The cytotoxic effects of essential oil and different extracts of *Pulicaria gnaphalodes* Boiss. on MDA-MB-468, Hela and K562 cancer cell lines

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Overview

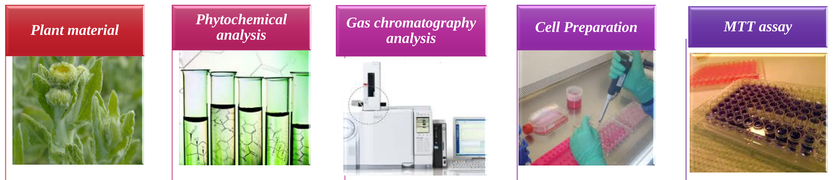
Several natural compounds have been identified for the treatment of malignancies. Due to a few safe drugs and the side effects caused by available chemotherapeutic agents, new drugs for treatment of malignancies are requested. The genus *Pulicaria* (Asteraceae) is represented by 100 species. Some compounds obtained from *Pulicaria* species have shown cytotoxic activity. The aim of this study was to evaluate the in vitro cytotoxic activity of different extracts and essential oil of *Pulicaria gnaphalodes* against Hela, K562 and MDA-MB-468 cancer cell lines.

Introdution

The genus *Pulicaria* belongs to the Asteraceae or Compositae family. *Pulicaria crispa* in Arabian traditional medicine is used as anti-inflammatory, insect repellent and herbal tea. *P. odora* also is used as traditional remedy for women after childbirth. Many compounds including phenolic derivatives, monoterpene derivatives, sesquiterpenes, diterpenes, flavonoids, triterpenes and steroids from the essential oils of the genus *Pulicaria* were isolated. According to the previous reports, flavonoids and sesquiterpenoids are the dominant constituents within this genus.

The pulicanone, pulicanol, pulicanarals A and B, and pulioplopanone were isolated from *P. canariensis* also have shown cytotoxic activity. *P.crispa* had no significant cytotoxic activity against K562 cells or antiviral activity but some compounds isolated from petroleum ether extract of *P. crispa* have shown cytotoxic effects. Other biological activities such as spasmolytic and anti-inflammatory effects were reported in *P. glutinosa* and *P. guestii* , respectively. In addition, in our previous studies the leishmanicidal activity of *P. gnaphalodes* essential oil had been evaluated against *Leishmania major* with an IC_{50} 0f 0.125 µl/ml .The present study was designed to evaluate the cytotoxic effects of different extracts and essential oil of the *Pulicaria gnaphalodes* Boiss. on three cancer cell lines including MDA-MB-468, Hela and K562 using MTT assay.

Methods



Plant collection and identification

P. gnaphalodes was collected from Tabas region and identified .The aerial parts of the plant were dried, powdered and extracted by methanol, petroleum ether and dichloromethane in different dilutions using percolator. Phenolic and tannin contents were measured by folin-ciocalteu method.

GC-Mass analysis of essential oil

The oil of P. *gnaphalodes* was analyzed using GC-Mass. The carrier gas was helium with a flow rate of 2 ml/min, the oven temperature for first 4 min was kept at 60°C and then increased at a rate of 4°C/min until reached to a temperature of 280°C, injector temperatures were set at 250°C. Mass spectrometer condition was as follow: ionized potential 70 eV, source temperature 200°C. Identification was based on retention data and computer matching as well as by comparison of electron-impact-mass spectra (EI-MS) with those relevant reference samples and the literature.

Cytotoxic evaluation

Hela , K562 and MDA-MB-468 were cultured in RPMI 1640 medium and grown in a humidified atmosphere using a CO_2 incubator providing 5% CO2 at 37 °C. Extracts were exposed to the cell lines in 3 different concentrations for 48 hours. This step was repeated for essential oil with the concentrations of 0.1, 0.2 and 0.5 µl/ml. The inhibitory effect of plant extracts and essential oil on cell growth was assessed using MTT assay. The absorbance of different wells- which directly correlate to the cell survival was measured at 570 nm by an ELISA plate reader and then the cell survivals were calculated.

Results

Determination of phenolic and tannin contents:

The highest amount of phenolic compounds were determined in methanol as a polar solvent and petroleum ether as a nonpolar solvent contained less amounts of phenols.

Table 1. Total phenolic and tannin contents of different extracts of *P. gnaphalodes* Boiss.

Extracts contents (mg/ml)	methanol	DCM: methanol (9:1)	DCM	Petroleum ether
Total phenolics	19.20	16.25	14.90	10.25
Total tannins	10.39	7.56	7.86	5.51

***GC-MS results*:**

Table 2. Major chemical composition of the essential oil of *P. gnaphalodes* Boiss.

Compounds	Type of compound	Rt	KI	(%)
A-pinene	Hydrocarbon monoterpene	3.82	0939	2.14
1,8-cineole	Alcoholic monoterpene	6.29	1031	5.9
Δ- terpinen	Hydrocarbon monoterpene	6.54	1060	1.42
Filifolone	Bicyclic hydrocarbon ketone	7.76	1082	2.47
Chrysanthenone	Monoterpene ketone	8.46	1128	6.42
Terpinen-4-ol	Alcoholic monoterpene	9.99	1177	3.46
A-terpineol	Alcoholic monoterpene	10.15	1189	3.51
Myrtenol	Alcoholic monoterpene	10.58	1196	1.90
trans-geraniol	Alcoholic monoterpene	11.61	1230	3.53
Neral	Aldehyde monoterpene	11.95	1238	1.13
Geraniol	Alcoholic monoterpene	12.42	1253	1.93
Geranial	Aldehyde monoterpene	12.88	1267	1.32
Thymol	Phenolic compound	13.58	1290	1.64
Δ-cadinene	Hydrocarbon sesquiterpene	20.21	1524	1.23
A-cadinene	Hydrocarbon sesquiterpene	24.77	1539	1.87

***Cytotoxic results*:**

Cytotoxicity of the plant extracts and essential oil were measured on 3 human cancer cell lines using MTT assay. Results are shown in figures 1 to 4.

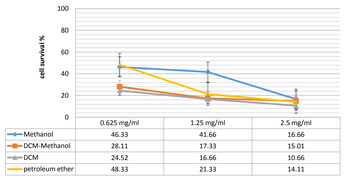
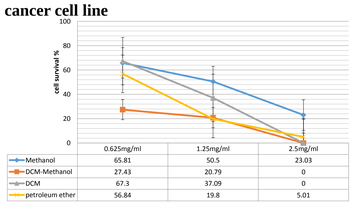


Fig. 1. Cytotoxic activity of different concentrations of the extracts on MDA-MB-468 cancer cell line



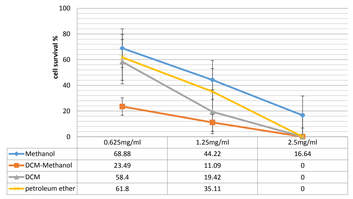


Fig. 2. Cytotoxic activity of different concentrations of the extracts on Hela cancer cell line

Fig. 3. Cytotoxic activity of different concentrations of the extracts on K562 cancer cell line

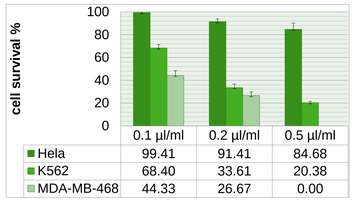


Fig. 4. Cytotoxic activity of *Pulicaria gnaphalodes* essential oil on different cancer cell lines

Malignancies are one of the most pernicious diseases of the present century, so that many cytotoxic evaluation studies on different plants have done in order to find novel antitumor ingredients. *Pulicaria* species are one of such plants that showed different isolated compounds with a wide range of biological activities .

Terpenoids are between the toxic constituents of the plants and generally extracted by non polar solvents . In this regard, the results obtained from the present study showed that the extracts of non polar solvents have more cytotoxic effects than polar ones, and those with higher polarity showed less cytotoxic effects against all tested cell lines (Fig. 1-3). In addition different sensitivity of cell lines toward tested extracts can be seen. Here it is remarkable that the extract of a mixed solvent like DCM: methanol (9:1) is the most cytotoxic extract against almost all tested cancer cell lines (IC_{50}<0.625 mg/ml). It seems that this semi-polar solvent maybe can extracts high amounts of sesquiterpene compounds that make it the best combination of solvents for extraction of cytotoxic agents.

In the present study cytotoxic activity of *P. gnaphalodes* essential oil was evaluated against three different cancer cell lines. Hela cells were resistant to the tested concentrations of this plant essential oil (IC_{50}>0.5 µl/ml), while K562 as a leukemia and MDA-MB-468 as a breast carcinoma cell line showed a good response to the tested compounds cause of different susceptibility of cells. GC-MS analysis showed that the major components of *P. gnaphalodes* essential oil are monoterpenes such as α-pinene, 1,8-cineole, terpinen-4-ol and α-terpineol. 1,8-cineole, as a monoterpene, demonstrated moderate cytotoxicity in HeLa, K-562, and some other cell lines . In the case of α-pinene cytotoxicity was comparable to doxorubicin against MDA-MB-468 cells . Terpinen-4-ol has shown cytotoxicity against HeLa and K-562 . In the present study we proposed these compounds are responsible for cytotoxic activity of *P. gnaphalodes* essential oil against tested cell lines.

Conclusion

It can be concluded that the essential oil of *P. gnaphalodes* Boiss. worth to be a new candidate for further cytotoxic evaluation via isolating some active ingredients and different extracts of this plant are less cytotoxic than essential oil.

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