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Cytotoxic activity of *Achillea arabica* Kotschy against renal cancer cell lines

Güneş Güniz GÜNGÖR[1], Fatma TOSUN[2*]

1 Istanbul Medipol University, Graduate School of Health Sciences, Department of Pharmacognosy, Istanbul, Türkiye
2 Istanbul Medipol University, School of Pharmacy, Department of Pharmacognosy, Istanbul, Türkiye

ABSTRACT

Achillea arabica Kotschy, belonging to the Asteraceae family, is a plant whose biological activities, such as wound healing, antimicrobial, anticancer, and antioxidant properties, have been evidenced by studies. It has been investigated for its cytotoxic activity in various cell lines, including AGS, MCF7, SW742, SKLC6, A375, PLC/PRF/5, HT29, and HepG2. However, no study has been conducted on any cell line related to kidney cancer. In this study, the cytotoxic activities of the extracts prepared from the root and aerial parts of the plant with dichloromethane, ethyl acetate, and methanol were investigated against A498 and UO31 kidney cancer cell lines. The highest activity was observed in the dichloromethane extract of the aerial parts of the plant on both cancer cell lines. The dichloromethane extract of the aerial parts showed 63% inhibition on the A498 cell line and 56% inhibition on the UO31 cell line at a concentration of 25 μg/mL. The results we have obtained have been of a quality that will lead to new research in this context.

Keywords: cytotoxic activity, *Achillea arabica*, renal cancer

INTRODUCTION

Cancer is one of the leading causes of death worldwide[1]. It is also a disease with typical properties such as abnormal cell growth, invasion, metastasis and mu-

* Corresponding author: Fatma TOSUN
E-mail: ftosun@medipol.edu.tr
ORCIDs:
Güneş Güniz GÜNGÖR: 0009-0002-0911-0946
Fatma TOSUN: 0000-0003-2533-5141
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tations[2]. According to the studies, 2,001.140 new cancer cases and 611,720 cancer deaths are expected to occur in 2024. The fact that most of the drugs in cancer treatment often have side effects such as ischemic heart disease, vomiting, bone marrow suppression, myelosuppression, liver dysfunction, fatigue and hypertension, which further hinder the treatment process, has led researchers to search for different treatments[3]. Therefore, the discovery of new treatment strategies or chemotherapeutics with minimal or no side effects that are easily accessible, cost-effective, and more effective for treating this deadly disease has become one of the major goals in cancer therapy[4]. In addition, chemotherapy treatment significantly reduces the patient's quality of life as it damages normal cells. Therefore, it is very important to search for new anti-tumor agents that are specific to tumor cells[5].

Throughout the ages, it is known that people have utilized nature to meet their basic needs. This has also been the case for the use of natural products as medicines in the treatment of various diseases[6]. Plant extracts and plant-derived natural compounds such as glycosides, alkaloids, tannins, terpenes, coumarins and flavonoids are known to inhibit the growth of cancer cells such as kidney, lung, breast and colorectal cancer cells[7,8]. Kidney cancer (Renal Cell Carcinoma-RCC) is the second most common type of cancer of the urinary system[9]. Currently, RCC constitutes approximately 2-3% of all adult malignancies worldwide and ranks as the 12th most common malignancy and the third most common urogenital cancer[10]. RCC has a high metastatic potential to the lung, liver, bone, head and neck[11]. Approximately 30% of RCC cases are diagnosed at an advanced or metastatic stage, and according to the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) criteria, nearly 80% of these patients belong to the intermediate or low risk group[12]. Due to limited treatment options, less than 40% of patients survive for ≥5 years after diagnosis. Additionally, the lack of routine adjuvant therapy in the clinic is a key reason for the recurrence of kidney cancer, as kidney cancer is resistant to both chemotherapy and radiotherapy. Therefore, there is a need to search for new compounds and develop targeted therapies for kidney cancer[13].

The *Achillea* genus includes 138 accepted species distributed in Europe, Asia, North Africa, North and Central America, Alaska, and Greenland[14]. The genus *Achillea* is represented by 50 species in Turkey[15]. *Achillea* species were reported to contain polyphenols, flavonoids, phenolic and quinic acid derivatives, sesquiterpenes lactones and essential oils; and to have anticancer, antioxidant, antimicrobial, anti-inflammatory, analgesic, antipyretic, antidiabetic, antihelmintic and antihypertensive activities[16-24].

Previous studies have shown that the extracts of *Achillea arabica* have anticancer, antimicrobial, antioxidant, anti-inflammatory, anticholinesterase, analgesic, anxiolytic and wound-healing effects[17,23-30] and contain phenolic compounds, flavonoids, sesquiterpenes lactones and essential oils[17,29-38].

When cytotoxic activity studies conducted on different cell lines were compiled to determine the anticancer efficacy of *A. arabica*, no study was found to be conducted on the A498 and UO31 kidney cancer cell lines.

METHODOLOGY

Plant material

Achillea arabica Kotschy used in this study was collected from Adana-Saimbeyli region on June 12, 2016. Herbarium specimens of the plant identified by Prof. Mecit Vural are registered in Istanbul University Faculty of Pharmacy Herbarium (ISTE 115056).

Preparation of extracts

The extraction process was conducted using solvents of increasing polarity, as they selectively consume compounds with different chemical structures based on their polarities. Additionally, considering the possibility of degradation with heat, the extraction process was carried out at room temperature. The pulverized plant material was successively macerated with dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) at room temperature. After each extraction with a solvent, the plant material was dried to remove the solvent completely and then subjected to extraction with the next solvent. After consumption, the extracts filtered through filter paper were concentrated using a rotary evaporator at 40°C.

Cytotoxicity assay on cancer cells

In-vitro 2Day XTT cytotoxic activity test[39] was applied to extracts of the roots and aerial parts. The 2Day XTT bioactivity assay is an *in-vitro* colorimetric cytotoxic activity test. XTT bioactivity assays were performed at the NCI MTP Experiment Development and Imaging Department. Kidney cancer cell lines (A498, UO31) were used in XTT cytotoxic activity tests. Sanguinarine chloride hydrate was used as a control in the experiment. The assay was performed as described previously[8].

RESULTS and DISCUSSION

In this study, the *Achillea arabica* plant was investigated for the first time in terms of cytotoxic activity on A498 and UO31 cell lines in kidney cancer. The

extract yields obtained by DCM, EtOAc and MeOH extraction of the aerial parts and roots of the plant are given in Table 1. The yield of the extract is also important for the calculation of the plant material to be used for the subsequent isolation of the active compounds.

As a result of the cytotoxic activity studies of the extracts obtained from the aerial parts and roots of the plant, the % inhibition values they showed on A498 and UO31 kidney cancer cell lines are given in Table 2.

Table 1. % yield obtained by extraction of the aerial parts and roots of *A. arabica*

	yield (a/a) %	
Extracts	**Aerial parts**	**Roots**
1	1.32%	1.18%
2	0.69%	0.68%
3	3.64%	3.36%

1: DCM extract; 2: EtOAc extract; 3: MeOH extract

Table 2. Cytotoxic activities of the extracts (25 ug/mL concentration)

	inhibition %					
	A498			UO31		
Plant parts	1	2	3	1	2	3
Roots	40	45	54	43	53	53
Aerial parts	63	41	46	56	43	54

1: DCM extract; 2: EtOAc extract; 3: MeOH extract

Cytotoxic activity was determined on the kidney cancer cell lines (A498 and UO31) by *in-vitro* 2Day XTT cytotoxic activity assay. On the A498 cancer cell line, the root MeOH extract, and the aerial parts DCM extract exhibited over 50% inhibition at a concentration of 25 µg/mL, while on the UO31 cell line, the root EtOAc and MeOH extracts, as well as the aerial parts DCM and MeOH extracts, showed over 50% inhibition at a concentration of 25 µg/mL. The highest activity on both cancer cell lines was observed with the DCM extract of the aerial parts. The DCM extract of the aerial parts exhibited 63% inhibition on the A498 cell line and 56% inhibition on the UO31 cell line at a concentration of 25 µg/mL.

In recent years, the therapeutic potential of medicinal plant extracts in preventing and treating cancer has attracted scientists' attention. Approximately 75% of approved cancer drugs have been developed based on agents of natural source. Plant-derived active compounds support treatment at all stages of cancer and are multi-targeted and non-toxic[40].

Previously, *A. arabica* has been tested against HeLa (cervical cancer), AGS (gastric adenocarcinoma), MCF7 (breast cancer), SW742 (colorectal adenocarcinoma) SKLC6 (lung cancer), A375 (skin cancer), PLC/PRF/5 (liver cancer)[24,30]. In another study, *A. arabica* (*A. biebersteinii*) extracts prepared with hexane, chloroform, and methanol were found to be effective in HT-29 (colorectal carcinoma) cell line, increased the activity of the 5-FU compound used in treatment, induced apoptosis by regulating PTEN/Akt/mTOR signaling pathway, and inhibited angiogenesis[41]. Ag-NPs were synthesized using *A. arabica* (*A. biebersteinii*) flower extract has been reported to induce apoptosis on MCF-7 cell line and can be considered a potential chemotherapeutic agent in treating breast cancer[42]. This study represents the first investigation on the cytotoxic activity of *A. arabica* on A498 and UO31 cell lines.

Previous studies reported that the extracts of the *Achillea* species exhibited cytotoxic activities against different cancer cell lines[17-22,43,44], but there are no reports in the literature dealing with the cytotoxic activities of the *Achillea* species on the renal cancer cell lines. Therefore, our study is important in terms of being the first cytotoxic activity study on the kidney cancer cell lines not only on the *A. arabica* species but also on the *Achillea* genus and contributed to the literature on this regard.

In conclusion, bioactivity-guided fractionation of the DCM extract of the aerial parts, exhibited the highest activity on both cancer cell lines, is planned to isolate and identify the compounds responsible for the activity.

STATEMENT OF ETHICS

There is no ethical statement provided.

CONFLICT OF INTEREST STATEMENT

The authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

These authors contributed to the work equally.

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