RESEARCH ARTICLE

In-vitro Evaluation of Antioxidant Activity of Glabridin in Combination with Oroxylin-A

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ABSTRACT

There has been a significant increase in interest in the study of free radicals due to shifting dietary and environmental patterns. Our bodies can become exposed to diverse physiochemical circumstances, pathogenic states, or distinct endogenous systems that produce free radicals. Oxidative stress is a condition caused by the body's exposure to free radicals. We have to give the body an antioxidant source when it can't handle more oxidative stress. A material that prevents oxidative damage to a target molecule, which is mostly brought on by free radicals, is referred to be an antioxidant. Antioxidants are essential for treating numerous ailments or are used as a supportive treatment for a wide range of disorders brought on by or made worse by oxidative stress. Glabridin and oroxylin-A are well-known active phytoconstituents with high anti-obesity activity. Antioxidants' hypolipidemic action and potential are very helpful in the treatment of disorders associated with obesity. The current investigation attempts to assess the antioxidant capacity of both oroxylin-A and glaciridin. Ascorbic acid was utilized as the reference antioxidant agent for comparison while evaluating the antioxidant activity. According to the findings, both active ingredients exhibit substantial antioxidant activity more than 65% greater than regular ascorbic acid. When combined with oroxylin-A, glabridin exhibits even greater antioxidant activity more than 80% higher than that of regular ascorbic acid.

Keywords: Glabridin, Oroxylin-A, Free radicals, Antioxidant.

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INTRODUCTION

Free radicals, which can harm any cell in the body they come into contact with and have dangerous effects, are a regular part of modern life.[1,2] The primary cause of all the harm that free radicals bring to the organism is a condition of elevated oxidative stress brought on by the radicals.[3,4] Even though the body contains defenses against oxidative stress, the issue worsens if exposure exceeds the body's tolerance.[5-7] Antioxidants are chemicals that help counteract the negative effects of free radicals.[8,9] A class of substances known as antioxidants work to neutralize or stop free radicals and other reactive species, preventing the oxidative potential of these substances from damaging cells or tissues.[10-12] Numerous natural materials have had their antioxidant properties investigated.[13-16] To identify more effective substitute sources for the treatment of illnesses linked to oxidative stress brought on by free radicals, it is still necessary to assess novel compounds for their antioxidant capacity. Glabridin from *Glycyrrhiza glabra* and oroxylin-A from *Oroxylum indicum* are well known to have anti-hyperlipidaemic activity and anti-obesity activity.[17-20] Oxidative stress can lead to obesity as well as be its cause. Prolonged obesity can initiate internal processes through multiple biochemical pathways that lead to stress (oxidative). These include oxidative phosphorylation, polyols and hexosamines pathways, PKC activation, and superoxide production by Nox.[21-23] Stress (Oxidative) may be a reason of obesity because it alters food intake and encourages the deposition of white adipose tissue. Moreover, obesity has been demonstrated to enhance stress (Oxidative); in fact, the accumulation of fat increases stress in the endoplasmic reticulum (ER) and Nox activity in adipocytes, amplifying reactive oxygen species (ROS).[24-25] Since glabridin and oroxyiln-A have anti-obesity activity and can be used in the treatment of obesity, they may also have antioxidant activity. They are previously shown to have antioxidant activity individually, so the present investigation was undertaken to investigate antioxidant effects in combination with *in-vitro* antioxidant evaluation. DPPH and hydrogen peroxide free radical scavenging methods were used to evaluate antioxidant activities. For comparison purposes, ascorbic acid was utilized as the standard antioxidant substance.

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MATERIALS AND METHODS

Materials

Glabridin & oroxylin-A were procured from Sigma Aldrich. Hydrogen peroxide, ascorbic acid, and DPPH from KJ Fine Chemicals, Mumbai, India. Other chemicals used were exactly as supplied and were of AR grade.

Antioxidant Activity

Evaluation of DPPH scavenging activity

By dissolving or dispersing each in distilled water, ascorbic acid, glabridin, and oroxylin-A solutions were created with 5 to 250 mcg/mL dilutions. A total of seven dilutions were prepared in this experiment to evaluate the antioxidant activity. About 100 mL of ethanol was mixed with four milligrams of DPPH to make a 0.004% w/v solution. The mixture was allowed to create the DPPH radical by being placed in a dark room for the entire night. An aliquot was filled with 0.1 mL test sample both alone and in combination at various doses with 3 mL of the 0.004% DPPH solution in ethanol. After 30 minutes of settling at room temperature, the mixture was well shaken. The de-colorization of DPPH was observed using the absorbance of 517 nm. The control was made with 0.1 mL of distilled water rather than the test sample. The %inhibition was estimated using the formula:

$$Inhibitory\ Ratio = \frac{(A0 - A1)}{A0}$$

A0: Absorbance of control;
*A1: A*bsorbance of test sample

H_2O_2 scavenging activity

Using the same test and standard dilutions, hydrogen peroxide scavenging activity was used to estimate the antioxidant potential of oroxylin-A & glabridin. Solution of H_2O_2 (0.002% v/v) prepared by adding distilled water to a volumetric flask that held 100 µL of 30% hydrogen peroxide. From here, 1-mL of H_2O_2 was moved to a flask, where it was combined with 100 mL of distilled water.

During the investigation, a fresh solution of buffer (Phosphate) (pH 7.4) was made.

- *Solution (A)*

A total of 276 mg of NaH2PO4 was weighed and added distilled water to the beaker to get the volume 100 mL.

- *Solution (B)*

The capacity was makeup to 100 mL with water and the mixture was transferred after 568 mg of Na2HPO4 had been weighed.

The pH was raised to 7.4 by combining 12 mL of solution (A) & 88 mL of solution (B) from the previously mentioned solutions. For the free radical experiment, freshly manufactured solutions of peroxidase 0.1 mg/mL and phenol red 0.2 mg/mL were also made. Two milligrams of phenol red and one mg of Horseradish peroxidase (HRP) dissolved in 10 mL of 100 mM buffer Mix. The standard and test solutions were mixed with 0.8 mL of buffer & 100 µL of 0.002% H_2O_2. The mixture was then pre-incubated for 10 minutes at 37°C for each 100 µL of solution. About 1-mL of phenol red dye with HRP mixture was added to this reaction mixture. About 15 minutes later, 1-M NaOH in volume of 50 µL was added, and the reading was immediately observed at 610 nm. The control was made with 0.1 mL of distilled water rather than the test or standard solution. The test sample's %inhibition of radical was computed using the same formula as the DPPH scavenging evaluation that was previously discussed.

RESULTS

DPPH Scavenging Activity

Free radical scavenging activity of either or both of the substances, glabridin and oroxylin-A, was found to be significantly active. The combined antioxidant potential of oroxylin and glaciridin was significantly greater than the individual compounds' respective capacities. Furthermore, it was discovered that the antioxidant activity of normal ascorbic acid and both glabridin and oroxylin-A was dose-dependent. When compared to ascorbic acid, glabridin and oroxylin-A displayed roughly 60 and 65% of antioxidant activity, respectively. Figure 1 presents the findings.

H_2O_2 Scavenging Activity

To combine the results of antioxidant potential, the potency of both oroxylin-A and glabridin alone and jointly was

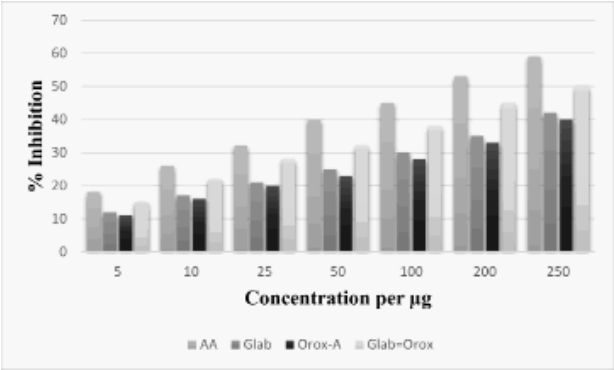

Figure 1: Antioxidant activity of glabridin and oroxylin-A using DPPH scavenging

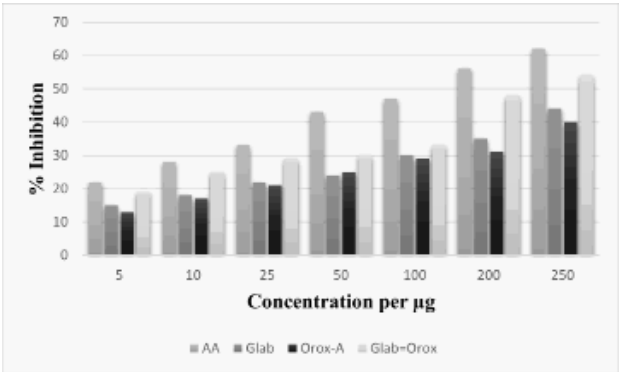

Figure 2: Antioxidant activity of glabridin and oroxylin-A using H_2O_2 scavenging

compared with hydrogen peroxide's antioxidant potential. The results are displayed in Figure 2. The findings show that oroxylin-A and glabridin have strong antioxidant action. In a hydrogen peroxide scavenging experiment, the combination of glabridin and oroxylin-A exhibited more than 85% antioxidant activity when compared to ascorbic acid. The outcomes also demonstrate that the combination of oroxylin-A and glabridin has a comparatively higher antioxidant capability than either compound alone.

CONCLUSION

The study's findings support the strong antioxidant activity of the active phytoconstituents, oroxylin-A and glabridin, found in traditionally utilized herbal plants. Furthermore, the activity is similar to that of ascorbic acid since, when examined individually, both glabridin and oroxylin-A exhibit more than 60% of the antioxidant activity of ascorbic acid, and when evaluated in combination, they exhibit more than 85% of the antioxidant activity. One possible explanation for their therapeutic advantages could be their antioxidant capacity. More activities impacted by antioxidant capacity, such as antineoplastic, hematinic, anti-diabetic, or protective properties, need to be investigated for these strong phytochemicals.

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