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Cytotoxic Activity of Asphodelus Microcarpus as Amain Food of Spalax Leucodon Egyptiacus

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Abstract

The present study aimed to know if the food of Spalax leucodon Egyptiacus has a role in its resistance to cancer. In vitro cytotoxic effects of the main food of Spalax leucodon Egyptiacus as a daily meal that was Asphodelus microcarpus which planted in Marsa Matroh governorate Eldabaa region .We use two fractions of the extract of this plant (Ethyl Acetate &Precipitate), cell viability and cytotoxic activities were assessed using The sulforhodamine B(SRB) assay, IC50 values were estimated for the A375 melanoma. We revealed that the IC50 for the A375 melanoma was 83 ug/ml for Ethyl Acetate (EtOAc) fraction, 65ug/ml for Precipitate (PPT) fraction after 48 hours of treatment. Asphodelus microcarpus fraction has considerable cytotoxic effects on the human melanoma cell line , its show effect on cell line A375 human melanoma ,further studies are required to emphasizes the relation between resistant cancer in Spalax leucodon Egyptiacus and its main food.

Keywords: Asphodelus microcarpus, A375 Melanoma Cell Line, Cytotoxicity, Spalax leucodon Egyptiacus

Introduction

The use of plants for therapeutic purposes, such as the treatment of fungi, bacteria, and viruses, as well as the treatment of cancerous tumors, is not new and this

appears in the papyri and Pharaonic temples. And the matter has evolved and reached the point that many drugs depend heavily on medicinal plants (**Neven, 2010**).

The importance of plants is due to the active substances and secondary plant metabolites in one or more parts of these plants contained in them such as alkaloids, glycosides, corticosteroids, essential oils, etc. (state of art) These components have biological activity effects (**Aysegul Peksel et al., 2013**).

Anticancer natural products act on multiple pathways to suppressor tumor growth by induction of apoptosis, down-regulation of genes, anti-metastatic activity, and inhibition of microtubule function or alteration of the structure of DNA. A wide variety of mechanisms exist for anticancer activity (**Massinissa et al., 2018**).

The genus Asphodelus comprises 187 genera and 2500 species. It is a circum-Mediterranean genus, which includes five sections and is represented by 16 species that belongs to the family Asphodelaceae (Liliaceae previously) that include several medicinal plants (**Amalia et al., 2016**).

Asphodelus microcarpus (Asphodelaceae) is an herb with roots of several spindle-shaped tubers, distributed over the Mediterranean region. The plants are hardy herbaceous perennials with narrow tufted radical leaves and an elongated stem bearing a handsome spike of white or yellow flowers, a perennial herb, 1 m high, usually on alluvial soil. Distrib. Occasional, locally dominant in the lower forest and upper moist-steppe zones of Iraq and Mediterranean Europe to Greece, Cyprus, Turkey, Syria, Lebanon, Palestine, Jorden, Sinai, Egypt, N. Africa. (**Mohamed et al. 2014**). Asphodelus is vital for the defense system as it increases the WBC (white blood cells) count.

Spalax leucodon Egyptians is lesser blind mole rat it lives on the north coast in El-Dabaa zone; Marsa Matrouh Governorate in Egypt (**Tammam & Omar, 2009**). Spalax is highly resistant to cancer. For >40 years they did not observe any spontaneous tumors among thousands of individual's carcinogens failed to induce tumors in Spalax (**Shams et al., 2014**).

The main food of Spalax leucodon Egyptians is Asphodelus microcarpus, the main question in this study is if the food of Spalax leucodon Egyptiacus has a role in its resistance to cancer? .To get an answer human melanoma cell line A375 was treated with A.microcarpus to investigate its effect.

Materials and methods

Collecting plant

The tubers of A. microcarpus Salzm. et Vivi (Asphodelaceae) (**Fig. 1**) were collected from 70 km West of Marsa Matrouh, Egypt, in February 2021. The plant was identified by Dr. **Mohamed F. Azzazy**, professor of plant ecology at the surveys of natural resources department, Environmental Studies, and Research Institute, University of Sadat City. A voucher specimen (AM 21) was placed in surveys of the natural resources department.

Fig. 1. Asphodelus microcarpus.

Extraction

Fresh tubers' plant material was cut into little pieces with a knife before being combined with 2.5 L of 80% methanol. The mixture was allowed to macerate for 24 hours with regular shaking before filtering on cellulose filter paper. The technique was repeated three times until the plant material was exhausted. The filtrate was collected and concentrated under vacuum at 40 °C until it reached 1L volume, after which it was partitioned with n-hexane (500 mL × 3), dichloromethane (500 mL × 4), and ethyl acetate (500 mL × 6) to give 0.21 g, 1.95 g, and 5.21 g, respectively., A yellowish-red precipitate (PPT) was generated during the partitioning process that did not dissolve in the remaining aqueous portion. It was taken apart and weighed 7.68 g.

Cell culture

The cell line human melanoma A375 was purchased from Nawah Scientific Inc., (Mokatam, and Cairo, Egypt). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) media supplemented with 100 mg /mL of streptomycin, 100 units/mL of penicillin, and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO2 atmosphere at 37 °C.

Cell viability

(SRB) sulforhodamine B assay was used to determine cell viability. In 96 well plates, aliquots of 100 µL cell suspension (5x10^3cells) were put and incubated in full media for 24h. Another aliquot of 100 µL media containing extracts (EtOAc raction and PPT) were treated Cells with various concentrations (100−0.01ug/mL). After 72h of extracts exposure, cells were fixed by replacing media with 150 µL of 10% Trichloroacetic acid (TCA) and incubated at 4°C for 1h. The TCA solution was removed, and the cells were washed five times with distilled water. Aliquots of 70 µL SRB solution (0.4%w/v) were added and incubated at room temperature for 10 minutes in the dark. Plates were washed three times with 1% acetic acid air-dried overnight. The absorbance was measured at 540 nm using a BMGLABTECH®-FLUO star Omega microplate reader (Ortenberg, Germany) after, 150µL of TRIS (10 mM) was introduced to breakdown protein-bound SRB stain(**Skehan et al., 1990**).

Results

According to TLC, we found the EtOAc fraction highly amount of anthraquinones so we chose it as mainly fraction which we tested the effect of our plant extract on human melanoma A375, and another one is the precipitate which precipitates after fractionation we consider it as a fraction.

The SRB assay is an anionic amino xanthene dye that forms an electrostatic complex with the basic amino acid residues of proteins under moderately acid conditions, which provides a sensitive linear response. The color development is rapid and stable and is readily measured at absorbance between 560 and 580nm. Human melanoma A375 cancer cell lines had different sensitivities to the various concentrations of fractions concentration. The viability of the cells was evaluated, using SRB. The maximum inhibitory concentration (IC50) of the Asphodelus microcarpus fractions EtOAc &PPT for A375 human melanoma cells was 83ug/mL for EtOAc fraction, 65ug/mL for PPT fraction after 48 hours of treatment.

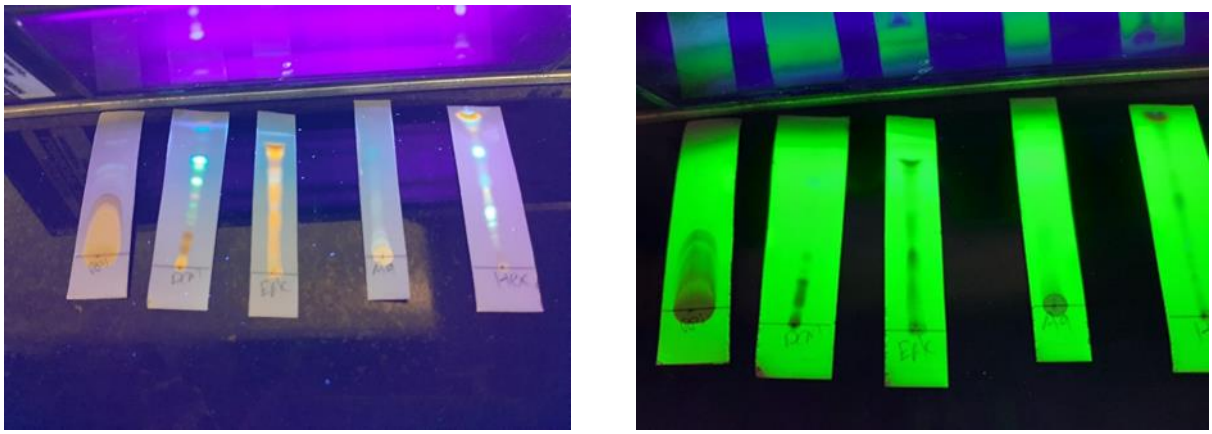

Fig. 4. TLC profile of methanolic extract, dichloromethane fraction, ethyl acetate fraction, n-hexane fraction, precipitate. Infrared spectroscopy was carried out on a Varian 800 FTIR Scimitar series utilizing a PIKE MIracle™ cell with KOH-loaded lenses.

At the mentioned concentration, 50% of the cells in the plate lost their proliferation and viability, and about 50% of the cultured cells lost their viability (**Figures 5 and 6 and Table 1**).

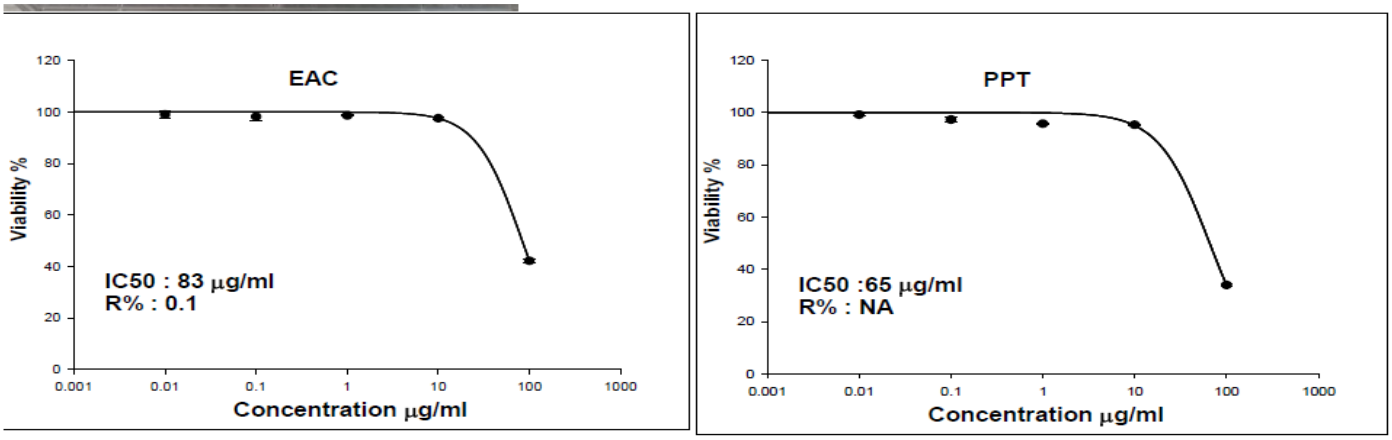

Fig. 5. Asphodelus microcarpus tuber fractions exhibited selective cytotoxic effects on the human melanoma cell line. The IC50 value of the EtOAc fraction in the human melanoma cell line was 83 ug/mL, while the IC50 value of the PPT fraction in the human melanoma cell line was calculated as 65 ug/mL.

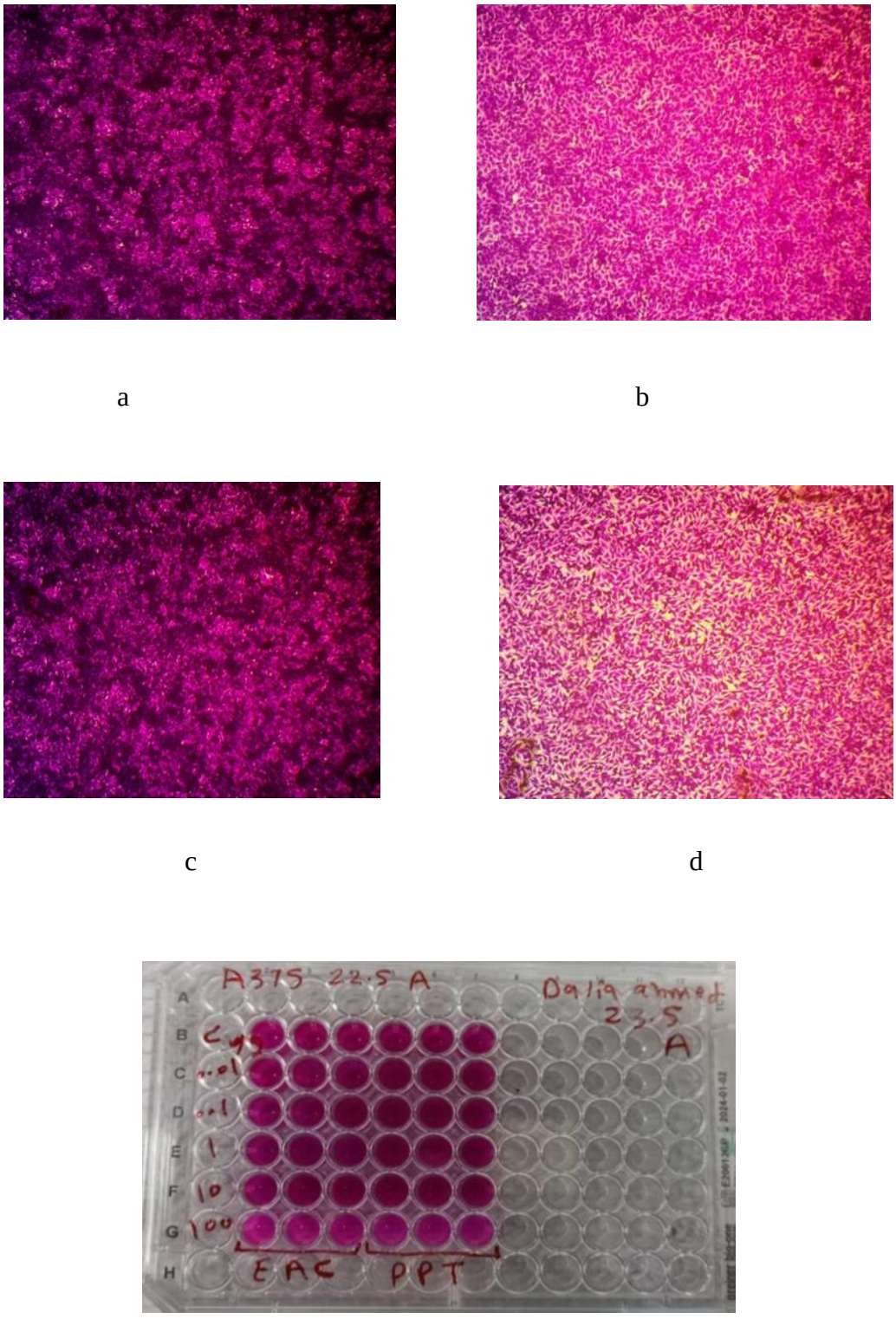

Fig. 6. Cytotoxic viability of A. microcarpus EtOAc fraction with concentrate 0.01 ug/mL'a'&100ug/mL'b' and PPT with concentrate 0.01 ug/mL'c'& 0.02 100ug/mL'd' on A375 melanoma cells and plates 'e'

Table 1. Value of IC50 of Asphodelus microcarpus fractions EtOAc &PPT on Human melanomaA375 using SRB assay.

Cell line	**Treatment**	**IC50 ug/ml**
A375	EtOAc fraction	83ug/ml
A375	PPT fraction	65ug/ml

Discussion

Asphodelus microcarpus considers the main food of Spalax leucodon Egyptiacus (**Tammam & Omar, 2009; Omar & Mohamed, 2013; Tammam et al., 2016**).

All parts of the plant have been studied , the leave was studied by (**El-Ghaly, 2017**), tuber was studied by (**El-Seedi, 2007; Ghoneim et al,. 2013; Hashem et al., 2019; Hammouda et al., 1972**) a whole plant by (**Ahmed M. Aboul-Enein et al., 2011**) seeds by (**Mohammed et al., 2019**). In using methanol 80 % as solvent were studied by (**Rawaa Al-Kayali1 et al. 2016; Kitaz Adawia, 2017; Abuhamdah et al., 2013**).

Some studies use crude extract (**Gürbüz et al., 2003; Aboul-Enein et al., 2011; Al-Kayali1 et al., 2016; Alhage and Elbitar, 2020; Mohammed et al., 2020**) but anther studies use fractions of extract (**El-Ghaly, 2017; El-Seed, 2007; Ghoneim et al., 2014; Petrillo et al., 2016; Di Petrillo et al., 2017; Abdellatef et al., 2021**). This study use fractions to clarify the accurate for the main reason to resistant cancer.

Roots of Asphodelus microcarpus were mainly reported to have anthraquinone and its derivatives.. The two fractions was considered in this paper EtOAc &PPT which have a high amount of anthraquinone according to TLC test, because anthraquinone is a potent anticancer compound (**Mohammed et al. 2020; Brijesh Tripathi, et al., 2014; El-Ghaly, 2017; Ghoneim et al., 2013**). The cytotoxicity of the EtOAc &PPT fraction of A. microcarpus tuber against the human melanoma A375 cell line was assessed for the first time in this work using the SRB assay most work used MTT (**Cattaneo et al., 2015; Groshi et al., 2017; Marrelli et al., 2021**) or trypan blue (**Özlem & Tülay, 2013; Gaber et al., 2015**) but the most important characteristic of SRB assay that SRB staining is independent of cell metabolic activity (**Vichai & Kirtikara, 2006**).

Melanoma is one of the most common and malignant forms of skin cancer that originates from melanocyte cells **(Sparsa et al., 2010**) .This study used human melanoma cell line A375 because A.microcarpus has a potent effect on the skin (**Di Petrillo et al., 2016**) anti-inflammatory, So we test its effect on human melanoma to make a whole result on skin.

Therefore, it can be considered that the presence of anthraquinone is an important reason for the effect of the plant on cancer cells; this effect confirms that Spalax leucodon Egyptiacus resistance to cancer may be due to its daily food of this plant as a main food.

Conclusion

Nutrition and food intake play an essential part in cancer treatment. This study examined whether A. micrpcarpus tubers were responsible for Spalax leucodon resistant cancer Investigation of the major anthraquinone content was detected in ethyl acetate fraction beside a precipitate formed during fractionation process. Referring to the results of the toxicity test we can be say that the plant has an effect on the toxicity of cancer cells and the daily feeding of the animal from it is a reason for its resistance to cancer. The experiment of using the plant in the daily food of the human being is suggested. LC-MS/MS profiling of the ethyl acetate fraction and PPT was suggested to identify the accurate component of anticancer.

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