Antimicrobial and Antimutagenic Properties of *Physalis alkekengi* L.

Selcuk Ceker[1]*, Hatıce Ogutcu[2], Guleray Agar[3] and Meryem S. Koseoglu[4]

1 Department of Medical Biology, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Türkiye. selcuk.ceker@ibu.edu.tr

2 Department of Field Corps, Faculty of Agriculture, Kırsehir Ahi Evran University, Kırsehir, Türkiye. hogutcu@ahievran.edu.tr

3 Department of Biology, Faculty of Science, Atatürk University, Erzurum, Türkiye. gagar@atauni.edu.tr

4 Department of Biology, Faculty of Science, Atatürk University, Erzurum, Türkiye. sengulm@atauni.edu.tr

Abstract

The application of herbal medicines is increasing day by day. Some bioactive compounds of medicinal plant have various therapeutic effects. In this study, the antimicrobial and antimutagenic of the *Physalis alkekengi*, used for various therapeutic effects were investigated. The methanol and water extracts of *P. alkekengi* L. were analyzed antimutagenic and antimicrobial activity. In the findings obtained, we determined that *P. alkekengi* L. has antimutagenic activity, especially at a concentration of 75 µL. In addition, antimicrobial activity of methanol and water extracts from of *P. alkekengi* fruits was examined against human pathogenic Gram positive and Gram-negative bacteria and yeasts. Methanol and water extracts of *P. alkekengi* L. showed varying degrees of inhibitor effects (15 mm40 mm, 20mm-30 mm respectively) on the growth of the diverse pathogenic strains tested. Methanol extract of *P. alkekengi* L. showed higher activity than commercial antibiotics in *Staphylococcus epidermis, Bacillus cereus, Proteus vulgaris and Pseudomonas aeruginosa* (30 mm, 40 mm, 25 mm, 30 mm, respectively). These findings indicated that the fruit extract of *P. alkekengi* L. has potential application as an ingredient in pharmaceuticals.

Keywords: *Physalis alkekengi* L., Antimutagenic, SCE, Antimicrobial.

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**Corresponding author:* Selcuk CEKER,
Department of Medical Biology, Bolu Abant Izzet Baysal University, 14300, Bolu, Türkiye.
E-mail: selcuk.ceker@ibu.edu.tr
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1. Introduction

Physalis alkekengi-L is a member of the genus Physalis in the Solanaceae family. It naturally spreads in Europe and Asia. It can grow from 30 to 60 cm. Stem simple or branched and briefly hairy at the top. The spiral-shaped leaves are 6 to 12 cm long and 4 to 9 cm wide. The flowers are white, with a five-lobed crown. The swollen calyx is orange red in color and surrounds the fruit. The flowers are greenish-white and heretical (Ge, Duan, Fang, Zhang, & Wang, 2009; Naseri, Mohammadian, & Pharm, 2008). Common names are Güveyfeneri, Gelinfeneri, Bride Grass, Kembel Grass, Kambil Grass, Kandil Grass, Red Slacking, Curd Grass, Wild Pepper, Patlanaç, Kızılyörük. Ripe fruits can be consumed raw or cooked. It contains twice the vitamin C of lemon (Namjoyan, Jahangiri, Azemi, Arkian, & Mousavi, 2015).

It is stated in the literature that *Physalis* genus are a rich source of phytochemicals required for the production and development of drugs (Jalali, Nejad, Ebadi, & Laey, 2009). It is anti-inflammatory, antipyretic, cough suppressant and expectorant. It has been used in the treatment of urinary and skin disease (Namjoyan et al., 2015). It relieves earache. It is used in cases of asthma and shortness of breath. It is effective in urinary tract infections. Its seeds are used to reduce intestinal parasites. It is used in cases of painful urination. It is used as a diuretic, antiseptic, liver corrector and sedative (Shekar Forosh, ChangiziAshtiyaniS, & Attari, 2012). *P. alkekengi* L. is a traditional medicinal herb. It is frequently used in the prevention and treatment processes of many diseases, including cough, leishmaniasis, sore throat, tonsillitis, pharyngitis, eczema, tumors, and hepatitis. Lately, some researchers reported that *P. alkekengi* L. have antitumor, antioxidant, antibacterial, anti-inflammatory, and cytotoxic properties (Ankrah et al., 2003; Choi & Hwang, 2003; Jacobo-Herrera et al., 2006; N. B. Pinto et al., 2010; M. B. Soares, Bellintani,

Ribeiro, Tomassini, & dos Santos, 2003; M. B. P. Soares et al., 2006). These biological activities of *P. alkekengi* L. have been associated with a high level of polyphenol compounds such as physalins, neophysalins, alkaloids, polysaccharides, and flavonoids. Nevertheless, there is no information about antimutagenic properties of *P. alkekengi* species. However very few papers are available regarding antibacterial and antifungal properties of *P. alkekengi*. That's why, in this study we aimed assayed for antibacterial, antifungal and antimutagenic activities the methanol and water extracts from *P. alkekengi* aerial parts.

2. Materials and Methods

2.1. Preparation of the extract

Aerial parts (fruits) of *P. alkekengi* L. plants were collected from Tortum / Erzurum in August 2020. A Soxhlet extractor was used for this process. The aerial parts of newly collected *P. alkekengi* L. (100 g) were treated with water and methanol (1 l) for 72 hours at a suitable temperature not exceeding the boiling point of the solvent. The resulting extracts were vacuum concentrated with a rotary evaporator (60°C) after filtering through Whatman filter paper (no. 1). The extracts were then lyophilized and stored in the dark (+4°C) until use.

2.2. Antimicrobial Activity

The water and methanolic aerial parts extracts (fruits) of *P. alkekengi* L. (PWE and PME) were investigated for its antimicrobial activities against pathogenic Gram positive bacteria (*Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228, *Bacillus cereus* RSKK 863) and Gram negative bacteria (*Salmonella typhi* H NCTC901.8394, *Proteus vulgaris* RSKK 96026, *Pseudomonas aeroginosa* ATCC27853) and yeast (*Candida albicans* Y-1200-NIH) by well diffusion method (Koçoğlu, Ogutcu, & Hayvalı, 2019; Nithya, Gnanalakshmi, & Pandian, 2011; Rubab et al., 2021). Pathogenic bacteria and yeast tested for resistance against four antibiotics and one antifungal (Kanamycin, Sulfamethoxazole, Ampicillin, Amoxicillin, Nystatin) (Nartop, Sarı, Altundaş, & Öğütcü, 2012; Xiang et al., 2018).

2.3. Determination of Antimutagenic Activity: Sister Chromatid Exchange Assay

Whole blood samples were taken from four nonsmoking healthy donors between the ages of 22 and 25. Whole blood samples (0.5 ml) were cultured in 6 ml of culture medium with phytohemagglutinin. Aflatoxin B_1 (AFB_1; 5 µM) and extracts of *P. alkekengi* L. (in concentrations of 25; 50; 75; 100; 125 and 150 µl/mL) were added to the cultures just before incubation. After these applications, the sister chromatid exchange (SCE) method, which is detailed in Ceker et al., was applied (Ceker et al., 2018).

2.4. Statistical analysis

In this study, three replicates of all experiment groups were accomplished for the reliability of the data. The data of each experiment groups were analyzed with SPSS 18.0 version using one-way analysis of variance. Significance was determined by Duncan's test. The level of significance was regarded $p<0.05$ for all statistical analysis.

3. Results

3.1. Antimutagenic Activity

We analyzed of SCE after treatment with different concentrations extracts of *P. alkekengi* L. (PME and PWE) (25, 50, 75, 100, 125 and 150 µl/ mL) against AFB_1. According to the results obtained; It has been determined that PME and PWE exhibit antimutagenic properties against the mutagenic effect of AFB_1. In terms of their antimutagenic effect, when a comparison is made between PME and PWE, it has been determined that PME has more antimutagenic properties. Particularly, it was observed that PME was most effective at a concentration of 100 µl/ mL (Table 1) ($p<0.05$).

3.2. Antimicrobial Activity

The antimicrobial activity of PWE and PME was examined against human pathogenic Gram positive and Gram-negative bacteria and yeasts. PME and PWE showed varying degrees of inhibitor effects (15 mm-40 mm, 20mm-30 mm respectively) on the growth of the diverse pathogenic strains tested when a dose of 20 mg/ml was used. Antimicrobial activities of plant extract of were compared with four commercial antibiotics and one anticandidal (Table 2).

Table 1. The antimutagenic effects of *P. alkekengi* L. extracts

	Concentrations (µl/ mL)	**SCE / Cell ± SE**	
		PME	**PWE**
Control	- - -	6.01 ± 0.13[a]	
AFB1	5 µM	7.72 ± 0.16[e]	
P. alkekengi L. extracts	75	6.10 ± 0.19[a]	6.18 ± 0.32[a]
AFB1 + *P. alkekengi* L. extracts	25	6.92 ± 0.24[c]	7.10 ± 0.45[d]
	50	6.75 ± 0.15[c]	6.98 ± 0.36[cd]
	75	6.54 ± 0.27[b]	6.93 ± 0.62[c]
	100	6.28 ± 0.33[a]	6.84 ± 0.48[c]
	125	6.63± 0.21[b]	6.80 ± 0.70[c]
	150	6.69 ± 0.29[bc]	6.92 ± 0.62[c]

* AFB1 (5 µM) was used as positive controls for human lymphocytes.
* [a-e] Means ± SE; values not sharing a common superscript are significantly different ($p<0.05$) as determined by Duncan test.

Table 2. The antimicrobial activities of *P. alkekengi* L. extracts

		PWE	**PME**	**AMP 10***	**SXT 25**	**AMC 30**	**K 30**	**NYS 100**
Microorganisms								
Gr (+)	*S. epidermis*	20	30	26	25	27	25	N
	S. aureus	20	30	30	24	30	25	N
	B. cereus	30	40	23	25	20	28	N
Gr (-)	*P. aeruginosa*	-	30	8	18	15	14	N
	S. typhi H	-	15	11	17	19	20	N
	P. vulgaris	-	25	17	19	20	21	N
Yeast	*C.albicans*	-	25	N	N	N	N	20

*Standard reagents (diameter of zone inhibition (mm). SXT25, sulfamethoxazole 25 µg; AMP10, Ampicillin 10 µg; NYS100, Nystatin 100 µg; K30, Kanamycin 30 µg; AMC30, Amoxycillin 30 µg; N: not tried.

The antimicrobial activity results of PWE and PME are given in Table 2. Accordingly, PWE showed higher inhibitory activity only in B. cereus (30mm) than all antibiotics. However, it did not show activity on other pathogenic microorganisms.

P. aeruginosa is a leading cause of nosocomial infections, can increase resistance to various antibiotics and cause high mortality and morbidity due to infections (Hanberger et al., 1999; Nadaroglu, Alayli, Ceker, Ogutcu, & Agar, 2020). PME showed higher activity than commercial antibiotics in *S. epidermis*, *B. cereus*, *P. vulgaris* and *P. aeruginosa* (30 mm, 40 mm, 25 mm, 30 mm, respectively) (Table 2). *B. cereus* is known as opportunist pathogen and is associated with food-borne illness (Öğütçü et al., 2017; Sarı et al., 2013). In addition, this PME showed the same inhibition activity as AMC and AMP in *S. aureus* (30 mm), but higher activity than SXT (24 mm) and K30 (25 mm). In addition, PWE did not show inhibitory activity in *C. albicans* (25 mm), while PME showed higher activity than anticandidal (Table 2). Systemic fungal infections (including *C. albicans*) have emerged as important causes of mortality and morbidity in immunocompromised patients (organ or ligament transplantation, cancer chemotherapy, adjuvants) (Altundas, Erdogan, Ögütcü, Kizil, & Agar, 2016; Nartop et al., 2019; Şakıyan, Özdemir, & Öğütcü, 2014).

4. Discussion

PWE and PME was studied against six bacterial and one fungal pathogenic strain. As a result, acquired, it is that accomplish the extracts were much more effective in Gr (+) bacteria than Gr (-) bacteria (Table 2). Helvacı et al., (2010) according to results, the methanol extract of *P. alkekengi* has strongly antibacterial activity against Gram positive bacteria (Helvacı et al., 2010). The possible reason for this may be the presence of the outer impermeable membrane, the presence of the periplasmic space, thin peptidoglycan monolayer and cell wall composition in Gram negative bacteria, so the activity is observed less or narrowly (Afzal, Ullah, Hussain, & Rukh, 2017).

Shu et al., (2016) according to results they determined that 50-EFP not only inhibited the growth (MIC) of *S. aureus* and *P. aeruginosa* strains, but also killed them (MBC) in vitro (Shu et al., 2016). While in other reports ten new sucrose esters, physacingose A–J (1−10), were isolated from the aerial parts of *P. alkekengi* var. franchetii under the guidance of 1H NMR spectroscopy. Ten isolates were evaluated for antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*, and it was determined that they did not show activity against *E. coli* but showed varying degrees of antibacterial activity against the other three bacteria (Zhang et al., 2016). In our study, PME was more effective in *P. aeruginosa* (30 mm) and *S. aureus* (30 mm) than the inhibition effect determined by other researchers (Table 2). Other reported significant resistance to Gr (+) bacteria, especially *B. cereus*, *B. subtilis*, and *E. faecalis*, ranging from 28.2 ± 1.8 to 31.8 ± 1.9 at 500 µg/mL/disc. showed a zone of inhibition. This extract inhibits the growth of Gr (-) bacteria, particularly *P. aeruginosa*, *K. pneumoniae* and *E. coli*, with zones of inhibition ranging from 14.6 ± 1.1 to 16.6 ± 3.2 at 500 µg/mL/disc. inhibited. Again, the extract exhibited a moderate effect on the fungi tested, producing a zone of inhibition diameter of 9.6 ± 2.2 to 14.2 ± 0.8 mm, depending on the susceptibility of the fungi (Helvacı et al., 2010). In our study, PME showed a very high inhibition activity both in Gr (+) B. cereus (40 mm), Gr (-) *P. aeruginosa* (30 mm) and fungi C. albicans (25 mm) (Table 2).

Also, this study is the first to elucidate the antimutagenic activity of *P. alkekengi* L. In previous studies showed that *P. alkekengi* extract has various pharmacological characteristics including antimicrobial and anti-oxidative activities (Helvacı et al., 2010), anticancer (Zhu et al., 2016), anti-inflammatory (Hong et al., 2015). Previous phytochemical studies of *P. alkekengi* L. var franchetii identified steroids (Sun, Jiang, & Cheng, 2021), flavonoids (Peng et al., 2015), phenylpropanoids (Chen, Xia, Liu, Xie, & Qiu, 2014), glycosides (Shu et al., 2021). Among them physalins are main components from extract of the *alkekengi* var. franchetii Physalin compounds are considered of great medicinal value since these biological activities, such as antimicrobial, antitumor, antioxidant anti-inflammatory, immunomodulatory, immunosuppressive, cytotoxic, trypanocidal, and molluscicidal effects (Januário et al., 2002; Khan, Bakht, & Shafi, 2016; Li et al., 2012; L. A. Pinto et al., 2016; M. B. Soares et al., 2003; Wang et al., 2021).

In our results showed that *P. alkekengi* extracts exhibited antimutagenic activity. This antimutagenic activity; It is thought to be due to the antioxidant properties of the substance. Recently, several studies showed that the fruit extract of *P. alkekengi* are -a high antioxidant capacity and it is used to treat various diseases (Helvacı et al., 2010; Liu et al., 2019). *Physalis* extracts is associated with a high level of polyphenol compounds (like ascorbic acid) and flavonoids (Medina-Medrano et al., 2015).

Considering the properties of *P. alkekengi* in the literature and the results of our study into account, use of the fruit of *P. alkekengi* and extract of *P. alkekengi* for medical purposes might be useful as they have potential anti genotoxic properties. However more

studies are needed; especially, their doses and analysis of compounds.

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