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NON-VOLATILE CHEMICAL COMPOSITION OF SAFFRON NATIVE TO JAMMU AND KASHMIR

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ABSTRACT

The current study was aimed to analyse the non-volatile chemical profile of saffron indigenous to the Valley of Kashmir. The prepared methanolic extract was subjected to LC-MS/MS analysis and 30 compounds were identified, mainly crocins, picrocrocin, kaempferols, hesperetin, malvidin-3-O-beta-D-galactoside, caffeic acid, yohimbinic acid etc. The compounds were found to be mainly flavonoids and phenolic in nature, these small bioactive molecules have potential to cure and prevent different diseases by playing role as anti-oxidant, antitumor, cytotoxic, anti-apoptotic, antidepressant etc. The complex chemical profile of saffron enables this spice to be used by multiple industries as a major ingredient in different formulations including pharmaceutical, cosmaceutical, neutraceuticals and aromaceuticals.

I.INTRODUCTION

The dried stigmas of fascinating crop of *Crocus sativus* are the source of world's costliest spice in the form of Saffron. The most important saffron-producing countries are Iran, followed by India, Greece, Morocco, Italy and Spain [1]. There are excellent uses of saffron known throughout the history both as an exotic condiment as well as a preventive or therapeutic agent with cytotoxic, antioxidant, antimicrobial, antidiabetic, antidepressive etc. properties [2,3]. The chemical composition of saffron is very diverse in the form of volatile and non-volatile compounds, indeed the diverse chemical profile of it is gaining much more interest among the researchers for its scientific exploitation. Saffron contains marker chemical compounds in the form of apocarotenoids such as crocin (for yellowness), picrocrocin (for flavor) and safranal (for aroma). These three marker compounds form integral parameters for detection of saffron quality using ISO-3632 clause. The various non-volatile components identified majorly are crocin, α-crocin, carotenoids (alpha and beta carotenes, lycopene and zeaxanthine, crocetin, and picrocrocin. Different qualities of saffron are available in the world depending upon the methods of harvesting, processing and packaging in their respective regions; due to these reasons there is expected dynamism present with respect to the chemical composition of saffron [1]. Also, crocin and

picrocin, the two main non-volatile components are highly sensitive to light and air which demands a proper drying method to be followed to retain the maximum chemical components in it.

The saffron of Pampore Kashmir, India is the world famous because of its intense dark reddish stigmas and aroma. There are thousands of people involved in the production and trade of this cash crop within the Kashmir Valley and challenges faced by the saffron industry are immense due to uncontrolled urbanization of saffron land, inappropriate marketing structure, adulteration, lesser production caused by pollution of cement factories etc. Indeed, because of white collar saffron fraud in Kashmir, this industry is dying a silent death as this is a major bottleneck which hampers its cultivation, trade and motivation among growers. There is a paramount importance to explore the chemical composition of saffron in a robust way and to provide a systematic approach for identification of its components, this will lead further opportunity to isolate and characterize the potential biomolecules of saffron indigenous to Kashmir; further, quality of saffron is a major concern and determination of chemical profile will act as a fingerprint for identification of genuine and fake saffron. More importantly, such measures are vital so as to prove scientifically the potential saffron components present within the native cultivars of Kashmir. Besides, there is no concrete literature available about analysis of Pampore Kashmir saffron composition using LC-MS analytical technique.

Therefore, keeping in view the importance of deciphering the chemical composition of saffron located in different regions of the world, the current study was under taken to analyze non-volatile chemical composition of saffron indigenous to Pampore Kashmir, India using Liquid Chromatography/mass spectrometry (LC/MS) technique.

II. MATERIALS AND METHODS

Plant Material Collection and Drying Procedure: Saffron flowers were handpicked early morning in their closed conditions during Oct-Nov 2015 from the world famous saffron karewas of Pampore Kashmir. The samples were placed in air zipper pouch polythene bags; stigmatic portion of flowers were manually separated back in the Laboratory at 24 ^{0}C and an already defined microwave (1000w, 4 mins) drying procedure was followed in the Department of Chemistry, University of Kashmir [4].

Sample Preparation: The dried saffron sample of 0.5 gram was weighed, grind into powder using electric blender and immediately allowed for cold extraction in 5 ml of HPLC grade methanol (Merck, Mumbai, India) by using shaker incubator in dark at 25 ^{0}C for 3 days [2]. The extract so obtained was filtered using Whatman filter paper; allowed to reduce further in volume using rotary evaporator and extract was stored in light protected screw capped brown colored bottles at 5°C until further processing.

Liquid Chromatography/Mass Spectrometry (LC/MS-MS) analysis: The prepared saffron sample was subjected to Liquid Chromatography Mass Spectroscopy (LC/MS) for determination of its non-volatile chemical composition using already defined method with slight modifications [5]. Ten microliters of filtered extract was injected into an Agilent 1100 HPLC chromatograph (Palo Alto, CA) equipped with UHPLC Dionex C18 RP Acclaim 120 Å, 2.1 × 150 mm, 3.0 μm column (Dionex, USA) thermostated at 20 °C. The solvents were Water

(acidified with formic acid, 0.1%) (A) and acetonitrile (B) with UV at 325 nm, 0.2 mL/min flow rate, gradient mobile system start with 0.2min at 1% ACN and 99% water (1% acetic acid) to 75% ACN at 16th min, this was brought to 100% ACN at 19th min to 5% ACN at 21st min and was maintained at same condition till run ends at 25^{th} min. The Spectra were recorded using MSMS Bruker Q-II TOF spectrophotometer; the MS conditions were electrospray ionization (ESI) in Positive and negative ion modes, Nebulizer 30.5 psi with 6.0 l/min N_2 flow, m/z range: 50-1500 m/z, Capillary voltage 4500 V, dry heater temperature at 280°C.

III. RESULTS AND DISCUSSION

The non-volatile chemical profiling of Indian saffron samples was analysed using LC-MS/MS technique. Names of the identified compounds and their respective molecular masses are listed in Table 1. There were total 30 compounds identified from methanolic extract of saffron, majority of such compounds were found during Electron Spray Ionization negative mode (ESI^-) as compared to ESI^+ mode. The study have shown that there are 8 classes of crocins found with different molecular weights i.e. **Compound 1, 12, 20, 21, 24,25 and 27**, the chromatogram of representative class of crocin (Molecular weight-976.4) is presented under Fig1.A. The compound responsible majorly for taste of saffron was observed during ESI^- mode in the form of Picrocrocin; possessing molecular weight of 330.3 (**Compound 2**) and its chromatogram is depicted in Fig.1B. Different types of phenolic and flavonoid compounds were identified in saffron extract such as Chrysoeriol 7-O-glucoside, Kaempferol trihexoside, Kaempferol diglycoside, Caffeic acid and hexose derivative, Hesperetin, Kaempferol-3-O-glucoside, Malvidin-3-O-beta-D-galactoside, 3-Hydroxy-3',4',5'-trimethoxyflavone, Petunidin-3-O-beta-glucopyranoside, 3-Hydroxy-3',4',5'-trimethoxyflavone, Apigenin-7-O-neohesperidoside, 3,7-Dimethoxy-3-hydroxyflavone, 6-O-pentosyl luteolin, Kaempferol-3-O-rutinoside-7-O-gluose I, Rhamnosyl luteolin derivative, Iristectorin A, Eriodictyol-7-O-glucoside, Haploside D (flavonoid) and Vitexin. Besides, an alkaloid compound in the form of Yohimbinic acid (**Compound 4**) was found to be present in saffron with molecular weight of 340.2 and its chromatogram is presented under Fig.1 C, this is the first report mentioning the presence of Yohimbinic acid in Indian saffron. The idemtification of the chemical compounds was done using blend of three resources i.e. Agilent Chemstation software, Massbank [6] and comparison of chromatograms with previous literature [5, 7,8].

Saffron has been shown to be beneficial in the possible treatment of various diseases and thus possesses potential biological properties such as anti-oxidant, antitumor, cytotoxic, anti-apoptotic, antidepressant, cardiovascular disorders, analgesic, asthma, aphrodisiac, anti-diabetic, anti-inflammatory, chemopreventive, gynaecological purposes, fairness enhancer etc. [9, 10,11]. The complex chemical composition of saffron comprising of range of compounds like apocarotenoids (Crocin, picrocrocin and safranal), monoterpenes, flavonoids, phenolics, anthocyanins, glycosides and alkalides, Yohimbinic acid etc. [12]. Furthermore, LC-ESI-MS as a technique is largely used nowadays for qualitative fingerprint of herbal extracts and particularly for phenolic compounds [8]. Polyphenolics and flavonoid compounds are important biologically active natural constituents that have positive effects on human health such as antioxidant property,

protective effect against cardiovascular diseases, highly effective scavengers of free radicals [8] without any toxicity or adverse events.

IV. CONCLUSION

30 compounds were identified in saffron samples native to Kashmir, these small bioactive molecules have potential to cure and prevent different diseases by playing role as anti-oxidant, antitumor, cytotoxic, anti-apoptotic, antidepressant etc. Based on these findings of this study; it is suggested that dietary intake of small amounts of natural sources of antioxidants such as saffron could decrease prevalence of different diseases; also isolation and characterization of chemical profile of saffron could act added novel dimensions for its sustainable use towards food, pharmaceutical, neutraceutical and cosmaceutical industries.

V.ACKNOWLEDGEMENT

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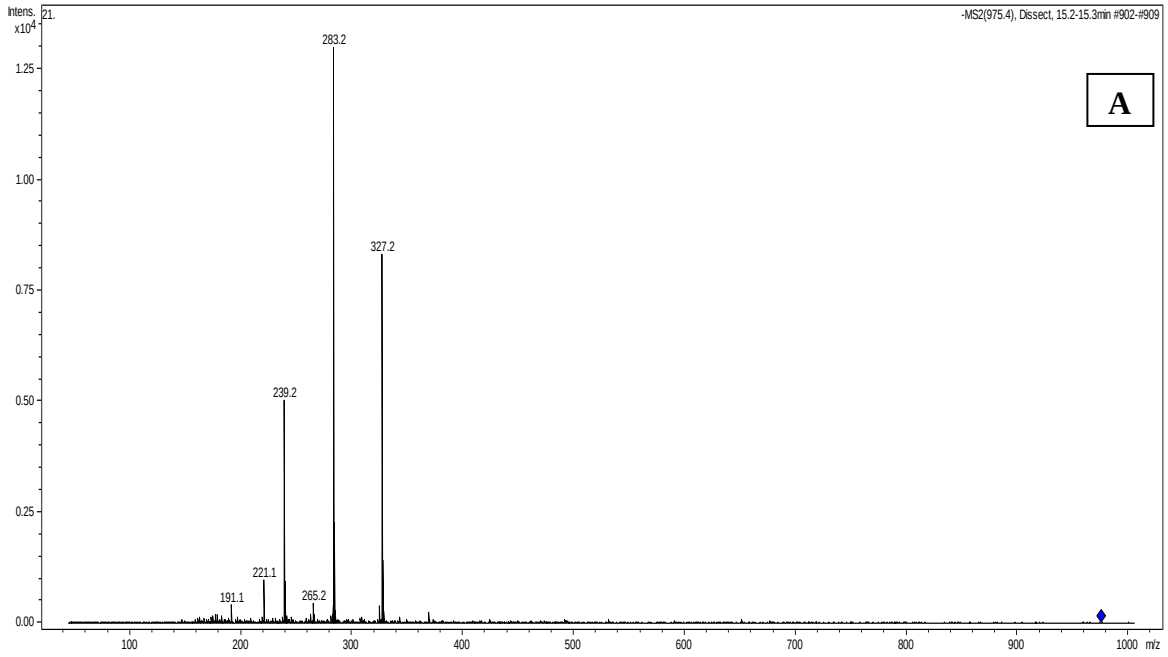
Table 1: LC-MS/MS analysis of Indian Saffron

Sr.No.	Compound Name	Molecular Weight	ESI Mode
1	Crocin	976.4	Negative
2	Picrcrocin	330.3	Negative
3	Chrysoeriol 7-O-glucoside	462.2	Positive
4	Yohimbinic acid	340.2	Negative
5	Vitexin	432.1	Negative
6	Kaempferol trihexoside	772.2	Negative
7	4',5,7-trimethyl ether of vitexin	472.7	Negative
8	Kaempferol diglycoside	610.2	Positive
9	Caffeic acid and hexose derivative	474.2	Negative
10	Hesperetin	302.1	Negative
11	Kaempferol-3-O-glucoside	448.1	Negative
12	Crocin class	668.2	Negative
13	Iristectorin A	492.3	Negative
14	Malvidin-3-O-beta-D-galactoside	493.3	Negative
15	Eriodictyol-7-O-glucoside	450.3	Negative
16	3-Hydroxy-3',4',5'-trimethoxyflavone	976.4	Negative
17	Petunidin-3-O-beta-glucopyranoside	479.2	Negative

18	Haploside D	682.3	Negative
19	3-Hydroxy-3',4',5'-trimethoxyflavone	329.2	Negative
20	Crocin class	814.3	Negative
21	Crocin class	652.3	Negative
22	Crocetin	328.2	Positive
23	Rhamnosyl luteolin derivative	712.3	Negative
24	Crocin class	639.3	Negative
25	Crocin class	594.3	Negative
26	Apigenin-7-O-neohesperidoside	578.3	Negative
27	Crocin class	476.3	Negative
28	3',7-Dimethoxy-3-hydroxyflavone	298.2	Negative
29	6-O-pentosyl luteolin	484.3	Negative
30	Kaempferol-3-O-rutinoside-7-O-gluose I	771.2	Negative



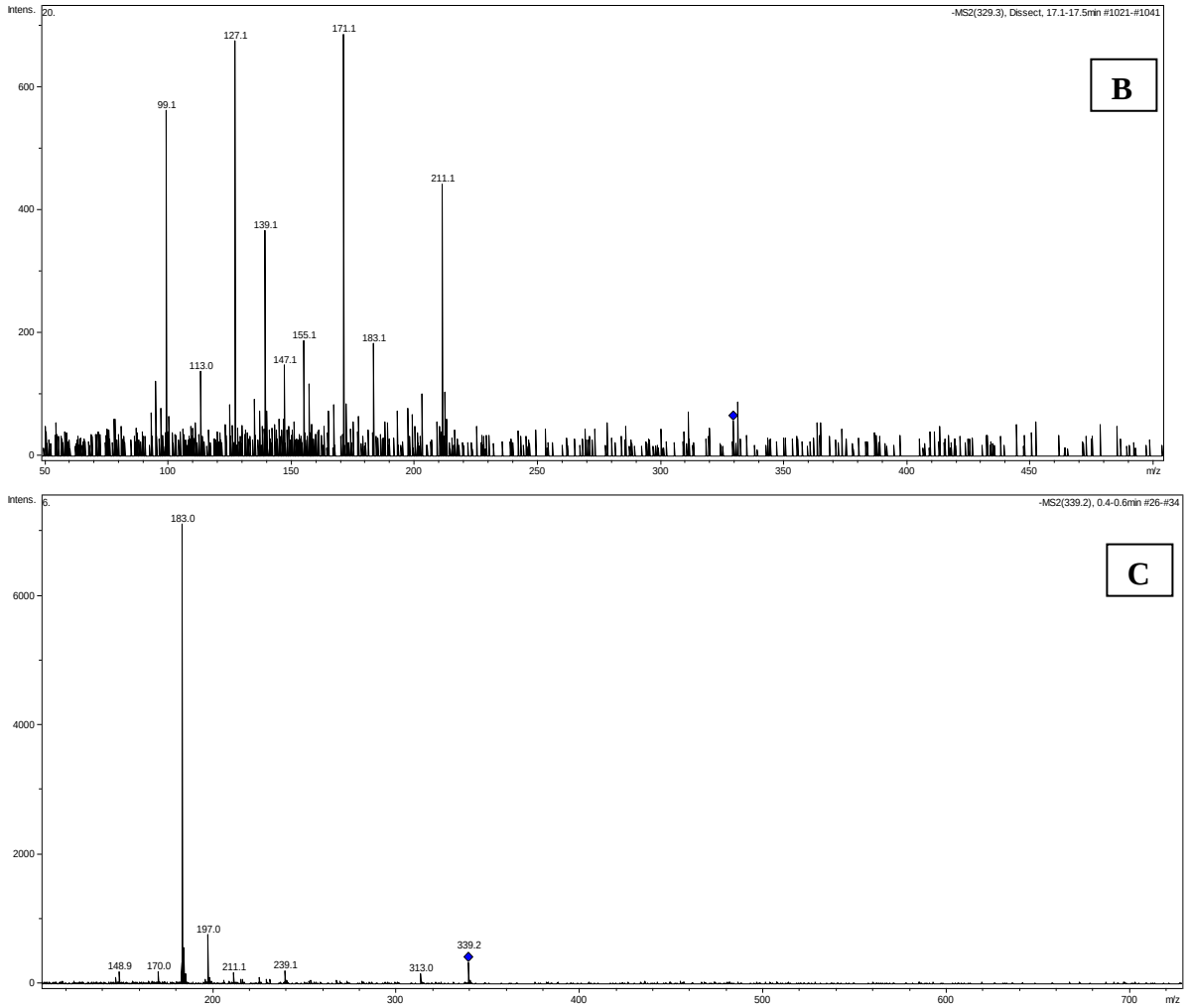


Table 1: A) Crocin (B) Picrocrocin (C) Yohimbinic acid

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