Original article

Effect of various concentrations of *Crocus sativus* and *Cannabis sativa* extracts on luminescent biosensor *Escherichia coli* SM10 S1

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Abstract

Introduction and objective: The potential risk of application of high dosage of traditional medicinal plants has not been fully understood. Appropriate microbial biosensors have been constructed for monitoring the toxicity of many harmful chemical compounds. The aim of this research was to see how effective are the different concentration of two medicinal plants extracts (*Crocus sativus* (saffron) and *Cannabis sativa*) on a bioluminescent marker system indicating their side effects.

Materials and methods: The stability and light intensity of *Escherichia coli SM10 λpir* were previously characterized and confirmed. Several concentrations of saffron and cannabis water extracts were prepared. The light intensity was measured for a mixture of 450µl of aqueous saffron extract and 50µl of biosensor using a luminometer.

Results: Results showed gradual decrease on light output in the way that luminescence decreased from 538859 RLU/s for 0.001g/ml aqueous saffron extract to 4830 RLU/s for 0.2g/ml concentration. Although induced increase in bioluminescence was observed for low concentration (0.001 and 0.01 v/v) of cannabis extract 0.25 and 1v/v concentration showed significant decrease in bioluminescent activity. Calculation of % INH of luminescent indicated the correct sensitivity of luminescent biosensor *E. coli* SM10 S1 to various concentration of saffron and cannabis extracts.

Conclusion: The results show the appropriate interaction of constructed biosensor to different concentrations which can be used for further investigation on other ranges of concentrations. Application of luminescent microbial biosensor for investigation of the quality of products such as saffron and cannabis is new.

Keywords: Medicinal plants; Microbial biosensor; Bioluminescent activity; Side effects

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Introduction

Many people in developing countries frequently use herbal medicines as a primary health care resource, mainly because western pharmaceuticals and health care are expensive. Also on cultural and spiritual point of views, herbal medicines are more acceptable where 60% of South Africans consult traditional healers [1]. Moreover, the main proportion of many drugs is originated from constituents of herbal medicines.

Regardless of the usage of plant originated medicines, there are some documents indicating that medicinal plants are potentially toxic and carcinogenic. For example, the safe use of the genotoxic plant extracts, such as methanol extracts of *Helichrysum simillimum* DC. (Asteraceae) is under question and more experiments on its mutagenicity and overall biological properties have to be applied [2]. Because that the traditional medicinal plants are usually safe for use, few investigations have been focused on the subject of toxicological tests which is required for modern pharmaceutical compounds.

Toxicological side effects of any chemicals such as constituents of medicinal plants can be investigated with various experiments. Classical methods such as the *Salmonella typhimurium* mutagenicity assay (the *Ames test*) have been used for three decades. There has been a continuous progress on additional toxicological tests based on the application of genetically engineered microorganisms [3-4]. Microbial biosensors can be defined as analytical devices which contain a biological sensor, such as a micro-organism or an enzyme, providing an information-linked response to a specific property (e.g. the presence of a toxic chemical), via a suitable transducer (e.g. electrochemical, optical) [5].

Genetically modified microbial biosensors may be either non-specific (metabolic) or specific (catabolic). Specific biosensors contain reporter genes downstream of a strong constitutive promoter. Elgorashi *et al.* [6] used VITOTOX® tests to investigate the potential mutagenic effects of plants used in South African traditional medicine. The VITOTOX® test is based on *S. typhimurium* strain TA104 *rec*N2-4 that contains the *lux* operon of *Vibrio fisheri* under transcriptional control of the *rec*N gene, which is part of the SOS system.

Incubation of the bacteria in the presence of a genotoxic compound results in the derepression of the *rec*N promoter, and hence expression of the *lux* operon. This expression finally results in light production as a function of genotoxicity [7]. Non-specific (metabolic) bioluminescent microbial biosensors encompass two broad categories; naturally luminescent, for example *V. harveyi*, and genetically modified, for example *E. coli* SM10 S1 which was used in this study. Two whole cell *E. coli* luminescent biosensors (strain DPD2794 and DE135) were also previously used to determine the antibacterial actions of 16 herbal tinctures [8].

In this study a general luminescent bacterial biosensor, *E. coli* SM10 S1 was used to investigate the effect of two different medicinal plant extracts on general physiological metabolism of the cell. *E. coli* SM10 S1 has been genetically engineered to harbour *luxAB* gene encoding bacterial luciferase enzyme (Mashreghi *et al.* Unpublished work).

Selection of Khorasan medical plants

Saffron: *Crocus sativus L.* known as saffron is one of the traditional plants which has many medicinal properties. *C. sativus* belongs to the Phylum *Mangnoliophyta*, Order *Mangoliopsdia* and family *Iridaceae.* Iran is a major growing region for this famous medicinal herb. Saffron contains

more than 150 volatile and aromatic-yielding compounds including safranal (insecticide and pesticide), carotenoids and riboflavins [9]. *Cannabis sativa*: belongs to the Phylum *Mangnoliophyta*, Order *Mangoliopsdia* and family *Cannabaceae*. *C. sativa* has been used as tinctures, teas, ointments and also is useful for the treatment of old cancers and mammary tumors. *C. sativa* is native to central Asia and long cultivated in Asia which contains about sixty cannabinoids, tetrahydrocannabionl (THC), cannabidiol (CBD), dronabinol, terpenes and sesquiterpenes [10].

Materials and methods

Saffron extraction and analysis

To prepare aqueous saffron extract, dry stigmas were ground in a sterile mortar and deionized sterile water was added to make various concentrations (1, 2, 10, 20, 100, and 200mg/ml). All concentrations were heated in warm water at 50°C for 5mins and analyzed on the basis of the light output measured immediately by a luminometer (FB12, Berthold Germany).

Cannabis sativa extraction and analysis

To prepare *C. sativa* ethanolic extract, 10g dried plant material was ground to a powder and extraction was followed using Sookseleh method. Extracting solvent was 80% ethanol prepared from high purity ethyl alcohol in the ratio of 1:15 powder: alcohol. The plant material was air dried overnight and various concentrations (0.1, 0.01, 0.001, 0.25, 0.5, 0.75 and 1%) were prepared in deionized water.

Real-Time biosensing using bioluminescent E. coli SM10 S1

Escherichia coli SM10 S1 with *lux*AB gene fused in its chromosomes was maintained in 70% glycerol at -78°C. Prior to the assays, the stock cultures were transferred to 250ml flasks containing 50ml sterile Luria Bertani (LB) medium (Merck, Germany) and incubated for 12h at 37°C on an orbital shaking incubator at 150 rpm.

The effect of the extracts on *E. coli* SM10 S1 (microbial luminescent biosensor) was determined on the basis of the procedure of Bhattacharyya *et al*. [11]. The assay protocols were adapted from previously described standard methods [12-14] and was carried out as described below. The bioassays for metabolic biosensors were performed in 1.5ml luminometer cuvettes with a total reaction volume of 500µl containing 450µl extracts and 50µl of the respective biosensor. Then, 10µl of a freshly prepared 10% decanal solution was added and luminescence was measured. Controls (+) contained 450µl deionized water, 50µl of the respective biosensor and 10µl of 10% decanal solution. Control (-) contained 500µl deionized water and 10µl of 10% decanal solution.

Results

The RLU (relative light per unit) ratio of the *E. coli* SM10 S1 exposed to saffron extracts at differing concentrations (1mg/ml to 200mg/ml) is summarized in figure 1. Luminescent strain showed decreased bioluminescence in response to the stress caused by saffron extracts at the concentrations as high as 1mg/ml. The RLU ratio of bioluminescent *E. coli* SM10 S1 exposed to concentration of 1mg/ml was 5388859.3 but in 200mg/ml concentration, RLU ratio changed to 4830.33 about 99.91% reduction in intensity of light output. Both high and low concentrations decreased the RLU ratios and made stress to strain but in various levels.

The RLU ratios in high saffron concentrations (100 and 200mg/ml) were significantly lower than low concentration, and there was no significant difference in the effect of plant extracts at 1 and 2mg/ml saffron extract compared to the control.

These results indicate that this biosensing panel was sensitive to the bioactive effects caused by high concentration of saffron water extracts.

Concentrations of 0.001% and 0.01% of *C. sativa* extract, increased bioluminescence in strain SM10 S1 (Fig. 2). Increased bioluminescence in strain SM10 S1 showed no stable response to other concentrations. At a concentration of 0.1 and 0.25% of Cannabis extract, slight decrease in RLU ratio of bioluminescence in strain SM10 S1 was detected; however, in higher concentrations (0.25% to 1%), significant decrease was observed compared to the control. The decrease in bacterial luminescence (INH%) due to addition of various concentration of plant extracts was determined using the following equations [15]:

INH % = 100- (luminescence of the test/ luminescence of the control) × 100

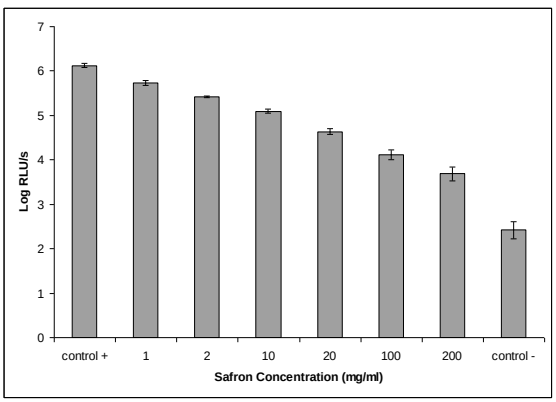

Fig. 1: Effect of different concentrations of saffron water extract on luminescence activity of *E. coli* SM10 S1

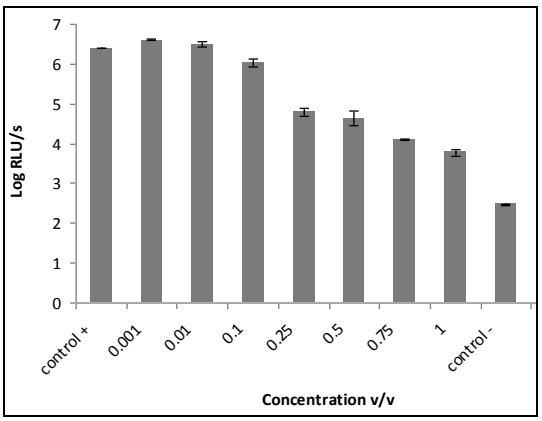

Fig. 2: Effect of different concentration**s** of *Cannabis sativa* extract on luminescence activity of *E. coli* SM10 S1

The results are shown in figure 3 for saffron and figure 4 for cannabis extracts. The results showed that inhibition of luminescence had a sharp increase in the presence of 1 to 20mg/ml saffron extract and remained more or less constant in high concentration of 100 and 200mg/ml (Fig. 3). Similar changes were seen about the effect of various concentrations of cannabis extract on inhibition of luminescence of the bioreporter strain of *E. coli* SM10 S1 (Fig. 4).

In the present study, a slight increase was observed for the bioluminescence of strain SM10 S1 at lower concentrations of *C. sativus* extract (0.001 and 0.01%), but no significant increase of RLU ratio when exposed to higher concentrations, unlike the behavior seen for the saffron extract. Increased RLU ratio of the strain SM10 S1 in the presence of 0.001 and 0.1% Cannabis extract indicated that induction of the general stress-response enhanced the RLU ratio of strain SM10 S1 at the above concentrations. The increased RLU ratio of strain SM10 S1 indicated the possibility that light emission was induced by both 0.001 and 0.01% concentration.

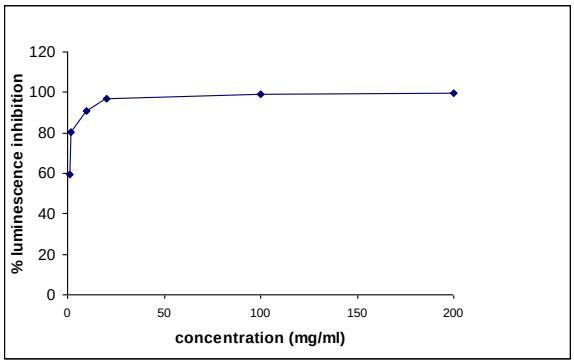

Fig. 3: Inhibition of relative luminescence levels of *E. coli* SM10 S1 by the presence of various concentrations of saffron extract

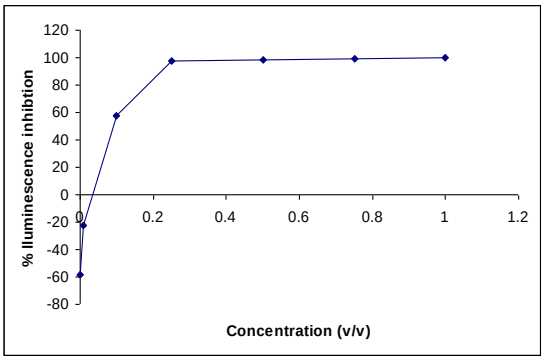

Fig. 4: Inhibition of relative luminescence levels of *E. coli* SM10 S1 by the presence of various concentration**s** of cannabis extract

Discussion

Microbial whole-cell biosensors could be ideal candidates for investigation of medicinal plants side effects because of their simple application, low cost and fast response. These devices mostly have been used for environmental toxicity assessment or their bioluminescent genes (*lux* or *luc* genes) applied as reporter genes [16-17]. Also drug industry could benefit from biosensor technology to assess the side effects of different concentrations or high dose of new products with unknown implications. This research assessed the effects of different concentrations of water and ethanolic extracts of two native Khorasan medicinal plants using a biosensor.

The reduction in RLU ratios of the strain SM10 S1 could be attributed to inhibition of cellular metabolism required for the production of energy or reduction of power [18]. In a similar manner, Schmidt, *et al.* [19] investigated the potential toxicity effect of different concentrations of green tea extracts on rat hepatocytes. They treated the rat hepatocytes in primary culture with various hydro-alcoholic green tea extracts. Their results indicated that all green tea extracts examined enhanced resazurin reduction significantly at a concentration of 100-500µg/ml medium, while a significant decrease was observed at 1-3mg/ml. (Resazurin is used mainly as an oxidation-reduction indicator in cell viability assays for bacteria and mammalian cells).

We found that in various concentrations of saffron and cannabis extracts, a certain range of concentration (10-200mg/ml for saffron and 0.25-1% for cannabis) had more significant effects on bioluminescent activity. The comparison of the effect of various concentrations of green tea extracts on rat hepatotoxicity with the effect of saffron and cannabis extracts on bioluminescent activity shows that both tests are similarly valuable. Bioluminescent biosensors are much easier to work with, not as expensive as the tissue culture methods.

In a research performed by Santa Maria *et al.* [20] investigation on the toxicity of the compounds of guarana (*Pauliniac cupana*) was undertaken. Several nonalcoholic drinks contain guarana extracts which keep people awake and are used by people demanding routine exercise. However, there is not enough information about the potential side effects of guarana. To find out such information and answer the questions about the appropriate use of such compounds, Santa Maria *et al.* [20] used several methods such as Microtox assay based on luminescent bacterium, *Photobacterium phosphoreum.* They concluded that the concentration of guarana

is of critical importance in its cytotoxic activity and high doses could be harmful to human health.

In comparison, high concentrations of saffron and cannabis extracts had more inhibitory effects on luminescence activity of genetically engineered *E. coli* SM10 S1 indicating that high dosage of such compounds can be harmful to any biological systems and may have toxic side effects. Several researches have been performed on the application of genetically and non-genetically engineered luminescent bacteria for toxicity tests of different compounds [21-23].

Conclusion

In conclusion, *E. coli* SM10 S1 responded correctly and in a logical manner to various concentrations of saffron and cannabis. Therefore, whole cell non-specific luminescent biosensors such as *E. coli* SM10 S1 facilitate a prior prediction of the toxic concentrations of some medicinal plant extracts.

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References

1) Taylor JLS, Elgorashi EE, Maes A, *et al*. Investigating the safety of plants used in South African traditional medicine: Testing for genotoxicity in the micronucleus and alkaline comet assays. *Environ Mol Mutagen.* 2003; 42: 144-54.
2) Verschaeve L, Van Staden J. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. *J Ethnopharmacol.* 2008; 119: 575-87.
3) Mashreghi M. Biosafety issues in biotechnology and engineering of microorganisms. *Iran J Biotechnol.* 2007; 5: 194-200.
4) Mashreghi M, Prosser JI. Survival and activity of Lux-marked phenanthrene-degrading *Pseudomonas stutzeri* P16 under different conditions. *Iran J Sci Technol Trans Sci.* 2006; 30: 71-80.
5) Dennison MJ, Turner APF. Biosensors for environmental monitoring. *Biotechno Adv.* 1995; 13: 1-12.
6) Elgorashi EE, Taylor JLS, Maes A, van Staden J, De Kimpe N, Verschaeve L. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. *Toxicol Lett.* 2003; 143: 195-207.
7) van der Lelie D, Regniers L, Borremans B, Provosst A, Verschaeve L. The VITOTOX(R) test, an SOS bioluminescence *Salmonella typhimurium* test to measure genotoxicity kinetics. *Mut Res Gen Toxicol Environ Mut.* 1997; 389: 279-90.
8) Watt K, Christofi N, Young R. The detection of antibacterial actions of whole herb tinctures using luminescent *Escherichia coli*. *Phytother Res.* 2007; 21: 1193-9.
9) Sastry, LVL, Srinivasan M, Subrahmanyan V. Saffron (*Crocus sativus* Linn.). *J Sci Ind Res.* 1955; 14A: 178-84.
10) Turner CE, Elsohly MA, Boeren EG. Constituents of *Cannabis sativa* L. XVII. a review of the natural constituents. *J Nat Prod.* 1980; 43: 169-234.
11) Bhattacharyya J, Read D, Amos S, Dooley S, Killham K, Paton GI. Biosensor-based diagnostics of contaminated groundwater: assessment and remediation strategy. *Environ Pollut.* 2005; 134: 485-92.
12) Bundy JG, Campbell CD, Paton GI. Comparison of response of six different luminescent bacterial bioassays to

bioremediation of five contrasting oils. *J Enviro Monit.* 2001; 3: 404-10.
13) Shingleton JT, Applegate BM, Nagel AC, Bienkowski PR, Sayler GS. Induction of the tod operon by trichloroethylene in *Pseudomonas putida* TVA8. *Appl Environ Microbiol.* 1998; 64: 5049-52.
14) Stitcher P, Jaspers MC, Stemmler K, Harms H, Zehnder AJ, van der Meer JR. Development and characterization of a whole-cell bioluminescent sensor for bioavailable middle-chain alkanes in contaminated groundwater samples. *Appl Environ Microbiol.* 1997; 63: 4053-60.
15) Parvez S, Venkataraman C, Mukherji S. A review on advantages of implementing luminescence inhibition test (*Vibrio fischeri*) for acute toxicity prediction of chemicals. *Environ Int.* 2006; 32: 265-8.
16) Hansen LH, Sorensen SJ. The use of whole-cell biosensors to detect and quantify compounds or conditions affecting biological systems. *Microb Ecol.* 2001; 42: 483-94.
17) Stoyanov JV, Magnani D, Solioz M. Measurement of cytoplasmic copper, silver, and gold with a lux biosensor shows copper and silver, but not gold, efflux by the CopA ATPase of *Escherichia coli*. *FEBS Lett.* 2003; 546: 391-4.
18) Chatterjee J, Meighen EA. Biotechnological applications of bacterial bioluminescence (lux) genes. *Photochem Photobiol.*1995; 62: 641-50.
19) Schmidt M, Schmitz HJ, Baumgart A, *et al*. Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. *Food Chem Toxicol.* 2005; 43: 307-14.
20) Santa Maria A, Lopez A, Diaz MM, Munoz-Mingarro D, Pozuelo JM. Evaluation of the toxicity of guarana with in vitro bioassays. *Ecotoxicol Environ Saf.* 1998; 39: 164-7.
21) Horswell J, Dickson SJ. Use of biosensors to screen urine samples for potentially toxic chemicals. *J Anal Toxicol.* 2003; 27: 372-6.
22) Ramanathan S, Ensor M, Daunert S. Bacterial biosensors for monitoring toxic metals. *Trends Biotechnol.* 1997; 15: 500-6.
23) Ramanathan S, Shi WP, Rosen BP, Daunert S. Sensing antimonite and arsenite at the subattomole level with genetically engineered bioluminescent bacteria. *Anal Chem.*1997; 69: 3380-4.