

Antimutagenic Activity of Essential Oil and Crude Extract of *Phlomis fruticosa*

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Abstract

The present study was carried out to investigate the antimutagenic activity of essential oil and crude extract of *Phlomis fruticosa*. This species belongs to the family Lamiaceae which includes a number of species with medical and pharmaceutical properties. The *Escherichia coli* K12 reversion assay for identifying antimutagens was used. The number of spontaneous and UV-induced Arg⁺ revertants, as well as cell survival was monitored in the presence of non-toxic concentrations of the essential oil. The number of UV-induced revertants was slightly increased in the presence of essential oil. The ethanol extract demonstrated a significant effect on viable cells. Study on pharmacological and phytochemical activities as well as antimutagenic potential against some chemical mutagens of the essential oils, total extract and their different fractions and constituents of *Ph. fruticosa* is in progress.

Keywords: Antimutagenicity, *E. coli* K12 test, essential oil, ethanol extract, Lamiaceae, *Phlomis fruticosa*

Introduction

The study of plant metabolites to obtain medication for the cure of different diseases has led to the isolation of several types of chemical products with therapeutic properties. Many of plants have as constituents chemical substances that react with genetic material and whose mutagenic and/or carcinogenic activity is affected by individual differences in the dose-response relation, metabolic degradation and cell damage repair (Mortin, 1980). Many plant substances may also have cell-protecting properties that modulate genotoxicity (Kada et al., 1985). As part of a broad screening study

of phytochemical analysis of plants belonging to the family Lamiaceae, we are currently investigating the antimutagenicity of *Ph. fruticosa* L., an eastern Mediterranean native shrub (DeFilips, 1972). In the present study we investigated the antimutagenic activity of essential oil and crude extract of *Ph. fruticosa*. Several *Phlomis* species are used in herbal medicine, for diseases of the respiratory tract or externally for treatment of wounds (Bucar et al., 1997). Traditionally, infusion of the leaves was used as a tonic drink (Tammaro & Xepapadakis, 1986). In Italy the leaves are used as a poultice on wounds (Tammaro, 1984). Analysis of a methanol extract of medicinal plant *Phlomis armeniaca* showed cytotoxic and cytostatic activities (Saracoglu et al., 1995). The essential oil and ethanol extract of *Ph. fruticosa* possessed antimicrobial activity (Ristić et al., 2000). *Ph. fruticosa* L. has not been investigated in this manner previously.

Materials and methods

Plant material

The aerial parts of *Phlomis fruticosa* L. were collected near Bar, Monte Negro, Yugoslavia, in October 1997. A voucher specimen (No. 407197) has been deposited in the Herbarium of the Institute of Botany and Botanical Garden, Faculty of Biology, Belgrade University.

Isolation and identification of essential oil

The leaves were dried, powdered and stored until further use. The oil was isolated after 2 h by the Clevenger method

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(European Pharmacopeia, 1975). Yield of essential oil was 0.06%. Qualitative data were determined by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Analytical GC – A Varian model 3400 chromatograph equipped with a split/splitless injector (250 °C) and a 30 m × 0.32 mm DB – Wax fused silica capillary column and FID (300 °C), was used for GC and GC/MS measurements. The column was temperature programmed as follows: 50 °C (3 min) and then 50–200 °C/min carrier gas 3 ml H₂/min. Peak areas were calculated electronically by a Varian DS –604 data station. GC/MS – The gas chromatography was connected via an open split interface and a fused silica capillary (at 250 °C) to the ion source of a Finnigan MAT 8230 mass spectrometer, equipped with a PD. 11/74 computer. Working conditions: carrier gas 2 ml He/min; other GC conditions as above. MS: ion source (electron impact) 170 °C, 70 eV.

Preparation of leaf extract

Powdered leaves (30 g) were exposed for 24 h to 70% ethanol at room temperature. After that period, the mixture was filtered and the filtrate was dried under vacuum. A stock solution was prepared containing 1 mg/ml dried evaporated extract in ethanol.

Bacterial strain

Escherichia coli K12, strain SY252, relevant marker *argE3*, was used in this work (Knezević & Simić, 1982). The strain was chosen from the laboratory collection (Laboratory of Microbiology, Faculty of Biology) for the detection of induced mutagenesis.

Media and growth conditions

The bacteria were cultured overnight in LB medium at 37 °C. The semi-enriched minimal agar medium (SEM) for survival and mutation assays contained 1 g (NH₄)₂SO₄, 10 g KH₂PO₄, 0.1 g MgSO₄ · 7 H₂O, 0.5 g trisodium citrate · 2 H₂O per liter adjusted to pH 7.0 with 1 M NaOH supplemented with 0.4% D-glucose, 1.5% Difco bacto agar and 3% (v/v) NB solution.

Antimutagenicity test

The *Escherichia coli* K12 reversion assay (test A) for identifying antimutagens was used (Vuković-Gačić & Simić, 1993). The overnight culture of *E. coli* SY252 *argE3* strain was washed by centrifugation and resuspended in 0.01 M MgSO₄ giving a similar titer. UV-irradiation was carried out with a “Camag” germicidal lamp with maximum output of 254 nm. Cells were irradiated at a thickness of less than 1 mm. Samples (0.1 ml) of UV-irradiated cells, appropriately diluted for the determination cellular viability and two-fold diluted for the determination of mutagenesis, were spread on

SEM agar plates with and without bioantimutagen. The number of Arg⁺ revertants and viable cells was determined after incubation at 37 °C for 72 h.

Results and discussion

The results of chemical analysis of essential oils composition of *Ph. fruticosa* are given in Table 1. The compounds identified have been arranged according to their RRt. Yield of essential oil was 0.04%. Thirty compounds were detected out of which 21 were identified by GC and GC/MS methods. Determination of one sesquiterpene alcohol is in progress. The main compounds were α-pinene, 1,8-cineole and caryophyllene.

Table 2 presents the response to the *E. coli* antimutagenicity assay obtained for the *Ph. fruticosa* essential oils using UV-irradiation to avoid possible interaction between chemical mutagens and antimutagens (Simić et al., 1997). The table presents the mutagenic index calculated in relation to the spontaneous induction values for the negative control. At the lowest investigated concentration (0.25 μl/plate) it can be seen that cell viability is 40%, while at the highest concentration (1 μl/plate) viability of cells is higher (65%). Mutagenic index was highest at the concentration of 1 μl/plate. All the results revealed an absence of antimutagenicity, but presented a tendency towards low mutagenic activity.

Table 3 presents the antimutagenic effect of the ethanol

Table 1. The composition of the essential oils of *Phlomis fruticosa*.

Components	%	RRt (min.)
α-pinene	56.6	8.172
α-thujene	2.3	8.104
camphene	t	9.607
β-pinene	1.0	9.820
β-myrcene	0.6	11.874
limonene	2.2	12.664
1,8-cineole	10.4	12.826
2-hexanol	0.4	13.049
β-ocimene	t	13.419
γ-terpinene	t	13.708
β-cymene	t	14.485
α-thujone	1.4	18.509
camphor	0.3	20.007
β-caryophyllene	2.0	22.716
terpinene-4-ol	0.4	22.961
α-humulene	0.2	24.243
α-curcumene	0.2	25.964
2-phenylethanol	0.9	29.033
sesq.terp.alc.	0.4	32.316

t-trace, concentration less than 0.1%.

Table 2. The effects of essential oils of *Ph. fruticosa* on the UV-induced mutagenicity on *E. coli* SY252.

Conc. (μ l/plate)	Viable cells/plate		V %	Arg ⁺ revertants/plate		I %
	-UV*	+UV**		-UV*	+UV**	
0	33	185	55	19	52	
EtOH	48	215	45	22	48	
0.25	43	165	40	16	60	-25
0.5	38	175	46	22	61	-27
0.75	35	210	60	23	58	-20
1	37	240	65	23	47	3

Dose of irradiance was 24 J/m².

Experiments were repeated three times.

The numbers represent the average of duplicate samples from three experiments.

V – Viability of cells.

%I = $(1 - Nt/Nc) \times 100$; Nc = control samples; Nt = sample with essential oil.

* dilution 10⁻⁶.

** dilution 10⁻⁵.

Table 3. Effects of the EtOH extract of *Ph. fruticosa* on the UV-induced mutagenicity on *E. coli* SY252.

Conc. (μ g/plate)	Viable cells/plate		V %	Arg ⁺ revertants/plate		I %
	-UV*	+UV**		-UV*	+UV**	
0	43	120	36	14	63	
EtOH	25	150	60	15	63	
0.25	24	120	50	13	62	2
0.5	24	120	40	16	72	-14
0.75	28	100	35	17	69	-10
1	280	140	5	16	68	-8

Dose of irradiance was 24 J/m².

Experiments were repeated three times.

The numbers represent the average of duplicate samples from three experiments.

V – Viability of cells.

%I = $(1 - Nt/Nc) \times 100$; Nc = control samples; Nt = sample with extract.

* dilution 10⁻⁶.

** dilution 10⁻⁵.

extract of *Ph. fruticosa*. Viability of cells decreased with higher concentrations, at the highest concentration (1 μ g/plate) the viability was only 5%. In Table 3 it can be seen that number of viable cells was very high (280). It can be presumed that the low percentage of cell viability (5%) of this concentration (1 μ g/plate) occurred because of this great number of viable cells per plate. The ethanol extract at a concentration of 1 μ g/plate had a stimulatory effect on viable cells. From these results it can be concluded that the ethanol extract had no antimutagenic activity, but it was observed that the use of high doses might cause an increase of cell viability. This interesting result requires further investigation of antimutagenicity and different mechanisms of actions of the ethanol extract of *Ph. fruticosa* as a potential antimutagen.

Further studies on pharmacological and toxicological activities of the extract, fractions and constituents of *P. fruticosa* are in progress.

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