

Comparison of antioxidant activity of extract from roots of licorice (*Glycyrrhiza glabra* L.) to commercial antioxidants in 2% hydroquinone cream

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Synopsis

Powdered dry roots of licorice (*Glycyrrhiza glabra* L.) were extracted with methanol. Licorice extract was tested for antioxidative activity in comparison with antioxidants (sodium metabisulfite and BHT) at 0.1%, 0.5%, 1.0%, and 2.0% w/w in 2% w/w hydroquinone cream. The systems were incubated in a dark room at $25^{\circ} \pm 0.5^{\circ}\text{C}$ and $45^{\circ} \pm 0.5^{\circ}\text{C}$ for three months. The physical stability and the percentages of hydroquinone remaining after two weeks and one, two, and three months were determined by UV spectrophotometer at 294 nm according to official standard procedures. The experiment revealed that oxidation degradation of hydroquinone was accelerated by heat even with the existence of antioxidants. The higher percentages of remaining hydroquinone were observed for higher antioxidant concentration but showed lower physical stability in the formulation in the presence of commercial antioxidants, especially in the cases of 1.0% and 2.0% BHT. In the third month, at $25^{\circ} \pm 0.5^{\circ}\text{C}$ and $45^{\circ} \pm 0.5^{\circ}\text{C}$, the extract demonstrated more antioxidant activity from two other commercial antioxidants at all concentrations, with about 43–53% and 34–46%, respectively, more hydroquinone remaining than in the control system ($p < 0.001$). In the third month, the preparation containing 0.1%, 0.5%, 1.0%, and 2.0% extract gave good physical formulation stability with about 72%, 76%, 78%, and 81% hydroquinone remaining at $25^{\circ} \pm 0.5^{\circ}\text{C}$ and 51%, 55%, 60%, and 63% hydroquinone remaining at $45^{\circ} \pm 0.5^{\circ}\text{C}$, respectively. This suggested the possibility of using a licorice extract at 0.5% and 1.0% as an effective natural antioxidant for substances that are oxidation-susceptible.

INTRODUCTION

One of the most important characteristics of many cosmetic products is stability. Hydroquinone, a hypopigmenting agent employed percutaneously to lighten localized areas of hyperpigmented skin such as blemishes, lentigo, melasma, chloasma, and freckles, is known for its high oxidative reactivity. It is one of the chemicals that are difficult to

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stabilize. It becomes brown in air due to oxidation. One gram of hydroquinone is soluble in 17 ml of water and freely soluble in alcohol. In antifreckle and hair dyeing cosmetic products, a law limits its content to 2% w/w (1). Most hydroquinone products used in creams, gels, lotions, and ointments are locally manufactured in Iran.

Being located in a tropical area, some Iranian people suffer from solar effects on their skin. Freckles are one of these effects. Hydroquinone is one of the cheapest hypopigmenting agents available in the Iranian market, which most middle and lower class Iranian people have preferred to use along with sunscreens products. However, with low stability and the side effects of allergy and irritation from hydroquinone for many consumers, the Cosmetic Control Division of Iran has special concerns for these products.

In 1995, the FDA and the Toxicology Division of the Department of Medical Sciences, Ministry of Health, in Thailand reported that six out of 20 samples collected from the market contained more hydroquinone than the allowed amount. This was excused because of the high instability of hydroquinone, and most manufacturers had put the excess hydroquinone in their products in order to maintain a constant amount of 2.0% during storage (1).

Recently, many researchers have been involved in finding means to prevent or delay deterioration by oxidative reactions in cosmetic preparations. A variety of antioxidants, both from natural sources and synthetic processes, are available in the market. In Iran, antioxidants that are usually incorporated in hydroquinone formulations are sodium metabisulfite (SM), BHT (butylated hydroxy toluene), BHA (butylated hydroxy anisole), ascorbic acid, vitamin E, citric acid, and/or combinations of these chemicals. Their shelf life is about one year. Hydroquinone itself has been used as an antioxidant in combination with others in the concentration of 0.05–0.1% (2). Various disadvantages of some of these antioxidants have caused concern. For example, an application of BHA and BHT is now restricted in many countries, since undesirable effects from these additives on the enzymes of the liver and lungs can occur. Occasionally, the antioxidant ability of vitamin E is less active (1). More recently, research has focused on developing safer and more effective antioxidants from natural sources, such as *Rosmarinus officinalis* L., *Piper* spp., *Geranium pratensis*, *Geum urbanum*, *Viola tricolor*, *Rumex acetosa*, *Ilex paraguensis*, *Rosa* sp., licorice root, cinnamon, ginger rhizomes, *Capsicum* spp. and green tea (3–10). Some of the chemical constituents (e.g., polyphenolic flavonoids) of *Glycyrrhiza glabra* have been identified as antioxidant agents; it is possible that the synergistic effects of flavonoid mixtures may be responsible for the high activity observed in licorice extract (6,11–16). However, a study of the antioxidative activity of the extract from *Glycyrrhiza glabra* has never been performed for cosmetic preparations. *Glycyrrhiza glabra* L. is good for skin eruptions, including dermatitis, eczema, pruritus, and cysts. It has also anti-inflammatory, anti-infecting, antiseptic, antibacterial, antihepatotoxic, antiviral, and antiphlogistic properties. It is also used for gastric and duodenal ulcer (17). *Glycyrrhiza glabra* L. is native to Eurasia and cultivated in Europe (Spain, Italy, France, etc), the Middle East (Syria, Iran, Turkey, Iraq, etc), and Asia (e.g., China). The parts used are the dried roots collected in the fall (18). Hydroquinone, which is known for its high sensitivity to oxidation, has been chosen as an indicator for comparison of the antioxidative activity of licorice extract to commercial antioxidants in the form of 2% w/w hydroquinone cream.

MATERIALS AND METHODS

EXTRACTION

An amount of 250 g of the powdered dry roots of *Glycyrrhiza glabra* L. was soaked in 2500 ml of methanol (Merck, Germany) for 24 hrs. The mixture was filtered, and the filtrate was evaporated to give a yield of 21.0% w/w of dried licorice extract.

MATERIALS

Cetyl alcohol, white petrolatum, mineral oil, Tween 80, NaOH, propylene glycol, butylated hydroxy toluene (BHT), and sodium metabisulfite (MS) were purchased from Merck (Germany). Methyl and propyl paraben were provided by Kech's (USA). Pemulen TR1 was received from the B.F. Goodrich Co. (USA). Deionized water was freshly prepared.

PREPARATION OF TEST SAMPLES

Hydroquinone cream was freshly prepared. The pemulen TR1 (2% w/w) was wetted in preserved water for 24 hr and dispersed with a double-bladed mixer (Ika-Werk, Germany) in 500 rpm for 10 min in a water bath at 75°C. Separately, cetyl alcohol (3% w/w), white petrolatum (8% w/w), mineral oil (8% w/w), and Tween 80 (1% w/w) were melted in a water bath at 70°C. The latter was added to the aqueous phase, and after neutralizing by NaOH solution (18% w/w) to pH 6.2, the mixture was stirred

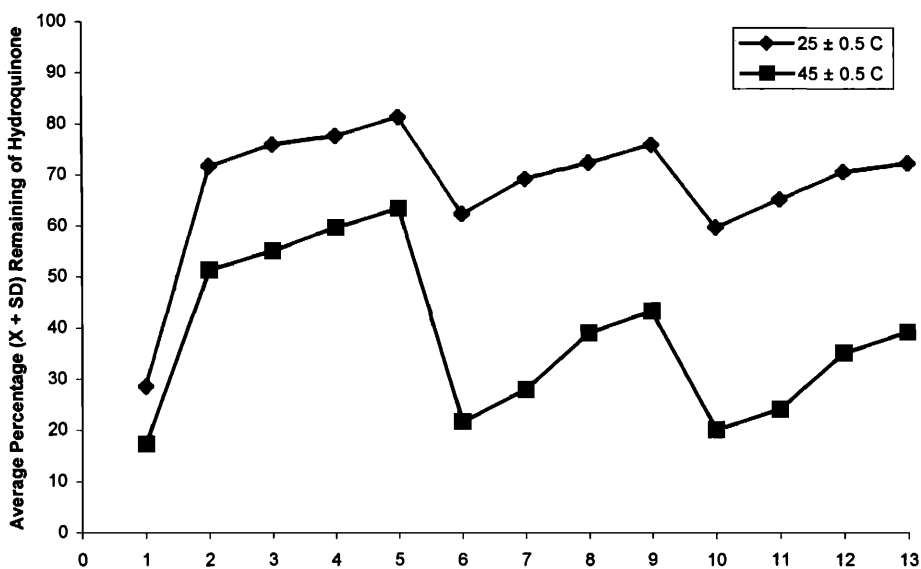


Figure 1. Comparison of the average percentage of hydroquinone remaining after incubation at 25° ± 0.5°C and 45° ± 0.5°C for three months. 1: CB (cream base) + HY (hydroquinone). 2: CB + HY + ext. (0.1%). 3: CB + HY + ext. (0.5%). 4: CB + HY + ext. (1%). 5: CB + HY + ext. (2%). 6: CB + HY + SM (0.1%). 7: CB + HY + SM (0.5%). 8: CB + HY + SM (1%). 9: CB + HY + SM (2%). 10: CB + HY + BHT (0.1%). 11: CB + HY + BHT (0.5%). 12: CB + HY + BHT (1%). 13: CB + HY + BHT (2%).

constantly until an emulsion formed. The 2% hydroquinone solution in propylene glycol was added to the cream at 40°C, and the resulting mixture was stirred while cooling to room temperature. The incorporation of licorice extract or commercial antioxidants to the formulation during preparation depends on the solubility properties. The extract (0.1%, 0.5%, 1.0%, and 2.0% w/w), levigated by propylene glycol (5% w/w) and BHT (0.1%, 0.5%, 1.0%, and 2.0% w/w), was in the oil phase, whereas sodium metabisulfite (0.1%, 0.5%, 1.0%, and 2.0% w/w) was in the water phase. The control was 2% hydroquinone cream without the extract or any commercial antioxidants.

ANTIOXIDATIVE ACTIVITY STUDY

A 10-g sample was put into a 20-ml, tightly screw-capped, test tube. One set of test samples was incubated at $45^{\circ} \pm 0.5^{\circ}\text{C}$ in an incubator (Fanazma Incubator, Iran) for three months to evaluate formulation stability. Another set was kept in a dark room at $25^{\circ} \pm 0.5^{\circ}\text{C}$ for three months. Samples at each concentration of the extract and commercial antioxidants were done in triplicate. Physical stability behaviors, i.e., changes in color and separation of emulsion, were observed optically every week. For the determination of the average percentages of hydroquinone remaining at $25^{\circ} \pm 0.5^{\circ}\text{C}$ and at $45^{\circ} \pm 0.5^{\circ}\text{C}$ after two weeks and after one, two, and three months, one gram of the tested samples was extracted with methanol and the amount of hydroquinone was measured by a UV spectrophotometer (Spectronic Genesys 2, USA) at 294 nm, according to the official standard hydroquinone assay (1,19).

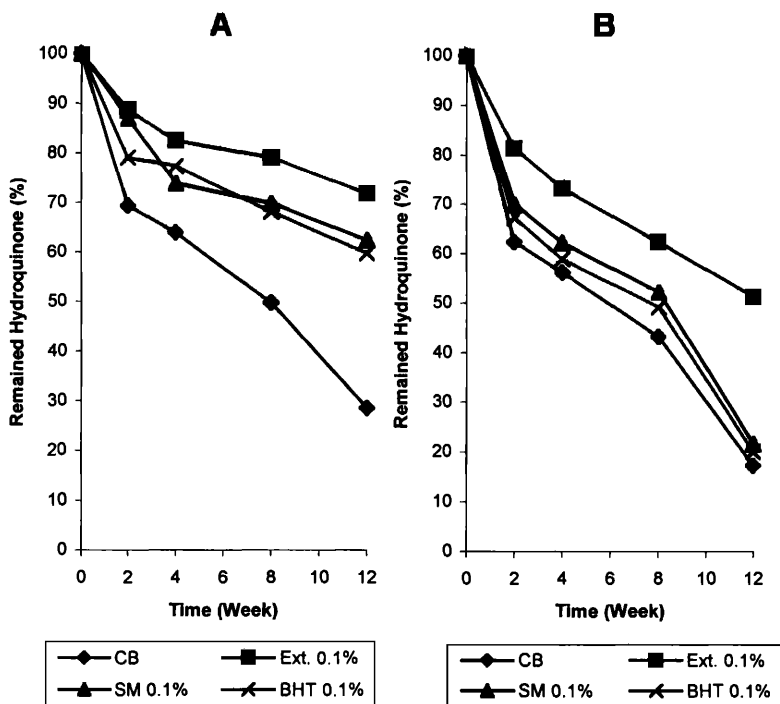


Figure 2. Formulation stability study of 2% w/w hydroquinone cream containing 0.1% extract and commercial antioxidants incubated at $25^{\circ} \pm 0.5^{\circ}\text{C}$ (A) and $45^{\circ} \pm 0.5^{\circ}\text{C}$ (B) for three months.

STATISTICAL ANALYSIS

ANOVA testing followed a Tukey test used to determine significant differences between groups, and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Powdered dry roots gave a yield of 21.0% w/w of dried licorice extract. The licorice extract is yellowish-brown in color. From physical observation (data not shown), the color of all sample systems was darker starting from the first week, with the darkest intensity in cream without any antioxidants (CB+HY) and the least dark in the system with 2.0% extract. The systems containing 2.0% sodium metabisulfite (SM) and 1.0% and 2.0% BHT showed emulsion instability. The 2.0% BHT cream started to crack from the first month, whereas 2.0% SM cream started to crack at the second month and continued. This may be due to the high concentrations of both antioxidants compared with the other test systems. BHT is a phenolic antioxidant for fatty acid and vegetable oil. Usually it is used at a level of 0.01–0.1% in cosmetics containing unsaturated materials (1). The extract systems at all concentrations (0.1%, 0.5%, 1.0%, and 2.0% w/w) were stable for three months.

When comparing the percentages of hydroquinone remaining after incubation at different temperatures for three months, the sodium metabisulfite, BHT, and licorice extract systems in all concentrations in the dark room at 25°C showed a higher remain-

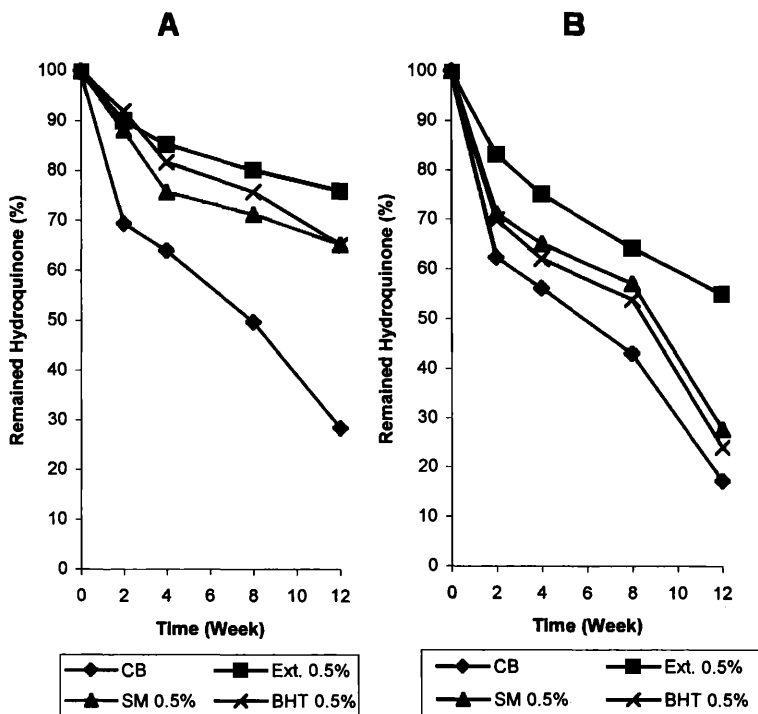


Figure 3. Formulation stability study of 2% w/w hydroquinone cream containing 0.5% extract and commercial antioxidants incubated at 25° ± 0.5°C (A) and 45° ± 0.5°C (B) for three months.

ing level of hydroquinone than those incubated at 45°C ($p < 0.05$). This indicated the effects of temperature in accelerating the oxidative degradation of hydroquinone (Figure 1). Figures 2–5 compare the average percentages of hydroquinone remaining in 2% w/w hydroquinone cream containing licorice extract and the commercial antioxidants kept at 25°C and 45°C for three months. Both water-soluble antioxidant (sodium metabisulfite) and oil-soluble antioxidant (BHT), as well as the extract at all concentrations, showed more hydroquinone remaining than in the control system after incubation at 25°C and 45°C for three months, with the exception of 0.1% SM and 0.1% BHT systems at 45°C ($p < 0.001$).

The difference in antioxidant activity between the systems of extract and SM or BHT at all concentrations was not significant at 25°C after two weeks ($p > 0.05$). This difference was not observed between the systems of extract and BHT at all concentrations at 25°C after one and two months ($p > 0.05$). The licorice extract at all concentrations showed more hydroquinone remaining than in the systems of SM and BHT at 25°C and 45°C after three months ($p < 0.05$). The comparison of antioxidant activity of licorice extract systems showed that there was no significant difference between 1.0% and 2.0% extract systems at 25°C and 45°C during a three-month period.

Both water-soluble and oil-soluble antioxidants and licorice extract in our study showed some protection from oxidative degradation in hydroquinone (but not 100% protection) during a three-month period. The extract systems were more effective than other com-

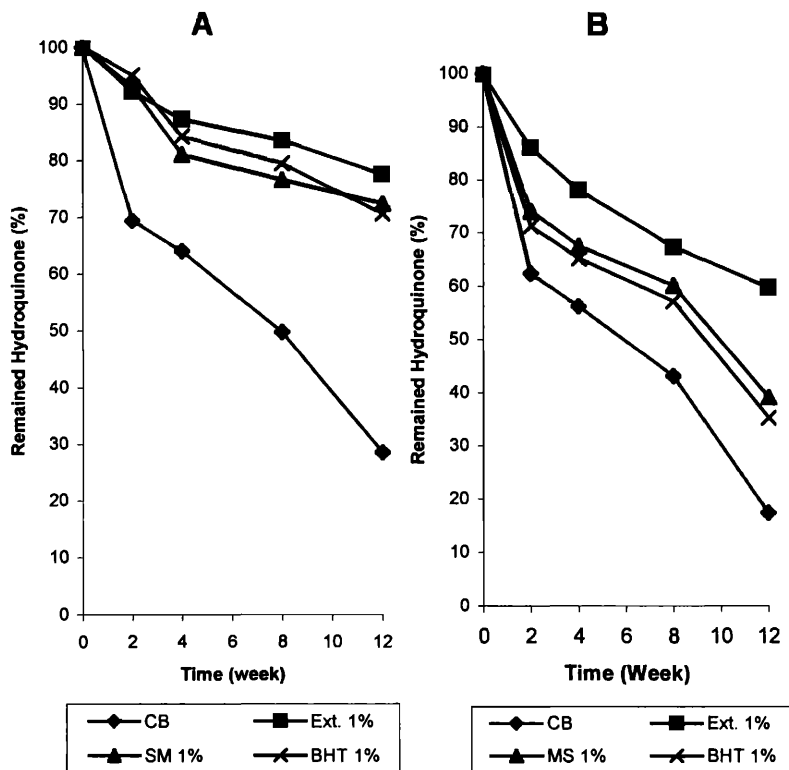


Figure 4. Formulation stability study of 2% w/w hydroquinone cream containing 1.0% extract and commercial antioxidants incubated at 25°C ± 0.5°C (A) and 45°C ± 0.5°C (B) for three months.

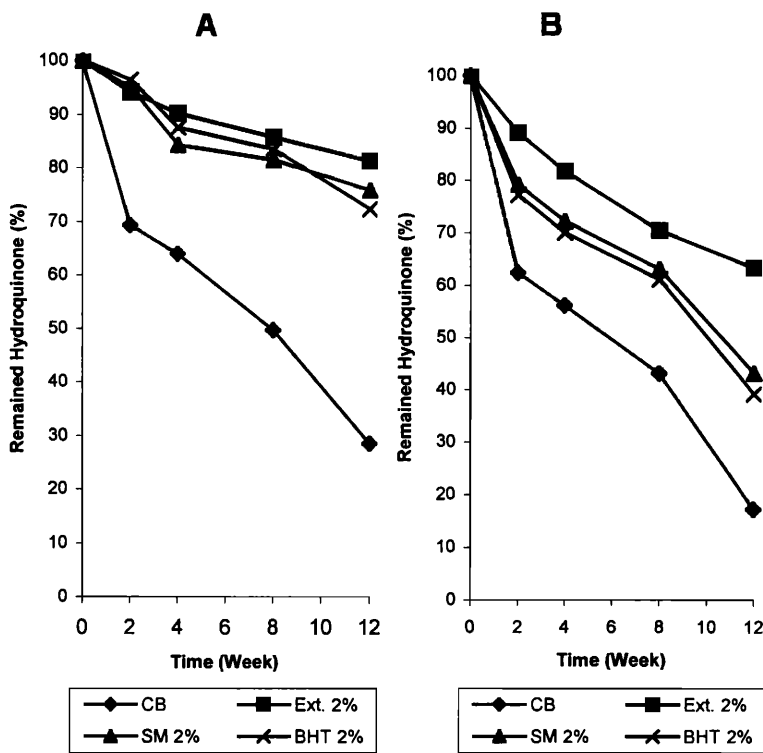


Figure 5. Formulation stability study of 2% w/w hydroquinone cream containing 2.0% extract and commercial antioxidants incubated at $25^{\circ} \pm 0.5^{\circ}\text{C}$ (A) and $45^{\circ} \pm 0.5^{\circ}\text{C}$ (B) for three months.

mercial antioxidants. According to our procedure, by incorporating hydroquinone after the cream was formed, and together with the solubility property of hydroquinone that is freely soluble in propylene glycol and slightly soluble in oil and water, we had expected that hydroquinone had been absorbed in both the oil and the water phases of the formulation. It is also evident that hydroquinone can be incorporated in the formulation in different ways. Besides our procedure, hydroquinone was used in the oil phase with laevo-ascorbic acid as antioxidant, and in the water phase with sodium metabisulfite and ascorbic and citric acids as antioxidants (1). This meant that the types of antioxidants selected for use in the formulation depended on the method of the incorporation of hydroquinone into the system. In our hydroquinone systems containing extract, the licorice acted as both water- and oil-soluble antioxidant agents and demonstrated significant protection from oxidative degradation in hydroquinone for three months, in comparison with sodium metabisulfite and BHT that were water-soluble and oil-soluble, respectively.

CONCLUSION

The licorice extract at 0.5% and 1.0% can be used as a double-action (both water- and oil-soluble) antioxidant, having 76% and 78% (at 25°C) and 55% and 60% (at 45°C) of hydroquinone remaining, respectively, after three months. These results indicate that

licorice extract is more effective than sodium metabisulfite and BHT, and may be used as a substitute for commercial antioxidants in oxidation-sensitive formulations.

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