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Prophage Induction in *Escherichia Coli* K-12(λ) by Some Plants from Iran

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ABSTRACT The test of induction of lambda-phage formation in lysogenic Escherichia coli K-12(λ) was used in a study of some medicinal plants from Iran. In this study, 19 plants from North-eastern of Iran (Turkmen Sahra) were chosen based on ethnobotanical information and screened for biologic activity. 100µg of dried extract dissolved in DMSO were spotted to the surface of the plates including E.coli, strain K12 (λ). Mitomycin C is used as positive control to check the efficiency of the experiment. The active extract cause the prophage to be released from the host genome and the phage reverts to the lytic mode and lyses cells. Production of plaque shows this effect. Extract from roots of *Ferula szowitsiana, Stachys turcomanica* Trautv, *Satureja mutica* Fisch, *Echium italicum, Hymenocrater platystegius, Glycyrrhiza glabra* showed positive effect. One of the 6 effective plants, *Ferula szowitsiana,* was chosen for fractionation and further studies. The induction test may provide a useful screen for the detection of potential DNA-reactive agents.

INTRODUCTION

Agents that interact with DNA have potential clinical utility for the treatment of cancer. Screening for anti-tumor agents requires assays that are rapid, sensitive, inexpensive, and amendable to large-scale screening. In vivo tumor test systems meet none of these criteria and mammalian cell culture assays are also slow and expensive. Therefore, in vitro microbiological assay systems are frequently used for the initial screening for anti-tumor agents (Bartus et al. 1984). Lwoff reported that Escherichia coli K-12, lysogenic for λ -phage could be induced by ultraviolet light and nitrogen mustard and called attention to an apparent correlation between induction capability and mutagenic and carcinogenic activities (Lwoff 1953). Later Geissler suggested that a correlation exists between the inducing activity of such agents and their mutagenic and carcinogenic properties (Geissler 1962). Lein et al. observed that the capability of antibiotics to induce lambda-phage formation in lysogenic Escherichia coli K-12 correlated with their ability to inhibit development of transplanted tumors in animals (Lein et al. 1962).

Evidence supporting a relationship between an agent's inducing capability and its anti-tumor activity was also given by Endo et al. who reported that tumor inhibitor derivatives of mitomycin C, nitrogen mustard and the antibiotic, carzinophilin were capable of inducing E. coli K-12(λ) (Endo et al. 1963). This procedure was utilized to determine the inducing capability of 109 purified antibiotics and 100 chemical agents (Price et al. 1964), N-nitrosamines (Jennifer et al. 1974) and N-nitroso compounds (Heinemann 1972). In the present report 19 plant extracts were tested for inducing activity. These plants were selected from Turkman Sahra area in Iran (Table 1). There have been few documents published on the study of Turkman Sahra medicinal plants.

There seems to be increasing possibility of finding biological activity among plants with recorded medicinal uses rather than from plants randomly selected. Therefore we have preferred to select plants with traditional medical uses (Betancur-Galvis et al. 2002).

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MATERIALS AND METHODS

Culture and Media: The lysogenic bacterium E.coli K-12(λ) were obtained from Iranian Research Organization for Science and Technol-

Table 1: List of taxa in the present study

Species name	*Plant family*	*Plant part*
Centaurea golestanica	*Asteraceae*	Aerial part
Dorema hyrcanum	*Apiaceae*	Aerial part
Ferula oopoda	*Apiaceae*	Root
Ferula szowitsiana	*Apiaceae*	Root / stem
Perovskia abrotanoides	*Lamiaceae*	Aerial part
Satureja mutica	*Lamiaceae*	Aerial part fisch
Leontice leontopetalum	*Berberidaceae*	Root
Caccinia macranthera var. macranthera	*Boraginaceae*	Root
Convolvulus commutatus	*Convolvulaceae*	Aerial part
Anchusa azurea Mill.	*Boraginaceae*	Aerial part
Biebersteinia multifida	*Geraniaceae*	Root
Bryonia aspera Steven ex leded	*Cucurbitaceae*	Root
Echium italicum L.	*Boraginaceae*	Aerial part
Glycyrrhiza glabra	*Fabaceae*	Aerial part
Hymenocrater platystegius	*Lamiaceae*	Aerial part
Marrubium anisodon K.Koch	*Lamiaceae*	Aerial part
Parrotia persica	*Hammamelidaceae*	bark
Stachys turcomanica Trautv	*Lamiaceae*	Aerial part
Zygophyllum fabago L.	*Zygophyllaceae*	Aerial part

ogy and maintained on nutrient agar. Nutrient broth and nutrient agar were purchased from Merck.

Plant Material: The plants were collected from Turkman Sahra and identified by A. Ghorbani. Voucher specimens of the plants were deposited in Shaheed Beheshti University of Medical Science, Traditional Medicine and Materia Medica Research Center, Tehran, Iran.

Chemicals: Dimethyl sulfoxide (DMSO) and mitomycin C were purchased from Sigma Chemical Co. Methanol was obtained from Merck.

Culture Preparation: 50 ml nutrient broth inoculated with E.coli K12 (λ) was incubated at 37ºC for 19 hours to obtain the optical density of 1 at 600 nm.

Extract Preparation: 5g of air-dried and powdered plant material was soaked in 50ml of methanol and shacked for 24hr. The mixture was filtered and concentrated by rotary evaporator and dried in a vacuum-oven at 40ºC. The solid residue was then dissolved in DMSO to obtain final extract concentrations of 20mg/ml.

Induction Test: Escherichia coli, strain K-12 (lysogenic for lambda phage) were incubated for 19 hours at 37ºC and 80 rpm in nutrient broth. 0.5 ml of this suspension was spread over a base plate containing 16ml of nutrient agar. 100µg of dried extract dissolved in DMSO were spotted to the surface of this plate. Plates were incubated overnight at 37 ºC and observed for plaques in the area adjacent to the sample or its toxicity zone. Compounds were tested on duplicate or triplicate plates and the tests were repeated several times, and positive control with amounts of 0.5, 0.25, 0.125µg of mitomycin C, a known inducer, were also included in each experiment to check the efficiency of system.

RESULT AND DISCUSSION

The general procedure used for detection of inducing activity with E-coli K12 (λ) was utilized. The active extract cause the prophage to be released from the host genome and the phage reverts to the lytic mode and lyses cells. Production of plaque shows this effect.

Results from the screening test are shown in table 2. Results clearly demonstrate that, at the concentrations employed extract of *Centaurea golestanica, Dorema hyrcanum, Ferula oopoda, Perovskia abrotanoides, Leontice leontopetalum, Caccinia var. macranthera, Cnvolvu-*

Table 2: Phage induction result

Methanolic extract	*Plaque observed*
Anchusa azurea Mill.	-
Biebersteinia multifida	-
Bryonia aspera Steven ex leded	-
Caccinia macranthera var. macranthera	-
Centaurea golestanica	-
Convolvulus commutatus	-
Dorema hyrcanum	-
Echium italicum	+
Ferula oopoda	-
Ferula szowitsiana	+
Glycyrrhiza glabra	+
Hymenocrater platystegius	+
Leontice leontopetalum	-
Marrubium anisodon K.Koch	-
Parrotia persica	-
Perovskia abrotanoides	-
Satureja mutica Fisch	+
Stachys turcomanica	+
Zygophyllum fabago L.	-

lus commutatus, Anchusa azurea Mill, Bieberstеinia multifida, Bryonia aspera Steven ex ledеd and *Marrubium anisodon* did not induce phage production but extract obtained from roots of *Ferula szowitsiana* and aerial part of *Satureja mutica fisch, Echium italicum, Glycyrrhiza glabra, Hymenocrater platystegius* were effecting inducers.

One of the 6 effective plants, *Ferula szowitsiana*, was chosen for fractionation and further studies.

These plants can be suitable candidate for investigation of cytotoxic and antiviral agents.

CONCLUSION

The induction test as described here is a simple, rapid, reliable, inexpensive and efficient testing for the detection of potential DNA-reactive agents.

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