoni CB, Branco CR, Botura MB, Branco A. In vitro photoprotective effects of *Marcetia taxifolia* ethanolic extract and its potential for sunscreen formulations. Rev Bras Farmacogn. 2015;25(4):413-8. doi: 10.1016/j.bjp.2015.07.013.
22. dos Santos Videira IF, Moura DF, Magina S. Mechanisms regulating melanogenesis. An Bras Dermatol. 2013;88(1):76-83. doi: 10.1590/s0365-05962013000100009.
23. Halaban R, Patton RS, Cheng E, Svedine S, Trombetta ES, Wahl ML, et al. Abnormal acidification of melanoma cells induces tyrosinase retention in the early secretory pathway. J Biol Chem. 2002;277(17):14821-8. doi: 10.1074/jbc.M111497200.
24. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, et al. A comprehensive review on tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2019;34(1):279-309. doi: 10.1080/14756366.2018.1545767.
25. Baxter LL, Pavan WJ. The etiology and molecular genetics of human pigmentation disorders. Wiley Interdiscip Rev Dev Biol. 2013;2(3):379-92. doi: 10.1002/wdev.72.
26. Zakhour R, El Jemly M, El Guourrami O, Nejjari R, Bouyahya A, Cherrah Y, et al. Preliminary phytochemical screening, acute toxicity and effect of *Albuca amoena* extracts on the central nervous system. J Herbmed Pharmacol. 2022;11(1):107-13. doi: 10.34172/jhp.2022.12.
27. Tan PH, Cheah YK, Jaganath IB, Son R. Bioprotective properties of three Malaysia *Phyllanthus* species: an investigation of the antioxidant and antimicrobial activities. Int Food Res J. 2011;18(3):887-93.
28. Ordoñez AAL, Gomez JD, Vattuone MA, lsla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. 2006;97(3):452-8. doi: 10.1016/j.foodchem.2005.05.024.
29. Miliauskas G, Venskutonis PR, van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004;85(2):231-7. doi: 10.1016/j.foodchem.2003.05.007.
30. Karray-Bouraoui N, Ksouri R, Falleh H, Rabhi M, Jaleel CA, Grignon C, et al. Effects of environment and development stage on phenolic content and antioxidant activities of *Mentha pulegium* L. J Food Biochem. 2010;34(Suppl 1):79-89. doi: 10.1111/j.1745-4514.2009.00303.x.
31. Şahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, et al. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. Food Control. 2004;15(7):549-57. doi: 10.1016/j.foodcont.2003.08.009.
32. Tuberoso CIG, Boban M, Bifulco E, Budimir D, Pirisi FM. Antioxidant capacity and vasodilatory properties of Mediterranean food: the case of *Cannonau* wine, myrtle berries liqueur and strawberry-tree honey. Food Chem. 2013;140(4):686-91. doi: 10.1016/j.foodchem.2012.09.071.
33. El Jemli M, Kamal R, Marmouzi I, Doukkali Z, Bouidida EH, Touati D, et al. Chemical composition, acute toxicity, antioxidant and anti-inflammatory activities of Moroccan *Tetraclinis articulata* L. J Tradit Complement Med. 2017;7(3):281-7. doi: 10.1016/j.jtcme.2016.06.006.
34. Ohikhena FU, Wintola OA, Afolayan AJ. Quantitative phytochemical constituents and antioxidant activities of the mistletoe, *Phragmanthera capitata* (Sprengel) balle extracted with different solvents. Pharmacognosy Res. 2018;10(1):16-23. doi: 10.4103/pr.pr_65_17.
35. Lee CJ, Chen LG, Chang TL, Ke WM, Lo YF, Wang CC. The correlation between skin-care effects and phytochemical contents in Lamiaceae plants. Food Chem. 2011;124(3):833-41. doi: 10.1016/j.foodchem.2010.07.003.
36. Bouyahya A, Lagrouh F, El Omari N, Bourais I, El Jemli M, Marmouzi I, et al. Essential oils of *Mentha viridis* rich phenolic compounds show important antioxidant, antidiabetic, dermatoprotective, antidermatophyte and antibacterial properties. Biocatal Agric Biotechnol. 2020;23:101471. doi: 10.1016/j.bcab.2019.101471
37. Bouothmany K, Bourhia M, Aoussar N, Attaleb M, Salamatullah AM, Nafidi HA, et al. Leaf extracts of *Cistus ladanifer* exhibit potent antioxidant and antiproliferative activities against liver, prostate and breast cancer cells. Appl Sci. 2022;12(17):8603. doi: 10.3390/app12160000.
38. Toty AA, Guessennd N, Bahi C, Kra AK, Tokore DA, Dosso M. Évaluation in-vitro de l'activité antibactérienne de l'extrait aqueux de l'écorce de tronc de *Harungana madagascariensis* sur la croissance de souches multi-résistantes. Bull Soc R Sci Liège. 2013;82:12-21.
39. Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: a review. Int J Mol Sci. 2021;22(7):3380. doi: 10.3390/ijms22073380.
40. Bedlovičová Z, Strapáč I, Baláž M, Salayová A. A brief overview on antioxidant activity determination of silver nanoparticles. Molecules. 2020;25(14):3191. doi: 10.3390/molecules25143191.
41. Aras A, Bursal E, Dogru M. UHPLC-ESI-MS/MS analyses for quantification of phenolic compounds of *Nepeta nuda* subsp. *lydiae*. J Appl Pharm Sci. 2016;6(11):9-13. doi: 10.7324/japs.2016.601102.
42. Awika JM, Rooney LW. Sorghum phytochemicals and their potential impact on human health. Phytochemistry. 2004;65(9):1199-221. doi: 10.1016/j.phytochem.2004.04.001.
43. Skotarczak K, Osmola-Mańkowska A, Lodyga M, Polańska A, Mazur M, Adamski Z. Photoprotection: facts and controversies. Eur Rev Med Pharmacol Sci. 2015;19(1):98-112.
44. Nunes AR, Vieira ÍGP, Queiroz DB, Alves Borges Leal AL, Maia Morais S, Muniz DF, et al. Use of flavonoids and cinnamates, the main photoprotectors with natural origin. Adv Pharmacol Sci. 2018;2018:5341487. doi: 10.1155/2018/5341487.
45. Tan EM, Hilbers M, Buma WJ. Excited-state dynamics of isolated and microsolvated cinnamate-based UV-B sunscreens. J Phys Chem Lett. 2014;5(14):2464-8. doi: 10.1021/jz501140b.
46. Lawrence KP, Long PF, Young AR. Mycosporine-like amino acids for skin photoprotection. Curr Med Chem.

2018;25(40):5512-27. doi: 10.2174/0929867324666170529124237.
47. Vieira MV, Pastrana LM, Fuciños P. Microalgae encapsulation systems for food, pharmaceutical and cosmetics applications. Mar Drugs. 2020;18(12):644. doi: 10.3390/md18120644.
48. Jangde R, Daharwal SJ. Herbal sunscreen: an overview. Res J Top Cosmet Sci. 2011;2(2):35-9.
49. Mansuri R, Diwan A, Kumar H, Dangwal K, Yadav D. Potential of natural compounds as sunscreen agents. Pharmacogn Rev. 2021;15(29):47-56. doi: 10.5530/phrev.2021.15.5.
50. Tahri D, Elhouiti F, Ouinten M, Yousfi M. Terpenoids of plants in arid environments. CABI Rev. 2022;17(1):1-12. doi: 10.1079/cabireviews202217001.
51. Hammiche V, Merad R, Azzouz M. Plantes Toxiques à Usage Médicinal du Pourtour Méditerranéen. Paris: Springer; 2013.
52. Dhyani P, Quispe C, Sharma E, Bahukhandi A, Sati P, Attri DC, et al. Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. Cancer Cell Int. 2022;22(1):206. doi: 10.1186/s12935-022-02624-9.
53. Zhang T, Chen W, Jiang X, Liu L, Wei K, Du H, et al. Anticancer effects and underlying mechanism of colchicine on human gastric cancer cell lines in vitro and in vivo. Biosci Rep. 2019;39(1):BSR20181802. doi: 10.1042/bsr20181802.
54. Cho JH, Joo YH, Shin EY, Park EJ, Kim MS. Anticancer effects of colchicine on hypopharyngeal cancer. Anticancer Res. 2017;37(11):6269-80. doi: 10.21873/anticanres.12078.
55. Bakar-Ateş F, Özmen N, Kaya-Sezginer E, Kurt EE. Effects of colchicine on cell cycle arrest and MMP-2 mRNA expression in MCF-7 breast adenocarcinoma cells. Turk Bull Hyg Exp Biol. 2018;75(3):239-44. doi: 10.5505/TurkHijyen.2018.22755.
56. Huang Z, Xu Y, Peng W. Colchicine induces apoptosis in HT-29 human colon cancer cells via the AKT and c-Jun N-terminal kinase signaling pathways. Mol Med Rep. 2015;12(4):5939-44. doi: 10.3892/mmr.2015.4222.
57. Ji X, Peng Q, Wang M. Anti-colon-cancer effects of polysaccharides: a mini-review of the mechanisms. Int J Biol Macromol. 2018;114:1127-33. doi: 10.1016/j.ijbiomac.2018.03.186.
58. Qin Z, Yuan X, Liu J, Shi Z, Cao L, Yang L, et al. *Albuca bracteata* polysaccharides attenuate AOM/DSS induced colon tumorigenesis via regulating oxidative stress, inflammation and gut microbiota in mice. Front Pharmacol. 2022;13:833077. doi: 10.3389/fphar.2022.833077.
59. Yuan X, Xue J, Tan Y, Yang Q, Qin Z, Bao X, et al. *Albuca bracteata* polysaccharides synergistically enhance the anti-tumor efficacy of 5-fluorouracil against colorectal cancer by modulating β-catenin signaling and intestinal flora. Front Pharmacol. 2021;12:736627. doi: 10.3389/fphar.2021.736627.
60. Aboufaras M, Selmaoui K, Ouzennou N. Use of complementary traditional phytotherapy to manage cancer in Morocco: a decade-long review of ethnopharmacological studies. J Herb Med. 2021;29:100494. doi: 10.1016/j.hermed.2021.100494.
61. Alami Merrouni I, Elachouri M. Anticancer medicinal plants used by Moroccan people: ethnobotanical, preclinical, phytochemical and clinical evidence. J Ethnopharmacol. 2021;266:113435. doi: 10.1016/j.jep.2020.113435.
62. Huang XF, Lin YY, Kong LY. Steroids from the roots of *Asparagus officinalis* and their cytotoxic activity. J Integr Plant Biol. 2008;50(6):717-22. doi: 10.1111/j.1744-7909.2008.00651.x.
63. El-Seedi HR, Burman R, Mansour A, Turki Z, Boulos L, Gullbo J, et al. The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: discovery of an active cardiac glycoside from *Urginea maritima*. J Ethnopharmacol. 2013;145(3):746-57. doi: 10.1016/j.jep.2012.12.007.
64. El-Bondkly EAM, Al Shammari B, El-Gendy M, Alsafari IA, El-Bondkly A, El-Shenawy FS, et al. Phytochemical screening, antifungal, and anticancer activities of medicinal plants *Thymelaea hirsuta*, *Urginea maritima*, and *Plantago albicans*. Biomed Res Int. 2022;2022:9544915. doi: 10.1155/2022/9544915.