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Antimitotic and antibacterial effects of the *Primula veris* L. flower extracts

Gamze Başbülbül, Ali Özmen*, H. Halil Biyik and Özge Şen

Adnan Menderes Üniversitesi, Fen-Edebiyat Fakültesi Biyoloji Bölümü Aydýn, Turkey.

Abstract — *Primula* is a plant genus which comprises about 400 species. It has been found in a number of pharmacological studies that primrose extracts are rich in saponins. Phenolic glycosides and saponins are characteristic compounds for the genus *Primula*. In this study several flower extracts from *Primula veris* L. has been tested for antibacterial activity and decoction from the flowers has been tested for antimitotic activity. Antibacterial activity was determined by the well diffusion method and *Allium cepa* L. has been used for evaluating cytotoxicity. Decoction of flowers was toxic on root number and root length in *A. cepa* L. and reduced the mitotic index significantly. All of the tested *P. veris* L. extracts showed inhibitory effect against both Gram positive and Gram negative microorganisms at varying degrees. The most effective fraction was found to be the ethanolic.

Key words: *Allium cepa* L., antibacterial, antimitotic, cytotoxicity, *Primula veris* L..

INTRODUCTION

Primula is a plant genus included about 400 species. Some of them are popular garden plants because of their colourful blossoms. Efficacy of primrose extracts which are rich in saponins have been demonstrated in a number of pharmacological studies, which has potent anti-asthmatic, anti-inflammatory and anti-viral properties. Phenolic glycosides and saponins are characteristic compounds for the genus *Primula* (Müller *et al.* 2005). Flavonoids may have existed in nature for over one billion years. Methoxyflavones have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors, precursors of toxic substances and have long been recognized to possess anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism (Huck *et al*. 2000). Ten lipophilic flavones were isolated from *Primula veris* L. in vitro cultures (Budzianowski *et al.* 2005). Two new flavonol glycosides have been identified and isolated from Italian *Primula* species (Fico *et al*. 2007). Otherwise *Primula veris* L. has a potential anxiolytic activity (Sufka *et al*. 2000). *Primula* species can also contains allergens (Paulsen *et al.* 2006) and some species are used traditionally to treat epilepsy and convulsions (Jager *et al*. 2006). Another *Primula* species has flavonoids that possessed strong cytostatic properties against HL 60 cells even at low concentrations (Tokalov *et al.* 2004). The biological effects of the genus *Primula* are evident. The aim of this study is to determine antimitotic and antibacterial effects of several flower extracts from *Primula veris* L..

MATERIAL AND METHOD

Extraction of flowers - Ether extract: 20 g of dried and milled flower were placed in a soxhlet cartridge and extracted with diethylether at 35^{0}C. After extraction Ether was evaporated by a rotary evaporator connected to a vacuum pump.

Ethanol extract: The residue in soxhlet cartridge has been dried and treated with ethanol in a shaker at room temperature. After extraction ethanol was evaporated.

Decoction - 50 g of dried and milled flower has been boiled in 1000 ml distilled water for 1 h. After boiling the extract was filtered and a part of this filtrate has been freeze-dried for preparing the Water extract. After freezing the water was removed by lyophilization.

* Corresponding author: phone: ++90 256 2128498; fax: ++90 256 2135379; e-mail: aozmen@adu.edu.tr

Antimitotic activity - Allium cepa has been used for evaluating cytotoxic properties since the early 1920's (GRANT 1982). This method is an easy and sensitive tool for measuring the total toxicity caused by chemical treatments as expressed by growth inhibition of the roots of onion bulbs. It has been reported that the results from Allium test fit in well in a test battery composed of prokaryotes and /or other eukaryotes (FISKESJÖ 1993). Small onion bulbs are carefully unscaled and cultivated on top of test tubes filled with the decoction of flowers. Water was used as a control. The test tubes were kept in an incubator at 24±2^{0}C and the test samples were changed daily. After 72 h the roots were counted and their lengths were measured for each onion. When the newly emerged roots measured 2.0 – 3.0 cm, they were fixed. The fixative solution was glacial acetic acid/absolute alcohol (1/3 v/v). The root tips were kept in aceto-alcohol solution for 24 h. After fixation, the slides were prepared for examination or the roots were transferred to %70 ethyl alcohols and stored in a refrigerator. For examination, the root tips were put into a watch glass to which 9 drops of aceto-orcein and 1 drop of 1 M HCl were added and warmed over a flame of spirit lamp for 2-3 min. These tips were kept at room temperature for 15-30 min. After removing the root caps from well-stained root tips, 1 mm of the mitotic zones were immersed in a drop of %45 acetic-acid on a clean slide and squashed under a cover glass. In order to spread the cells evenly on the surface of the slide, squashing was accomplished with a bouncing action by striking the cover glass with a match stick. MI was expressed in terms of divided cells/total cells. A statistical analysis was performed on the collected data. The means of the control and seed extracts were obtained from descriptive analysis and an Independet-samples test was performed to obtain P values.

Antibacterial activity - Antibacterial activity was determined by the well diffusion method. Muller Hinton agar plates were seeded with 24 h cultures of the bacterial strains. The inoculum was adjusted to 0.5 MacFarland turbidity standards (10^8 cfu/ml). Muller-Hinton Agar plates were inoculated with each of these bacterial suspensions using sterile swabs. The dried plant extracts were dissolved in sterile dimethylsulfoxide (DMSO) to give a final concentration of 100 mg/ml. Wells were cut into the agar and filled with 50 µl of the plant extracts. Sterile DMSO was used as negative control. Inoculated plates were incubated at 37°C for *Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 35218*, Enterococcus faecalis* ATCC 51299*, Proteus* sp., *Listeria* sp. , *Serratia marcescens* and at 30 °C for *Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 11778, *Pseudomonas fluorescens* DSMZ 50090, *B. sphaericus* DSMZ 396.

The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in duplicate and the mean of the diameter of the inhibition zones was calculated.

RESULTS AND DISCUSSION

Antimitotic activity - The root lengths and numbers from control and decoction are given in table 1. *Primula veris* L. flower decoction reduced significantly root number and root length when compared with control.

Table 1 — The average root lengths and numbers in control and in decoction after 72 h.

Extract	Average root numbers (±SD)	Average root lengths (mm) (±SD)
Control	35 (±4)	27.9 (±3.7)
Decoction	27.8 (±3.8)*	7.1 (±1.7)*

*Significant at 0.05 level

These results show that the extract from *Primula veris* L. flowers has inhibitory effects on root growth and length in *Allium cepa.* In conformity with human cell cytotoxicity (TOKALOV *et al.* 2004) it was found that *Primula veris* L. flower decoction has cytotoxic properties also in plant test systems.

Table 2 — The dividing and total cells that counted in microscopic observations and mitotic index (MI) in control and in decoction.

Extract	Total cells	Dividing cells	MI (±SD)
Control	10000	1617	%16 (± 1, 6)
Decoction	10000	516	%5 (± 1, 2)*

*Significant at 0.05 level

In table 2 the mitotic indexes are given for control and for decoction. It is evident that decoction of flowers reduced the mitotic index significantly. In conclusion antimitotic effect of plant is provided by substances which found in flower decoction.

In respect of this results, *Primula veris* L. flowers contains antimitotic constituents that can stop the

mitosis in anywhere of the cell cycle. Furthermore these constituents probably affect the cytoskeleton or tubulin polymerization or degradation.

Antibacterial activity:

Antibacterial activity of three different extracts of *Primula veris* L. has been evaluated *in vitro* against ten bacterial test species, which are known to cause some infections in humans. These results are given in table 3.

Traditionally, *Primula* extracts are prepared with water in folk medicine and especially consumed as *Primula* tea. Because of water extract found as potentially active fraction against many bacteria, results of this study support the traditional use of this herb. Additionally, there must be very active compounds in the other extracts while they show wide inhibitory spectrum.

The results of these antimicrobial screening confirms the potential of *Primula* herb for production of bioactive compounds. These findings are useful tools for rationalizing the use of medicinal plants in folk therapy. However, the phytochemical characterization of extracts and identification of biologically active compounds are necessary.

Table 3 — Diameters of inhibition zones.

Extracts	Zone of inhibition (mm)									
	S.aureus	*E.faecalis*	*B.cereus*	*B.sphaericus*	*M.luteus*	*S.marcescens*	*E.coli*	*Proteus sp.*	*Listeria sp.*	*P.fluorescens*
Ether	–	10	12	11	–	–	8	–	–	12
Ethanol	–	12	8	–	–	–	–	–	–	28
Water	–	12	10	8	–	8	–	–	–	20

All the tested extracts have inhibited both Gram positive and Gram negative bacterial species at varying degrees. Among the tested microorganisms *E. faecalis, B. cereus* and *Pseudomonas fluorescens* were inhibited by all extracts. Ether and water extracts have higher inhibitory spectrum from that of ethanol extract. None of the tested extracts did show inhibitory effect against *S. aureus, Proteus sp.* and *Listeria* sp. The biggest inhibition zone was observed with ethanol fraction.

It is known that *Primula* herb has antispasmodic, vermifuge, emetic and astringent effect in public medicine. However, there has been relatively few study in literature about antimicrobial and anticancer effects of this plant. Primin (2-methoxy-6-n-pentyl-1, 4-benzoquinone), a naturally occurring product obtained from *Primula obconica* has shown antimicrobial and antitumour properties (Brondaný *et al.* 2007). An other literature reports that water insoluble crude extracts from *Primula longipes* aerial parts has strong antimicrobial activity with low MIC values against both Gram positive and Gram negative bacteria (Buruk *et al.* 2006).

Antimycobacterial effect of *Primula* has also been investigated. Leaves and flower extracts of *Primula vulgaris* Huds. subsp. *sibthorpii* has shown to be active against *Mycobacterium tuberculosis* $H_{37}R_V$ (ATCC 27294) and extracts caused % 41 inhibition of *M. tuberculosis* (Tosun *et al.* 2005).

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