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A modified, economic, sensitive method for measuring total antioxidant capacities of human plasma and natural compounds using Indian saffron (*Crocus sativus*)

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Abstract

Background: Free radicals are involved in various human diseases that can possibly be prevented by antioxidants. There are many but rather expensive methods to determine total antioxidant capacity of human plasma (for endogenous antioxidant levels) or plant extracts/natural compounds (for antioxidant potential in terms of radical inhibiting or scavenging properties). We describe a simple, fast and economical 'crocin assay' using the Indian spice saffron.

Methods: In crocin assay, the extent of bleaching of crocin, a carotenoid from saffron, by peroxyl radicals generated by thermal decomposition of azo-initiator was measured. We examined its applicability to clinical samples and plant extracts.

Results: The cost of Indian saffron is almost 38 times less per unit dry weight compared to the 'Sigma' saffron. Yet, it gives 26 times better yield of crocin than that from 'Sigma' saffron. It was also shown that Indian saffron is rich in crocin. The total antioxidant capacity (TAC) values of human plasma from normal, healthy individuals, using Sigma as well as Indian crocin, expressed in terms of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC), were comparable. We have also demonstrated that crocin assay can be used for clinical samples such as plasmas from healthy and diabetic individuals. The antioxidant potentials, TEAC, of plant extracts and pure natural compounds by Indian and Sigma crocin assays were similar. Addition of uric acid to plasma induced a concentration-dependent response. The assay was compared to standard radical scavenging 1,1′-diphenyl-2-picrylhydrazyl (DPPH) assay and was found to match well, showing better sensitivity and hence validates this assay for natural compounds and clinical samples.

Abbreviations: AAPH, 2.2′-azobis (2-amidinopropane) dihydrochloride; ABTS, 2,2′-azobis-3-ethylbenzthiazoline-6-sulfonic acid; CVD, cardiovascular diseases; DPPH, 1,1′-diphenyl-2-picrylhydrazyl; FRAP, Ferric reducing antioxidant power; IC_{50}, Concentration which gives 50% inhibition; ORAC, Oxygen Radical Absorbance Capacity; PBS, Phosphate-buffered saline; TAC, Total antioxidant capacity; TEAC, Trolox equivalent antioxidant capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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Conclusions: Development of crocin assay using the Indian saffron is economical and sensitive method for measurement of total antioxidant capacities from human plasma as well as natural compounds and plant extracts.

Keywords: Crocin bleaching assay; Saffron; DPPH assay; Plasma antioxidant capability; Natural antioxidants; Uric acid

1. Introduction

Free radical-induced oxidative damage is involved with various human diseases such as cardiovascular diseases (CVD), neural disorders such as Alzheimer's and Parkinson's disease, diabetes and cancer. Antioxidants are substances that delays or inhibits oxidative damage when present in small quantities compared to an oxidizable substrate. Therefore, antioxidants can help in disease prevention by effectively quenching free radicals or inhibiting damage caused by them [1,2].

Many methods have been developed for measuring the total antioxidant capacity (TAC) in vitro or for measuring free radicals or their actions. These methods for measuring antioxidant capacity are mostly based on quenching of stable free radicals such as 1,1′-diphenyl-2-picrylhydrazyl (DPPH) [3], 2,2′-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) [4] by antioxidants; inhibition of lipid peroxidation [5,6]; ferric reducing antioxidant power (FRAP) [7]; fluorimetric measurement of β-phycoerythrin (in the ORAC, Oxygen Radical Absorbance Capacity assay) [8]; etc. These assays are useful due to their sensitivity and minimal sample preparation and thus are widely used in biochemical analysis of clinical samples [9–11]. However, they are time consuming and utilize expensive chemicals and/or instrumentation. Crocin assay is one such method, which measures the total antioxidant capacity (TAC) of the biological fluid or plant extracts and pure natural compounds [12]. Crocin is a water-soluble carotenoid derived from dry stigmata of *Crocus sativus* L. (saffron), and this pigment is responsible for the red color of saffron.

The rate constants of crocin with different radicals in aqueous solution are known [13]. Crocin is bleached both by reducing and oxidizing radicals but not by superoxide (O_2^-) and methyl (CH_3) radicals. The high molar absorptivity of 1.35×10^{-5} M^{-1} cm^{-1} at 440 nm allows for sensitive measurements, especially with alkoxyl and peroxyl radicals. Hence, in 'crocin assay', depletion of the absorbance of crocin is measured spectrophotometrically after radical attack induced by thermal decomposition of azo-initiator, 2.2′-azobis (2-amidinopropane) dihydrochloride (AAPH), at a constant lapse of time. This assay is particularly important for clinical tests because of the advantage of microplate-based technology, making it a rapid and precise assay for a number of samples at the same time. This simple spectrophotometric kinetic assay has been used for the measurement of total peroxyl radical-trapping capacity of antioxidants from complex mixtures of food components [14] and human plasma samples [12].

The present study is based on comparison of the bleaching of carotenoid crocin extracted from saffron from Sigma and an Indian local market. In India, saffron, the most expensive spice of the world, is cultivated largely at Kashmir in northern India. Saffron is used extensively in the Indian medicinal system, Ayurveda, to heal a variety of diseases such as arthritis, cold, asthma, acne, skin disorders, impotence and infertility [15]. It has been used in culinary purposes for cooking and coloring foods. In India, it is also employed in large quantities in auspicious occasions and weddings. Hence, it is easily available and at a lower cost as compared to that from chemical suppliers such as Sigma.

The previously reported crocin assay for measurement of antioxidant activities was based on the use of crocin isolated from saffron from Sigma. The results of the crocin assay are based on the effectiveness of the crocin extracted from saffron and its concentration in each batch. However, the cost of saffron, available commercially, is very high, i.e., US$ 37.7/g of saffron (Sigma 2004-5 catalogue), which is a large expense for clinical trials, especially from developing countries such as India.

We used saffron available in Indian markets as the initial raw material for extraction of crocin. Saffron