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RESEARCH

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Apoptosis and genotoxicity effects of *Zygophyllum album* and *Suaeda palaestina* extracts on Ehrlich solid carcinoma in mice

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Abstract

Background Various medicinal plants and their bioactive compounds exhibited promising anticancer activities by inducing apoptosis, inhibiting angiogenesis, and modulating several signaling pathways in cancer cells. This study aims to assess whether two medicinal plant extracts have anticancer properties, *Suaeda Palaestina* and *Zygophyllum album*.

Methods This study used Ehrlich solid tumor mice as its in vivo model. We divided male mice into five groups ($n=5$ per group). Group I was used as a control for Ehrlich ascites carcinoma (EAC). Groups 2 and 3 were given *Z. album* extract 180 mg/kg and 360 mg/kg body weight intraperitoneally. Groups 4 and 5 were given the same dose of *S. palaestina* and treated three times a week for 2 weeks, starting on day 10 after EAC implantation. After 3 weeks, we collected blood samples and thigh skeletal muscle, homogenized them, and processed them for analysis. The results showed that Ehrlich solid rats (EST) treated with low-dose dichloromethane extracts from *Z. album* and *S. palaestina* had significantly smaller tumor sizes than the control group. Protein expression levels of p53, caspase 3, and Bcl-2 were quantified by western blotting.

Results The extracts from both plants induced the hunger mechanism, leading to increased expression of p53 and caspase 3 and decreased expression of Bcl-2 at the protein level in EST mice treated with *Z. album* and *S. palaestina*. In addition, the comet assay indicated that these plants have a genotoxic potential for solid tumor cells. The T3 and T4 levels in EST blood samples revealed that both plants had significantly reduced the concentration of T3 and significantly increased T4 compared to the EST mice untreated group. Furthermore, these results showed that *Z. album* and *S. palaestina* had antiproliferative effects in EST mice through apoptosis-mediated genotoxicity.

Conclusions These findings indicated that *S. palaestina* and *Z. album* could be considered potential natural sources of anticancer agents.

Keywords Ehrlich solid tumor, Medicinal plants, *Suaeda palaestina*, Anticancer agent, *Zygophyllum album*, Apoptotic genes, Western blot, Comet assay, RIA

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Background

Cancer remains a formidable global challenge, with its complex etiology, rapid progression, and limited therapeutic options. The search for innovative and effective anticancer agents has led researchers to explore the untapped potential of natural sources, such as medicinal plants (Chowdhury et al. 2021). Nature's pharmacopeia

has long held the promise of novel compounds that could hold the key to combat this relentless disease (Noor et al. 2021). Medicinal plants are considered the backbone of folk medicine, which means that over 3 billion people in developing countries use them on a regular basis (Ahvazi et al. 2012). These medicinal plants are recognized as abundant sources of compounds and chemicals that can be used in the synthesis and development of drugs. Furthermore, these plants contribute significantly to the development of human cultures around the world (Ahvazi et al, (2012)**;** Singh and Kumar 2023).

Halophytes are recognized for their capacity to endure and counteract damaging ROS produced in a saline environment, as they have powerful antioxidant systems (Neeraj Kumar et al. 2019; Rahman et al. 2021). Surprisingly, antioxidants identified in halophytes reveal considerable biological activity, exceeding antioxidants from medical glycophytes and synthetic antioxidants (Lopes et al. 2023). These species are currently gaining popularity due to their high concentrations of bioactive compounds. With that in consideration, the halophytes *Suaeda palaestina* and *Zygophyllum album* could potentially be of interest.

Z. album is classified as a halophyte and shrubby plant and is included in the Zygophyllaceae family (Beier 2003) which consists of approximately 27 genera and 285 species generally restricted to arid and semiarid regions (Beier 2003). Indeed, many plants in this genus contain various bioactive potentials including antidiabetic, antioxidant, anti-inflammatory, antitumor, and antimicrobial effects (Tran et al. 2020). Zygophyllin, quinovic, and glycosides are the main components in Zygophyllum species, and they have been proven to have antipyretic and anti-inflammatory effects. This species has long been used as a cure for asthma, gout, and rheumatism (Mohammedi 2020).

The plant *Suaeda palaestina,* scientifically known as *S. palaestina,* belongs to the Chenopodiaceae family and is categorized as a halophyte shrub. The aforementioned plant is an annual herbaceous species that exhibits robust growth in Mediterranean salt marshes. It has been noted that this particular family demonstrates hypoglycemic and hypolipidemic actions (El-Attar et al. 2019a). According to recent studies (Bradford 1976; Singh et al. 2010), *S. maritima* is a good source of antioxidants and has antimicrobial activities.

In this study, we look into these plants' potential as new anticancer drugs. We look at how they affect tumor growth, apoptosis induction, genotoxicity, and how they interact with thyroid hormone to affect the progression of cancer.

Methods

Selection and collection plants

In March 2015, the Desert Research Center (DRC) Egypt collected samples of *Z. album* from the South Sinai and *S. palaestina* from the Borg El-Arab Alexandria. The identification of these plants was performed at the Flora and Phyto-Taxonomy Research Section, which is part of the Horticultural Research Institute of the Agricultural Research Center in Giza, Egypt.

Preparation of plant materials

The methodologies employed by Moustafa et al. (2014) were used with modifications as following: Fresh intact plants were gathered from several regions throughout Egypt. The plant components were dried at room temperature and stored in a dry area before use. The plants underwent a meticulous water washing process, followed by a drying period at room temperature in the absence of light. Subsequently, they were pulverized into a coarse powder using an electric grinder. The extraction of shoot powders (50 g) was conducted using Dichloromethane (CH_2Cl_2) as the solvent. The powders were immersed in 150 ml of CH_2Cl_2 solvent for 48 h at room temperature. At ambient temperature, the particles were submerged in 150 cc of CH_2Cl_2 solvent for 48 h. The plant extracts were collected dropwise and then filtered using Whatman No. 1 filter paper. The leftovers were submerged in 150 ml of solvent for 24 h and subsequently filtered once more. The samples were dehydrated, preserved in glass containers at − 20 °C, and then reconstituted in DMSO for analysis (Wartofsky and Burman 1982).

Animal experiment

The experiment involved Swiss albino male mice 8-weeks-old (20–25 g). They were grouped into five animals per cage. The animals were kept in controlled laboratory settings, with a temperature of 25 ± 2 °C, relative humidity ranging from 40 to 45%, and a 12-h dark/light cycle. Water and unrestricted access to a regular dry pellet diet were supplied. Before commencing the experiment, the mice were acclimated to laboratory conditions for 7 days. The animals were subjected to the guidelines set forth by the National Cancer Institute for the Care and Use of Laboratory Animals. The guidelines for the ethical use and maintenance of laboratory animals issued by the National Institutes of Health and authorized by the Scientific Committee of the Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt, were complied with in all procedures used in caring for rats and taking blood and tissue samples for this experiment.

Acute toxicity in mice

We used the OECD guideline 425 (2008) for chemical testing to determine the acute toxicity of plant extracts in Swiss albino mice. Mice administered oral injections of the extracts at doses up to 2 g/kg body weight showed no adverse effects.

Ehrlich solid-bearing tumors mice

For solid transplantation, the Ehrlich carcinoma cells received a fixed number of viable cells 0.2 mL Ehrlich ascites carcinoma (EAC) containing 2×10^6 cells/mouse were inoculated intramuscular into the femoral region of recipient male mouse and left for 9–15 days to allow tumor to grow.

Experimental design

The animals were divided into five groups: each of five animals. All groups were given EAC cells (2×10^6 cells/mouse), and this day served as day zero. Group one was the EAC's control group. Fourteen days after EAC transplantation (from day 10–23), groups 2 and 3 mice bearing solid Ehrlich tumor were injected three times weekly for 2 weeks intraperitoneally (IP) with *Z. album* extract (180 mg/kg and 360 mg/kg body weight), respectively. While groups 4 and 5 were injected IP by *S. palaestina* extract (180 mg/kg and 360 mg/kg body weight), respectively. After the second and fourth weeks of the experiment, the animals were beheaded, their right thighs were shaved, and around 40 mg of the articular capsule were extracted by rinsing the area with a salt solution. The material was ground into a fine powder in 5 L of a 50 mM Tris HCl buffer with a pH of 7.4. The solution also contained 0.1 M NaCl and 0.1% Triton X-100. Upon the conclusion of the experimental period, euthanasia of the mice was carried out using cervical dislocation, followed by necropsy procedures. Individual blood samples were collected from the inferior vena cava of the mice using heparin as an anticoagulant. After collection, the samples were mixed for 10–20 min and then centrifuged at 3000 RPM for 15 min. The resulting plasma was carefully separated and preserved at −80 °C for future analyses. Tumors obtained from the left thigh muscles of the mice were subjected to further biochemical and molecular analyses, such as Western blotting to examine apoptosis-related proteins (p53, Bcl-2, andcaspase-3) and comet assays to assess DNA damage.

Tumor assessment

After inoculating the tumor, the size of the left thigh in the lower limb of different experimental groups was determined using a digital electronic balance for weighing and a two-end electronic digital caliper from Switzerland for measuring. The measurements were conducted bi-daily until the 23th day, signifying the end of the experiment. The size of the tumor was later determined using the formula outlined by the formula below:

$$\begin{aligned}&\left(\text{The mean tumor weight of control group}\right.\\&\left.-\text{the mean tumor weight of treated group}\right)\\&/\left(\text{the mean tumor weight of control group}\right)\times 100.\end{aligned}$$

Western blotting analysis of EST cell apoptosis-related proteins

To assess the effects of *Z. album* and *S. palaestina* on apoptosis-related proteins in Ehrlich solid tumor mice, a series of laboratory procedures were conducted. Initially, tissue samples were lysed using RIPA buffer, and the protein concentration was determined using the Bradford method. This step was crucial for preparing the samples for further analysis.

The proteins were then separated using SDS-PAGE and transferred to a polyvinylidene fluoride membrane. This process allowed for the isolation and identification of specific proteins of interest. Subsequently, western blot analysis was performed to detect p53, Bcl-2, and caspase-3 proteins. This involved using specific primary antibodies and HRP-conjugated secondary antibodies to bind to these target proteins.

The final steps involved visualizing the protein bands using tetramethylbenzidine and analyzing them through densitometry using ImageJ software. To ensure accuracy, β-actin was used as a standard to normalize the density of each protein band. This comprehensive process enabled researchers to quantitatively analyze changes in apoptosis-related protein expression following treatment with the plant extracts, providing valuable insights into their potential anticancer mechanisms.

Comet assay

The comet test was employed to identify potential DNA damage following different treatments. Alkali-labile sites and DNA strand breakage are detected by measuring the migration of DNA from immobilized nuclear DNA (Singh et al. 2010). Microscope slides were coated with an agarose gel that immobilized Ehrlich solid tumor cells from the control and experimental groups. Placing the slides in a lytic solution allowed the DNA to remain trapped in the agarose while lysing and distributing the cell components. The slides were soaked in an alkaline solution for a period of time to denature the DNA. Strand breakage in denatured cellular DNA led to

supercoil relaxation; the number of breaks increased the degree of relaxation. Given enough relaxation, applying an electric field across the slides generated a motive force that allowed the charged DNA to migrate through the surrounding agarose away from the immobilized main bulk of nuclear DNA. After electrophoresis, the slides were rinsed in neutral buffer, and ethanol was used to fix the gel and its contents. The fixed-slide DNA was stained with GelRed, a fluorescent DNA-specific stain. Stained slides were evaluated under a fluorescent microscope. The migration of DNA was measured using image analysis software, which determined various parameters of the comet, i.e., tail length, percentage of DNA in tail, and tail moment = % DNA in tail × tail length.

Radioimmunoassay (RIA): thyroid hormones

RIA is the most widely implemented nuclear medicine technique. It is an in vitro method that does not expose the patient to radiation. However, this is not the only reason it is so widely used. To develop a radioimmunoassay, antibodies specific for the hormone to be measured, radioisotopically labeled hormone and pure hormone are required. The principle underlying the method is as follows, labeled hormone will be competed with unlabeled hormone for binding sites on the antibody to the hormone. If varying amounts of unlabeled hormone are added to constant amounts of antiserum and labeled hormone, the percentage of labeled hormone binding to antibody will be inversely proportional to the amount of unlabeled hormone present in the system.

A standard curve used concentrations of standards with a limited and fixed amount of labeled antigens and antibodies. The quantity of radioactivity associated with the bound fraction is inversely proportional to the concentration of unlabeled antigen. The interpolation of the standard curve estimates the antigen concentration in the sample.

The RIA procedures of thyroid hormones (T_3 and T_4) described according to Kaptein et al. (1981) and Wartofsky and Burman (1982) were used.

Statistical analysis

Means were statistically compared using the GraphPad prism 6 program with one-way analysis of variance (ANOVA) which was carried out to test any significant difference between treatments at $P < 0.05$.

Results

Effect of *Z. album* and *S. palaestina* on tumor growth

To evaluate the potential antitumor activity of *Z. album* and *S. palaestina* extracts in vivo, the Ehrlich solid tumour (EST) mice model was generated by intramuscular injection of EAC cells into mice. After injected intratumorally (IT) by *Z. album* and *S. palaestina* at 180 mg and 360 mg/kg body weight three times a week for two weeks, both extracts were found to significantly inhibit tumor growth in a dose-dependent manner. The tumour weight was 3.67 ± 0.17 g of the control group, while those of mice treated with low and high concentrations of *Z. album* were reduced to 3.05 ± 0.104 g and 2.25 ± 0.12 g, respectively, and the tumor weight of mice treated with low and high concentrations of *S. palaestina* was 2.50 ± 0.10 g and 2.25 ± 0.07 g (Tables 1 and 2). The tumour inhibition% was 17%, 32%, and 38.7%, 60.8%, respectively (Fig. 1a and b). It seemed that S. palaestina extract impacted more pronounced tumour inhibition than *Z. album* at either concentration.

Table 1 Effect of *Z. album* extract (two concentrations) on tumor growth of EST mice model

Group	Tumor weight (g)	Inhibition rate %
Untreated control	3.6750 ± 0.17^{a}	–
Z. album 180 mg/kg	3.0500 ± 0.10^{b}	17
Z. album 360 mg/kg	2.2500 ± 0.12^{c}	32

Results are expressed as mean ± SE

[a,b,c] Different superscript letters in the same column of tumor weight showed significance difference at $P < 0.05$.by Duncan's new multiple range test

Table 2 Effect of *S. palaestina* extract (two concentrations) on tumor growth of EST mice model

Group	Tumor weight (g)	Inhibition rate %
Untreated control	3.67 ± 0.17^{a}	–
S. palaestina 180 mg/kg	2.50 ± 0.10^{b}	38.7
S. palaestina 360 mg/kg	1.44 ± 0.07^{c}	60.8

Results are expressed as mean ± SE

[a,b,c] Different superscript letters in the same column of tumor weight showed significance difference at $P < 0.05$.by Duncan's new multiple range test

Determination of the apoptosis in Ehrlich solid tumor (EST)

Western blot analysis was performed to evaluate changes in the levels of P53, Bcl2, and Caspase 3 proteins in the EST mice following treatment by *Z. album* and *S. palaestina* in comparison with untreated EST. The expression levels of P53 and Caspase 3 proteins were significantly up-regulated with relative densities of 3.25 ± 0.19 and 5.40 ± 0.28 for p53 2.96 ± 0.15 and 5.23 ± 0.21 for Caspase 3 after *Z. album* (180 mg/kg and 360 mg/kg) treated, respectively, in comparison with untreated EST group with 0.97 ± 0.8 for p53 and 0.92 ± 0.09 for caspase-3 (Fig. 2a), while the expression level of Bcl-2 was down-regulated after treatment by *Z. album* with relative

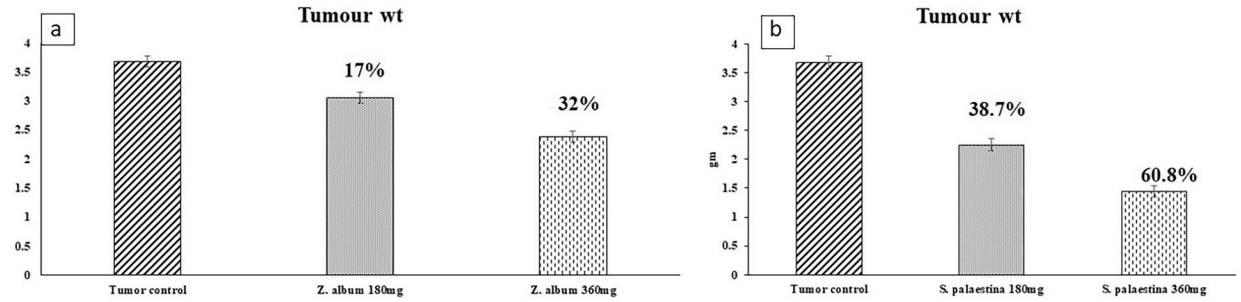

Fig. 1 **a** The tumor inhibition % of *Z. album* extract (180 mg and 360 mg/kg) on tumor growth of EST mice model. **b** Diagram for the tumor inhibition % of *S. palaestina* extract (180 mg and 360 mg/kg) on tumor growth of EST mice model

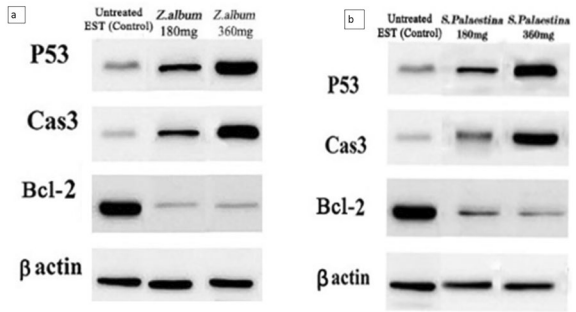

Fig. 2 **a** Western blot profiles of the effect of *Z. album* extract on protein expression of p53, Bcl-2, and Caspase 3 in EST cells compared with the (untreated) control group. **b** Western blot profiles of the effect of *S. palaestina* extract on protein expression of p53, Bcl-2, and Caspase 3 in EST cells compared with the (untreated) control group

densities of 0.31 ± 0.02 in 180mg/kg and 0.25 ± 0.02 in 360mg/kg treatments (Fig. 2a and Table 3). Regarding *Z. album*. When *S. palaestina* (180 mg/kg and 360 mg/kg) was used, the expression levels of P53 and caspase-3 proteins were significantly up-regulated with relative densities of 2.73 ± 0.17 and 4.61 ± 0.23 for p53 and 2.11 ± 0.10 and 4.50 ± 0.20 for caspase 3, respectively, while the expression level of Bcl-2 was down-regulated with relative densities of 0.57 ± 0.04 of 180 mg/kg and 0.36 ± 0.03 of 360 mg/kg treatments (Fig. 2b) and (Table 4).

Table 3 Band quantification of p53, caspase 3 and bcl-2 proteins expression after treatment by *Z. album* extract with two concentrations

Group	P53 protein expression	Caspases 3 protein expression	Bcl-2 protein expression
	Relative density	Relative density	Relative density
Untreated EST	1.00±0.08	1.00±0.09	1.00±0.07
Z. album (180 mg)	3.25±0.19	2.96 ± 0.15	0.31±0.02
Z. album (360 mg)	5.40±0.28	5.23±0.21	0.25±0.02

Assessment of thyroid hormones in EST

In our results, we found that the T3 level was substantially increased in EST blood cells (70.24%) and T4 decreased in EST blood cells (58.5%) compared with normal control blood cells. In addition, the treatment of the EST group with *S. palaestina* revealed a significant decrease in the levels of T3 by 27% at the dose of 180 mg/kg, while the reduction was 34% at dose 360 mg/kg compared with untreated EST group (Fig. 3a). On the contrary, *S. palaestina* at doses 180 and 360 mg/kg induced a substantial increase of T4 level by 154% and 169%, respectively, compared to EST mice untreated treatment (Fig. 3b).

Similarly, the treatment of mice bearing solid tumor with *Z. album* revealed a significant decrease in the levels of T3 by 34.5% at dose 180 mg/ kg, while the reduction was 35% at dose 360 mg/kg (Fig. 3c). Whereas that of mice with *Z. album* revealed a significant increase of T4

Table 4 Band quantification of p53, caspase 3 and bcl-2 proteins expression after treatment by *S. palaestina* extract with two concentrations

Group	P53 protein expression	Caspase 3 protein expression	Bcl-2 protein expression
	Relative density	Relative density	Relative density
Untreated EST	1.00± 0.08	1.00±0.09	1.00 ± 0.07
S. palaestina (180 mg)	2.73± 0.17	2.11±0.10	0.57±0.04
S. palaestina (360 mg)	4.61± 0.23	4.50±0.20	0.36±0.03

Table 5 Detection of DNA fragmentation by the comet assay, assessed as tail moment and tail length in Ehrlich solid tumor (EST) mice treated with two *Zygophyllum album* extracts (180 mg/kg and 360 mg/kg)

Group	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
EST untreated (control)	3	97	2.19 ± 0.11^{c}	1.9	4.16
Z. album (180 mg/kg	19	81	7.19 ± 0.38^{b}	5.28	37.96
Z. album (360 mg/kg	30	70	9.74 ± 0.42^{a}	8.37	81.52

Different superscript letters in the same column of tail length showed significance difference at $P < 0.05$ by Duncan's new multiple range test

Table 6 Detection of DNA fragmentation by the comet assay, assessed as tail moment and tail length in Ehrlich solid tumor (EST) mice treated with two *Suaeda palaestina* extract (180 mg/kg and 360 mg/kg)

Group	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
EST untreated (control)	3	97	2.19 ± 0.11^{c}	1.9	4.16
S. palaestina (180 mg/kg)	16	84	6.51 ± 0.30^{b}	4.6	29.95
S. palaestina (360 mg/kg)	21	79	7.68 ± 0.32^{a}	5.92	45.47

Different superscript letters in the same column of tail length showed significance difference at $P < 0.05$ by Duncan's new multiple range test

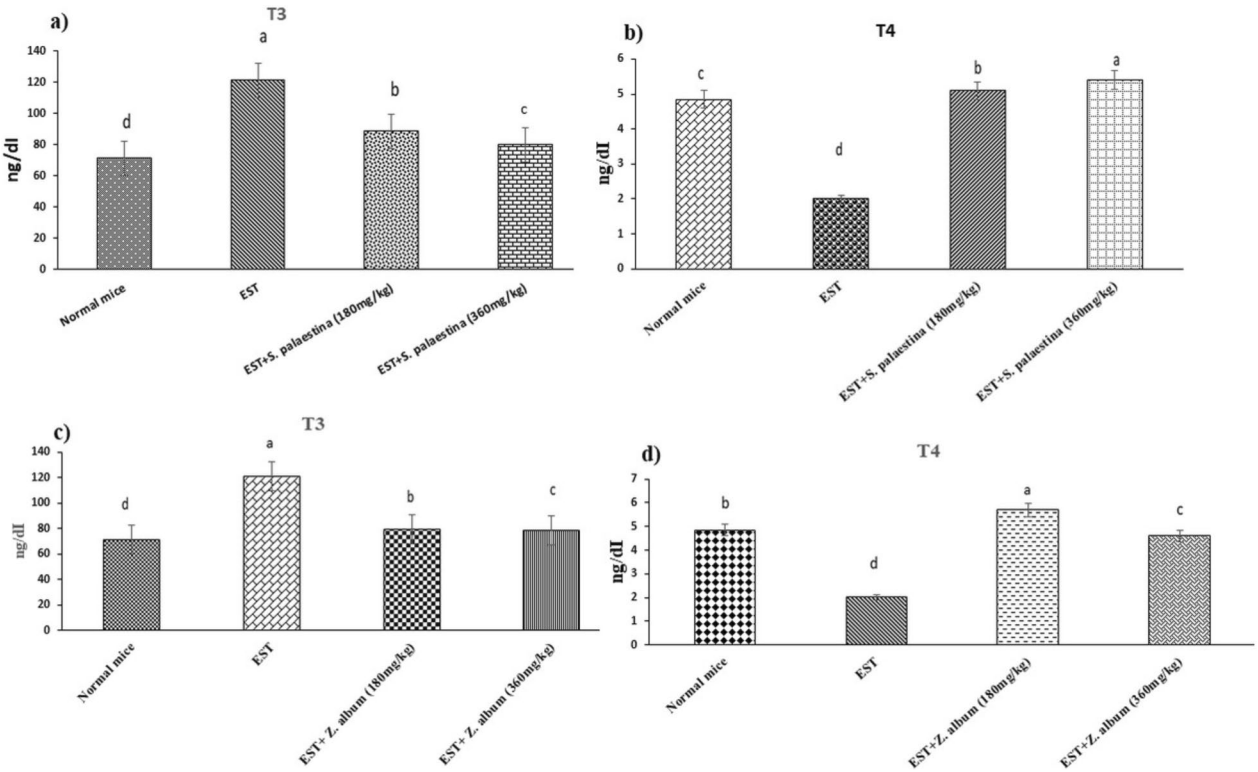

Fig. 3 **a** Diagram for the effect of *S. palaestina* on T3 in mice Ehrlich solid tumor, by Duncan's test as a post hoc test. **b** Diagram for the effect of *S. palaestina* on T4 in mice Ehrlich solid tumor, by Duncan's test as a post hoc test. **c** Diagram for the effect of *Z. album* on T3 in Ehrlich solid tumor mice, by Duncan's test as a post hoc test. **d** Diagram for the effect of *Z. album* on T4 in Ehrlich solid tumor mice, by Duncan's test as a post hoc test

at doses 180 mg/ml (184%) and 360 mg/kg (129%) compared to EST mice untreated treatment (Fig. 3d).

Assessment of DNA fragmentation

In this study, the assessment of the DNA fragmentation in an Ehrlich solid tumor cell exposed to the *Zygophyllum album* and *Suaeda palaestina* was determined. Data presented in Tables 5 and 6 show the levels of DNA fragmentation parameters; tailed%, untailed %, tails length, tail DNA% and tail moment. The assessed *Z. album* and *S. palaestina* caused significant DNA fragmentation. Treatment-dependent DNA fragmentation in EST cells was observed after *Z. album and S. palaestina* treatments and was compared with that of untreated EST control (Figs. 4A and 5A). Both extracts induced considerable DNA fragmentation even at low concentration (180 mg/kg) as demonstrated by the DNA tail presence (Figs. 4B and 5B). The tail length exhibited the DNA fragmentation which was treated with 360 mg/kg doses (Figs. 4C and 5C). Our results also revealed that *Z. album* caused significant DNA fragmentations, as evidenced by the comet lengths 7.19 ± 0.38 in 180 mg/kg and 9.74 ± 0.42 μm in 360 mg/kg which were much more than that in the untreated control (2.19 ± 0.11 μm) group, while by the comet length of *S. palaestina* 6.51 ± 0.30 in 180mg/kg and 7.68 ± 0.32 at 360 mg/kg. The high doses of *Z. album* and *S. palaestina* (360 mg) inflicted significantly higher fragmentation effect on DNA of EST cells than low doses (180 mg) (Figs. 4D and 5D); tail DNA percentage was 1.9% in control, 5.92% and 8.37% in high dose (360 mg) and 4.6% and 5.28% in low dose (180 mg), respectively.

Discussion

The results of this study provide valuable insights into the potential anticancer activity of *Z. album* and *S. palaestina* extracts on Ehrlich solid tumor (EST) in a murine model. The discussion below elaborates on the implications of the findings and their significance in the context of cancer research and therapy.

The observed reduction in tumor size in EST mice treated with *Z. album* and *S. palaestina* extracts highlights the potential of these plant-derived compounds as effective agents against solid tumors. The significant decrease in tumor size, particularly with high doses

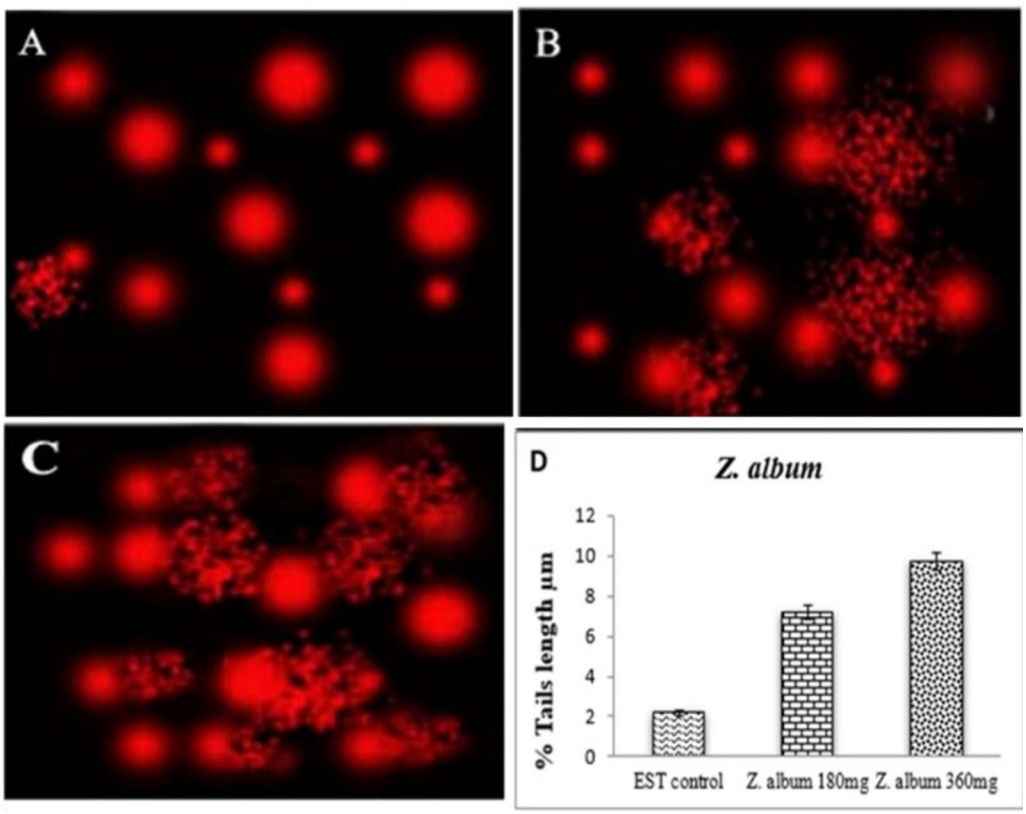

Fig. 4 Comet images representing DNA fragmentation in Ehrlich solid tumor (EST) cells. **A** represents DNA strand breaks of untreated EST, while **B** represents EST after treatment with 180 mg/kg of *Z. album* extract, and **C** represents EST after the exposure to 360 mg/kg of *Z. album* extract. **D** Diagram for the extent of DNA fragmentation expressed in terms of comet % tail length

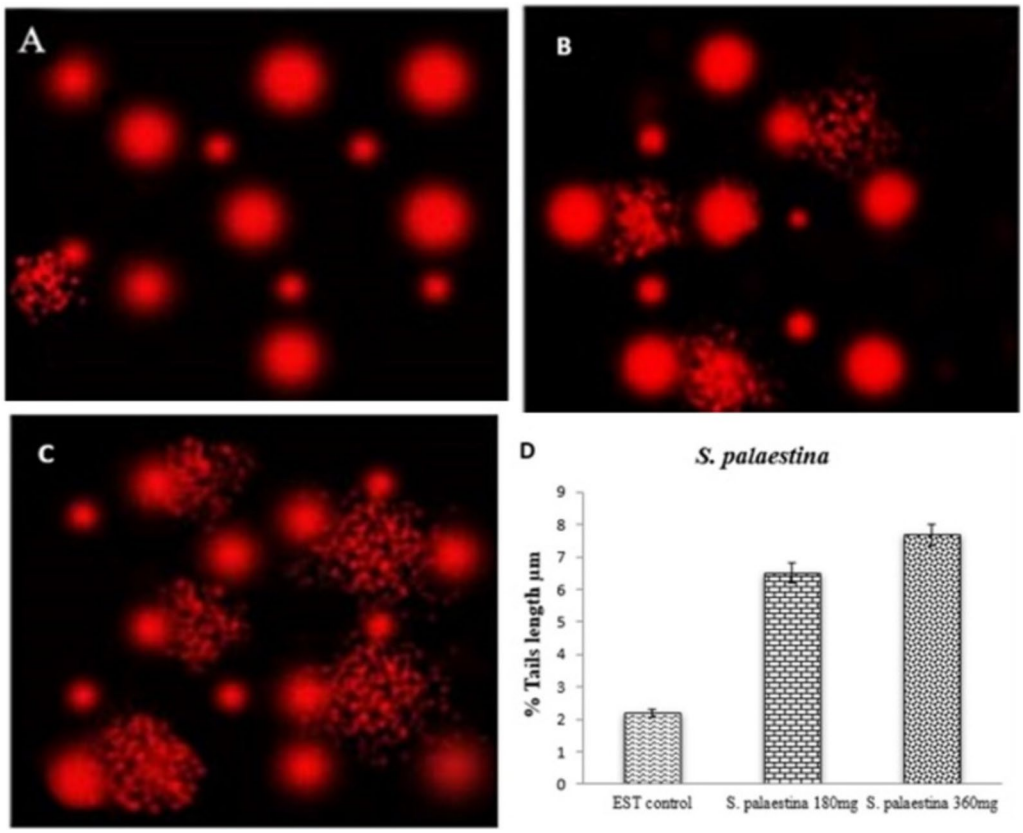

Fig. 5 Comet images representing DNA fragmentation in Ehrlich solid tumor (EST) cells. **A** represents DNA strand breaks of untreated EST, while **B** represents EST after treatment with 180 mg/kg of *S. palaestina* extract, and **C** represents EST after the exposure to 360 mg/kg of *S. palaestina* extract. **D** Diagram for the extent of DNA fragmentation expressed in terms of comet % tail length

of these extracts, underscores their promising role in inhibiting tumor growth. The dose-dependent response indicates a correlation between the concentration of the extracts and their antitumor effects.

The ability of natural compounds, such as those found in *Z. album* and *S. palaestina*, to hinder tumor progression is of great interest in cancer research. These findings align with previous studies demonstrating the antiproliferative and anticancer properties of phytochemicals, providing a foundation for further investigation into the mechanisms underlying these effects (Feitelson et al. 2015; Sabeel et al. 2023).

The up-regulation of p53 and Caspase 3, coupled with the down-regulation of Bcl-2, in the treated EST mice indicates that both *Z. album* and *S. palaestina* extracts induce apoptosis in tumor cells. P53 is an essential tumor suppressor protein that controls cell cycle arrest and initiates apoptosis in response to cellular stress and DNA damage. Caspase 3, the key effector caspase, is responsible for executing the final steps of apoptosis. The down-regulation of the anti-apoptotic protein Bcl-2 further supports the pro-apoptotic effects of these extracts. The observed changes in protein expression levels reflect a shift toward apoptosis in tumor cells, suggesting that *Z. album* and *S. palaestina* extracts may trigger programmed cell death in EST. These results are consistent with the literature, which emphasizes the pivotal role of p53, Caspase 3, and Bcl-2 in the balance between cell survival and apoptosis in cancer cells. The findings of this study corroborate the potential of plant-derived compounds as agents capable of modulating these crucial apoptotic pathways. These results confirmed that the *Z. album* and *S. palaestina* have definitely induced apoptosis in EST cells mechanism mainly through the overexpression of p53 and caspase genes, whereas Bcl-2 was down-regulated. Moreover, these results corresponded almost perfectly with those of mRNA expression levels on HepG2 cells by RT-PCR (El-Attar et al. 2019b).

The genotoxic potential of *Z. album* and *S. palaestina* extracts on EST cells is evident from the significant DNA fragmentation observed in the comet assay. Even at low concentrations, both plant extracts induced notable DNA fragmentation, which was characterized by the presence of DNA tails in the comet assay. The increased tail length

with higher doses of the extracts underscores their genotoxic effects.

DNA fragmentation is a hallmark of apoptotic cell death, and the extent of fragmentation correlates with the severity of cellular damage. These results indicate that the extracts induce genotoxicity in EST cells, further supporting their role in promoting apoptosis. The genotoxic potential of these extracts aligns with the findings of previous studies on natural compounds' ability to induce DNA damage in cancer cells as a mechanism of tumor suppression. These results indicate that *Z. album* and *S. palaestina* have marked antitumor potentials on the basis of their ability to cause severe DNA fragmentation to EST cells as compared to the negative control.

Dolai et al. (2016) and El Sayed et al. (2020) reported that the effect of Anthocephalus cadamba extract on Ehrlich ascites carcinoma (EAC) cells may cause DNA fragmentation in EAC cells. It is observed that an aqueous extract of *Fagonia cretica* induced DNA fragmentation in MCF-7 cell lines by Abd-El-Moneim et al. (2020). Ezz El-Din et al. (2018) noted that treatment of doxorubicin-exposed male mice with *Z. album* extract caused significant decreases in the chromosomal aberrations either in the spermatocyte cells or in bone marrow, as well as decreased the malondialdehyde activity, the micronucleus formation, and the rate of DNA fragmentation. The alterations in thyroid hormone levels, specifically the decrease in T3 and the increase in T4, in the blood samples of EST mice following treatment with *Z. album* and *S. palaestina* extracts are intriguing. The role of thyroid hormones in cancer development and progression has been a subject of debate in the scientific community. The observed changes may indicate a potential link between thyroid hormones and breast cancer pathogenesis. Several studies have shed light on the complex interplay between thyroid hormones and mammary gland biology, as well as their potential implications for breast cancer. The relationship between thyroid hormones and the mammary gland offers multiple perspectives emphasized by Aziz and Mehta (2015) and Ren and Zhu (2022). They underscored the influence of these hormones on processes such as differentiation and lobular growth, which bear resemblance to the effects of estrogen. Additionally, research findings have implicated thyroid hormones in the development of breast cancer. Breast cancer cell lines have provided further insights into the potential role of specific thyroid hormones. T3, in particular, has been linked to the promotion of tumor proliferation and increased cell proliferation effects (Krashin et al. 2019). These observations suggest that T3 may contribute to the development and progression of breast cancer. Krashin et al. (2019) reported a compelling connection between survival outcomes and induced hypothyroidism in mice following the implantation of murine mammary adenocarcinoma cells. These observations underscore the potential impact of thyroid hormones on the course of cancer therapy and survival.

Furthermore, conventional chemotherapeutic agents have demonstrated limitations due to their non-selective action on both cancer and normal cells, resulting in undesirable side effects. This has prompted the exploration of natural bioactive agents as a promising approach to cancer prevention. These natural agents offer selective cytotoxicity against cancer cells while exhibiting favorable safety and efficacy profiles (Laskowski et al. 2022).

The collective body of research on thyroid hormones and their intricate involvement in breast cancer underscores the need for further investigation into the mechanisms and clinical implications of thyroid hormone modulation in cancer therapy. These studies have laid the foundation for exploring innovative therapeutic strategies and natural agents in the quest for effective and selective cancer treatments.

The findings are consistent with prior research suggesting that thyroid hormones can influence cancer outcomes. The increase in T4 levels, in particular, may have implications for cell proliferation, as T4 has been associated with promoting proliferation in breast cancer cells. These results warrant further investigation into the intricate relationship between thyroid hormones and breast cancer. The results of this work regarding the effects of *Z. album or S. palaestina* administration revealed significantly reduced concentrations of T3, and significant increase of T4 compared to the Ehrlich solid tumor mice group. Thyroid hormones, T3 and T4, are crucial for survival; they are involved in the processes of growth, metabolism, and development, as well as proliferation and metastasis (Liu et al. 2019).

Conclusions

Treatment of Ehrlich solid tumors with *Z. album* or *S. palaestina* extracts resulted in a significant tumor size reduction. The study showed decreased tumor weight and volume, indicating marked inhibitory effects on tumor cells.

Molecular analysis revealed up-regulation of p53 and caspase 3, along with down-regulation of Bcl-2, suggesting the progression of apoptosis. Comet tests showed that both plant extracts caused DNA fragmentation in EST cells after they were treated with them, which is more evidence that they can kill tumors.

Radioimmunoassay tests on EST blood samples showed reduced T3 and increased T4 levels compared to untreated mice, indicating a possible relationship between thyroid hormones and breast cancer.

These findings suggest that both *Z. album* and *S. palaestina* extracts could be promising herbal therapeutic agents for cancer treatment, warranting further investigation.

Abbreviations

EAC	Ehrlich ascites carcinoma
IP	Intraperitoneally
EST	Ehrlich solid mice
T3	Triiodothyronine
T4	Thyroxine
Bcl2	B-cell lymphoma 2
RIA	Radioimmunoassay
CH_2Cl_2	Dichloromethane
RIPA	Radioimmunoprecipitation assay
SDS-PAGE	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
mRNA	Messenger of ribonucleic acid
HepG2	Hepatoblastoma cell line
RT-PCR	Reverse transcription polymerase chain reaction
MCF-7	Human breast cancer cells

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Declarations

Ethics approval and consent to participate
The animals were subjected to the guidelines set forth by the National Cancer Institute for the Care and Use of Laboratory Animals. The guidelines for the ethical use and maintenance of laboratory animals issued by the National Institutes of Health and authorized by the Scientific Committee of the Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt, were complied with in all procedures used in caring for rats and taking blood and tissue samples for this experiment.

Consent for publications
Not applicable.

Competing interests
The author declares that they have no competing interests.

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References

Abd-El-Moneim OM, Abd El-Rahim AH, Mohamed AAER et al. (2020) Enhancement effects of ethanolic extract of *Fagonia cretica* on Bisphenol A (BPA)-induced genotoxicity and biochemical changes in rats. Bull Natl Res Cent 44:1–13. https://doi.org/10.1186/s42269-020-00295-y

Ahvazi M, Khalighi-Sigaroodi F, Charkhchiyan MM, Mojab F, Mozaffarian VA, Zakeri H (2012) Introduction of medicinal plants species with the most traditional usage in Alamut region. Iran J Pharma Res 11(1):185

Aziz S, Mehta R (2016) Technical aspects of toxicological immunohistochemistry, system specific biomarkers. New York, Springer. https://doi.org/10.1007/978-1-4939-1516-3

Beier BA (2003) Phylogeny and taxonomy of subfamily Zygophylloideae (Zygophyllaceae) with special reference to the genus fagonia. Doctoral dissertation, Acta Universitatis Upsaliensis

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Chowdhury P, Ghosh U, Samanta K et al. (2021) Bioactive nanotherapeutic trends to combat triple negative breast cancer. Bioact Mater 6:3269–3287. https://doi.org/10.1016/j.bioactmat.2021.02.037

Dolai N, Islam A, Haldar PK (2016) Methanolic extract of *Anthocephalus cadamba* induces apoptosis in Ehrlich ascites carcinoma cells in experimental mice. Indian J Pharmacol 48:445. https://doi.org/10.4103/0253-7613.186190

El-Attar MM, Abdel-Tawab FM, Awad AA et al. (2019a) Anti-cancer effects of *Zygophyllum album* and *Suaeda palaestina* extracts on human liver cancer cell lines. Egypt J Genet Cytol 48(1):77–90

El-Attar MM, Awad AA, Abdel-Tawab FM et al. (2019b) Assessment of cytotoxic and anticancer activity of *Zygophyllum album* and *Suaeda palastina* extracts on human liver cancer cell lines. Arab Univ J Agric Sci 27:539–544. https://doi.org/10.21608/ajs.2019.43663

El-Din AE, Ali MHM, Ahmed ES et al. (2018) Ameliorative effect of *Zygophyllum album* extract against hepatotoxicity induced by doxorubicin in male mice. Int J Pharmacol 14:421–427. https://doi.org/10.3923/ijp.2018.421.427

El Sayed RAA, Hanafy ZEM, Abd El Fattah HF, Mohamed AK (2020) Possible antioxidant and anticancer effects of plant extracts from *Anastatica hierochuntica*, *Lepidium sativum* and *Carica papaya* against Ehrlich ascites carcinoma cells. Cancer Biol 10:1–16. https://doi.org/10.7537/marscbj100120.01

Feitelson MA, Arzumanyan A, Kulathinal RJ et al. (2015) Sustained proliferation in cancer: mechanisms and novel therapeutic targets. In: Seminars in cancer biology. Elsevier, pp S25–S54. https://doi.org/10.1016/j.semcancer.2015.02.006

Kaptein EM, Macintyre SS, Weiner JM et al. (1981) Free thyroxine estimates in nonthyroidal illness: comparison of eight methods. J Clin Endocrinol Metab 52:1073–1077. https://doi.org/10.1210/jcem-52-6-1073

Krashin E, Piekiełko-Witkowska A, Ellis M, Ashur-Fabian O (2019) Thyroid hormones and cancer: a comprehensive review of preclinical and clinical studies. Front Endocrinol (Lausanne) 10:1–23. https://doi.org/10.3389/fendo.2019.00059

Laskowski TJ, Biederstädt A, Rezvani K (2022) Natural killer cells in antitumour adoptive cell immunotherapy. Nat Rev Cancer 22:557–575. https://doi.org/10.1038/s41568-022-00491-0

Liu Y-C, Yeh C-T, Lin K-H (2019) Molecular functions of thyroid hormone signaling in regulation of cancer progression and anti-apoptosis. Int J Mol Sci 20:4986. https://doi.org/10.3390/ijms20204986

Lopes M, Sanches-Silva A, Castilho M et al. (2023) Halophytes as source of bioactive phenolic compounds and their potential applications. Crit Rev Food Sci Nutr 63:1078–1101. https://doi.org/10.1080/10408398.2021.1959295

Mohammedi Z (2020) Phytochemical, antidiabetic and therapeutic properties of zygophyllum. Herb Med J. https://doi.org/10.22087/hmj.v5i4.813

Moustafa SM, Menshawi BM, Wassel GM et al. (2014) Screening of some plants in Egypt for their cytotoxicity against four human cancer cell lines. Int J Pharm Tech Res 6:1074–1084

Neeraj Kumar NK, Shubham Lamba SL, Ashwani Kumar AK (2019) Antioxidant defence in halophytes under high salinity. In: Halophytes and climate change: adaptive mechanisms and potential uses. Cabi Wallingford UK, pp 196–208. https://doi.org/10.1079/9781786394330.0196

Noor F, Noor A, Ishaq AR (2021) Recent advances in diagnostic and therapeutic approaches for breast cancer: a comprehensive review. Curr Pharm Des 27:2344–2365. https://doi.org/10.2174/1381612827666210303141416

Rahman MM, Mostofa MG, Keya SS (2021) Adaptive mechanisms of halophytes and their potential in improving salinity tolerance in plants. Int J Mol Sci 22:10733. https://doi.org/10.3390/ijms221910733

Ren B, Zhu Y (2022) A new perspective on thyroid hormones: crosstalk with reproductive hormones in females. Int J Mol Sci 23:2708. https://doi.org/10.3390/ijms23052708

Sabeel Z, Liang Y, Hao M, Ying L, Guo R, Chen R, Yang Z et al. (2023) A comprehensive review of antitumor properties of Angelica species and their antitumor-responsible constituents and the underlying molecular mechanisms involved in tumor inhibition. Phytother Res 37(5):2187–2211. https://doi.org/10.1002/ptr.7841

Singh A, Kumar A (2023) Medicinal plants: a future of modern medical system. Frontiers 10:214–232

Singh RK, Mishra SK, Kumar N, Singh AK (2010) Assessment of DNA damage by comet assay in lymphocytes of workers occupationally exposed to petroleum fumes. Int J Genet 2:18

Tran N, Pham B, Le L (2020) Bioactive compounds in anti-diabetic plants: from herbal medicine to modern drug discovery. Biology (Basel) 9:252. https://doi.org/10.3390/biology9090252

Wartofsky L, Burman KD (1982) Alterations in thyroid function in patients with systemic illness: the "euthyroid sick syndrome." Endocr Rev 3:164–217. https://doi.org/10.1210/edrv-3-2-164

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