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Biological Activity of Quercetin-3-*O*-Glucoside, a Known Plant Flavonoid[1]

Seyed Mehdi Razavi[a,2], Saber Zahri[a], Gholamreza Zarrini[b], Hossein Nazemiyeh[c], and Sariyeh Mohammadi[a]

[a] *Department of Biology, Faculty of Sciences, University of Mohaghegh-Ardabili, Ardabil, Iran*

[b] *Depatrment of Animal Sciences, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran*

[c] *Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medicinal Sciences, Tabriz, Iran*

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Abstract—Cytotoxic, phytotoxic, antimicrobial and antioxidant effects of quercetin 3-*O*-glucoside (Q3G) isolated by HPLC from aerial parts of *Prangos ferulaceae* was studied by MTT assay, lettuce germination assay, disk diffusion and DPPH method. Our results showed that Q3G exhibits high antioxidant effect with RC_{50} of 22 μg/mL, it has low cytotoxicity and no antibacterial effects. Q3G exhibits high phytotoxic effect with IC_{50} value of 282.7 μg/ml, as well. It is assumed that Q3G does not play a defense role in plants and it may act as an allelopatic agent.

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INTRODUCTION

Plants produce a large group of organic compounds that appears to have no direct function in growth and development and are known as secondary products. These compounds have been suggested to have important ecological functions in plants. They protect plants against being eaten by herbivores and against being infected by microbial pathogens. They serve as attractants for pollinators and seed dispersing animals and as agent for plant-plant competition, too [1]. On the other hand, because of high biological activities, plant secondary products are used in drugs composition. Quercetin-3-*O*-glucoside (Q3G) is the most known plant secondary product that is widespread in the majority of plant families. High concentration of Q3G and other derivatives of quercetin are present in apple, tea, onions and cratagus. It has been repeatedly shown that quercetin and its glycosilated derivatives can inhibit several key enzymes, e.g., phospholipase A_2 and C, tyrosin protein kinases, lipoxygenase, cyclooxygenase, cyclic nucleotide phosphodiesterase and cytochrome P_{450} systems. Thus, it shows antihistamine, antiinflammatory and anticarcinogenic properties and may also help to reduce symptoms like fatigue, depression, anxiety, coronary heart disease and cancer [2, 3].

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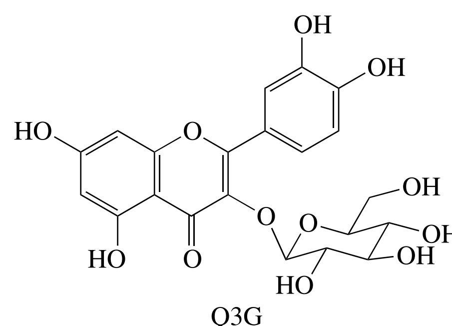
[2] Corresponding author; e-mail: razavi694@gmail.com

[3] Abbreviations:



The aim of the present study was to evaluate cytotoxic, phytotoxic, antimicrobial and antioxidant effects of Q3G.

RESULTS AND DISCUSSION

The cytotoxic assay results indicated that Q3G could reduce the viability of cell lines. We consider compounds as cytotoxic when they reduce the viability of cells to less than 50%. By assuming this, Q3G was cytotoxic to McCoy cell line at concentration higher than 215.2 mg/ml. It is noticeable that IC_{50} value for Q3G was 215.2 mg/ml.

Q3G reduces in a concentration-dependent manner seed germination of lettuce (characteristic plant for allelopatic studies). The IC_{50} were 282.78 μg/ml for germination inhibition. It is obvious that G3P exhibited high phytotoxic effects against lettuce (figure).

Our results showed that Q3G exhibited no activity against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Candida kefyr.*

Q3G exhibited high antioxidant activity in DPPH assay with RC50 value of 22 µg/ml.

In conclusion, because of weak antimicrobial and noncytotoxic activity of Q3G, it is assumed that this compound has no defense role in plants and could not protect plants against pathogens and herbivores. Phytotoxic potential of Q3G proved that it could serve as allelopatic compound for plants. On the other hand, high antioxidant effect of Q3G, make makes it potent for defense against oxidative stress and free radical scavenging activities in plants. Many foods are rich in Q3G and human daily intake ranges between 10–20 mg. Q3G can transport across the intestinal brush border membrane by sodium dependent glucose transporter. Thus, it exhibited biological effects more efficient than other derivatives of quercetin [2]. Because of powerful antioxidant potential, Q3G can be considered as a natural anticarcinogenic agent. Because of high antioxidant potential, Q3G and some other flavonoids have important effects on cancer chemoprevention. The chemopreventive properties of flavonoides are generally believed to reflect their ability to scavenge endogenous ROS. In spite of cytotoxic properties of Q3G in vitro condition, it may interfere in several steps that lead to the development of malignant tumors including protecting DNA from oxidative damages, inhibiting carcinogen and activating detoxifying systems [4]. In conclusion, mega doses of Q3G in daily foods, may interfere with anticancer drugs.

It was reported that some quercetin derivatives such as quercetin–3-*O*-rhamnoside exhibited strong antibacterial effects [5], but we didn't find any antibacterial and antifungal activity for Q3G. Thus, it is assumed that sugar moiety of flavonoides play important role in their biological activities.

A survey of literature showed that Q3G can inhibit seed germination and growth of *Arabidopsis thaliana* and it can inhibit conidial germination of *Neurospora crassa* [6].

EXPERIMENTAL

Extraction, Isolation and Identification

Dried powdered aerial parts of *Prangos ferulaceae* L. (200 g) were Soxhlet-extracted successively, with *n*-hexane, dichloromethane and methanol. The methanolic extract was concentrated under reduced pressure at 45°C to yield a residue (15 g). This residue (2 × 4 g) was fractionated on an SPE-C18 cartridge using different methanol-water mixtures (2 : 8, 4 : 6; 8 : 2; 10 : 0 MeOH : H_2O). The 40% methanol fraction (1.8 g) was subjected to preparative HPLC using methanol and water gradient as follows: 0–75 min, 35% methanol; 75–82 min, 45% methanol; 83 min, 35% methanol; 90 min. The flow-rate was 20 ml min^{-1}. The yield of Q3G was 31 mg, R_t 24.5 min. It was identified by comparison of its physical and spectroscopic data (m.p., UV, ^{1}H NMR and ^{13}C NMR) with those previously reported in the literature [7].

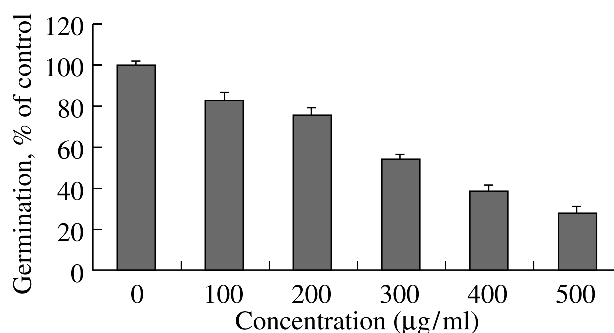


Fig. Effects of different concentration of Q3G on lettuce seed germination.

Cytotoxicity Assay

McCoy cell lines (Pasteur, C123) were grown in RPMI 1640 (Gibco, no. 51800–019) medium. Each 500 ml of the medium consisted of 5.2 g RPMI powder, 1 g of sodium bicarbonate, 1% w/v of penicillin/streptomycin and supplemente with 10% heat-inactivated fetal calf serum (FCS) in deionised water [8]. Completed medium was sterilized by filtering through 0.22 µm microbiological filters (Art no 11107-25). Cell line was maintained in a humidified atmosphere of 5% CO_2 at 37°C.

The stock solutions of Q3G were prepared by dissolving the compound in deionized water (100 µL). The final concentration of the compound was 100, 500, 750 and 1000 mg/ml. Cells were plated in the appropriate media on 20-well plates in a 500 µl total volume at a density of 6×10^5 cell/ml. Triplicate wells were treated with media containing different concentration of Q3G. The plates were incubated at 37°C in 5% CO_2 for 16 h. For evaluating of cell viability, each well was supplemented with 50 µl of a 5 mg/ml solution of 3-(4.5-dimethylthiazol–2-yl)–2.5-diphenyl tetrazolium bromide (MTT) in uncompleted media. The media was carefully removed from each well and 1 ml of deionized water and 1 mM glycine buffer was added [9]. The plates were gently agitated until the color reaction was uniform and the OD_{570} was determined using a microplate reader. The amount of MTT converted to formazan is a sign of the number of viable cells. Media-only treated cells served as the indicator of 100% cell viability. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the control in the MTT assay. Viability percentage was evaluated as $OD_{treatment}/OD_{control}$ [10].

Phytotoxic Assay

Lettuce (*Lactuca sativa* L. CV. Varamin) seeds were used to test germination response to different concentration of Q3G. Sterile deionized water was used to dissolve Q3G to generate six concentrations ranging 100, 200, 300, 400 and 500 μg/ml of the compound. All seeds were surface sterilized with sodium hypochloride (1%). Four replicates, each of 25 seed, were prepared for each treatment using sterile Petri dishes (90 mm) lined with one sterile filter paper (Whatman, number 2). 5 ml of different concentration of Q3G was added to each given 5 ml of deionized water. Prepared plates were then placed in a germination cabinet at 25°C in the dark. Germination was deemed to occur only after the radicle had protruded beyond the seed coat by at least 1mm. After 1 week, in the each treatment, germination percentage was determined and root length was measured [6].

Antimicrobial Assay

The antibacterial and antifungal activities of Q3G were determined against *E. coli* (PTCC 1047), *St. epidermis* (PTCC 1114), *B. cereus* (ATCC 10876), *P. aeruginosa* (ATCC 27853) and *C. kefyr* (ATCC 38296) by the disc diffusion method (Lorain 1996). Muller—Hinton agar (MHA) (oxoid) and sabouraued dextrose agar (SDA) was used to bacterial and fungal strains respectively. The filter paper discs (6 mm in diameter) were individually impregnated with 10 μl of 100 mg/ml the Q3G stock solution and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37°C for 24 h and at 30°C for 48 h for fungal strains. The diameters of inhibition zones were measured in millimeters. All the tests were performed in duplicate. Gentamicin (30 μg) served as positive control [11].

Antioxidant Assay

Serial dilutions were carried out with the stock solutions (1 mg/ml) of Q3G to obtain concentrations 0.5, 0.25, 0.175, 0.087, 0.043, 0.021, 0.010, 0.005, 0.002 and 0.001 mg/ml. Diluted solutions (5 mL each) were mixed with 5 ml of 2.2-diphenyl–1-picryl hydrazyl (DPPH, Sigma) and allowed to stand for 3 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each concentration. The RC_{50} value, which is the concentration of the test material that reduced 50% of free radical concentration, was calculated as μg/ml [12].

Statistical Analysis

In all assays, SPSS 11.5 software was used for statistical analysis. Analysis of variance (ANOVA) followed by Duncan test was used to see the difference amongst various groups. The significance level was set at $p < 0.05$.

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