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Chemical Characterization, α-glucosidase Inhibitory and Cytotoxic Properties of *Crocus Pallasii* Stigmas

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ABSTRACT

The *Crocus* genus has been reported to possess diverse biological properties. In the present investigation, chemical characterization, α-glucosidase inhibitory and cytotoxic properties of stigmas from *Crocus pallasii* (*C. pallasii*) have been studied. Chemical composition was investigated by GC-MS, LC-MS and HPLC. The alpha-glucosidase inhibitory activity of the stigmas ethanolic extract of *C. pallasii* was examined by an enzyme assay. The extract's cytotoxicity against HepG2 liver cancer cells was assessed using the MTT test. Fatty acids were the predominant volatile compounds within the ethanolic extract of *C. pallasii* stigmas. Safranal and crocin, which are the bioactive compounds of saffron, were detected in the ethanolic extract of *C. pallasii* stigmas. The amount of safranal and crocin identified by HPLC were 0.27 and 14.23 mg/g of dry extract, respectively. The ethanolic extract of *C. pallasii* stigmas and crocin inhibited α-glucosidase and IC_{50} values were 4.77 and 1.8 mg/mL respectively. Both ethanolic extract of *C. pallasii* stigmas and crocin showed uncompetitive type of α-glucosidase inhibition. Stigmas ethanolic extract of *C. pallasii* also showed toxicity against HepG2 liver cancer cell line. Based on the above findings, *C. pallasii* might be considered as an interesting candidate to enhance the quality of products in the functional foods, beverages, drinks, pharmaceutical and cosmeceutical industries.

Keywords: *Crocus*, Liver cancer, α-glucosidase, Diabetes, Metabolome, GC-MS, LC-MS

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INTRODUCTION

Metabolites are substances produced as a result of metabolic reactions, essential for the survival and growth of plants [1]. Plant cells are capable of synthesizing two distinct categories of metabolites, namely primary metabolites and secondary metabolites. Primary metabolites have a direct role in the processes of development and metabolism, encompassing carbohydrates, lipids, proteins, and nucleic acids. The secondary metabolites such as alkaloids, phenolic, essential oils, steroids, lignin, tannins, and flavonoids, are derived from primary metabolites and are considered the secondary intermediate and final products of primary metabolism. One of the key functions of secondary metabolites is to protect plants against environmental stresses. Consequently, their production is frequently stimulated by events such as pathogen damage [2].

Crocus pallasii subsp. *haussknechtii* (Boiss. & Reut. ex Maw) B.Mathew is the closest relative of *C. sativus* (saffron) [3]. Recent research has demonstrated that saffron and its derivatives can influence hyperglycemia, exhibiting anti-diabetic properties [4]. Glucosidase enzymes play a crucial role in breaking down starch into simple sugars, contributing to elevated blood glucose levels. Alpha-glucosidase inhibitors (AGIs) are employed for the management of type 2 diabetes mellitus, either as monotherapy or in conjunction with other antidiabetic medications [5]. Very few reports have been published on the metabolites and biological activity of *C. pallasii.* Moudi et al. (2020) reported that the corm extract of *C. pallasii* contain three phenolic compounds including chicoric acid, chlorogenic acid and syringic acid and two flavonoid compounds including kaempferol and apigenin. Moreover, methanol extract of leaves and corm of *C. pallasii* had antimicrobial activity [6]. According to a study carried out by Mosaddegh et al., *C. pallasii* exhibits anti-angiogenesis activity and induces cell death in HUV-EC-C cells [7]. Hamzeloo-moghadam et al. reported the cytotoxic activity of *C. pallasii* against A-549 human lung carcinoma cells [8]. Shakeri et al. reported that corm of *C. pallasii* induce cell death in MDA-MB-231 and MCF-7 human breast cancer cells [9]. The identification of secondary metabolites of the *C. pallasii* is crucial for medical and food industries, but no comprehensive study has been conducted on its chemical compounds. Therefore, the goal of this study is to metabolomics analysis of *C. pallasii* stigma as well as its anti-diabetic and cytotoxicity potential.

MATERIALS AND METHODS

Plant Collection and Extraction

The specimen of *C. pallasii* was collected in Sanandaj, Iran, in the fall 2019. A voucher specimen of *C. pallasii* subsp. *haussknechtii* (Boiss. & Reut. ex Maw) B.Mathew (12543 HKS) identified by Hosein Maroofi has been deposited in the HKS herbarium of

Kurdistan Agricultural and Natural Resources Research and Education Center, Sanandaj, Iran. The stigmas were isolated and dried under shade, thereafter grinded, and soaked in ethanol for 24 hours. The suspension was filtered and concentrated using rotary evaporation. The ethanol solvent was then dried to determine its dry weight [10].

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

The extracts were dissoleved in methanol and subjected to GC-MS analysis using an Agilent 6890 series gas chromatograph equipped with a Varian column. The mobile phase was helium, with a flow rate of 1 mL/min. The temperature was first set at 60°C and then increased to 280°C. The injection volume was 1µL. An electron beam with energy of 70 electron volts (eV) was employed for ion generation. The detected masses ranged from 40 to 600 m/z. The identification of the constituents in the extracts was done by determining their retention time and retention index and then compared with data in the NIST and Wiley databases.

Liquid Chromatography with Mass Spectrometry (LC-MS)

The separation procedure was conducted using a chromatographic method on an Atlantis T3-C18 analytical column with dimensions of 3 m in length and 2.1 mm in diameter. The column was eluted with a mobile phase consisting of 95% methanol and 5% water containing 0.1% formic acid, at a flow rate of 0.15 mL/min. The column temperature was set at 45°C. The injection volume was 10 µL, and the length of the analysis was 30 minutes. The experimental setup involved the utilization of electrospray ionization (ESI) in combination with a triple quadrupole mass analyzer operating in the positive and negative modes. The spray voltage was set at 4500 V. The nebulizer gas utilized in the experiment was nitrogen (N2 Garde 5) with pressure of 40 psi and source temperature of 120 °C. The energy of the impact was measured to be 35 electron volts (eV).

Chemometric Data Analysis

The regions of interest multivariate curve resolution (ROIMCR) method, which combines regions of interest and Multivariate Curve Resolution-Altrenating Least Squares (MCR-ALS) methods, is used to compress and filter data on LC-MS full scan data without losing essential information on mass accuracy and spectral resolution. The region-of-interest (ROI) method creates a data matrix suitable for multivariate modeling, including mass traces and intensities of the same m/z values. The output parameters include a vector, a matrix of data comprising mass spectrometry (MS) spectra at each retention time scan, and a cell array. All ROI m/z values with significant traces are organized in the MSROI data matrices. MCR-ALS analysis uses a bilinear decomposition approach to resolve profiles of components in mixtures without providing information about their compositions. The method decomposes the instrumental response matrix to the concentration/elution profiles and the pure spectra of the components present in a mixture. The peak area and heights of each elution profile can be related to the concentration of components [11].

High-performance Liquid Chromatography (HPLC)

The 20 microliters of extract were passed through a column (Eurospher II 100-5 C18, 25 cm length and 4.6 mm inner diameter). Methanol and acetonitrile were used as carrier phase with a flow rate of 1 mL/min. Crocin and safranal prepared from Sigma-Aldrich company, were used for drawing standard curves. The absorbance of crocin and safranal were obtained at 440 and 320 nm, respectively. Finally, the amount of crocin and safranal was calculated in mg/g of dry extract.

Enzyme Assay

The assay was conducted on the activity of α-glucosidase by the modified Pistia-Brueggeman method with brief changes [12]. The assay involved adding phosphate buffer, plant extract or crocin, enzyme solution of alpha-glucosidase, and substrate to 96-well micro plates. The absorbance was measured at 405 nm in three replicates, with negative controls present. The percentage of enzyme inhibition was calculated using the equation % inhibition = $100 \times [(OD_{control}-OD_{test})/OD_{control}]$. The IC_{50} value was calculated using linear regression analysis of each percent inhibition plot. Kinetic analysis was performed to determine the type of stigmas extract or crocin's inhibition against alpha-glucosidase activity. The inhibition was measured using four different concentrations of p-NPG as substrates in the absence and the presence of stigmas extract or crocin. The type of inhibition and kinetic parameters were determined by Lineweaver-Burk plot analysis based on Michaelis-Menten kinetics.

MTT Assay

The MTT assay was employed to assess the cytotoxicity of the extracts against cancer cells, as reported in a previous study [13]. 10,000 cells were evenly distributed in 96-well plates and after taking morphology were incubated with varying doses of the extract for 24 and 48 hours. Then the culture medium was removed and the MTT solution was added at a concentration of 0.5 mg/mL. The plate was incubated for three hours at 37oC under 5% CO2 pressure. The viable cells convert MTT solution into formazan crystals, which were then dissolved in dimethylsulfoxide (DMSO). The absorbance at 570 nm in each well was quantified using a microplate reader. The IC50 value, which represents the concentration at which 50% of cancer cells survive, was determined by regression analysis of the dose-response curve

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Table 1 Chemical profile (GC-MS) of *C. pallasii* stigmas ethanol extract. Safranal and fatty acids are written in bold type.

Retention time (min)	Component Name	% of total
6.99	2(5H)-Furanone	2.830
14.58	Safranal	4.251
26.00	Palmitic acid	7.030
27.94	Linoleic acid	5.784
28.02	Oleic acid	39.472
28.29	Stearic acid	10.380
31.52	Isopropyl linoleate	5.461
31.59	9,17-Octadecadienal	10.581
31.67	Ethyl linoleate	6.527
Total%		92.316

Table 2 Metabolites in the extract of *C. pallasii* based on LC-MS resolved by MCR-ALS method in comparison with the extract of *C. sativus*.

Mode	Name a	Experimental mass b	Adduct c	Rt d	peak area e (*C. sativus*)	peak area e (*C. pallasii*)
$(ESI)^-$						
		36		1	0.47	2.38
	3,5,5-trimethyl-1,4-cyclohexadion-2-ene (III)	133	M-H_2O-H	1	5.49	0.29
	2,6,6-trimethyl-3-oxo-1-cyclohexen-1-carboxaldehyde	165	M-H	1	0.77	0.89
		188		1.11	0.05	0.73
		168		2	6.09	0.46
		482		2	1.23	0.34
		319		2.5	1.46	9.64
		480		2.71	4.43	0.004
	Chlorogenic acid	117	M-3H	3.8	2.83	1.09
		230		4	0.98	0.88
		330		4	4.28	3.94
		198		4.08	6.97	3.26
		542		4.19	0.02	0.81
		197		4.5	2.43	2.75
		385		4.5	0.01	0.04
		385		4.5	0.03	4.02
	Isophorone	183	M+FA+H	5	0.01	0.01
		246		5	0.50	3.13
		271		5	5.46	0.02
		257		5.2	0.86	0.77
		643		5.2	0.80	5.43
		385		5.3	0.29	0.08
		169		5.5	7.56	0.19
		182		5.5	3.47	2.09
	Tricrocin	406	M-2H	5.8	0.14	6.05
	Safranal	185	M+Cl	5.98	3.90	1.60
	4-(2,2,6,-trimethyl-cyclohexan-1-yl)-3-buten-2-one	384	2M-H	6.3	0.02	1.00
		890		6.5	0.16	4.77
		184		7.8	0.99	0.53
		354		8.2	1.92	0.19
		114		9	1.44	0.02
$(ESI)^+$						
		45		1.5	0.09	4.99
	Isophorone	47	M+3H	1.5	3.50	4.06
		55		1.5	0.12	5.07
	4H-pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl-	56	M+2H+Na	1.5	0.44	0.06
	2,6,6-trimethyl-3-oxo-1-cyclohexen-1-carboxaldehyde	56	M+3H	1.5	0.10	7.41
		698		1.5	0.03	3.55
	3,5,5-trimethyl-4-hydroxy-1-cyclohexanon-2-ene (I)	60	M+2H+Na	5.5	0.03	0.01
		244		7.3	0.02	8.79
	Osthole	245	M+H	7.3	0.06	3.39
		435		8.5	0.01	5.37
	Isopimpinellin	515	2M+Na	8.5	0.03	2.40
		572		8.5	0.01	4.33
	Pyrogallol	75	M+H+Na	9	0.76	4.96
		365		9.2	0.02	1.16
		409		9.2	0.07	17.95
		486		9.2	0.02	0.88
		199		9.5	0.004	0.37
	3,5,5-trimethyl-2-hydroxy-1,4-cyclohexadion-2-ene (IV)	213	M+2Na+H	9.5	0.03	0.06
		285		9.5	0.004	8.45
	Crocin 3	226	M+2H+Na	10	0.39	9.41

[a] The name of the identified chemical compounds.
[b] The extracted mass of chemical compounds (m/z).
[c] The identified adducts.
[d] The retention times of the elution peak maximum.
[e] The area under each of the resolved chromatographic profiles in C matrices by MCR-ALS.
[f] Formic acid

Statistical Analysis

The study used three independent experiments and SPSS software to analyze the data, revealing a statistically significant difference between two groups with a *p* value less than 0.05. MATLAB R2023b (The Mathworks Inc., https://www.mathworks.com) was used to perform MCR-ALS using the MCR-ALS toolbox, which is openly accessible from http://www.mcrals.info. The MATLAB functions for the ROI process can be found at: https://cidtransfer.cid.csic.es/descarga.php?enlace1=298348e5b34daf9e844835352bafa6%2045250ee1.

RESULTS

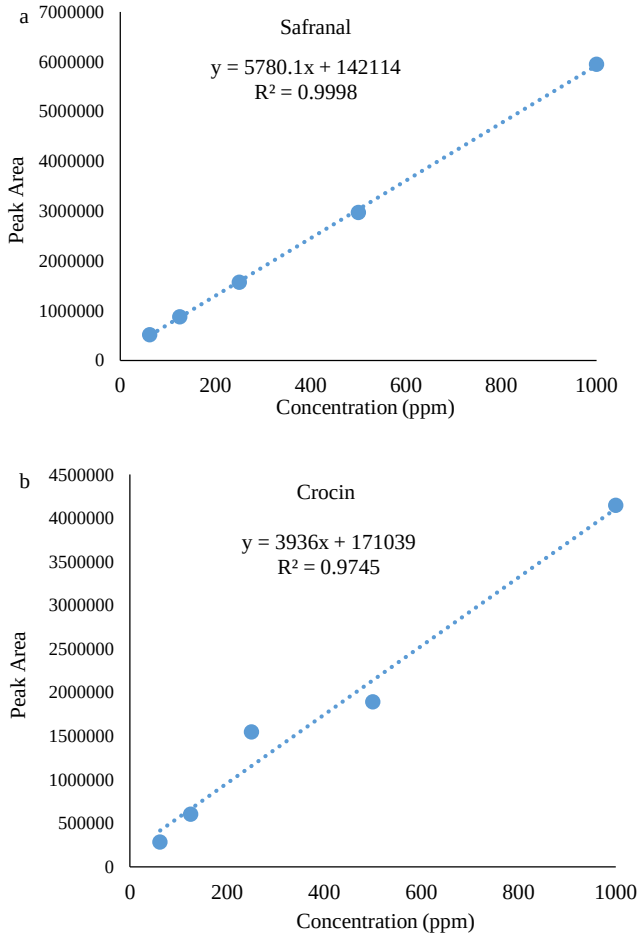

Fig. 1 Calibration curves for safranal (a) and crocin (b) by HPLC. The peak area was plotted against the concentration of the standards (62, 125, 250, 500 and 1000 ppm).

GC-MS Analysis of the Stigmas Ethanolic Extract C. Pallasii

The chromatographic profile of the stigmas ethanolic extract of *C. pallasii* was determined using GC-MS analysis. The volatile constituents were identified, and their relative abundances were measured. The results are presented in Table 1. The chemical composition analysis of the ethanolic extract of *C. pallasii* stigmas revealed the presence of nine chemical compounds, which accounted for 92.316% of the total compounds. Fatty acids were the predominant volatile compounds within the ethanolic extract of *C. pallasii* stigmas. Oleic acid was the most abundant fatty acid followed by 9,17-octadecadienal, stearic acid, palmitic acid, ethyl linoleate, linoleic acid and isopropyl linoleate. Safranal, which is one of the most important bioactive compounds of saffron, was detected in the ethanolic extract of *C. pallasii* stigma.

Untargeted Metabolomics Analysis of the Stigma Ethanolic Extract of C. pallasii by LC-MS

The MCR-ALS approach was utilized to examine the LC-MS data sets of the *C. sativus* and *C. pallasii* extracts in the positive and negative MS ionization modes. A total of 53 metabolites were detected. In Table 2, the components obtained by the MCR-ALS technique for *C. sativus* and *C. pallasii* are presented. The provided table presents the compounds' names, molecular mass, identified adducts, retention times, and peak areas for samples of *C. sativus* and *C. pallasii*. Fragmentation patterns of the main components identified in *C. pallasii* stigmas by LC-MS analysis were provided in Table 3.

Table 3 Fragmentation patterns of main components identified in *C. pallasii* stigmas by LC-MS analysis.

Compound Name	Experimental Mass (m/z)	Fragment Ions (m/z)
Isophorone	183	155, 125, 95
Chlorogenic Acid	117	99, 81, 53
Safranal	185	150, 123, 95
Oleic Acid	282	264, 222, 149
Crocin	406	357, 297, 179
Osthole	245	229, 201, 147
Isopimpinellin	515	473, 439, 359
Pyrogallol	75	57, 45, 29

Quantitative Analysis of Crocin and Safranal in the Stigmas Ethanolic Extract of C. pallasii by HPLC

The HPLC analysis of the stigmas ethanolic extract of *C. pallasii* showed the identification of safranal and crocin. To quantify these compounds, calibration curves for safranal and crocin were generated by preparing standard solutions of known concentrations (Fig. 1). Table 4 shows the quantities of these compounds in the extract. The amount of safranal and crocin obtained by HPLC was 0.27 and 14.23 mg/g of dry extract, respectively.

Table 4 HPLC analysis of safranal and crocin in the stigmas ethanolic extract of *C. pallasii*.

Compound	Concentration (mg/g dry extract)
Safranal	0.27 ± 0.08
Crocin	14.23 ± 0.05

α-glucosidase Inhibitory Activity of Ethanolic Extract of C. pallasii

Figure1 shows the results of α-glucosidase inhibitory activity of ethanolic stigmas extract of *C. pallasii* and crocin in 5 concentrations. Several concentrations were used to determine the IC_{50} value of the extract and crocin, which were 4.77 and 1.8 mg/mL respectively. To determine the type of inhibition, Double reciprocal plots were drawn for both the ethanolic stigmas extract and crocin in concentration with highest inhibitory effect (Fig. 2). So both of the ethanolic stigmas extract of *C. pallasii* and crocin showed uncompetitive type of α-glucosidase inhibition. Finally, enzyme kinetic parameters (K_m, V_{max}) were calculated for α-glucosidase in the absence and presence of ethanolic stigmas extract of *C. pallasii* and crocin from related double reciprocal plots (Table 5).

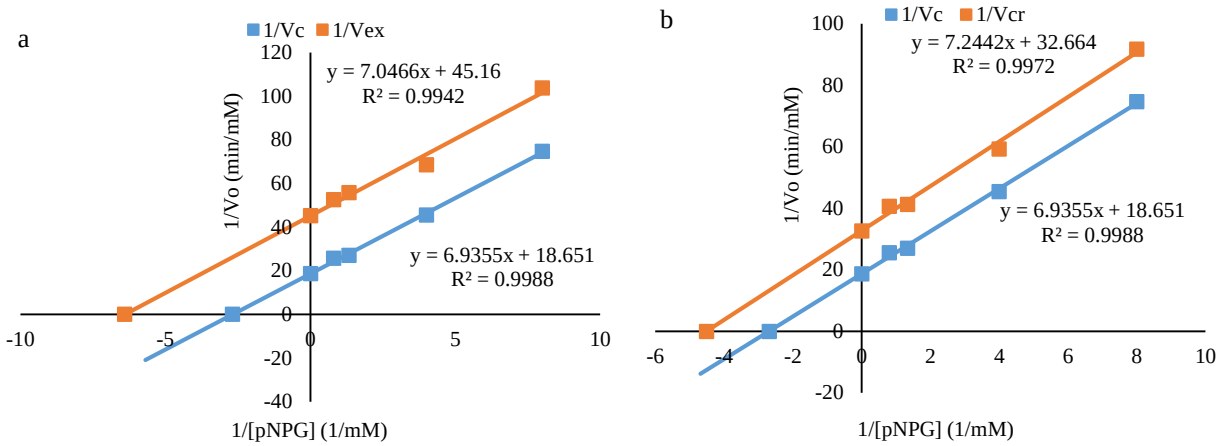

Fig. 2 Double reciprocal plot for α-glucosidase inhibition by ethanolic stigmas extract of C. pallasii (5 mg/mL) (a) and crocin (2 mg/mL) (b)

Table 5 Kinetics parameters for α-Glucosidase inhibition by ethanolic stigmas extract of *C. pallasii* and crocin.

Sample	K_m (mM)	V_{max} (mM/min)	K_i (mg/mL)	(IC_{50} mg/mL)	Inhibition Type
Control	0.37	0.05	-	-	-
Crocin	0.22	0.03	2.94	1.8	Uncompetitive
C. pallasii	0.16	0.02	3.66	4.77	Uncompetitive

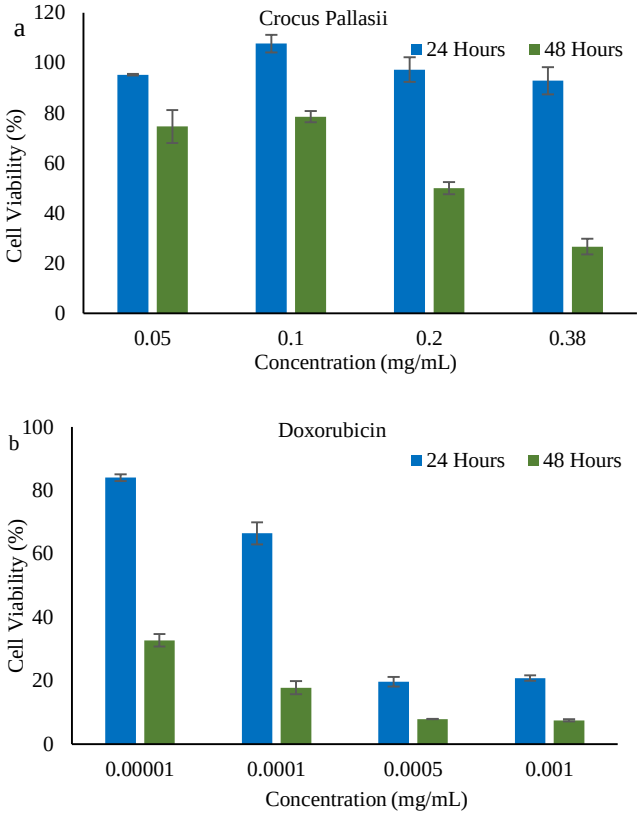

Fig. 3 Viability of HepG2 liver cancer cells exposed to various concentrations of doxorubicin (a) and stigmas ethanolic extract of *C. pallasii* (b) during incubation periods of 24 and 48 hours. Data are means of three independent replicates ± SD; *: P< 0.05 for the significant differences between 24 and 48 h treatment.

Cytotoxicity Evaluation of the Stigmas Ethanolic Extract of C. pallasii Against HepG2 Cancer Cell

MTT was employed to evaluate the influence of stigmas ethanol extract of *C. pallasii* for 24 and 48 hours on the viability of HepG2 liver cancer cells. Based on the results, stigmas extract of *C. pallasii* had no inhibitory effect on the cell proliferation in 24 hours (Fig. 3 and Table 6). That's why IC_{50} value cannot be calculated. Despite 24 hours, stigmas extract of *C. pallasii* had inhibitory effect on the cell proliferation in 48 hours with the IC_{50} value of 0.19 ± 0.01 mg/mL on HepG2 cells. Doxorubicin was employed as a positive control.

Table 6 The *in vitro* cytotoxic IC_{50} values on HepG2 liver cancer cell line.

Treatment	IC_{50} (mg/mL) ± SD	
	24 Hours	48 Hours
Doxorubicin	0.15 ± 0.03	0.00047 ± 0.00021
C. pallasii	-	0.19 ± 0.01

DISCUSSION

The chemical component, α-glucosidase inhibitory, and cytotoxic properties of *C. pallasii* stigmas were investigated. According to GC-MS analysis, fatty acids and safranal were the most volatile compounds in the ethanolic extract. Loizzo and collaborators (2015) identified 4H-pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl, osthol, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, methyl palmitate, palmitic acid, xanthotoxin, methyl oleate, methyl stearate, methyl arachidate and isopimpinellin in ethanol stigmas extract of *C. cancellatus* subsp. *damascenus* by GC-MS analysis [14]. In this study, a total of 20 and 32 components were identified in the extract using LC-MS positive and negative ESI modes, respectively. Tricrocin and crocin 3 were detected in the ethanolic stigma extract of *C. pallasii*, respectively, in the negative and positive modes of LC-MS analysis. Crocin, in general term, includes Crocin-I (Crocetin-di-beta -D-gentiobiosyl ester), Crocin-II (Crocetin-beta-D-gentiobiosyl-beta-D-glucosyl ester or tricrocin), Crocin-III (Crocetin-mono-beta-D-gentiobiosyl ester), Crocin-IV (beta-D-monoglucoside ester of monomethyl alpha-crocetin) [15]. Numerous research have provided evidence about the biological activities of crocins [16]. According to Algandaby's (2016) findings, crocin exhibits notable antioxidant and anti-inflammatory properties, hence safeguarding against liver fibrosis produced by thioacetamide in mice [17]. According to a research conducted by Liou et al. (2018), it was shown that crocin has anti-photodamage and cytoprotective properties, leading to improvements in visual performance [18]. The study conducted by Boussabbeh et al. (2016) documented the capacity of crocin to mitigate the deleterious impacts of patulin, which is classified as a secondary toxic metabolite [19]. Zarghami and collaborators (1971) described the identification of seven volatile constituents of saffron as isophorone (3,5,5-trimethyl-2-cyclohexen-1-one),

3,5,5-trimethyl-4-hydroxy-1-cyclohexanon-Zene (I), 3,5,5-trimethyl-1,4-cyclohexadione (II), 3,5,5-trimethyl-1,4-cyclohexadion-2-ene (III), 3,5,5-trimethyl-2-hydroxy-l&cyclohexadion-2-ene(IV), 2,6,6-trimethyl-4-hydroxy-l-cyclohexene-I-carboxaldehyde (V), and 2,4,4-trimethyl-3-formyl-6-hydroxy-2,5-cyclohexadien-l-one (VI). Isophorone and the compounds I, II, III, and IV all possess nine carbons [20]. 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde, namely safranal is a terpene aldehyde [21]. Isophorone is an α,β-unsaturated cyclic ketone that has been isolated from the volatiles of cranberries (*Vuccinium macrocarpon Ait*) [20]. In this study, Isophorone was detected in the stigmas of *C. pallasii* in both positive and negative mode. Safranal was detected in the extract by negative mode electrospray as well as GC-MS. Tarantilis and collaborators (1997) isolated 2,6,6-trimethyl-3-oxo-1-cyclohexen-1-carboxaldehyde and 4-(2,2,6,-trimethyl-cyclohexan-1-yl)-3-buten-2-one from the saffron by steam distillation (SD) and micro-steam distillation extraction (MSDE) techniques, respectively and identified them by gas chromatography-mass spectrometry (GC-MS) [21]. 4-(2,2,6,-trimethyl-cyclohexan-1-yl)-3-buten-2-one was detected in the stigmas of *C. pallasii* by negative mode electrospray. Chlorogenic acid and pyrogallol were other detected compounds in the stigmas of *C. pallasii* by LC-MS. Chlorogenic acid, referred to as coffee tannic acid and 3-caffeoylquinic acid, is a polyphenolic phenylacrylate chemical that is soluble in water and synthesized by several plant species. Chlorogenic acid is believed to have various biological activities, such as antioxidant, liver and kidney protection, antibacterial, antitumor, regulation of glucose metabolism and lipid metabolism, anti-inflammatory, protection of the nervous system, and action on blood vessels [22]. Mykhailenko and collaborators (2021) identified chlorogenic acid (0.31 ± 0.004 mg/g) in the methanolic extract of *C. sativus* leaves by HPLC [23]. Karimi and collaborators (2010) examined the phenolic and flavonoid compounds of methanolic saffron stigmas extract by reversed phase (RP)-HPLC. The findings of the study identified gallic acid as the main phenolic component and pyrogallol as the main flavonoid compound, with concentrations of 1.82 ± 0.02 and 1.4 ± 0.05 mg/g dry sample, respectively [24].

The principal components of saffron are crocin and safranal, which have a range of biological functions, including anti-inflammatory and antioxidant properties [25, 26]. The amounts of safranal and crocin in the extract were 0.27 and 14.23 mg/g of dry extract, respectively. Caballero-Ortega and collaborators (2007) quantified the 10 major saffron compounds in eleven certified saffron samples (*C. sativus* L.). Similar to our results, the amount of safranal obtained by HPLC in 11 different saffron (*C. sativus* L.) sources was in the range of 0.22–1.29 mg/g of stigmas [27].

The suppression of the digestion of complex polysaccharides by pancreatic α-amylase and intestinal α-glucosidase has been proposed as a viable approach for the control of type II diabetes [28]. The present study provides evidence for the ability of the stigma extract of *C. pallasii* to act as an α-glucosidase inhibitor. *C. pallasii* stigmas extract and crocin, its active metabolite, inhibited α-glucosidase activity with IC_{50} values of 4.77 and 1.8 mg/mL, respectively. In the inhibition kinetic analysis, *C. pallasii* extract and crocin had uncompetitive inhibitory effects on α-glucosidase. Gardenia yellow is a mixture of total crocetin derivatives. In a recent study conducted by Ren et al. (2022), it was found that Gardenia yellow (with crocin HPLC >90% and crocetin HPLC >9%) exhibits competitive inhibition towards α-amylase and α-glucosidase [29]. Some identified compounds in *C. pallasii* stigmas extract, such as crocin, oleic acid, linoleic acid, chlorogenic acid, osthole, and pyrogallol, could justify the observed α-glucosidase inhibition [4]. *In vitro* studies showed that oleic and linoleic acids are more effective than acarbose in suppressing α-glucosidase activity [30]. Chlorogenic acid, a phenolic compound in plant-based diets, inhibits α-amylase and α-alpha glucosidase [31]. Osthole inhibits α-glucosidase activity with an IC_{50} value of 0.95 mg/mL [32].

The assessment of cytotoxicity has significant importance in the process of validating newly discovered active chemicals or plant extracts derived from botanical sources [33]. Results showed that the extract from *C. pallasii* exhibits cytotoxic action against the HepG2 liver cancer cell line. It was found that osthol suppresses the proliferation of HeLa cells in a way that is dependent on both time and dose [34]. Crocin and safranal have a variety of pharmacological effects, including antioxidant [35], immunity enhancement, and antitumorigenic properties [36, 37].

In conclusion, the study revealed that *C. pallasii* stigmas contain compounds like safranal and crocin, which have been found to possess cytotoxicity activity against HepG2 liver cancer cell and α-glucosidase inhibitory activity. These compounds, similar to saffron, could improve the quality of functional foods, beverages, pharmaceuticals, and cosmeceutical goods.

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Footnotes

Authors' contributions: Raheleh Shakeri, Mohammad Ali Zarei and Mahsa Naghavi Sheikholeslami conceived and planned the study; Taban Ibrahim Abdalqadir performed the experiments; Jalal Khorshidi collected and identified specimens; Raheleh Shakeri, Mohammad Ali Zarei and Mahsa Naghavi Sheikholeslami analyzed data. Raheleh Shakeri wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of Interests

The author(s) declare no potential conflicts of interest concerning this article's research, authorship, and/or publication.

Ethical Approval

Not applicable

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