



ASSESSMENT OF CYTOTOXIC AND ANTICANCER ACTIVITY OF *Zygophyllum album* AND *Suaeda palastina* EXTRACTS ON HUMAN LIVER CANCER CELL LINES

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ABSTRACT

In this study, anticancer activity of *Zygophyllum album* and *Suaeda palastina* extracts was evaluated. Dichloromethane, methanol and hot water were used as solvents for extraction. Results indicated that the highest half-maximal inhibitory concentration (IC₅₀) on human lung carcinoma (A549) cell lines was achieved by dichloromethane extracts of *Z. album* and *S. palastina* (70.48 µg/ml and 34.82 µg/ml respectively) compared to methanolic and hot water extracts. Furthermore, dichloromethane extracts of both plants had anti-proliferative effect and highly cytotoxicity on human cancer cells. IC₅₀ of *Z. album* was 27.74 µg/ml in the human hepatocellular carcinoma (HepG2), while IC₅₀ of *S. palaestina* was 30.76 µg/ml with no cytotoxic activity on normal cell lines. In conclusion, these results suggest that *Z. album* and *S. palaestina* could be a good candidate species as a natural source of anticancer agents.

Keywords: Medicinal plants, cytotoxic activity, *zygophyllum album*, *Suaeda palaestina*, anticancer agent, MTT assay, human liver cancer cell line

1. INTRODUCTION

Traditional medicines derived mainly from plants still play major roles in the management of various diseases (Karunanayake and Tennekoon, 1993; Narayana and Dobriyal, 2000). Medicinal plants are currently used as raw

materials for extraction of active ingredients which are used in the synthesis of several drugs including cancer disease (Rasool Hassan, 2012). In addition to, the World Health Organization (WHO) has reported that about 80% of the world population relies on conventional medicine for their primary health care (Latif et al 2014). Out of a total of 250,000 plant species present on earth, approximately one thousand have anticancer activities (Bibi et al 2012).

Cancer is one of the most dangerous diseases in humans and presently there is a considerable amount of new anticancer agents derived from natural products like medicinal plants (Sharma et al 2011). Globally, about 1 in 6 deaths is due to cancer, whereas cancer is the second leading cause of death worldwide, as well as it is responsible for an evaluated 9.6 million deaths in 2018. (WHO, 2018). The potential of using medicinal plants as anticancer drugs was recognized in 1950's by U.S National Cancer Institute (NCI). Since 1950, major contributions have been made for finding out naturally existing anticancer drugs (Sharma et al 2011). Moreover, about 60% of the actually used anticancer drugs have been extracted from natural products, mostly of plant origin (Latif et al 2014)

Zygophyllum album is a halophyte and shrubby plant belonging to Zygophyllaceae family Tackholm, (1977) which includes about 27 genera and 285 species frequently restricted to arid and semi-arid areas (Beier et al 2003). Indeed, many plants belonging to this genus have anti-inflammatory,

molluscicidal, and expectorant activities (**Hassanean et al 1993**). The main constituents described from *Zygophyllum* species are zygophyllin, quinic acid, and glycosides, which have been demonstrated to have anti-inflammatory and anti-pyretic activity.

Suaeda palaestina is a halophyte and shrubby plant belonging to the chenopodiaceae family, which is an annual herbaceous plant particularly abundant in the Mediterranean salt marshes. Hypoglycaemic and hypolipidaemic activities have been reported in this family. Regarding *Suaeda palaestina*, no further studies were conducted on its biological activities (**Oueslati et al 2012**).

This study aimed to assess the potential anti-carcinogenic of the two Egyptian medicinal plants; *Zygophyllum album* and *Suaeda palaestina* using human cell lines.

2. MATERIALS AND METHODS

2.1 . Plant selection and collection

Zygophyllum album and *suaeda palaestina* samples were collected from South Sinai and Borg el-Arab Alexandria (Egypt), respectively, in March 2015 by Desert Research Center (DRC). These halophytes were identified at the Flora and Phyto-Taxonomy Research Section, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt.

2.2. Collection and preparation of plant materials

The methods and protocols followed by **Seham et al (2014)** were used with modification as will be described in the following:

Fresh whole plants were collected from different localities in Egypt. The plant material was dried at ambient temperature and stored in a dry place prior to use. The plant was washed well with water, dried at room temperature in the dark, and then ground in an electric grinder to give a coarse powder.

Shoot powders (50 g) were extracted by using several solvents with increasing polarity (dichloromethane, methanol, and hot water, respectively).

The powders were soaked in 150ml solvents at room temperature for 48h. The plant extracts were collected drop wise and filtered using Whatman No.1 filter paper. The residues were soaked in

150ml of Dichloromethane for 24h and filtered it again. The extracts were then dried and finally placed in glass vials and stored at -20° C and the extracts were resuspended in 1% dimethyl sulphoxide (DMSO) before testing.

2.3. Measurement of cytotoxic activity by MTT assay

Human lung carcinoma (A549), hepatocellular carcinoma (HepG2) and normal liver (THLE2) cells at the exponential growth phase were seeded at a density of 1×10^4 cells per well (100 μ L/well) onto 96-well plate (Falcon, Franklin Lakes, NJ) in DMEM medium (GIBCO, Grand Island, New York, USA; Cat.no. A1049101). The cell density was adjusted by trypan blue exclusion method. *Zygophyllum album* and *Suaeda palaestina* were added with different concentrations (ranged from 0 to 200 μ g/mL) for 24 hour at 37 °C in a 5% CO₂ with 95% humidity incubator. In addition, different concentrations of cisplatin as reference chemotherapeutic drug were added and the microplates were incubated for a further 48 hour in DMEM medium (200 μ L). The medium was washed gently twice with ice-cold phosphate buffer saline (PBS) and a volume of 200 μ L MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole, (Molecular probes, Eugene, Oregon, USA; Cat.no.V-13154)] was added to each well. The microplate was incubated at 37 °C for another 4 hours in CO₂ incubator. About 180 μ L medium/MTT was removed and 100 μ L of acidified isopropanol were added per well to solubilize the formazan produced. Finally, the microplate was incubated with shaking for 15 minutes. The absorbance of each well was measured at 630 nm using a microplate reader (ELX800, Biokit, Spain). Assays were performed in triplicate on three independent experiments. Sigmoidal and dose dependent curves were constructed to plot the results of the experiment. The concentration of the compounds inhibiting 50 % of cells (IC₅₀) was calculated using the sigmoidal curve.

2.4. 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 AND DISCUSSION

3.1. Screening for the best solvent for cytotoxic activity of each plant

The cytotoxicity of each extract was assessed against Human lung carcinoma (A-549) cell lines. The result showed that the dichloromethane extracts were significantly active against the human lung carcinoma with IC50 values of 70.48 µg/ml and 34.82 µg/ml for *Zygophyllum album* and *Suaeda palastina*, respectively (Table 1 and Figure 1, 2).

3.2. Anti-cancer activity of dichloromethane extracts of tested plants on HepG2 and THLE2 cell lines

The anticancer activity of *Z. album* and *S. palaestina* dichloromethane extracts were tested on HepG2 (Liver cancer cell) and THLE2 (normal cell) cell lines and was examined by MTT assay. *Z. album* crude extract inhibited the proliferation of human hepatocellular carcinoma (HepG2) which recorded an IC50 value of 27.74µg/ml, but low cytotoxic activity was observed against normal liver (THLE2) cells (IC50 = 1485 µg/ml) as shown in (Fig. 3).

Fig. (4) showed that *Suaeda palastina* crude extract has potent cytotoxicity of (HepG2) (IC50 = 30.76 µg/ml), while no cytotoxic activity was recognized against normal liver (THLE2) cells (IC50 = 1442 µg/ml). These results are comparable to that of cisplatin (standard anticancer drug) used as positive control on HepG2, (20.15µg/mL) and the IC50 value of normal liver THLE2 (645.7 µg/mL). These data suggest that *Z. album* and *S. palaestina* had marked influence on tumour cell viability and targeted liver (HepG2) and lung (A-549) carcinoma cell lines, indicating the presence of powerful cytotoxic components in the dichloromethane fractions. In addition, the extracts were not significantly cytotoxic against normal human liver (THLE2) cell lines. Oueslati et al (2012), Ksouri et al (2013) obtained comparable results for the medicinal halophyte *Suaeda fruticosa* and *Zygophyllum album* showing that dichloromethane extract recorded the highest anticancer activity against human lung carcinoma (A-549) and colon adenocarcinoma cell lines (DLD-1). Therefore, our results revealed that *Z. album* and *S. palaestina* have appreciable anti-tumour activity and could be considered as a potential source of anticancer compounds.

Conclusion

In conclusion, these results have established that *Z. album* and *S. palaestina* exhibited substantial anticancer activity against liver carcinoma cell lines. The data suggest that these medicinal halophytes might be valuable sources of bioactive secondary metabolites and a promising source of health products for functional food or nutraceutical industries.

Table 1. Cytotoxic activity of several extracts from *Zygophyllum album* and *Suaeda palastina* shoots against lung carcinoma (A-549) cell line. Each value represents the mean ± SD of three determinations.

Plant	Extracts	IC50 (µg/ml)
		A-549
<i>Zygophyllum album</i>	Dichloromethane	70.48
	Methanol	479.2
	Hot water	771.5
<i>Suaeda palastina</i>	Dichloromethane	34.82
	Methanol	4936
	Hot water	-

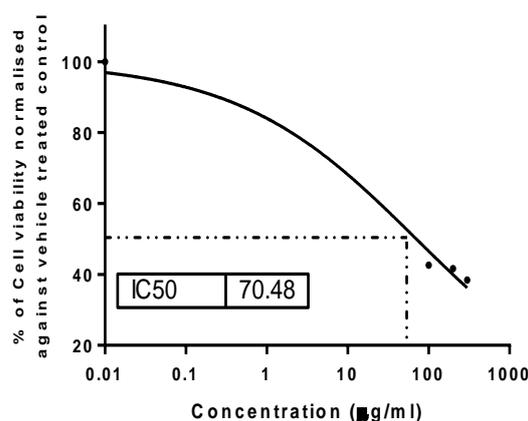


Fig. 1. Anti-proliferation effects of dichloromethane extract of *Zygophyllum album* on A-549 (lung cancer) cell lines.

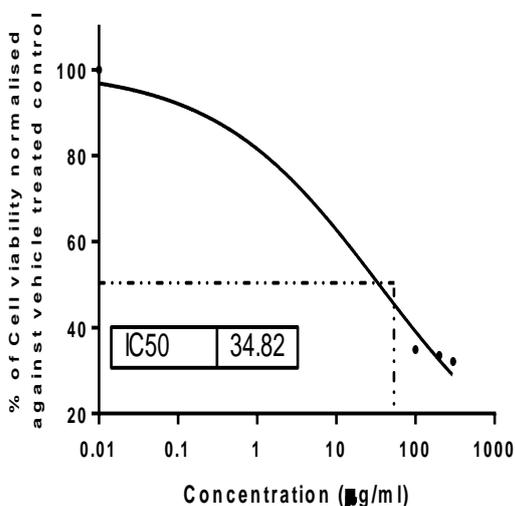


Fig. 2. Anti-proliferation effect of dichloromethane extract of *Suaeda palaestina* on A-549 (lung cancer) cell lines

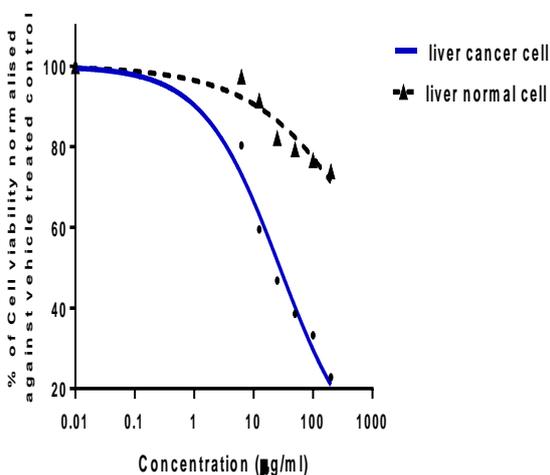


Fig. 3. Anti-cancer activity of *Zygophyllum album* crude extraction on HepG2 (human liver cancer cell line) and THLE2 (normal liver cells).

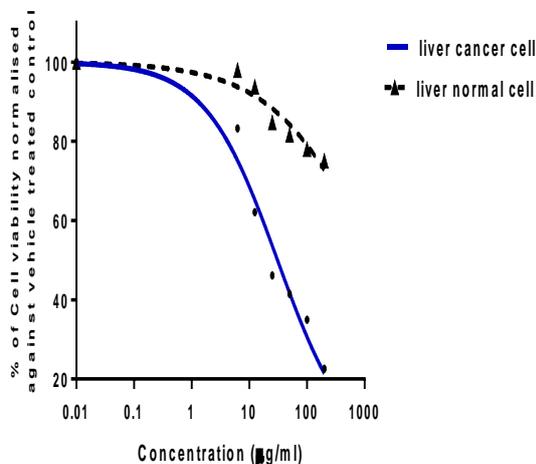


Fig. 4. Anti-cancer activity of *Suaeda palaestina* crude extraction on HepG2 (human liver cancer cell line) and THLE2 (normal liver cells).

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زىجوفلم ألبم والسوىده بلاستىنا تقىىم السمىة الخلوىة والنشاط المضاد للسرطان لمستخلصات على خطوط خلاىا سرطان الكبد فى الإنسان

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الخلاىا السرطانىة الكبدىة المخبترة بقىمة IC50
27.74 مىكروجرام/ مل لنبات *Z. album* ، فى حىن
كانت قىمة IC50 30.76 مىكروجرام/ مل لنبات *S. palaestina*
الطبىعىة للكبد (THLE2). وبذلك ىتبنى من النتائج أن
كلا من *Z. album* و *S. palaestina* تعد مصدر
طبىعى جىد للعوامل المضادة للسرطان.

الكلمات الءالءة: النباتات الطبىعىة، سوىده بلاستىنا،
زىجوفلم ألبم، النشاط السمى للخلاىا، عوامل مضاده
للسرطان، أختبار MTT، خطوط الخلاىا السرطانىة فى
الكبد للإنسان

الموجز

تم تقىىم النشاط المضاد للسرطان لمستخلصات كل
من الءاى كلورومىثان والمىثانول والماء الساخن لكل من
نباتات *Zygophyllum album* و *Suaeda palaestina*
وأشارت النتائج إلى أن المستخلصات
بمذىب الءاى كلورومىثان أعطت أعلى قىمة للتركىز
المثبط لنصف عدد الخلاىا (IC50) على خطوط
الخلاىا السرطانىة للرئة (lung A549) بقىمة 70.48
مىكروجرام/ مل ، 34.82 مىكروجرام/ مل على التوالى
مقارنة بمستخلصات المىثانول والماء الساخن.
بالإضافة إلى ذلك، أظهرت مستخلصات الءاى
كلورومىثان للنباتات على سرطان الكبد (HepG2)
تأثير مضاد للسرطان وسمىة خلوىة عالىة ضد خطوط

تحكىم: ا.ء عبد الوهاب حسن

ا.ء نجلاء محمد عبىد