



Anti-proliferative effect of *Cistus laurifolius* on human cervical adenocarcinoma (Hep2C), human muscle rhabdomyosarcoma (RD), mouse fibrosarcoma (Wehi 164) cell line

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Abstract

In the current study; it was tried to determine antiproliferative activity of extract obtained from *Cistus laurifolius* leaves on different cancer cell type such as human cervical adenocarcinoma cells (Hep2C), human muscle rhabdomyosarcoma cells (RD), mouse fibrosarcoma cells (Wehi 164) by MTT Assay. The results indicated that *Cistus laurifolius* inhibited, in a dose-dependent manner, the proliferation of Hep2C, RD, Wehi 164 cells. Maximum anti-proliferatif effects were observed in 1000 µg/ml, 500 µg/ml, 250µg/ml concentrations of extract. These findings suggest that *Cistus laurifolius* extract could reduce proliferatif effect of cancer cell lines. Their use as potential anticancer agent needs further studies.

Keywords: *Cistus laurifolius*, human cervical adenocarcinoma cells (Hep2C), human muscle rhabdomyosarcoma cells (RD), mouse fibrosarcoma cells (Wehi 164)

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***Cistus laurifolius*'un insan serviks karsinoma (Hep2C), insan kas rabdomyosarkoma (RD), fare fibrosarkoma (Wehi 164) hücre hatları üzerinde antiproliferatif etkisi**

Özet

Bu çalışmada; *Cistus laurifolius* ekstraktının; insan serviks karsinoma (Hep2C), insan kas rabdomyosarkoma (RD), fare fibrosarkoma (Wehi 164) hücre hatları üzerinde antiproliferatif aktivitesinin MTT testi aracılığı ile belirlenmesi amaçlanmıştır. Sonuç olarak, *Cistus laurifolius* ekstraktının doza bağlı bir şekilde, Hep2C, RD, Wehi 164 hücrelerinin proliferasyonunu inhibe ettiği tespit edilmiştir. Maksimum anti-proliferatif etki 1000 µg/ml, 500 µg/ml, 250µg/ml ekstrakt konsantrasyonlarında gözlenmiştir. Bu sonuçlar, *Cistus laurifolius*'nin kanser hücrelerinin proliferatif etkisini azaltabileceğini işaret etmektedir ve antikanser ajan olma potansiyeli konusunda daha ileri çalışmalar yapılmalıdır.

Anahtar kelimeler: *Cistus laurifolius*, insan serviks karsinoma (Hep2C), insan kas rabdomyosarkoma (RD), fare fibrosarkoma (Wehi 164)

1. Introduction

Cancer is a multistage process which transformation of normal cell to step by step pre-cancerous lesion and malignant tumors. These differentiations are due to the interaction between genetic factors and environmental effects such as chemical carcinogens (aflatoxin, asbestos, arsenic etc.), biological carcinogens (infections from certain bacteria, parasites, viruses, or etc.) and physical carcinogens (ionizing radiation and ultraviolet and etc) [1, 2]. Today, despite

considerable effort and treatment methods such as chemotherapy and radiotherapy, cancer is still important cause of death and morbidity on a global scale. Also these treatments have serious side effects such as diarrhea, pain, nausea, fatigue, vomiting and hair loss. For this reason, anti-cancer drug development studies maintain their importance and plant metabolites are seen options for anticancer drug development [3].

Plant metabolites had an essential role in traditional treatment with their complex mixture of valuable substance. Dry powders, extracts or different parts of them have been used for treatment of various disease include cancer [4,5]. Isolation or detection of plants active compounds lead to the discovery of so many drugs with therapeutic effect such as analgesic (morphine and codeine isolated from *Papaver somniferum* L.), antipyretic or fever reducing drug (quinine isolated from *Cinchona* sp.) the cardiac glycoside (digitoxin isolated *Digitalis* sp.), anticancer (paclitaxel isolated from *Taxus* sp., camptothecin or derivatives topotecan, irinotecan, belotecan, gimetecan, 9-Nitrocampothecin isolated from *Camptotheca acuminata*, epipodophyllotoxin, colchicine isolated from *Colchicum autumnale* L., vincristine and vinblastine isolated from *Catharanthus roseus*). Recently, a substantial ratio of clinically used anticancer drugs are from natural resources and have been approved for treatment of acute myeloid leukemia, choriocarcinoma, lung cancer, testicular and ovarian cancers, bladder cancer and lymphoma etc [6-9].

Despite drug development studies and extensive resources are devoted to reduce cancer incidence worldwide, cancer cases continue to spread and are expected to increase by 70% in the next 20 years. This reveals a fundamental need to develop more effective and reduced side effected anticancer drugs from new sources are the focus of interest. Several *Cistaceae* species are consumed in traditional medicine as antiulcerogenic, antiinflammatory, antimicrobial, wound healing, antioxidant [10-13]. Especially, traditional Turkey folk medicine *Cistus laurifolius* have been used for diabetes [14]. The antiproliferative potential of *C. creticus*, *C. libanotis*, *C. monspeliensis*, *C. villosus*, *C. salviifolius*, partially evaluated against variable cancer cell lines, but to the best of our knowledge, there are few researches about anticancer activity of *Cistus laurifolius* [15-16]. In the current study; it was tried to determine antiproliferative activity of *Cistus laurifolius* L. on different cancer cell type such as human cervical adenocarcinoma cells (Hep2C), human muscle rhabdomyosarcoma cells (RD), mouse fibrosarcoma cells (Wehi 164).

1. Materials and Methods

2.1. Plant Material and Extract Preparations

The fresh leaves of *Cistus laurifolius* L. were collected from Tandır province of Eskişehir, Turkey in in July, 2013. The identity of sample was authenticated from Herbarium of Anadolu University (ANES No. 15518). *Cistus laurifolius* L. extract was prepared according to the method of Sadhu et al. [17]. Leaves of *Cistus laurifolius* were firstly sun dried for 3 days and were further dried in an oven at $40\pm 5^{\circ}\text{C}$ for 48 h. The dried leaves were griddled. 40 gr powdered leaves boiled in 800 ml water for 60 minutes and the extract were evaporated by vacuum evaporator and lyophilisated. Lyophilisate stored at $+4^{\circ}\text{C}$ until using for MTT assay.

2.2. MTT assay

Human cervical adenocarcinoma cells (Hep2C), human muscle rhabdomyosarcoma cells (RD), mouse fibrosarcoma cells (Wehi 164) were cultured in Eagle's MEM medium, Dulbecco's Modified Eagle's Medium and RPMI 1640 medium, respectively. All culture medium supplemented with 100 U/ml penicillin and 100 $\mu\text{g/ml}$ streptomycin and 10% FBS, under the conditions of 5% CO_2 at 37°C . After cultivation, cells were harvested and plated in 96-well plates at 1.0×10^6 cells/mL in 100 μL . Allowed to adhere at 37°C in 5% CO_2 for 24 h. Thereafter, 24h incubation, mediums were replaced with 100 μL different concentration of *Cistus sp.* extract (1000 $\mu\text{g/ml}$, 500 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$, 125 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$, 12.5 $\mu\text{g/ml}$, 6.12 $\mu\text{g/ml}$, 3 $\mu\text{g/ml}$) diluted with 14mM DMSO (Dimethyl sulfoxid) and incubated 37°C in 5% CO_2 for 24 h. For positive and negative control; culture medium of cell and ammonium molybdate were used respectively. At the end of incubation period, 20 μl (at a concentration of 5 mg/ml) MTT (Methylthiazolyldiphenyl-tetrazolium bromide) was added to each well. After incubation for 3 hours, culture media in each well was aspirated and 100 μl of propanol was added to dissolve the formazan products. Cell viability was assessed by MTT assay; the absorbance was read at a wavelength of 570 nm with respect to the reference wavelength at 620 nm using a spectrophotometer as previously described [18]. Two replicates of MTT assay was evaluated and all cell lines treated with different concentration of *Cistus sp.* extract. The results were analyzed by Softmax Pro 7.1 Software, 4 Parameter Logistic Assay, dose response curves were created and IC50 values were determined.

3. Results

The cytotoxic effect of *Cistus laurifolius* extract on human cervical adenocarcinoma cells (Hep2C), human muscle rhabdomyosarcoma cells (RD) and mouse fibrosarcoma cells (Wehi 164) was determined by MTT assay. Cells were incubated for 24 h with the positive control and 1000, 500, 250, 125, 100, 50, 25, 12.5, 6.12, 3 µg/ml. Dose dependent cytotoxic activities were observed in all the tested cell lines. Higher concentration, (1000, 500, 250 µg/ml) of *Cistus laurifolius* L. extract showed a significant cytotoxic effect. DMSO which used as diluent and ≤125 µg/ml *Cistus laurifolius* L. extract concentrations treated Hep2C and RD cells exhibited no cytotoxic effect (Figure 1, 2). All concentration of *Cistus laurifolius* L. extract observed antiproliferative effect on Wehi 164 cell line (Figure 3).

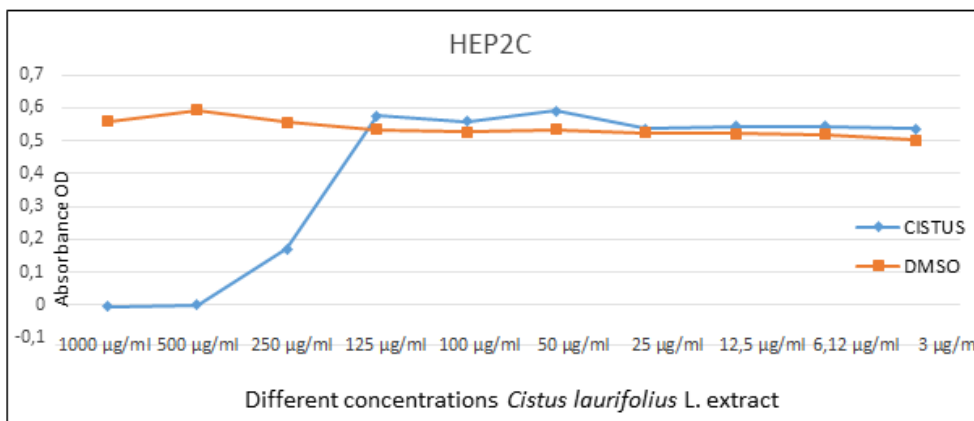


Figure 1. Effect of *Cistus laurifolius* extract on human cervical adenocarcinoma cells (Hep2C)

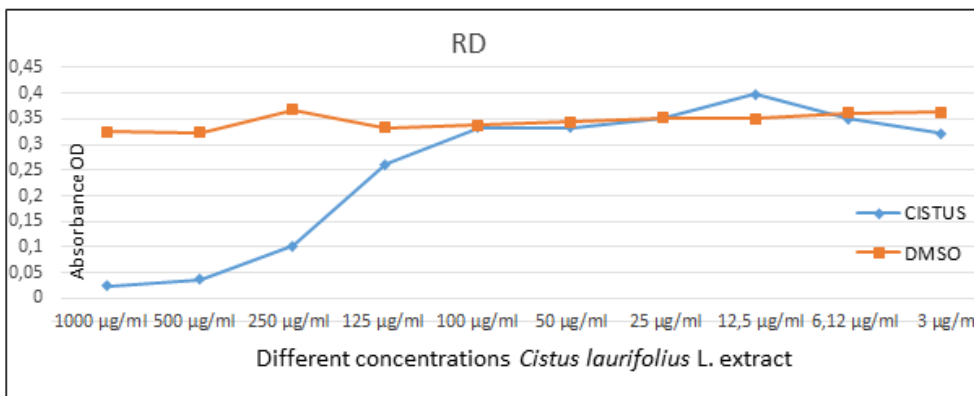


Figure 2. Effect of *Cistus laurifolius* extract on human muscle rhabdomyosarcoma cells (RD),

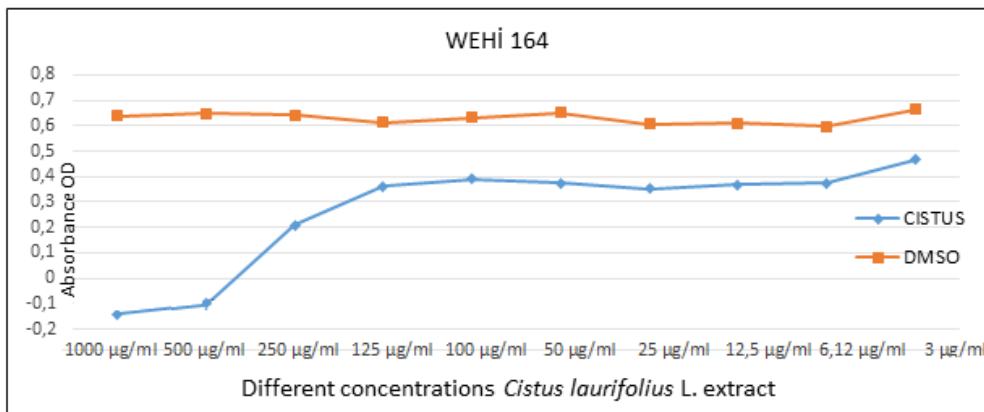


Figure 3. Effect of *Cistus laurifolius* extract on mouse fibrosarcoma cells (Wehi 164)

The results are reported in Table 1 as IC₅₀ values (µg/ml). *Cistus laurifolius* extract showed to be very toxic against all the tested cell lines with an IC₅₀ around 200 µg/ml against Hep2C and RD, while it was more effective on Wehi (IC₅₀ 127 µg/ml).

Table 1. IC₅₀ Values (µg/ml of extract) for Antiproliferative Activity of *Cistus laurifolius* Towards Different Cells

Cell Type	IC ₅₀ Values (µg/ml of extract)
Hep2C	216,76
RD	205,11
Wehi164	127,59

Higher concentration (1000 µg/ml, 500 µg/ml, 250 µg/ml) of extract exhibited very strong cytotoxic activity in all the tested cell lines, while all lower concentration up to 3 µg/ml of extract showed remarkable amount of cytotoxic activity on Wehi cell line. Plant extracts show IC₅₀ value > 300 ppm considered as non-effective and not potential agent for anticancer drug development studies [9].

4. Conclusions and discussion

The phytochemical studies *Cistus libanotis*, *Cistus villosus* and *Cistus monspeliensis* extracts highlight their antiproliferative activity on human melanoma (A-375) and human breast cancer (MCF-7) cells [19, 20]. *Cistus laurifolius* is used as complementary medicine against diabetes in Turkey [21]. It is also used as anti-cancer, expectorant, anti-cold and dysentery [22]. Studies based on content analysis revealed that; *Cistus* leaf contains 49, 33% water, 50,67% dry matter, 32,95% ash, 67,05% organic matter, 1,74% nitrogen, 10,87% protein, 9,4% fixed oil and 0,9% crude fiber [23]. Young shoots of *Cistus* contain 4,09% water, 95,91% dry matter, 33,53% ash, 66,46% organic matter, 0,68% nitrogen, 4,25% protein, 7,14% fixed oil, 1,44% crude fiber [24]. In the leaf known major compounds were identified as 3-*O*-methyl quercetin, 3,7-*O*-dimethyl quercetin, genkwanin, 3,7-*O*-dimethyl kaempferol, 3,4-*O*-dimethyl quercetin, apigenin, 3,4-*O*-dimethyl kaempferol, ellagic acid, β -sitosterol-3-*O*- β -glucoside, quercetin 3-*O*- β -rhamnoside, 5-*O*-*p*-coumaroyl quinic acid methyl ester, 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3- β -l-rhamnopyranosyloxy)-2-methoxyphenoxy]-1,3-propanediol and 2,3-dihydro-2-(4- β -l-rhamnopyranosyloxy-3- β -methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol [25].

In vitro cytotoxic activity of essential oil *Cistus ladanifer* was studied on human chronic myelogenous erythroleukemia (K562), human breast adenocarcinoma (MDA-MB-231 and MCF7), and human neuroblastoma cell lines (SH-SY5Y) and IC₅₀ results were 49.6, 90.0, 128.1 and 92.8 ppm respectively [9]. 0.5 mg/ml *Cistus incanus* L., *Cistus monspeliensis* L. extract was also induced a significant antiproliferative effect on normal human prostate cells (PZ-HPV-7) [26]. In another study, *C. villosus* and *C. libanotis* hexane extracts revealed no specific anticancer activity on murine monocyte/macrophages (J774.A1), human breast cancer cells (MCF7) and human melanoma cells (A-375). High antiproliferative activity of *C. monspeliensis* extract against A-375 cell line with IC₅₀ results 82.44 mg/ml at 24h and 52.44 mg/ml at 72h were determined [15]. To the best of our knowledge, there are no researches determined IC₅₀ results of *Cistus laurifolius*, therefore it is hard to compare our study findings with previously published research.

Previous studies reported that, rutin and gallic acid were determined as the main phenolic compounds of *Cistus laurifolius* [27]. Apigenin, quercetin and kaempferol are being increased in soils growing *C. ladanifer*. Also, in the extract of *C. libanotis*, quercetin 3,7,3',4'-tetramethyl ether (retusin) was detected as main compound. The major polyphenolic constituents of *Cistus incanus* extract were determined as myricetin glycosides (%61.8) and quercetin glycosides (%9.28). Revealed through previous studies that quercetin triggers many cellular actions such as p53 activation, induction of caspase-mediated apoptosis and cell cycle arrest [15, 24]. In other studies done the fact that aqueous extract of *Cistus laurifolius* has an acute antidiabetic effect and may be useful in controlling hyperglycemia

[28, 29, 30]. It could be considered to say that *Cistus laurifolius* shows protective effects by preventing oxidative stress, apoptosis, and inflammation related to diabetes-induced complications, thanks to its rich bioactive content [27].

In this study we evaluated the antiproliferative effect of lyophilised aqueous extracts from *Cistus laurifolius*. Since few documented results are available about different species of *Cistus* genus, our studies have been initiated to elucidate this question. Results of these research deserve detailed investigation of chemical composition of extract and determination of pathway or action mechanisms of antiproliferation.

Acknowledgements

This paper was presented at 4th National Botanical Congress. 06-09 July 2017 Afyon-Turkey.

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