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Research Article

The Effects of Garlic Extracts on Prostate Cancer

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Abstract

In the current study, garlic samples obtained from various provinces of Turkey, including Kastamonu, Balikesir, Edirne, and Aksaray, were analyzed for their chemical composition and biological activity. The organosulfur content of the prepared extracts was assessed using GC-MS. A total of seven compounds were identified across all samples, with 3-Vinyl-4H-1,2-dithiin and 2-Vinyl-4H-1,3-dithiin recognised as the predominant compounds. Additionally, the effects of these extracts at concentration of 25, 50, 75, 100, 150 and 200 µg/ml on benign prostatic hyperplasia (BPH), as well as on the LNCaP, DU145, and PC3 cell lines, were evaluated using MTT assays. Cytotoxic activity was detected in all samples examined. The most pronounced activity was recorded against the LNCaP cell lines, exhibiting varying IC_{50} values ranging from 23.91 to 48.84 µg/ml.

Keywords: Garlic, Organosulfur compounds, Prostate cancer

Sarımsak Ekstrelerinin Prostat Kanseri Üzerindeki Etkileri

Öz

Mevcut çalışmada, Kastamonu, Balıkesir, Edirne ve Aksaray olmak üzere Türkiye'nin çeşitli illerinden temin edilen sarımsak örnekleri kimyasal bileşimleri ve biyolojik aktiviteleri açısından analiz edildi. Hazırlanan ekstraktların organosülfür içeriği GC-MS kullanılarak değerlendirildi. Tüm örneklerde toplam yedi bileşik tanımlandı ve 3-Vinil-4H-1,2-ditiin ve 2-Vinil-4H-1,3-ditiin baskın bileşikler olarak tanındı. Ek olarak, bu ekstraktların iyi huylu prostat hiperplazisi (BPH) üzerindeki etkileri ve LNCaP, DU145 ve PC3 hücre hatları üzerindeki etkileri MTT analizleri kullanılarak değerlendirildi. İncelenen tüm örneklerde sitotoksik aktivite tespit edildi. En belirgin aktivite, 23,91 ila 48,84 µg/ml arasında değişen IC_{50} değerleri gösteren LNCaP hücre hatlarına karşı kaydedildi.

Anahtar Kelimeler: Sarımsak, Organosülfür bileşikleri, Prostat kanseri

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1. Introduction

The genus *Allium* L. comprises approximately 900 species and is recognized as a taxonomically complex and highly variable group, primarily distributed throughout the northern hemisphere. Historically, *Allium* was classified within the Liliaceae family; however, recent molecular studies conducted by the Angiosperm Phylogeny Group (APG) have led to a reevaluation of its taxonomic standing. Consequently, *Allium* has been reclassified into the Amaryllidaceae family, within the subfamily Allioideae and the tribe Allieae [1]. *Allium sativum*, commonly known as garlic, is the most widely cultivated and consumed vegetable in this genus. Apart from its culinary use, garlic exhibits a wide range of biological activities. This species significantly influences the cardiovascular system through its hypotensive, hypolipidemic, antiplatelet, and hypocholesterolemic effects. Additionally, it demonstrates anticarcinogenic properties by regulating cell proliferation, apoptosis, and immune responses [2].

The biological effects of garlic are primarily attributed to various chemical compounds present within the bulb, with a significant portion of research focusing on specific organosulfur constituents. Members of the *Allium* genus typically contain S-Alk(en)yl cysteine sulfoxides, which are non-protein sulfur-containing amino acids. These compounds are characterised by the absence of odor and function as precursors to the volatile and distinctive aromatic constituents of *Allium* plants [3].

(+)-S-allyl-L-cysteine sulfoxide (ACSO), commonly referred to as alliin, was the first stable precursor compound associated with garlic to be identified. It serves as the primary organosulfur component responsible for the release of most odorous volatiles when garlic is crushed or cut [4]. In addition to alliin, three other principal sulfoxide compounds have been identified in *Allium* species: (+)-S-methyl-L-cysteine sulfoxide (methiin, or MCSO), (+)-S-propyl-L-cysteine sulfoxide (propiin, or PCSO), and (+)-S-trans-1-propenyl-L-cysteine sulfoxide (isoalliin, or TPCSO) [5]. Allinase, a lyase enzyme, is released upon crushing, chopping, or grinding garlic. This enzyme catalyzes the breakdown of alliin, yielding pyruvate, ammonia, and sulfenic acids. Sulfenic acid, an unstable compound, undergoes self-condensation, ultimately forming allicin. Allicin, in turn, is highly reactive and decomposes into dithiins, polysulfides, and ajoenes through a process known as allylization [6].

Prostate cancer is one of the most prevalent cancers worlwide and ranks as the most frequent cancer among men in the United States, being the second leading cause of cancer-related fatalities in the country. The beneficial effects of garlic and its organosulfur compounds on prostate cancer have been substantiated through epidemiological studies, clinical trials, and experimental research [7]. In the current study, garlic samples were obtained from various regions, including Kastamonu, Balıkesir, Edirne, and Aksaray. The extracts derived from these samples were analyzed for their content of allicin-derived compounds. Furthermore, the effects of these extracts on benign prostatic hyperplasia (BPH), as well as on the LNCaP, DU145 and PC3 cell lines, were assessed.

2. Material and Methods

2.1. Plant Material

Garlic samples were provided from local producers (Kastamonu, Balıkesir, Edirne ve Aksaray) in June 2023.

2.2. Chemicals and Reagents

Hydrocarbon mixtures (C_7-C_{30} n-alkanes), diethyl ether, MTT (M5665) were purchased from Sigma Aldrich. RPMI 1640 (21875-034) and DMEM/F-12 (31330-038) were provided from Thermo. DMSO was purchased from VWR. were purchased from Sigma Aldrich.

2.3. Sample Preparation

Garlic samples (10 g) were crushed and left for thirty minutes to allow for the formation of organosulfur compounds through the reaction catalyzed by allinase. The samples were then transferred into falcon tube, and 30 ml of ether was added. The extraction process was conducted three times with constant stirring using a rotator. Subsequently, the samples were centrifuged for 30 minutes to isolate the supernatants. The liquid portion was then evaporated using a rotary evaporator under reduced pressure, and the resulting extracts were preserved at 4°C until further analysis.

2.4. GC-MS Analysis

The evaluation of the samples was conducted using GC-MS (Agilent 5977A Series) that had an HP-5MS Ultra Inert (Agilent) capillary column (60 m x 0.250 m x 0.25 μm). Information about the experimental procedure was provided in the previous study [8].

2.5. Cell Culture

BPH (Primary Prostate Epithelial Cell line), DU145 (Prostate Epithelial Cells cell line), PC3 (prostatic adenocarcinoma cell line) and LNCaP (human prostate adenocarcinoma cell line) were sourced from ATCC and stored in liquid nitrogen in our stock. For cytotoxicity assays, the cells were cultured in 100 mm dishes and passaged until reaching 70% confluence. The cultures were maintained in a humidified incubator at 37°C with 5% carbon dioxide.

2.6. Cytotoxicity assays (MTT)

To determine the IC_{50} values of extracts prepared in DMSO at concentrations of 25, 50, 75, 100, 150 and 200 μg/ml, cells were initially plated in 96-well culture plates, and cell viability assays were conducted after a 24-hour incubation period. Following a 48-hour exposure to the extracts, an MTT assay was carried out. A 0.5 mg/mL MTT reagent was added to the wells of the 96-well plate and incubated for 4 hours. After incubation, the medium was discarded, and 200 μL of DMSO was added to dissolve the formazan crystals. Absorbance was measured using a microplate reader at 570-690 nm, with blank wells (no cells) serving to account for any background absorbance. The IC_{50} values were calculated by comparing extract treated cells

versus control group, which is treated just equal volume of DMSO in which extract is applied, through regression analysis using GraphPad Prism version 10.16.

Results and Discussion

Ether extracts of garlic samples obtained from different localities in Türkiye were prepared and these extracts were evaluated in terms of chemical profile and biological activity. The following codes were assigned to extracts: GK for Kastamonu, GE for Edirne, GA for Aksaray, and GB for Balıkesir. The chemical profiles of the samples were analyzed using GC/MS, and the Total Ion Chromatograms (TIC) are illustrated in Figures 1 through 4.

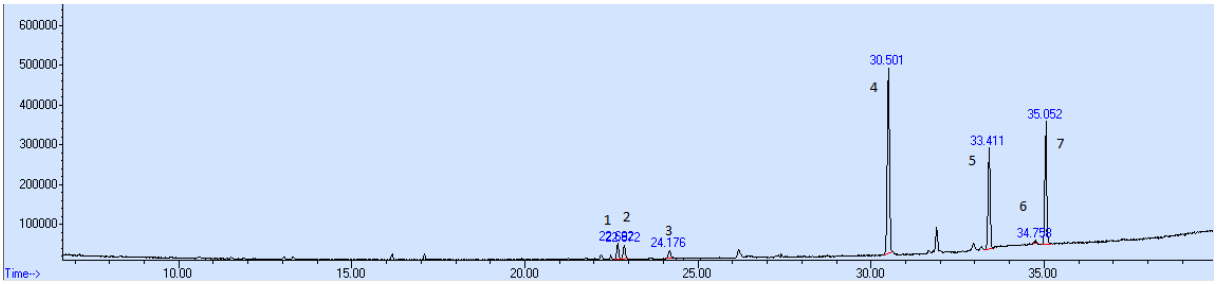

Figure 1. TIC of GK

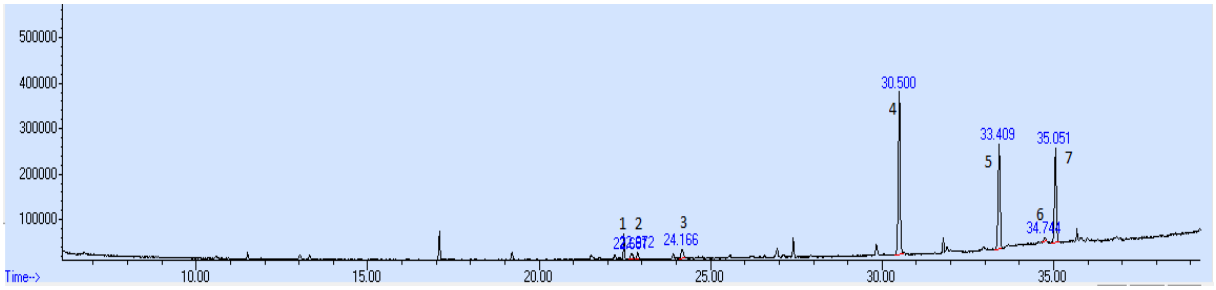

Figure 2. TIC of GE

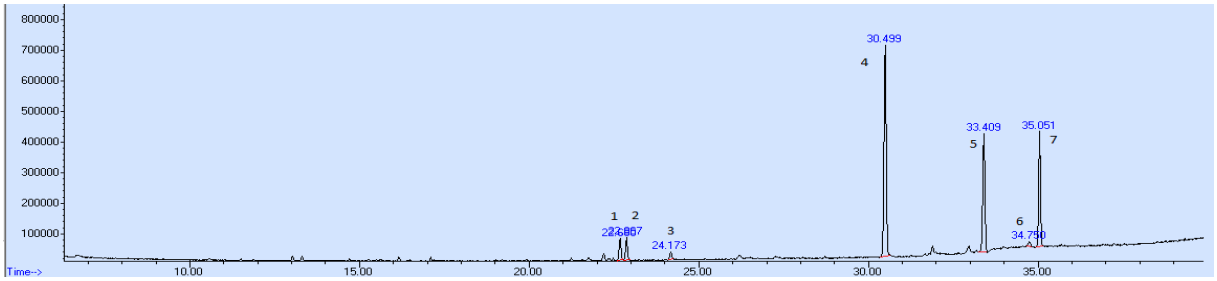

Figure 3. TIC of GA

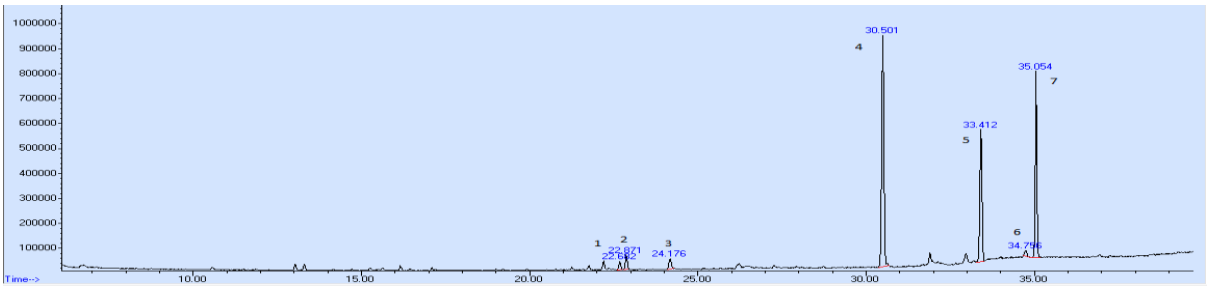

Figure 4. TIC of GB

The identification of individual organosulphur compounds in the samples was performed by comparing their RI values and mass spectra with those documented in the NIST 14 and Wiley libraries. In total, seven organosulfur compounds were identified in the analysed samples (Table 1).

Table 1. Organosulfur Compound Composition of Garlic Extracts from Different Regions of Türkiye.

No	RI[a]	RI[b]	Compound	GK(%)	GE(%)	GA(%)	GB(%)
1	1076	1077	Diallyl disulfide	3.28	1.76	3.93	1.31
2	1097	1097	Allyl(Z)-1-Propenyl disulfide	2.90	1.65	4.34	2.43
3	1141	1143	Allyl methyl trisulfide	2.51	2.42	1.91	1.87
4	1175	1175	3-Vinyl-4H-1,2-dithiin	37.38	37.63	39.82	38.70
5	1217	1217	2-Vinyl-4H-1,3-dithiine	23.53	24.97	24.44	24.43
6	1289	1289	Diallyl trisulfide	1.42	1.99	1.31	1.37
7	1517	1517	Butylhydroxytoluene	20.63	19.59	19.44	22.86
			Total %	91.65	90.01	95.19	92.97

[a]Retention index calculated on HP-5MS column using the homologous series; [b]Literature retention index found in NIST Chemical WebBook.

Among these, 3-Vinyl-4H-1,2-dithiin and 2-Vinyl-4H-1,3-dithiin were identified as the predominant compounds. Previous studies have reported these compounds as dominant in extraction methods, particularly when parameters such as temperature and pH were varied. The specific extraction conditions employed during the process influence the formation of different alliin-derived compounds [9-11]. In contrast, a study conducted on garlic samples collected from various regions of Türkiye, including Kastamonu and Aksaray, employed the Headspace-Gas Chromatography-Mass Spectrometry method to analyze volatile components, revealing diallyl disulphide as the predominant compound present in all samples [12].

The effects of the extracts on benign prostatic hyperplasia (BPH), as well as their cytotoxicity against LNCaP, DU145, and PC3 cell lines, were evaluated. The results are presented in Table 2.

Table 2. The Cytotoxic Activities Against BPH, LNCaP, DU145, and PC3 Cell Lines of Garlic Extracts from Different Regions of Türkiye (IC_{50} μg/mL).

Sample	BPH	LNCaP	DU145	PC3
GK	125.9	34.42	63.05	64.32
GE	77.91	25.28	35.99	37.99
GA	145.5	23.91	72.45	64.06
GB	140.3	48.84	93.70	76.91

The biological activities of extracts with comparable organosulfur content exhibited similar patterns, demonstrating varying degrees of efficacy across all tested cell lines. The most

pronounced cytotoxic activity was observed against the LNCaP cell lines in all samples. Moreover, the reduced cytotoxic in BPH cell lines, relative to other tested cell lines, indicates the potential selectivity of these extracts for targeting prostate cancer. The cytotoxic activity observed in all samples may be attributed to the presence of 3-Vinyl-4H-1,2-dithiin and 2-Vinyl-4H-1,3-dithiin, the principal constituents of the extracts. However, to date, no studies have specifically investigated the cytotoxic activity of these compounds against these particular cell lines. In contrast, the cytotoxic properties of minor components, such as diallyl disulfide and diallyl trisulfide, have been well documented in prostate cancer research. These compounds exert their effects through various mechanisms. In a comparative study, PC-3 prostate cancer cells were compared to noncancerous PNT1A cells, which displayed a greater resistance to cell death induced by diallyl trisulfide. The study reported that phosphorylation of p66Shc at serine 36 and the activation of extracellular signal-regulated kinase 1/2 were significantly lower in PNT1A cells compared to PC-3 cells [13]. Also Kim et al., 2007 reported that the treatment with diallyl trisulfide activates a mitochondria mediated apoptosis pathway in LNCaP, LNCaP-C81 and LNCaP-C4-2 cell lines regardless of their androgen responsiveness. In contrast, normal prostate epithelial cell line (PrEC) displayed markedly greater resistance to apoptosis induced by diallyl trisulfide [14]. The other minor compound diallyl disulfide has been shown to inhibit the proliferation of prostate cancer cells by inducing apoptosis [15]. Further research has demonstrated that diallyl disulfide induces cell cycle arrest at the G2/M transition in PC-3 cells by downregulating CDK1 expression. These findings indicate that diallyl disulfide suppresses the proliferation of prostate cancer cells through cell cycle regulation [16].

3. Conclusion

In conclusion, the current study presented the organosulfur contents and cytotoxic activities of garlic samples provided from different localities of Türkiye against BPH, LNCaP, DU145, and PC3 cell lines. The organosulfur content across all samples exhibited similarity, with 3-Vinyl-4H-1,2-dithiin and 2-Vinyl-4H-1,3-dithiin identified as the predominant compounds. This observation suggests that the employed extraction method was effective for these major compounds, regardless of the sample variations. Furthermore, the cytotoxic activities of the samples demonstrated high levels. The observed reduction in cytotoxic activity within the BPH cell lines, when compared to other cell lines, underscores the potential efficacy of the samples in targeting prostate cancer. Therefore, the findings of this study indicate that the effects of 3-Vinyl-4H-1,2-dithiin and 2-Vinyl-4H-1,3-dithiin compounds on prostate cancer require additional investigation using both in vitro and in vivo methods.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Furkan Kuznek: Preparation of garlic extracts, Recep İlhan-Gökçe Yıldırım Buharalıoğlu: Cytotoxic activity experiments (equal), Ahmet Emir: Analysing the samples, design and coordinating the study, writing the manuscript.

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