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ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE EFFECT OF HIPPOMARATHRUM MICROCARPUM PETROV (APIACEAE) GROWING IN TURKEY ON PC3 CANCER CELL PROLIFERATION

TÜRKİYE'DE YETİŞEN HIPPOMARATHRUM MICROCARPUM PETROV (APIACEAE)'UN PC3 KANSER HÜCRE PROLİFERASYONU ÜZERİNDEKİ ETKİSİ

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SUMMARY

Apiaceae is the 3rd largest family of Turkey with the largest genus, which is widely distributed throughout the world and represented in our country with 109 genera and 450 species. The genus Hippomarathrum has 28 species which are outspread in the North, Northwest, West and Central of Iran, Turkey, Caucasus and Iraq and one of this species H. microcarpum Petrov is a plant which grows wild in Turkey and is used as food by the people in Eastern Anatolia. In this study, the in vitro anticancer activity of the aqueous and ethanol extracts obtained from H. microcarpum was investigated on cancer cell proliferation. For this purpose human prostate (PC-3) cells were used and measurements were performed via MTT test. Aqueous and ethanol extracts obtained from aerial parts exhibited potent inhibitor effects on cell proliferation. Ethanol extract inhibited the proliferation of PC-3 cell at 24th hour with a 12.99 mg/mL IC_{50} value.

Keywords: *antiproliferative, Apiaceae, cancer, Hippomarathrum microcarpum, prostate*

ÖZET

Apiaceae tüm dünyada geniş bir yayılış gösteren ve ülkemizde 109 cins ve 450 türle temsil edilen, Türkiye'nin en çok cins içeren 3. büyük familyasıdır. Kuzey, Kuzeybatı, Batı ve Orta İran, Türkiye, Kafkaslar ve Irak'ta yayılış gösteren Hippomarathrum cinsinin dünyada 28 türe vardır ve bu türlerden biri olan H. microcarpum Petrov. Türkiye'de Doğu Anadolu'da, yabani olarak yetişen ve halk tarafından gıda olarak kullanılan bir bitkidir. Bu çalışmada, H. microcarpum'un su ve etanol ekstrelerinin PC3 insan prostat kanser hücre proliferasyonu üzerindeki etkilerinin incelenmesi amaçlanmıştır. Bu amaçla, insan prostat kanser (PC-3) hücreleri kullanılmış ve MTT testi ile sitotoksisite analizleri gerçekleştirilmiştir. Toprak üstü kısımlarından hazırlanan sulu ve etanollü ekstreler hücre proliferasyonu üzerinde güçlü

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inhibitör etki sergilemiştir. Etanollü ekstrenin 24 saatlik uygulama sonucunda PC-3 proliferasyonunu 12.99 mg/mL IC_{50} değeriyle inhibe ettiği saptanmıştır.

Anahtar kelimeler: *antiproliferatif, Apiaceae, Hippomarathrum microcarpum, kanser, prostat*

INTRODUCTION

Apiaceae is one of the biggest and cosmopolite family throughout the world and the majority of the worldwide species diversity is concentrated in Asia (some genera are exclusively Asiatic) [1]. The genus of *Hippomarathrum* Link is a member of Apiaceae family and it has five species that are *H. crassilobum* Boiss., *H. cristatum* (DC) Boiss., *H. microcarpum*, *H. scabrum* (Fenzl) Boiss., and *H. boissieri* Reuter et Hausskn. *Hippomarathrum* is 50-100 cm height and an erect, much-branched perennial genus. This genus is distributed in rocky slopes and fields [2]. The members of the genus have long been used as spice in ethnobotany [3]. *H. microcarpum* is a gray shrub filled with yellowish flowers [4] and it is reported that the coumarins and furanocoumarins found in the roots and fruits of this genus [5].

We aimed to investigate the antiproliferative effect of aqueous and ethanol extracts of *H. microcarpum* species on human prostate (PC-3) carcinoma cells since cancer has become an important cause of morbidity and mortality in the world [6]. According to the results of biomedical research covering the last 20 years, is quite in excess of information about actual the molecular events during carcinogenesis and signaling pathways involved in cancer progression. For many years, the results obtained from the studies, matrix metalloproteinase (MMP) enzymes, such as extracellular matrix proteinase, during the development of cancer has revealed that the main tool of the changes observed in the microenvironment [7, 8].

MATERIALS AND METHODS

Plant material

The plant was collected from the below mentioned locality by Songül Karakaya and Hayri Duman and identified by Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Department of Biology) and the voucher specimen is kept in AEF (Herbarium of Ankara University Faculty of Pharmacy).

Collection locality: *Hippomarathrum microcarpum*: Adana, south of Tufanbeyli, 13.07.2014 (AEF 26699).

Preparation of extracts

For the extraction procedure, 60.3456 g of aerial parts were grounded and macerated with 500 mL of distilled water for 4 h at temperatures between 30-35°C. Extract was filtered and then lyophilized by using Christ Gamma 2-16 LSC Freeze Dryer. 50, 2341 g of aerial parts were grounded and macerated (Heidolph MR3001, Germany) for 8 hours/3 days with ethanol in a water bath not exceeding 60°C using a Heidolph mechanical mixer (300 rpm). The extracts, filtered and concentrated till dryness using a rotary evaporator (Heidolph VV2000, Germany) and yielded 3.2865 g and 2.8816 g aqueous and ethanol extract, respectively.

Cell culture

Human prostate cancer cell line PC3 (CRL-1435) was purchased from American Type Culture Collection, Cell Biology, LGC Promochem, Wesel, Germany. The cells were maintained in DMEM (PAA Laboratories) media supplemented with 10% fetal bovine serum (FBS, Lonza), 1% penicillin/streptomycin (PAA, The Cell Culture Company) and 1% L-glutamine (PAA, Austria) and incubated in a 5% CO_2 humidified atmosphere at 37°C.

Cell viability assay

The effect of aqueous and ethanolic extracts of *Hippomarathrum microcarpum* on PC3 cell viability was measured by 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 180 µl of $5x10^4$ cells were plated in 96-well microtiter plates and then incubated overnight in a humidified atmosphere of 5% CO_2 in air at 37°C. After incubation, the cells were treated with *H. microcarpum* extracts (final concentrations were 0.5, 1, 5, 10 and 20 mg/mL) and incubated for 24 hours in order to assess the cell viability. Untreated cells were used as control. After incubation, the culture medium was removed and exchanged for a fresh one. 20 µl of MTT solution (5mg/mL in PBS, Sigma) was added per well and incubated at 37°C for 4 hours. The metabolically active cells reduced MTT dye to formazan crystals. The medium was then removed and the blue MTT-formazan was dissolved in DMSO (Merck). The extent of the reduction of MTT within the cells was quantified by measuring the absorbance at 540 nm with microplate reader (Thermo, Germany) and compared with untreated cells. Data were obtained from quadruplicate wells per condition and represented mean ± standard deviation (SD) of at two independent experiments.

RESULTS AND DISCUSSION

Effects on cell proliferation

In the study, the PC-3 human prostate carcinoma cells were treated with different concentrations of aqueous and ethanolic extracts of *H. microcarpum* and the cell viability was measured for 24 hour as described in the experimental part. The results of these measurements are shown in Figure 1 and 2.

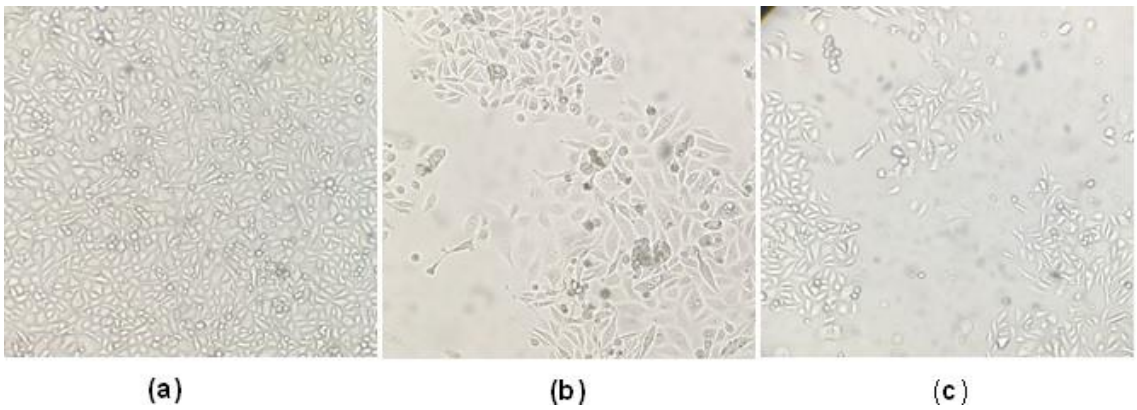

Figure 1. The growth pattern and morphology of PC-3 cells in control (a), aqueous (b) and ethanolic (c) extracts of *H. microcarpum* for 20 mg/mL concentration were examined under an inverted microscope (Leika, DM IL LED, Germany) with x100 scale. Photographs are representative fields of more than three independent experiments.

Cell viability was significantly lower for all treated concentrations of ethanolic and aqueous extracts when compared to control (Fig. 2). The aqueous extracts of 10 and 20 mg/mL concentrations (Fig. 2a) inhibited cell proliferation significantly as compared to 0.5 mg/mL treated group, whereas the cell viability was significantly lower in ethanolic extract treated groups at 1 mg/mL and higher concentrations when compared to 0.5 mg/mL (Fig. 2b).

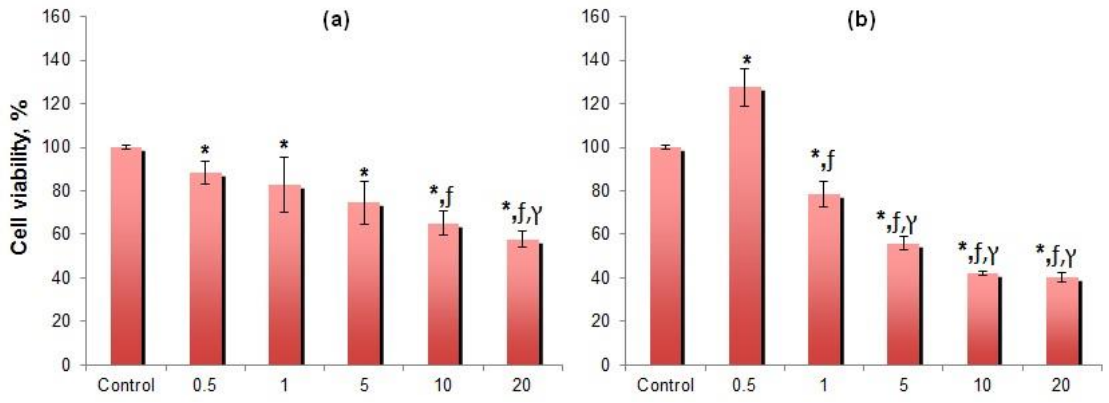

Figure 2. Effects of different concentrations of *H. microcarpum* extracts on viability of PC-3 were determined by MTT assay. The cells were seeded at $5x10^4$ cell/mL in complete DMEM medium and treated with different concentrations of aqueous (a) and ethanolic (b) extracts of *H. microcarpum* for

24 h. The results are expressed as percentage of live cells compared with untreated control. The data present the mean ± SD of four independent experiments. The differences are * from control, ^{f} from 0.5 mg/mL, ^{r} from 1.0 mg/mL ($p<0.05$).

Table 1. Effects of different concentrations of *H. microcarpum* (final concentrations were 0.5, 1, 5, 10 and 20 mg/mL) on viability of human PC-3 cells via MTT assay.

	Cell viability, % of control	**IC_{50} (mg/mL)**	**P values compared to control**
Aqueous extract (mg/mL)			
0.5	88.44±5.33		0.182
1	82.75±12.58		0.002
5	74.56±9.65	23.34	0.000
10	65.18±5.52		0.000
20	57.77±3.71		0.000
Ethanolic extract (mg/mL)			
0.5	127.46±8.66		0.000
1	78.63±5.89		0.000
5	55.95±2.96	12.99	0.000
10	42.12±0.92		0.000
20	40.39±2.22		0.000

In the PC-3 cells, aqueous extracts exhibited the highest cytotoxic effect with 23.34 mg/mL IC_{50} value at 24th hour, and we observed significant inhibition of cell proliferation at 20 and 10 mg/mL doses (57.77±3%, 65.18±5.52%, respectively, $p<0.05$). On the other hand, ethanolic extracts exhibited the highest cytotoxic effect with 12.99 mg/mL IC_{50} value at 24th hour, and we observed significant inhibition of cell proliferation at 20 and 10 mg/mL doses (40.39±2.22%, 42.12±0.92%, respectively, $p<0.05$).

Our results show that, although both extracts have significant effects on inhibition of cell proliferation, the ethanolic extract has more potent effect when compared to the aqueous extract. In the literature, there are no studies carried out on the cytotoxic and anticancer effects of *Hippomarathrum* species. However, cytotoxic properties of some coumarins such as isoimperatorin, xanthotoxin [9], felamidin [10], osthole, isoimperatorin, oxypeucedanin [11], umbelliferone [12], bergapten, isopimpinellin [13] and heraclenin [14] were also demonstrated against various tumor cells lines. Also coumarin has been reported that it was shown to possess anti-tumour and antimetastatic activity in rats [15]. The presence of coumarins such as isoimperatorin, bergapten, xanthotoxin, isopimpinellin (in

fruits); osthole, oxypeucedanin, heraclenin, oxypeucedanin hydrat (in roots) [16] and (+) prangenin (heraclenin), umbelliferone [17] has been reported in *H. microcarpum*.

As a consequence, we can conclude that *H. microcarpum* has promising effects against the proliferation of cancer cells, and may represent a herbal alternative to synthetic drugs based on its coumarin content. These data have supplied a wealth of information on the antiproliferative effect of *H. microcarpum*. Further studies are necessary to clarify the mechanisms underlying these effects and also to detect the responsible constituent(s).

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REFERENCES

1. Troia, A., Raimondo, F.M., Castellano, G., Spadaro, V. (2012). Morphological, karyological and taxonomic remarks on *Ferulago nodosa* (L.) Boiss. (Apiaceae). Plant Biosystems, 146, 330–337.
2. Ozer, H., Sokmen, M., Gulluce, M., Adıguzel, A., Sahin, F., Sokmen, A., Kılıc¸ H., Barıs, O. (2007). Chemical composition and antimicrobial and antioxidant Activities of the essential oil and methanol extract of *Hippomarathrum microcarpum* (Bieb.) from Turkey. Journal of Agriculture and Food Chemistry, 55, 937−942.
3. Baytop, T. (1997). *Türkçe Bitki Adları Sözlüğü (A Dictionary of Vernacular Names of Wild Plants of Turkey);* Turkish Language Society: Ankara, Turkey, p. 294.
4. Khalilzadeh, M.A., Tajbakhsh, M., Gholami F.A., Hosseinzadeh, M. (2007). Composition of the Essential oils of *Hippomarathrum microcarpum* (M. Bieb.) B. Fedtsch. and *Physospermum cornubiense* (L.) DC. from Iran. Journal of Essential Oil Research, 19, 567–568.
5. Sefidkon, F., Shaabani, A. (2003). Analysis of the Oil of *Hippomarathrum micocarpum* (M. Bm) B. Fedtsch. from Iran. Journal of Essential Oil Research, 15, 261-262.
6. Jemal, A., Tiwari, R.C., Murray, T., Ghafoor, A., Samuels, A., Ward, E., Feuer, E.J., Thun, M.J. (2004). Cancer statistics. CA: A Cancer Journal for Clinicians, 54, 9-29.
7. Kessenbrock, K., Plaks, V., Werb, Z. (2010). Matrix metalloproteinases: regulators of the tumor microenviroment. Cell, 141: 52-67.
8. Page-McCaw, A., Ewald, A.J., Werb, Z. (2007). Matrix metalloproteinases and the regulation of tissue remodeling. Nature Reviews Molecular Cell Biology, 8, 221–233.

9. Ameen, B.A.H. (2014). Phytochemical study and cytotoxic activity of *Ferulago angulata* (Schlecht) Boiss, from Kurdistan-region of Iraq. International Journal of Innovative Research in Advanced Engineering, 1(9), 1-5.
10. Rosselli, S., Maggio, A.M., Faraone, N., Spadaro, V., Morris-Natschke, S.L., Bastow, K.F., Lee, K.H., Bruno, M. (2009). The cytotoxic properties of natural coumarins isolated from roots of *Ferulago campestris* (Apiaceae) and of synthetic ester derivatives of aegelinol. Natural product communications, 4(12), 1701-1706.
11. Shokoohinia, Y., Hosseinzadeh, L., Alipour, M., Mostafaie, A., Motlagh H.R.M. (2014). Comparative evaluation of cytotoxic and apoptogenic effects of several coumarins on human cancer cell lines: Osthole induces apoptosis in p53-Deficient H1299 Cells. Advances in Pharmacological Sciences, 2014-2018.
12. Kıvçak, B., Mert, T. (2002). Antimicrobial and cytotoxic activities of *Ceratonia siliqua* L. extracts. The Turkish Journal of Biology, 26, 197-200.
13. Yang, L.L., Wang, M.C., Chen, L.G., Wang, C.C. (2003). Cytotoxic activity of coumarins from the fruits of *Cnidium monnieri* on leukemia cell lines. Planta Medica, 69(12), 1091-1095.
14. Setzer, W.N., Setzer, M.C., Schmidt, J.M., Moriarity, D.M., Vogler, B., Reeb, S., Holmes, A.M., Haber, W.A. (2000). Cytotoxic components from the bark of *Stauranthus perforatus* from Monteverde, Costa Rica. Planta Medica, 66(5), 493-494.
15. Maucher, A., Kager, M., Angerer, E.V. (1993). Evaluation of the antitumour activity of coumarin in prostate cancer models. Journal of Cancer Research and Clinical Oncology 119,150-154.
16. Pigulevskii, G.V., Dranitsyna, Y.A., Kerimov, S.S., Kozhina, I.S. (1967). Coumarins and Furocoumarins of *Hippomarathrum microcarpum.* Khimiya Prirodnykh Soedinenii, 3, 215.
17. Soedin, P. (1998). Coumarins: Plants, Structures, Properties. Chemistry of Natural Compounds, 34, 4.