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Original article

A survey about prophage induction ability in *Escherichia coli* K-12(λ) by ethnic medicinal plants of Kohgiluyeh va Boyerahmad, Iran

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Abstract

Background and objectives: There is a growing trend towards investigating natural products as sources of compounds with biological effects and many researches have been carried out in order to find effective medications against many diseases. Cancer is no exception and studies focusing on evaluating the effects of different materials on DNA, give valuable information in cancer researches and carcinogenicity studies; thus the present study was focused on evaluating the impact of medicinal plants from Kohgiluyeh va Boyerahmad province, Iran on DNA. **Methods**: Thirty five plant species collected have been investigated for prophage induction ability in *Escherichia coli* K-12(λ) through inductest. **Results**:The assay demonstrated that 8 plants were able to affect DNA. **Conclusion:** The results confirm the role of natural resources for biologic effects and what's more, potential drug candidates in new drug discovery.

Keywords*: Escherichia coli* K-12(λ), ethnopharmacology**,** inductest, Kohgiluyeh va Boyerahmad

Introduction

One of the most serious challenges of a cell is DNA damage due to the possibility of enhancing the risk for mutations or leading to cell death [1]. Screening tests which detect phage induction capacity are valuable because of the correlation between carcinogenicity, mutagenicity, phage induction and carcinostatic activity [2]. Some of the usual methods used for prescreening which are usually able to detect most of the components with established clinical utility, are cell culture based cytotoxicity assays. However, because cytotoxicity prescreens are nonspecific and they might detect compounds with a wide variety of mechanisms of action, they result in a large number of positive samples for *in vivo* screening. Some promising approaches have been reached through some more selective *in vitro* tests. The lysogenic induction assay is an example of these methods which has been introduced during the past decades. This assay detects compounds that interact with DNA or interfere with DNA synthesis, and a good correlation between the induction ability and anticancer activity has been reported [3]. Besides, it seems that there is a clear correlation between a compound's ability to induce prophage in lysogenic bacteria and its

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ability to inhibit development of transplanted tumors in rodents [4]. Unfortunately, the inductest is not able to detect all tumor-inhibitor substances, but it can find most of the inducing agents with antitumor activity which indicates that the test could be used to screen antineoplastic agents. The induction test offers a number of advantages, and unlike the screening systems that involve inhibition of animal tumors, it is rapid, inexpensive, and requires very small quantities of the test agent [5].

Medicinal plants are natural sources that are usually set for prescreening of cancer research using various methods. A large number of medicinal plants have been used by local people of Kohgiluyeh va Boyerahmad, a mountainous province in south-west of Iran and many of these species have not been examined for biological activities [6].

In the present study 35 species which have been used ethnomedicaly by people of the province have been examined for the ability to induce prophage induction in *Escherichia coli* K-12(λ).

Experimental

Medium and chemicals

Nutrient agar medium, nutrient broth, skim milk medium, glycerol and dimethyl sulfoxide (DMSO) were provided from Merck, Germany. Mitomycin C was obtained from Sigma, USA.

Bacteria

The lysogenic *E coli*. (k12) λ was obtained from Pasture Institute, Iran.

Plant material

The medicinal plants were collected from Kohgiluyeh va Boyerahmad province, Iran during 2009-2011. Each species was identified by botanists of the Traditional Medicine and Material Medica Reasearch Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. The voucher specimens of the plants were kept in the Herbarium of TMRC.

Extraction

The powdered sample of each species (5g) was macerated with methanol for 24 h. The filtrate was then concentrated and kept in refrigerator till the time of the inductest experiment.

Preparation of the extracts

The dried methanol extract of each species was dissolved in DMSO in concentration of 20 mg/mL prior to the experiment.

Prophage induction

Prophage induction was assessed according to Taghvaei *et al*. with some modifications [7]. *Escherichia coli*, strain K-12 (lysogenic for lambda phage) were incubated for 24 h at 37 ºC and 100 rpm in nutrient broth. A suspension was then spread over a petri dish of nutrient agar. A sample of each species was spotted on the surface of the petri dish. After an overnight incubation at 37 ºC, the samples were examined for possible plaques in the area adjacent to the spot. Each experiment was repeated in duplicate. Mitomycin C (0.5mg/mL) and DMSO were used as positive and negative controls, respectively.

Results and Discussion

The results of prophage induction assay are presented in table 1. Among the 35 examined species *Eryngium billardieri*, *Haussknechtia elymaitica*, *Hypericum perforatum*, *Satureja bachtiarica* and *Turgenia latifolia* presented the ability to induce prophage induction. These plants have been used by local people of Kohgiluye va Boyerahmad province, Iran to treat different ailments. *Eryngium billardieri* has been used orally to treat constipation, while oral preparations of *Haussknechtia elymaitica* have been utilized in diabetes and hypertension. Those who have suffered from menstrual problems would find benefit from infusions of *Hypericum perforatum* and infusions of *Turgenia latifolia* have demonstrated to be helpful as a remedy in urinary duct problems [8]. Considering the results of the present study, it was concluded that the methanol extract of the above five species could

Table 1. The prophage induction results of Kohgiluyeh and Boyerahmad province medicinal plants.

No.	Samples	Family	Parts used for extration	Induction effect
1	*Alcea calvertii* Boiss.	Malvaceae	Flower	-
2	*Alhagi pseudalhagi* (M.Bieb.) Desv.	Fabaceae	Aerial parts	-
3	*Amygdalus scoparia* Spach	Rosaceae	Aerial parts	-
4	*Arctium minus* (Hill) Bernh.	Asteraceae	Rhizome	-
5	*Astragalus ovinus* Boiss.	Fabaceae	Fruits	-
6	*Astragalus fasciculifolius* Boiss.	Fabaceae	Aerial parts	-
7	*Capparis spinosa* L.	Capparaceae	Aerial parts	-
8	*Cerasus microcarpa* Boiss.	Rosaceae	Young branches	-
9	*Chenopodium foliosum* Asch.	Chenopodiaceae	Aerial parts	-
10	*Cichorium intybus* L.	Asteraceae	Aerial parts	-
11	*Datura innoxia* Mill.	Solanaceae	Aerial parts	-
12	*Eremostachys adenantha* Jaub.& Spach	Lamiaceae	Aerial parts	-
13	*Eremostachys macrophylla* Montbret & Aucher	Lamiaceae	Aerial parts	-
14	*Eryngium billardierei* F. Delaroche	Apiaceae	Aerial parts	+
15	*Foeniculum vulgare* Mill.	Apiaceae	Aerial parts	-
16	*Fraxinus rotundifolia* Mill.	Oleaceae	Aerial parts	-
17	*Gentiana olivieri* Griseb.	Gentianaceae	Tuber	-
18	*Haussknechtia elymaitica* Boiss.	Apiaceae	Tuber	+
19	*Hyoscyamus reticulatus* L.	Solanaceae	Aerial parts	-
20	*Hypericum perforatum* L.	Hypericaceae	Whole plant	+
21	*Malva parviflora* L.	Malvaceae	Whole plant	-
22	*Marrubium astracanicum* Jacq.	Lamiaceae	Aerial parts	-
23	*Mindium laevigatum* (Vent.) Rech. f. & Schiman-Czeika	Campanulaceae	Aerial parts	-
24	*Nasturtium officinale* W.T.Aiton	Brassicaceae	Whole plant	-
25	*Nerium indicum* Mill.	Apocynaceae	Aerial parts	-
26	*Ricinus communis* L.	Euphorbiaceae	Aerial parts	-
27	*Rosa canina* L.	Rosaceae	Fruits	-
28	*Sanguisorba minor* Scop.	Rosaceae	Whole plant	-
29	*Satureja bachtiarica* Bunge	Lamiaceae	Whole plnat	+
30	*Tanacetum polycephalum* Sch.Bip.	Asteraceae	Aerial parts	-
31	*Teucrium polium* L.	Lamiaceae	Whole plant	-
32	*Thymus daenensis* Celak.	Lamiaceae	Whole plant	-
33	*Turgenia latifolia* Hoffm.	Apiaceae	Whole plant	+
34	*Urginea maritime* Baker	Liliaceae	Tuber	-
35	*Verbascum songaricum* Schrenk	Scrophulariaceae	Aerial parts	-

interact with DNA and might have cytotoxic effects which suggests further cytotoxicity studies, but the point is that if these medications have been and are being used by local people, they should be profoundly examined; moreover the people might be monitored for any ignored adverse effects that might be related to the toxic effects of these plants. It should also be kept in mind that the characteristics of cytotoxicity might have somehow changed or reduced during the preparation according to the local ethnomedical data. Literature review has also revealed that despite few data about the cytotoxicity of most of these species, some toxicological data has been reported. A review about the phytochemical constituents and pharmacological activities of the genus *Eryngium* has demonstrated that extracts or isolates from this genus have shown *in vitro* bioactivitities such as cytotoxicity against various human tumor cell lines [9]. Also, Roscetti *et al*. have shown that *Hypericum perforatum* flower extract have exhibited a significant concentration-dependent and long-lasting inhibition of cell growth, and has induced apoptotic cell death [10]. Besides, *Hypericum perforatum* methanol extract has been able to cause cell death induced by apoptosis in bladder cancer cells [11]. The presented results of

our study as well as the previous cytotoxicity evaluations suggest the necessity of further studies about the plants used ethnomedically in treatment of diseases.

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Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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