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Kumar Katragunta
University of Mississippi

Bharathi Avula
University of Mississippi

Ya-Hong Wang
University of Mississippi

Roy Upton
University of Mississippi

Ikhlas A. Khan
University of Mississippi

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Kumar Katragunta[a], Bharathi Avula[a], Yan-Hong Wang[a], Roy Upton[b], and Ikhlas A. Khan[a,c]

[a]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677 USA.
[b]American Herbal Pharmacopoeia, 3051 Brown's Lane, Soquel, CA 95073 USA.[c]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

ABSTRACT

Saffron, the most expensive spice in the world, is comprised of dried and dark red stigma of *Crocus sativus* L. flowers of the Iridaceae family. It is mainly used as a spice for imparting color, fragrance, and flavor to food, but its medicinal and dyeing properties are also well known. In the United States, saffron products are used as dietary supplements for mood elevation, relaxation, weight loss, and to increase metabolism. This paper describes two analytical methods for the determination of crocetin esters, picrocrocin, and safranal in saffron samples and dietary supplements for inclusion in a monograph under development by the American Herbal Pharmacopoeia. Method validation showed satisfactory results in linearity, precision and recovery. The content of picrocrocin, safranal and crocetin esters ranged from 0.6-10.2%, 0.02-0.22%, and 2.8-25.6%, respectively for thirty-seven stigma samples. Twenty-nine dietary supplements were analyzed. No saffron compounds were found in 16 (55%) of these products. Flower of Carthamus tinctorius and fruit of *Gardenia jasminoides* were the main adulterants detected in dietary supplements. Summarily, 60 compounds, including five standards were tentatively identified from saffron stigma, style, and petal samples using high resolution mass spectrometry. Principle component analysis was used to discriminate between saffron stigma samples and dietary supplements. The results indicated that commercial products are of variable quality and that this analytical method is suitable for quality assessment of a variety of both raw material and finished dietary supplements.

INTRODUCTION

- Saffron consists of the dried stigmas of *Crocus sativus* L. The stigmas have been used as a common spice to impart color, flavor, and fragrance to foods most commonly used in Middle Eastern and Spanish cuisine.
- The culinary qualities of the plant, namely color, flavor, and fragrance, are correlated with the putative medicinal properties specifically as reflected in the chemistry of the stigmas that contain picrocrocin, safranal, and crocetin esters [1-2].
- The methods reported herein include quantitative analysis of five compounds (two crocetin esters, *trans*-crocetin, safranal, and picrocrocin) for saffron whole flower and flower parts (stigma, style, petal) and dietary supplements using high-performance liquid chromatography-photodiode array detector (HPLC–PDA) and ultra-high-performance liquid chromatography-photodiode array detector-mass spectrometry (UHPLC-PDA-MS).
- The LC-QToF untargeted fast screening method and chemometrics analysis described in the present study were used to investigate the authenticity of saffron samples and dietary supplements. The presence of dyes in some commercial samples could also be detected establishing the usefulness of both methods for fostering compliance with good manufacturing practices for saffron.

CHROMATOGRAPHIC CONDITIONS

Liquid Chromatography-Time of flight mass spectrometry (LC-DAD/QToF-MS)

The liquid chromatographic system was an Agilent Series 1290 comprised of the following modular components: binary pump, a vacuum solvent degasser, an autosampler with 108-vial well-plate trays, a thermostatically controlled column compartment, and a diode array detector (DAD). The separation was achieved on a Poroshell 120 EC-C18 (150 × 2.1 mm, 2.7 µm) column. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) at a flow rate of 0.2 mL/min, with gradient elution of 0 min, 1% B; 3 min, 20% B; 25 min, 63% B; 30 min, 100% B. A 5-min wash followed each run with 100% B and an equilibration period of 5 min with 1% B. Two microliters of the sample were injected, and the column temperature was set at 40°C.

The mass spectrometric analysis was performed with a QToF-MS/MS (Model #G6530A, Agilent Technologies, Santa Clara, CA, USA) equipped with an ESI source with Jet Stream technology using the following parameters: drying gas (N2) flow rate, 11.0 L/min; drying gas temperature, 325°C; nebulizer, 30 psig, sheath gas temperature, 300°C; sheath gas flow, 11 L/min; capillary, 3500 V; skimmer, 65 V; and fragmentor voltage, 100 V. The acquisition was controlled by MassHunter Acquisition Software Ver. A.05.01 and the data were processed with MassHunter Qualitative software Ver. B.07.00, which provided a list of possible elemental formulas using the Generate Molecular Formula. Samples were analyzed in all-ion MS–MS mode, where experiment 1 was carried out with collision energy of zero and experiment two with a fixed collision energy of 40 eV. Accurate mass measurements were obtained by means of reference ion correction using reference masses at *m/z* 121.0509 (protonated purine) and 922.0098 [protonated hexakis(1H, 1H, 3H-tetrafluoropropoxy) phosphazine or HP-921] in positive ion mode.

REFERENCES

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[2] D'Archivio AA, Giannitto A, Maggi MA, Ruggieri F (2016) Geographical classification of Italian saffron (*Crocus sativus* L.) based on chemical constituents determined by high-performance liquid-chromatography and by using linear discriminant analysis. Food Chem 212:110–116.

RESULTS AND DISCUSSION

- All five compounds were detected in all authenticated stigma samples of saffron. The compounds from stigma of various samples were in the range of 0.6–9.9%.
- Of the twenty-nine dietary supplement products tested, only 11 (38%) showed a profile similar to authenticated saffron. Most of the products were labeled to contain either stigma powder or extract. Twelve (41%) preparations were not consistent with their labels in respect of the flower part and/or 18 (62%) preparations had no claimed concentrations of crocins or safranal. The remaining 11 products claiming to contain lepticrosalides showed variations in the detectable amounts whereas nine preparations did not meet the standards stated on their labels.
- This study demonstrates that multivariate statistical analysis (Fig. 2) using LC-QToF provides useful information in the quality assessment of saffron and select dietary supplements. It can be used as a powerful tool to profile and differentiate various phytochemical compositions among different samples.
- 15 of 29 dietary supplements tested showed the presence of hydroxysafflor yellow A, safflor yellow A, anydrosafflor yellow B, carthamin, and kaempferol derivatives, indicating the presence of safflower (*Carthamus tinctorius*). Geniposide, the main component of gardenia (*Gardenia jasminoides*) fruit was detected in two supplements indicating adulteration.

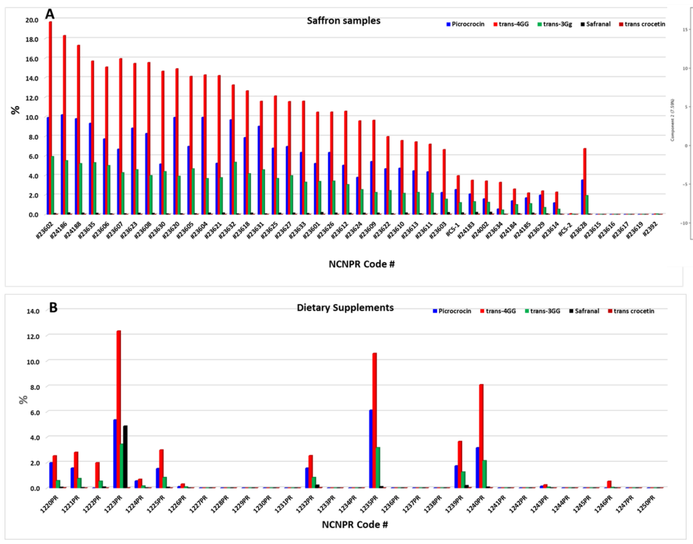

Fig. 1 Content (%, *w/w*) of five compounds in saffron samples (A) and dietary supplements (B)

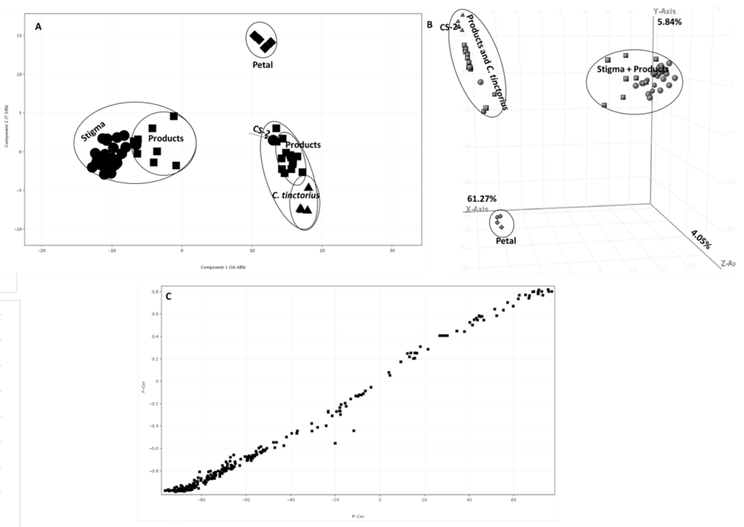

Fig. 2 (a) 2D PCA score plot, (b) 3D PLS-DA, (c) 2D loading plot showing the similarities and differences between saffron samples illustrating good separation according to positive ionization mode data (intensities of 231 MFs, intensity threshold 3000 cps)

CONCLUSIONS

- There are a plethora of analytical methods and standards currently available. Many of these lack the ability to simultaneously identify, quantify relevant compounds, and detect adulterants in a single analysis.
- The high cost of saffron as a spice, its potential health benefits, and investigation in clinical trials, together with its high propensity for adulteration require that appropriate identity and quality control standards be developed.
- The UHPLC-PDA-MS and HPLC–PDA methods developed can accomplish each of these analytical goals in a single analysis with shorter retention times, while maintaining good resolution.
- A nontargeted LC-QToF analysis that included key reference compounds was carried out to profile the chemical constituents of authentic saffron and twenty-nine dietary supplements.
- The tentative identification of marker and unknown compounds was performed by the accurate mass and fragment ions. PCA and PLS-DA were applied to the various samples as a quality assessment to differentiate their chemical profiles.
- Commercial products revealed significant product-to-product variations with 16 of 29 products not containing any of the saffron compounds analyzed.
- These analytical methods provide an alternative, fast, and accurate tool for the quality assessment of saffron raw material and select dietary supplements.

ACKNOWLEDGEMENTS

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