

CYTOTOXICITY STUDY OF *FERULA HERMONIS* BOISS

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يعتبر نبات شرش الزلوع من النباتات المشهورة والتي تنتشر على سفوح جبال حرمون الواقعة بين المنطقة الحدودية بين لبنان وسوريا ، فلقد استعمل هذا النبات كمقوى جنسي وبيع كمنتج عشبي طبي في العديد من الدول العربية ولكن لازالت الدراسات الإكلينيكية تحتاج إلى براهين على جودة وفاعلية وسلامة هذا المنتج الطبي ، ففي الأونة الأخيرة تم نشر العديد من الأبحاث عن الخواص الكيميائية لهذا النبات. في هذه الدراسة حاولنا أن نسلط الضوء على مستخلص هذا النبات وإجراء تجارب عن فاعلية هذه المستخلصات من حيث تأثيرها على الخلايا وقياس درجة السمية (cytotoxicity activity). يعتمد هذا الاختبار على وضع صبغة التترازوليم (وهي مادة صبغية صفراء تدوب في الماء حيث يحدث لها اختزال في وجود الخلايا الحية فقط) على الخلايا بعد تعرضها على المادة المراد اختبارها فتعطي لون بنفسجي لاينوب في المحاليل المائية " purple formazan product" حيث يمكن قياسه بالطيف الضوئي. فلقد تم استخدام الأثير البترولي وخلات الايثيل والميثانول كمذيبات ذات قوة أيونية مختلفة لاستخلاص هذا النبات. أخيرا من نتيجة الاختبار لمستخلصات النبات اتضح أن مستخلص الأثير البترولي أكثر سمية من مستخلص خلات الايثيل ومستخلص الميثانول يمكن من دراسة هذا النبات في المستقبل وذلك باستخلاص ومعرفة المادة السامة واختبارها كمركبات ضد الأورام.

Ferula hermonis Boiss (Apiaceae) is known in the Midleast as 'zallouh'. In the last decade, scientists paid attention to study chemistry and biological activity of the plant. In this study *Ferula*

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hermonis were collected and evaluated for cytotoxic activity. The plant was extracted using different polarity solvents: petroleum ether, ethyl acetate and methanol. The cytotoxic effect of the extracts was examined in-vitro on human cell line (stomach cancer cell line, SCL-40). The assay was based on incubation of tetrazolium dye (MTT; a yellow water-soluble tetrazolium dye that is reduced by live, but not dead cells) with the cells that pre-incubated with a tested substance. A purple formazan product that produced can be determined spectrophotometrically.

The result confirmed that the SCL-40 cells were much more sensitive to the petroleum extract than other extracts, which showed the prominent cytotoxicity. The plant showed pronounced cytotoxic activity will be further evaluated for the possible isolation of active antitumour compounds.

INTRODUCTION

Ferula hermonis is well known for its traditional use in impotence for both men and women. In the South of Lebanon *F. hermonis* is known as Shilsh-el-zallouh which means “hairy root” whilst in the north of Lebanon it is called Hashishat-al-kattira, meaning “herb of abundance”¹. *Ferula hermonis* has recently received much attention due to its commercial value as an aphrodisiac, and as an herbal alternative to Viagra²⁻⁵. Auzi and co workers⁴ have recently reported 17 sesquiterpene compounds from the seed of *F. hermonis*. While *Ferula* may achieve the same positive results as Viagra, Unfortunately, the plant doesn’t come with the drugs long list of harmful side effects that has been reported which include facial flushing, upset stomachs, headaches, heart complications, urinary tract infections, and abnormal vision².

Despite this fact, the plant is still sold in many herbal shops. In the current study the possible cytotoxicity of the plant was evaluated.

MATERIALS AND METHODS

Materials

Solvents; Petroleum ether 40-60°C, ethyl acetate (EtOAc), methanol (MeOH), and dimethylsulphoxide (DMSO) were obtained from Sigma Company.

Stomach cancer cell line (SCL-40). 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Molecular Probes Europe BV, The Netherlands), RPMI-1640 (Roswell Park Memorial Institute), 10% foetal calf serum (Sigma-Aldrich Co,UK), 1% penicillin/streptomycin and 1% L-glutamine (Sigma-Aldrich Co,UK), silica gel GF245 (Sigma-Aldrich Co,UK), 96 well microtiter plate (Tissue Culture) and Light microscope.

Plant material

The plant was collected from Hermon Mountain Lebanon and identified by the herbarium of Botany Department Faculty of Sciences, Elfateh University, Tripoli-Libya. A voucher specimen (FH2002) was deposited in the herbarium.

Extraction of plant material

350 gm of the powdered roots of Ferula plant was macerated successively in petroleum ether 40-60°C, EtOAc and methanol. The extracts were concentrated under reduced pressure using a rotary evaporator to get rid of the solvents. The amount of extracts was 20 g (oil), 12 g and 18 g for petroleum ether, EtOAc and methanol, respectively.

***In vitro* acute cytotoxicity assay**

Each extract was dissolved in 0.2 ml solvent DMSO for the *in vitro* for cytotoxicity evaluation against stomach cancer cell line (SCL-40)⁶.

Cells were cultured in RPMI-1640, supplemented with heat inactivated 10% foetal calf serum, 1% penicillin/streptomycin and 1% L-glutamine incubated at 37°C and 5% CO₂ at aseptic condition. The cytotoxicity was assessed by the measurement of the LD₅₀ value in a standard MTT.

The MTT assay was carried out as follows; the cells were seeded at a concentration of 1-2 x 10⁵ cells/well of a 96 well microtiter plate. Each concentration was replicated 6 times. The cells were treated with two-fold serial dilution of extracts, medium alone to which no sample was added

(positive control and produced 100% viability), and DMSO alone that was used as a negative control and produce 0% viability.

Samples were dissolved in 0.2 ml DMSO and made up to 1 ml with the medium. The sample solution was filtered and sterilized before being added to the cells. 100 µl of cells in medium was pipetted into each well, 90 µl of medium was added and the 10 µl of sample added accordingly.

After 24 hrs, the plates were viewed under the microscope to record visual observations and to check for any contamination. The medium was then removed gently and then 150 µl of fresh medium and 50 µl of sterile solution MTT (1 mg/ml medium) were added in each well.

The plates were incubated for 3-4 hrs; the well contents were carefully removed. At this stage the blue formazan crystal were visible at the bottom of wells, which had contained live cells. 100 µl DMSO was added to dissolve any crystals present. The plate was shaken on a shaker for 5 minutes and absorbance was determined at 570 nm.

Solvent and medium were also examined as control. The percent suppression in cell viability for the test sample was determined by comparing the absorbance of the test sample with the mean control value⁷. The mean suppression and standard errors were calculated (Figs. 1&2). The graphs were used to determine the LD₅₀, which is the concentration at which 50% cell death suppression occurred.

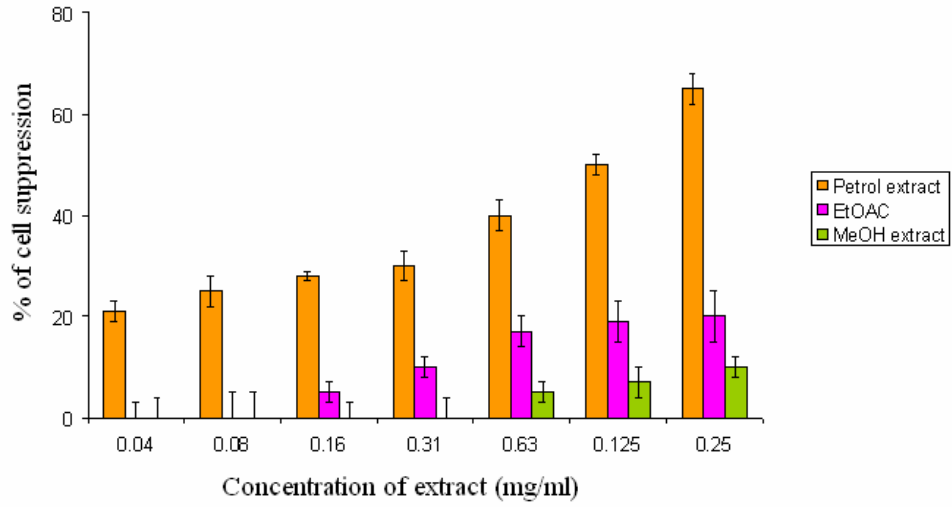


Fig. 1: The effect of extract concentration on the % of cell suppression.

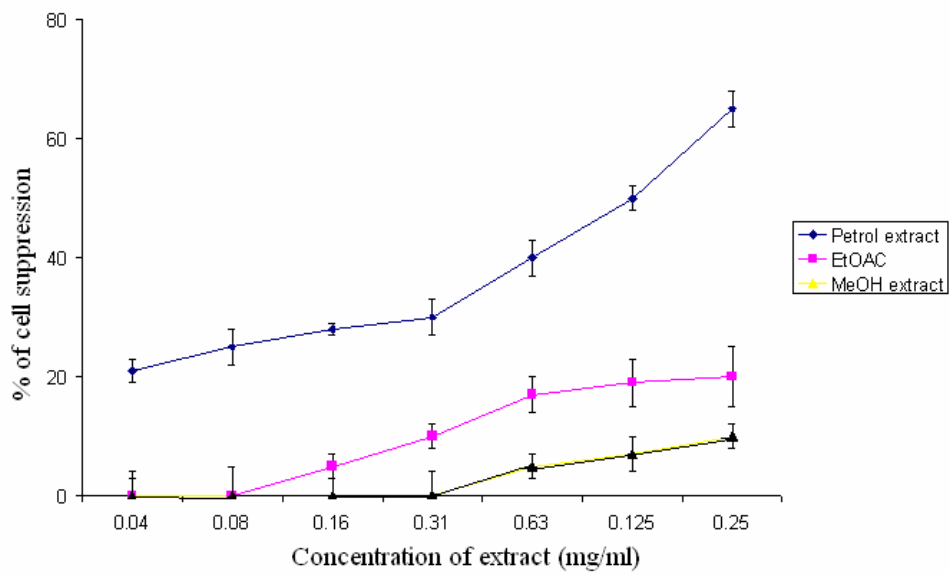


Fig. 2: IC₅₀ values of various *Ferula hermonis* extracts against SCL-40.

RESULTS AND DISCUSSION

The data in Table 1 showed that out of the three extracts, the petroleum extract at a concentration of 0.125 mg/ml causes 50% cell death suppression and was most cytotoxic. The EtOAc and methanol extracts at the concentration of 0.125 displayed cell deaths of 19% and 7% respectively (Figs. 1&2). Recent studies have demonstrated that the compounds commonly found in extract of *Ferula hermonis* with petroleum ether are sesquiterpenes⁴, therefore, It can be assumed that the sesquiterpenes might be responsible for cytotoxicity of *Ferula hermonis*. Further study is underway to isolate and examine pure active major compounds for cytotoxic effect and other pharmacological activity⁸.

Table 1: The % of the cell suppression by *Ferula hermonis* extracts against SCL-40 cells.

Concentration of the extract (mg/ml)	% of cell suppression		
	Petroleum extract	EtOAc extract	MeOH extract
0.04	21	ND	ND
0.08	25	ND	ND
0.16	28	5	ND
0.31	30	10	ND
0.63	40	17	5
0.125	50	19	7

ND= Non detected in the used concentrations

Conclusion

The present study supports the previous report for toxicity of *Ferula hermonis*². Therefore, the use of *F. hermonis* as an herbal medicine

should be under strict control to avoid any health complication.

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