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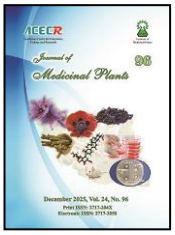

Review Article

In vitro antioxidant activity of *Crocus sativus* L.: A systematic review

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ABSTRACT

Background: *Crocus sativus* L., or saffron, is a valuable medicinal plant with a complex biochemical makeup that includes crocin, safranal, picrocrocin, flavonoids, and phenolic acids, compounds linked to a variety of pharmacological properties, including antioxidant activity. **Objective:** This systematic review summarizes existing evidence for the antioxidant activity of saffron stigma extracts *in vitro* and assesses the influence of methodological variables, including the type of assay, geographical origin, extraction method, and solvent type, on reported results. **Methods:** A comprehensive literature search was conducted using the PubMed, Scopus, and Web of Science databases to identify peer-reviewed original research articles published between January 2000 and April 2025. The review followed PRISMA guidelines, 28 studies were qualitatively synthesized. **Results:** DPPH assay was the most frequently used method (26 studies, ~92.86 %), followed by FRAP assay (11 studies, ~39.29 %) and ABTS assay (8 studies, ~28.57 %). Iranian saffron samples tended to exhibit high crocin content, which was frequently associated with elevated antioxidant potential, particularly in DPPH and ABTS assays. Maceration was the most frequent extraction method (19 studies, 67.86 %), yet ultrasound-assisted extraction (UAE) demonstrated improved extraction of crocin and total phenolics. Methanol was the most extensively used solvent (15 studies, ~53.57 %), yet water-based extractions combined with UAE were also found effective. **Conclusion:** These findings highlight the need for standardization of protocols to increase reproducibility and comparability across studies. The review provides evidence-based information to optimize the processing and utilization of saffron, thus augmenting its role as a valuable natural antioxidant useful in the food, pharmaceutical, and cosmetic industries.

Abbreviations: PRISMA, preferred reporting items for systematic reviews and meta-analyses; PICO, population, patient, or problem/intervention/comparison or control/outcome; UAE, ultrasound-assisted extraction; MAE, microwave-assisted extraction; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ABTS, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ORAC, oxygen radical absorbance capacity; TEAC, trolox equivalent antioxidant capacity; TPC, total phenolic content; OH, hydroxyl radical; NBT, nitroblue tetrazolium; FTC, ferric thiocyanate; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; TRAP, total radical-trapping antioxidant parameter; TAC, total antioxidant capacity

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1. Introduction

Crocus sativus L., or saffron, has been highly valued throughout the centuries not just as a spice but also on account of its medicinal and possible cosmetic uses [1, 2]. This geophyte perennial, which grows in Mediterranean climates, is characterized by a very specific biochemical pattern comprising glycosides, carotenoids, flavonoids, monoterpenes, and other derivatives [3]. These bioactive compounds are accountable for a wide variety of pharmacological effects, including antioxidant, anti-inflammatory, anticancer, antibacterial, and neuroprotective activity, making *C. sativus* an attractive candidate in food and pharmaceutical industries [4, 5]. *C. sativus* extracts increase antioxidant enzymes activity and reduce oxidative stress. Choice of antioxidant assay is crucial in quantifying the antioxidant activity of *C. sativus* extracts [6]. Most common *in vitro* tests, such as DPPH, ABTS, ORAC, and FRAP, are based on distinct reaction mechanisms and can have distinct sensitivities to various antioxidant compounds found in saffron extracts [1, 6-11]. The results of these assays can vary significantly due to several influencing factors. The geographical origin of saffron samples plays a crucial role in determining their antioxidant properties. Variations in climatic conditions, soil composition, and agricultural practices across regions such as Iran, Spain, and India can lead to differences in the concentration and composition of bioactive compounds. For instance, Iranian saffron is often cited for its superior quality and higher crocin content, which may enhance its antioxidant activity compared to saffron from other regions [12].

The antioxidant activity of *C. sativus* is strongly influenced by the extraction procedures used to yield its bioactive constituents [6, 13]. Various techniques, including maceration, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE), have been explored. Research indicates that the combination of UAE and MAE can yield higher antioxidant activity compared to traditional methods [14]. The type of solvent used during extraction also affects the solubility and yield of different antioxidant compounds. [1, 6]. Common solvents include ethanol, methanol, water, and acetone, each with varying polarities that influence the extraction efficiency of specific bioactive compounds. For example, methanol/water mixtures have been shown to extract higher levels of total phenolic content and antioxidant activity compared to other solvents [14]. Given the extensive variability in measurements of antioxidant activity, due to geographical origin, assay type, extraction method, solvent type, and units of measurement, there is a critical need for a systematic review that summarizes and critically analyzes existing literature. This synthesis will allow patterns and correlations between antioxidant outcomes and these key factors to be identified. Therefore, this systematic review aims to synthesize current evidence on the *in vitro* antioxidant activity of *C. sativus* stigma extracts, with a specific focus on how methodological variables including geographical origin, assay type, solvent type, and extraction type influence reported outcomes.

2. Materials and methods

The systematic review of *in vitro* antioxidant activity on *Crocus sativus* L. (saffron) was carried out using the PRISMA-P criteria as a guide [15-17]. The objective was to synthesize current evidence regarding the *in vitro* antioxidant activity of *C. sativus* stigma extracts, with a focus on assay types,

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geographical origin, extraction methods, solvent types, and reported correlations between bioactive constituents and antioxidant outcomes.

2.1. Literature Search Strategy

A comprehensive literature search was performed using three major electronic databases: PubMed, Scopus, and Web of Science (WOS), to identify peer-reviewed original research articles published between January 2000 and April 2025. No language restrictions were applied during the initial search; however, only studies published in English were included in the final analysis due to resource limitations. The search strategy was structured according to the PICO framework and utilized a combination of controlled vocabulary (e.g., MeSH terms) and free-text keywords relevant to saffron and antioxidant activity (Table 1). The final search string is presented in Table 2. Studies were selected based on predefined inclusion and exclusion criteria aligned with the objectives of the review.

2.2. Study Selection Process

All retrieved records were imported into EndNote (version 21.3) for deduplication and preliminary screening. Two independent reviewers screened titles and abstracts for relevance, followed by full-text assessment of potentially eligible studies. The screening process resulted in a total of 160 records, which were reduced to 123 after removing duplicates. Of these, 48 full-text articles were assessed for eligibility, and 28 met the inclusion criteria for qualitative synthesis. A PRISMA flow diagram illustrates the study selection process (Fig. 1).

2.3. Data extraction

Following selection of literature against the inclusion and exclusion criteria of the study, data were collected according to the purpose of the study. A table was designed to extract data from included studies. The table contained the author(s) and publication year, specific study characteristics, geographical origin, assay type, measuring unit, solvent/extract type, extraction method, and correlation findings (if reported).

Table 1. Resource search strategy in scientific databases

Time limitation	**Till 26 April 2025**	
Language limitation	**English**	**Number of studies**
Database	**Search strategy**	
PubMed	*Crocus sativus* [MeSH Terms]) AND ((("Antioxidant activity"[MeSH Terms]) OR ("Antioxidant properties"[Title/Abstract])) OR ("Antioxidant capacity"[Title/Abstract]))	54
WOS	(((((((((TS=("*Crocus sativus*")) OR TS=("saffron*")) AND TS=("Antioxidant activity")) OR TS=("Anti-Oxidant activity")) OR TS=("Antioxidant properties")) OR TS=("Anti-Oxidant properties")) OR TS=("Antioxidant effect*")) OR TS=("Anti-Oxidant effect*")) OR TS=("Antioxidant capacity")) OR TS=("Anti-Oxidant capacity")	47

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Table 1. Resource search strategy in scientific databases (Continued)

Time limitation	**Till 26 April 2025**	
Language limitation	**English**	**Number of studies**
Database	**Search strategy**	
Scopus	TITLE-ABS-KEY("*Crocus sativus*") OR TITLE-ABS-KEY(saffron*) AND TITLE-ABS-KEY("Antioxidant activity") OR TITLE-ABS-KEY("Anti-Oxidant activity") OR TITLE-ABS-KEY("Antioxidant properties") OR TITLE-ABS-KEY("Anti-Oxidant properties") OR TITLE-ABS-KEY("Antioxidant capacity") OR TITLE-ABS-KEY("Anti-Oxidant capacity")	59

Table 2. PICOs, inclusion criteria and exclusion criteria applied to database search

PICOs	**Inclusion criteria**	**Exclusion criteria**
Population	Original research studies investigating the antioxidant activity of extracts derived specifically from the stigmas of *Crocus sativus* L. (saffron).	Studies focusing on plant parts other than saffron stigmas (e.g., leaves, tepals, corms), or on animal models exclusively.
Intervention	Application of saffron stigma extracts (any solvent: aqueous, alcoholic, hydroalcoholic, etc.) assessed for antioxidant activity using *in vitro* methods.	Studies evaluating antioxidant activity of saffron supplements, by-products, or formulations (e.g., cosmetics, pharmaceuticals), rather than crude stigma extracts.
Comparison	Studies with or without a control/comparator group (e.g., ascorbic acid, BHT, Trolox, or untreated controls)	Not applicable
Outcome	Quantitative measurement of antioxidant activity (e.g., DPPH, ABTS, FRAP, reducing power, β-carotene bleaching, or other validated antioxidant assays).	Studies lacking quantitative assessment of antioxidant activity or reporting only qualitative results.
Study design	Peer-reviewed original research articles.	Review articles, book chapters, conference abstracts, and studies for which the full text is unavailable.

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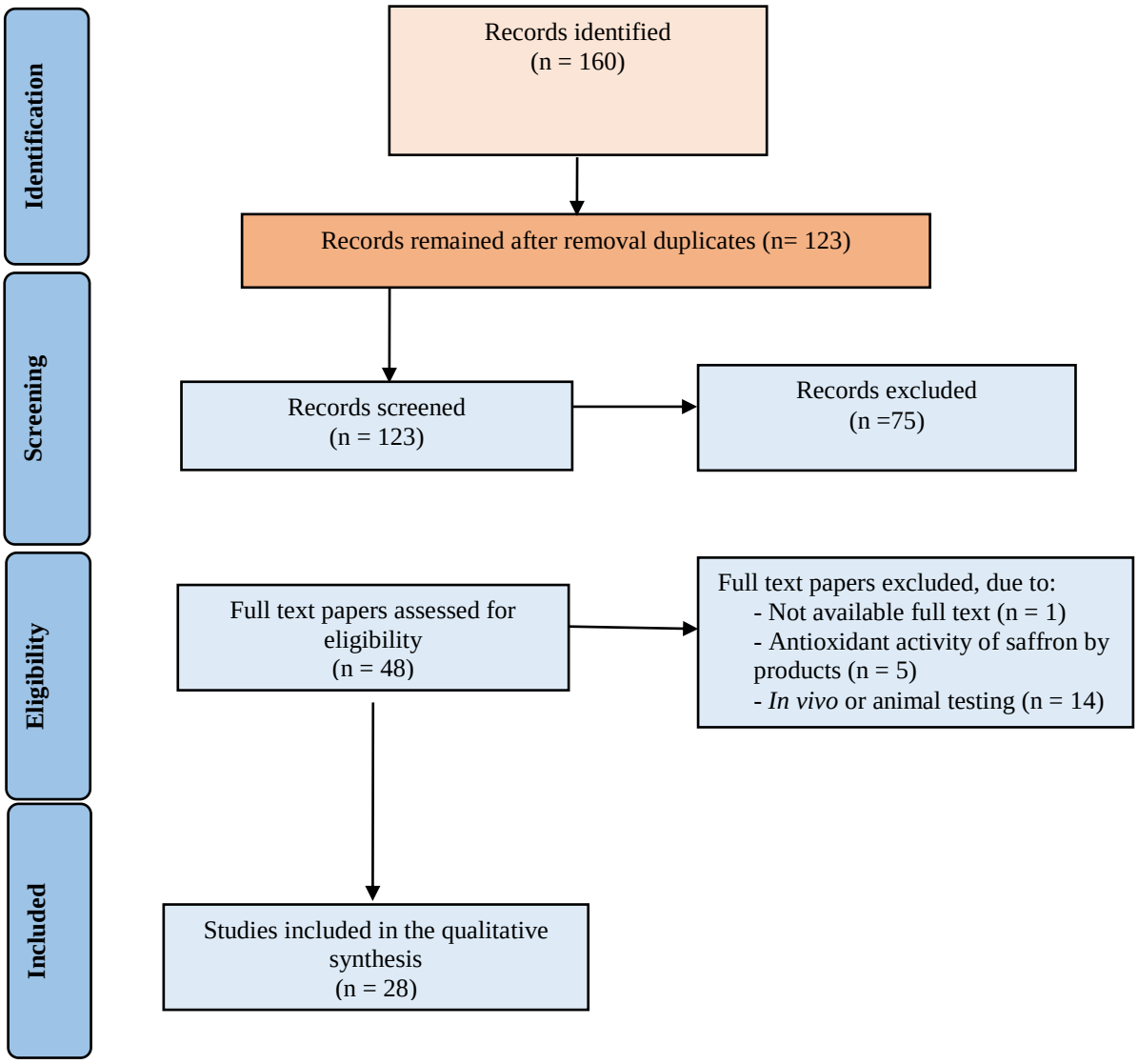

Fig. 1. PRISMA flow diagram for searching resources

2.3. Data extraction

Following selection of literature against the inclusion and exclusion criteria of the study, data were collected according to the purpose of the study. A table was designed to extract data from included studies. The table contained the author(s) and publication year, specific study characteristics, geographical origin, assay type, measuring unit, solvent/extract type, extraction method, and correlation findings (if reported).

3. Results

This systematic review included 28 peer-reviewed original research articles that investigated the *in vitro* antioxidant activity of *Crocus sativus* L. stigma extracts. The studies exhibited considerable heterogeneity in methodological approaches, including assay types, solvents, extraction techniques, and geographical origins of saffron samples (Table 3).

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Table 3. Findings from the literature review conducted as part of this study

No.	Author, year	Specific study characteristics	Geographical origin	Assay (Unit)	Solvent/ Extraction	Correlation
1	Alizadeh – Salteh, 2017	Compared antioxidant activity across years and plant parts (stigma and petal)	Iran	DPPH (% scavenging)	Methanol/ Maceration	-
2	Anuar *et al*., 2017	Imported saffron samples from Iran, Spain, and Kashmir, Quantified color parameters and phytochemical composition	Malaysia	DPPH (% scavenging)	Methanol/ Maceration	Positive correlation between DPPH scavenging activity and flavonoid content (e.g., quercetin and kaempferol)
3	Assimopoulou *et al.*, 2005	Evaluated the main bioactive constituents: crocin and safranal	Greece	DPPH (EC_{50} (mg sample/mg DPPH))	Methanol/Soxhlet	-
4	Baba *et al*., 2015	Comparative evaluation of phytochemical analysis in stigma, corm, and leaf tissues	India	DPPH (% inhibition) FRAP (Absorbance at 700 nm) NBT (% inhibition)	Ethanol, Water/Maceration	-
5	Behdani and Hoshyar, 2016	Total phenolic content of organic versus conventional saffron stigma, Quantifid crocin, picrocrocin, and safranal	Iran	DPPH (% scavenging) FRAP (mol Fe^{2+}/g DW)	Distilled water/Maceration	-
6	Benkerroum *et al.*, 2024	Comparative evaluation of phenolic content, antioxidant, and antibacterial activities of stigmas vs. petals	Morocco	DPPH (IC_{50} (µg/mL), Inhibition (%))	Methanol/ Maceration	Strong positive correlation between TPC and antioxidant activity in stigmas (r = 0.962)

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Table 3. Findings from the literature review conducted as part of this study (Continued)

No.	Author, year	Specific study characteristics	Geographical origin	Assay (Unit)	Solvent/ Extraction	Correlation
7	Cerdá-Bernad *et al.*, 2022	Compared total phenolic content and volatile compounds of different parts (petals and stigmas)	Spain	DPPH (mmol TE/100 g DW) ABTS (mmol TE/100 g DW) FRAP (mmol TE/100 g DW)	Methanol/Ultrasound-assisted extraction	-
8	Chatterjee *et al.*, 2005	Developed a modified, cost-effective assay for measuring total antioxidant capacity (TAC) in human plasma and plant extracts	India	DPPH (µmol TE/l)	Ethanol, Distilled water/Maceration	-
9	Chen *et al*., 2008	Investigated the relationship between antioxidant activity and crocin content, evaluated the antioxidant potential of: crocin isolated from *Crocus sativus* and the extract of stigmas	China	DPPH (mg α-tocopherol E./g) Lipid peroxidation inhibition (mg α-tocopherol E./g)	Hydroethanol/Maceration	No correlation between crocin content and antioxidant activity
10	Drioiche *et al.*, 2023	Evaluated the phytochemical composition using different extraction methods	Morocco	DPPH (EC_{50} (µg/mL)) FRAP (EC_{50} (µg/mL)) TAC (mg ascorbic acid equivalent per gram of extract (mg EAA/g))	Water, Hydroethanol/Decoction, Soxhlet water extraction, Soxhlet hydroethanolic extraction	Perfect correlation between phenolic content and antioxidant activity across all tested methods.

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Table 3. Findings from the literature review conducted as part of this study (Continued)

No.	Author, year	Specific study characteristics	Geographical origin	Assay (Unit)	Solvent/Extraction	Correlation
11	Gismondi *et al.*, 2012	Focused on identifying the biochemical components and quality classification	Italy	DPPH (IC_{50} (mg/mL)) FRAP (mmol/g DW)	Water (ddH_2O), Methanol/Maceration	-
12	Jadouali *et al*., 2017	Evaluated polyphenols content and flavonoids in different floral parts: petals, stamens, styles, and whole flowers with different solvents	Morocco	DPPH (IC_{50} (µg/mL), % inhibition) Reducing power assay (Absorbance at 700 nm)	Ethanol, Methanol, Water/Maceration	-
13	Kabiri *et al*., 2022	Evaluated of aromatic compounds, quality parameters, from different regions	Morocco	DPPH (% inhibition) FRAP (mmol Fe (II)/g DW) ABTS (mmol AAE/g DW)	Methanol/Ultrasound assisted extraction	Moderate positive correlation between crocin content and antioxidant activity (especially with ABTS)
14	Karimi *et al*., 2010	Evaluated phenolic and flavonoid content of saffron stigma in different solvents	Iran	DPPH (% scavenging) FRAP (% inhibition)	Ethanol, Methanol, Boiling water/Maceration	-
15	Kosar *et al*., 2007	Evaluated the volatile composition, coloring compounds, of saffron cultivated in two regions	Turkey	ABTS (µmol/g DW)	Methanol/Maceration	-

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Table 3. Findings from the literature review conducted as part of this study (Continued)

No.	Author, year	Specific study characteristics	Geographical origin	Assay (Unit)	Solvent/Extraction	Correlation
16	Muzaffar *et al.*, 2015	Evaluated proximate composition from two ecogeographical zones	India	DPPH (% inhibition) Reducing power assay (Absorbance at 700 nm) ABTS (% inhibition) FRAP (mmol/kg) Metal Chelating Activity (% chelating) Lipid Peroxidation (% inhibition)	Methanol/Maceration	-
17	Ouahhoud *et al.*	Compared antioxidant potential across different plant parts (stigmas, tepals, leaves)	Morocco	DPPH (IC_{50} (µg/mL)) FRAP (µmol Fe^{2+}/g) B-carotene (% oxidation)	Hydroethanol/Maceration	-
18	Papandreou *et al.*	Investigates the inhibitory effects on amyloid-β (Aβ) fibrillogenesis of the extract	Greece	ABTS (mmol/kg DW (equivalent to Trolox)) FRAP (µmol ascorbic acid equivalent)	Hydromethanol/Maceration	-
19	Parray *et al.*, 2015	Evaluated antibacterial activity in stigmas and callus cultures derived from corm slices	India	DPPH (% inhibition) OH (% inhibition) FTC (% inhibition) TBA (% inhibition) TBARS (% inhibition)	Methanol (Soxhlet)	-

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Table 3. Findings from the literature review conducted as part of this study (Continued)

No.	Author, year	Specific study characteristics	Geographical origin	Assay (Unit)	Solvent/ Extraction	Correlation
20	Pellegrini *et al*., 2006	Evaluated the total antioxidant capacity (TAC) of a wide range of plant-based foods	Italy	Trolox Equivalent Antioxidant Capacity (TEAC) (mmol Trolox equivalents/kg) Total Radical-Trapping Antioxidant Parameter (TRAP) (mmol Trolox equivalents/kg) FRAP (mmol Fe^{2+}/kg)	Water (Maceration)	-
21	Rahaiee *et al.*, 2015	Compared phytochemical constituents using different solvents	Iran	DPPH (% inhibition)	Ethanol, Methanol, Water (Maceration)	Positive correlation was found between the antioxidant activity, total phenolic (r^2 = 0.991) and flavonoid (r^2 = 0.996) contents
22	Ramadan *et al.*, 2012	Evaluated safety in ethanolic extracts	Egypt	DPPH (% inhibition)	Ethanol (Maceration)	-
23	Samaha *et al*., 2022	Evaluated cytotoxic effects of saffron cultivated in Lebanon compared to other geographical origins (Italy, Iran, and India).	Labanon	DPPH (% inhibition) ABTS (% inhibition)	Methanol (Maceration, Microwave-Assisted Extraction)	-

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Table 3. Findings from the literature review conducted as part of this study (Continued)

No.	Author, year	Specific study characteristics	Geographical origin	Assay (Unit)	Assay (Unit)	Correlation
24	Tong *et al*., 2015	Evaluated of different drying methods (sunlight, hot air, vacuum, microwave) on chemical composition	China	DPPH (IC_{50} (mg/mL))	Hydromethanol (Ultrasonic assisted extraction)	Strong positive correlation between Trans-4-GG and antioxidant activity
25	Urbani *et al.*, 2016	12 commercial saffron powder samples from Italian market focused on trans-crocin-4, picrocrocin, and safranal content	Italy	DPPH (% inhibition or µmol TE/g) ABTS (µmol TE/g)	Methanolic extracts, with and without alkaline hydrolysis/Ultrasonic assisted extraction	ABTS strongly correlated with trans-crocin-4 content (R^2 = 0.5732)
26	Zaazaa *et al*., 2021	Investigated the antidiabetic, and antibacterial activities	Morocco	DPPH (IC_{50} (µg/mL))	Boiling distilled Water/Infusion	-
27	Zhang *et al*., 2019	polysaccharide content, and crocin content in saffron samples collected from seven geographical regions	China	DPPH (% inhibition) OH (% scavenging) SOD (% scavenging) Reducing power assay (Absorbance at 700 nm)_	Water for polysaccharide extraction, 50% Ethanol for crocin/ethanol extract preparation/Ultrasound-assisted extraction	Crocin correlated strongly with: -DPPH (r = 0.993) -OH (r = 0.974) -Reducing power (r = 0.986)
28	Zheng *et al*., 2018	Evaluated total phenolics and antioxidants profiles of commonly consumed edible flowers	China	DPPH (µmol TE/g DW) ABTS (µmol TE/g DW)	acetone/water/acetic acid (70:29.5:0.5)/Maceration	Antioxidant capacity is significantly related to the TPC values. Whereas, TFC value and antioxidant capacities of flowers exhibit a poor relation

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3.1. Geographical Origin

The included studies originated from 11 countries: Morocco (n = 6, 21.4 %), Iran (n = 4, 14.3 %), India (n = 4, 14.3 %), China (n = 4, 14.3 %), Italy (n = 3, 10.7 %), Greece (n = 2, 7.1 %), and Turkey, Egypt, Lebanon, Spain, and Malaysia (n = 1 each, 3.6 %) (Fig. 2).

3.2. Assay Type

The DPPH assay was the most commonly employed method (26 studies, 92.9 %), followed by FRAP (11 studies, 39.3 %) and ABTS (8 studies, 28.6 %). Less frequently used assays included reducing power (3 studies, 10.7 %), lipid peroxidation and OH assays (2 studies each, 7.1 %), and a range of others such as NBT, metal chelating, FTC, TBA, TBARS, SOD, TEAC, TRAP, and TAC (each in 1 study, 3.6 %) (Fig. 3).

3.3. Solvent Type

Methanol was used in 15 studies (~53.57 %), water in 10 studies (~35.71 %), ethanol in 7 studies (~25.00 %), hydroethanol in 3 studies (~10.71 %), and hydromethanol in 2 studies (~7.14 %) (Fig. 4).

3.4. Extraction Method

Maceration was applied in 19 studies (67.86 %), ultrasound-assisted extraction (UAE) in 5 studies (~17.86 %), and Soxhlet extraction in 3 studies (~14.29 %) (Fig. 5). The heatmap results (Fig. 6) show that maceration was most commonly used in Iran and India, while UAE was more prevalent in China.

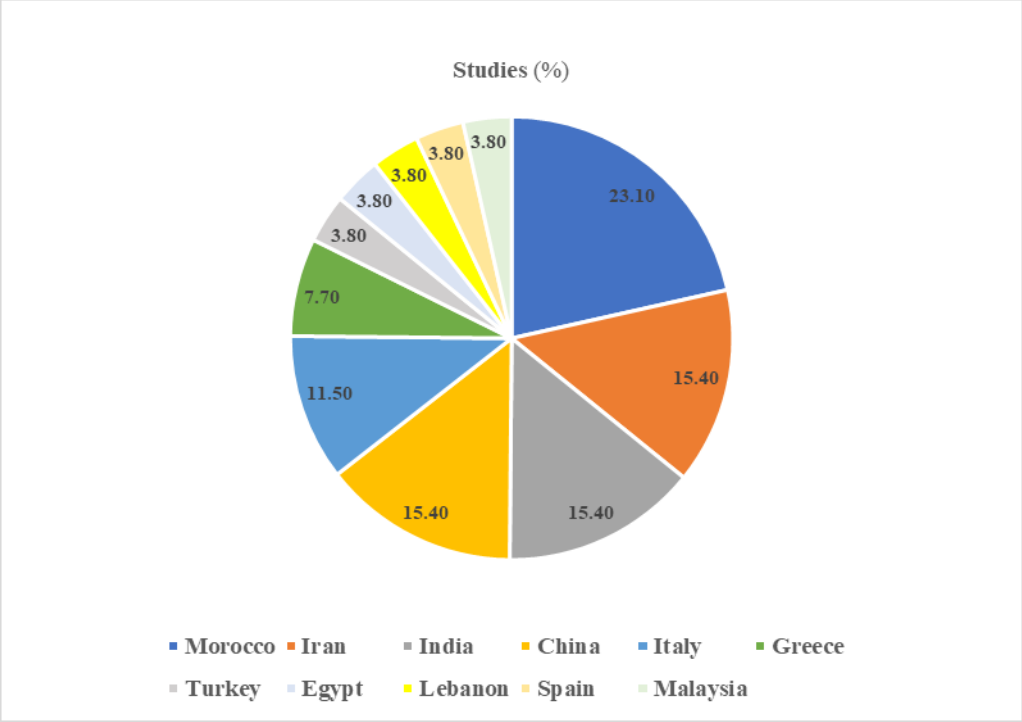

Fig. 2. Distribution of countries in reviewed studies

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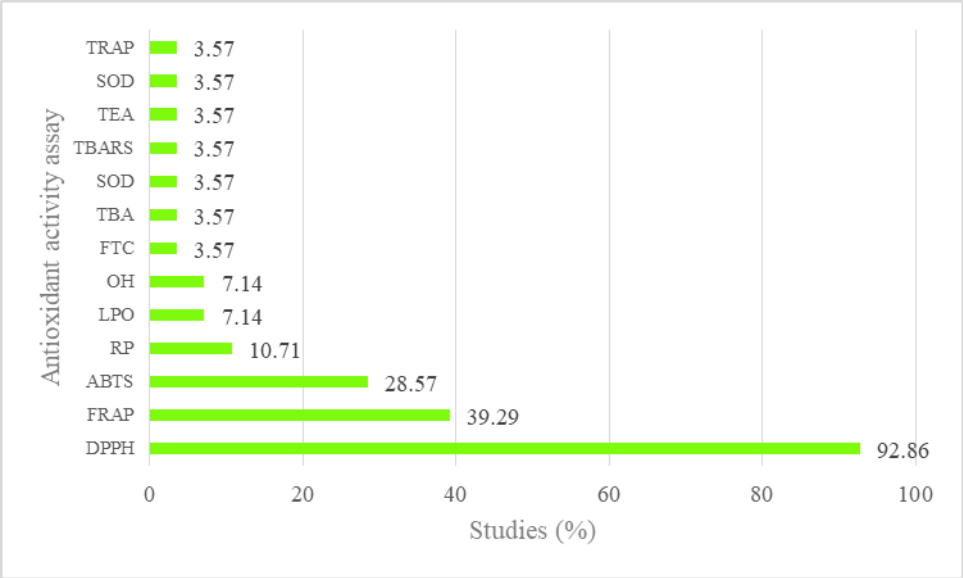

Fig. 3. Assay type distribution in reviewed studies

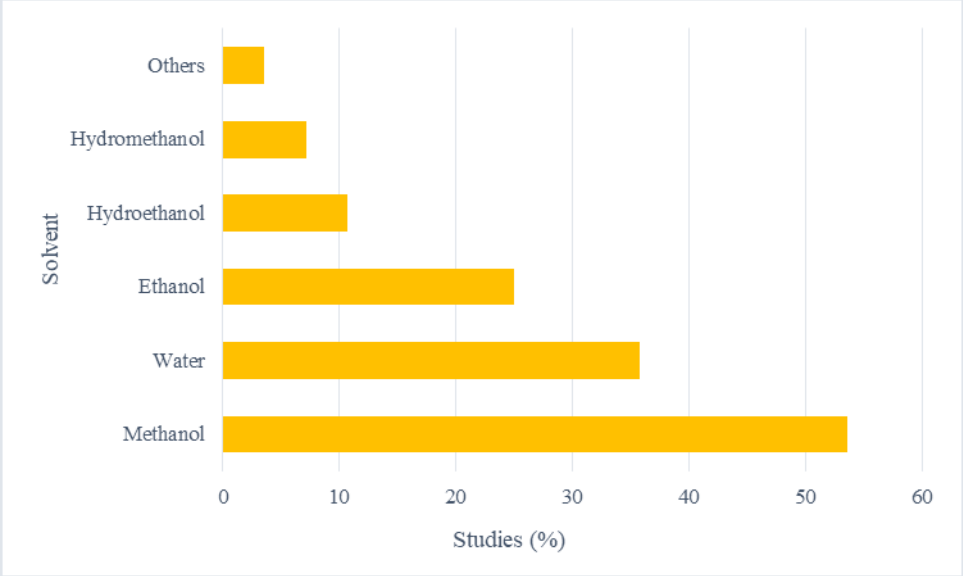

Fig. 4. Solvent usage in reviewed studies

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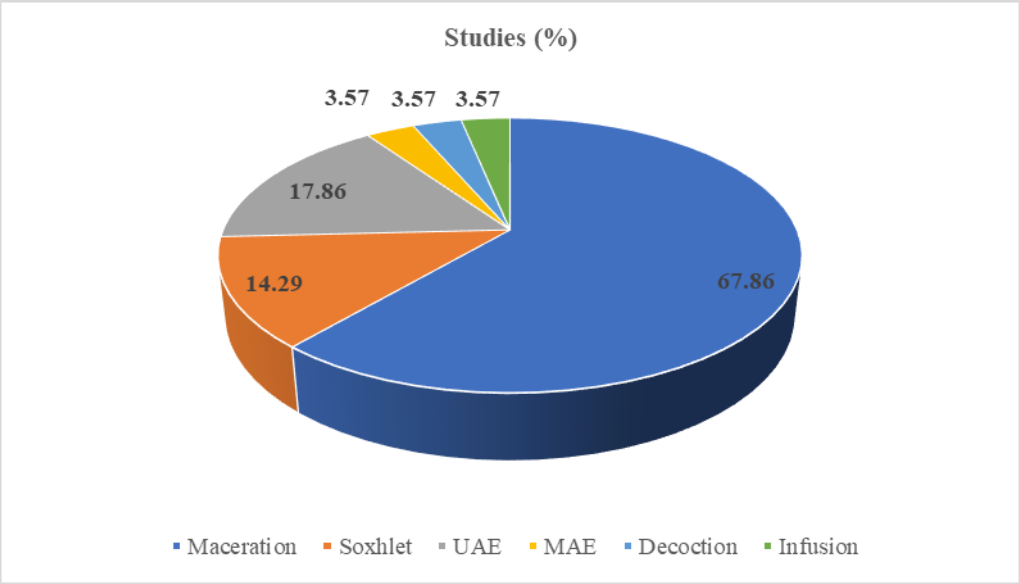

Fig. 5. Extraction method distribution in reviewed studies

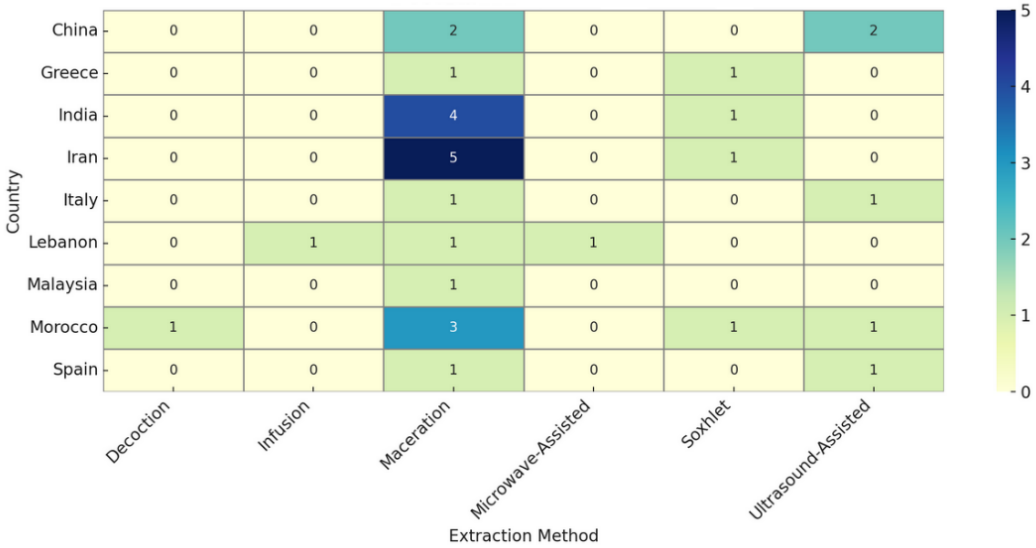

Fig. 6. Heatmap of extraction method by geographical origin

3.5. Correlations of Bioactive Compounds with Antioxidant Activity

Some studies reported correlations between antioxidant activity and levels of crocin, safranal, picrocrocin, and total phenolics. In certain studies, no correlation was observed between crocin levels and antioxidant activity. In others, moderate to high correlations were reported. Total phenolic content (TPC) showed consistent positive correlations with antioxidant activity across multiple studies. One study reported a correlation coefficient of r = 1.0 between TPC and antioxidant activity. Flavonoid content did not consistently correlate with antioxidant activity.

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4. Discussion

The findings of this systematic review underscore the potent *in vitro* antioxidant capacity of *Crocus sativus* stigma extracts, primarily attributed to its rich phytochemical profile—including crocin, picrocrocin, safranal, flavonoids, and phenolic acids. However, the magnitude and interpretation of antioxidant activity varied significantly across studies, largely due to methodological inconsistencies.

County of study was identified as one factor that affects the antioxidant activity of saffron. Research from various countries showed different levels of antioxidant activity, possibly due to the variation in climatic conditions, soil types, agricultural practices, and post-harvest treatment. For instance, Iranian saffron samples tended to have high concentrations of crocin, a compound that is highly correlated with enhanced antioxidant potential most particularly in ABTS and DPPH assays [18, 19]. Moroccan saffron, however, tended to exhibit close correlations between total phenolic content (TPC) and antioxidant activity, particularly when extraction was conducted using hydroethanolic solvents [20]. Indian research has mainly focused on the comparative evaluation of different plant parts, where it has been observed that stigmas displayed significantly better antioxidant activity compared to corms or leaves [21]. Among the 28 studies examined, the DPPH method stands out as the most widely used and prevalent technique, accounting for 26 studies. This finding aligns with general observations in phytochemical research, where DPPH is favored as a primary screening tool due to its simplicity, speed, and reliability. The mechanism of this test is based on the ability of antioxidant compounds to neutralize the stable DPPH radical. Following DPPH, the FRAP test was utilized in 11 studies and ABTS in 8 studies. These two methods are also extensively employed in antioxidant research, each covering different mechanisms for evaluating antioxidant capacity. FRAP measures the reducing power of compounds, while ABTS, similar to DPPH, operates on a radical scavenging principle but can be performed over a broader pH range [22]. Furthermore, a smaller number of studies employed other methods such as the reducing power assay, lipid peroxidation and OH assays. The diversity in these methods indicates researchers' efforts to comprehensively assess the antioxidant activity of *C. sativus* from various aspects. Other less frequently used methods include NBT, metal chelating activity, FTC, TBA, TBARS, SOD, TEAC, TRAP, and TAC, each addressing specific facets of the antioxidant mechanism. The predominance of DPPH and FRAP/ABTS in this study confirms the importance of these assays as gold standards in the *in vitro* evaluation of antioxidant activity. However, discrepancies in the results obtained from these assays can arise due to their distinct reaction mechanisms and varying sensitivities to the diverse antioxidant compounds present in *C. sativus* extracts. Therefore, it is recommended to employ a combination of multiple methods with different mechanisms for a comprehensive evaluation of antioxidant activity, to gain a more complete understanding of the extracts' antioxidant potential. This approach is particularly crucial given the complex biochemical composition of *C. sativus*, which includes glycosides, carotenoids, flavonoids, and monoterpenes. Solvent choice played a critical role in the management of the yield and constitution of antioxidant substances extracted from saffron stigmas. Methanol, water and ethanol were the most common solvents used followed by hydroethanol, hydromethanol.

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Methanol extracts were more likely to yield antioxidant activity, particularly in DPPH and FRAP assays, perhaps due to the fact that methanol has the ability to extract a larger range of polar and semi-polar antioxidants such as flavonoids and phenolics [23, 24]. However, water-based extractions proved to be highly effective, particularly when coupled with ultrasound-assisted extraction (UAE), and indicated that green methods can represent effective alternatives without compromising antioxidant yield [25, 26]. Notably, various studies determined that a positive relationship between total phenolic content (TPC) and antioxidant activity using methanol/water solvent blends occurred, therefore highlighting the role of solvent polarity in influencing extraction efficiency [14, 20]. The extraction procedure had a strong impact on the assayed antioxidant activity of saffron. Maceration was the most prevalent method, followed by ultrasound-assisted extraction or UAE and Soxhlet extraction. There has been a growing trend in using UAE in recent years, with studies revealing its excellence in extraction yield and greater antioxidant activity over conventional maceration [27-29]. Specifically, UAE method showed association with high content of crocin and total phenolic compounds, which are key contributors to antioxidant activity. Tong *et al*. confirmed a strong positive correlation between trans-4-GG (a derivative glycoside) and antioxidant activity, indicating the potential of newer extraction methods in increasing the recovery of bioactive constituents [28]. Likewise, Zhang *et al.* showed that UAE method greatly enhanced the extraction of crocin and polysaccharides, which both exhibited high correlations with DPPH scavenging activity [18]. The heatmap results (Fig. 6) show that maceration remains the world's most prevalent method of saffron extraction, particularly in Iran and India. However, there is a movement towards more efficient methods like UAE in various countries, including China. The distinction is due to the current traditional practices and the eventual shift towards newer, advanced extraction methods in saffron research worldwide. A number of studies have tried to establish a correlation between antioxidant activity and the level of certain bioactive compounds like crocin, safranal, picrocrocin, and total phenolics. Although crocin has generally been considered to be the principal antioxidant compound in saffron, the correlation between its level and antioxidant activity was not always linear. For example, Chen *et al.* reported no correlation between crocin levels and antioxidant activity [30], while other reports indicated moderate to high correlations, especially in the ABTS and DPPH assays [18, 27]. Total phenolic content (TPC) has, however, consistently demonstrated positive correlations with antioxidant activity in various test protocols. Drioiche *et al.* found an absolute correlation (r = 1.0) of TPC with antioxidant activity across all the extraction methods tested, pointing to the crucial role of phenolic compounds in the antioxidant character of saffron [20]. Other studies emphasized the role of phenolic acids and flavonoids in contributing to total antioxidant capacity, whereas the flavonoid content by itself did not consistently exhibit high correlations [8, 9, 31]. The current systematic review highlights significant variation in methodological approaches, such as the selection of assays, solvents, extraction methods, and reporting, all of which collectively influence reproducibility and comparability of outcomes. To take this field further, standard protocols that define best practices in saffron

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extraction and determination of its antioxidant activity are necessary. Follow-up research studies ought to take into consideration the utilization of multi-assay approaches to ensure the whole antioxidant activity spectrum is covered and the employment of rigorously validated quantitative techniques for quantifying the bioactive components. Moreover, careful documentation of experimental parameters, such as solvent concentration, temperature, extraction time, and sample preparation, would significantly enhance the reproducibility and validity of the data.

5. Conclusion

This systematic review points to the marked *in vitro* antioxidant activity of *Crocus sativus* L. stigma extracts, which is attributable to a large extent to bioactive molecules like crocin, picrocrocin, safranal, flavonoids, and phenolic acids. Nevertheless, the degree of antioxidant activity differs greatly among studies, with a strong reliance on methodological disparities, especially in relation to assay selection, geographical origin, extraction procedures, and solvent polarity. The most commonly utilized assays were the DPPH, FRAP, and ABTS, with the most common one being DPPH; however, a multi-assay strategy is suggested to enable a broader evaluation. Iranian and Chinese saffron samples tended to have greater antioxidant activity, an indication of the climatic conditions and agricultural methods. Techniques such as ultrasound-assisted extraction (UAE) were more efficient in the extraction of antioxidant compounds than traditional maceration methods. Solvent choice was also important, with methanol and hydroalcoholic mixtures being optimal for maximum antioxidant activity, although aqueous extracts were also promising when combined with advanced methodologies. Correlation studies revealed total phenolic content to consistently exhibit high positive correlations with antioxidant activity, whereas crocin presented variable correlations based on the assay and sample preparation technique. The results emphasize the importance of standardization in future studies in order to enhance reproducibility and comparability to the greatest extent. In general, *C. sativus* L. presents high antioxidant activity, hence its potential applications in the food, pharmaceutical, and cosmetic industries. It is important to note that while these findings offer robust methodological guidance for future research and preclinical development, they do not constitute definitive evidence for the efficacy of specific saffron-based products or their direct clinical outcomes in human health.

Authors' contributions

Corresponding author conceived the title of study and searching the databases. The screening of articles based on inclusion and exclusion criteria and data extraction were performed by all three authors.

Conflicts of interest

The authors declare no competing interests.

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