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DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of crude extract and various fractions of *Crocus sativus*

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Abstract

Oxidative stress is one of the major etiologic factors that form the basis of wide range of chronic and degenerative diseases. The antioxidant compounds found in medicinal plants have, therefore, gained an extensive academic interest because of their potential therapeutic value. In the DPPH (2,2-diphenyl-1-picrylhydrazyl), assay, the radical-scavenging activity of the crude methanolic extract and solvent-partitioned extracts of the dried stigmas *of Crocus sativus L.* were examined. The crude extract was then subjected to consecutive liquid liquid partitioning using n- hexane, chloroform, ethyl acetate, methanol and n-butanol to give separate fractions. Antioxidant activity was assessed at concentrations ranging from 10 to 100 µg/mL. All the fractions had concentration-dependent radical-scavenging activity. The polar fractions including the n-butanol and methanol fractions showed the greatest percentage scavenging of up to 91.35%. Conversely, the non-polar fractions had relatively low activity. These results highlight the central role of solvent polarity in the effectiveness of bioactive compound extraction. The strong antioxidant activity of the polar fractions of saffron is an indicator of the potential of the fractions as natural sources of antioxidants with possible uses in the nutraceutical and pharmaceutical industries.

KEYWORDS

Crocus sativus L., antioxidant activity, DPPH assay, phenolic compounds, solvent fractions, n-butanol extract

1.0 INTRODUCTION

Azadirachta indica, Oxidative stress is a primary predisposing factor in the pathogenesis and progression of a continuum of chronic and degenerative diseases, such as oncological, cardiovascular, neurodegenerative, and metabolic diseases [1,2]. This disturbance is caused by an imbalance between overproduction of reactive oxygen species (ROS) and the reduced ability of the endogenous antioxidant defence system, eventually leading to the occurrence of oxidative lipid- protein and nucleic acid damage [1]. Therefore, development of effective antioxidant compounds that are capable of trapping free radicals has drawn a lot of academic attention.

Synthetic antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) are widely used in the food and pharmaceutical industries; however, increasing doubts about their long-term safety, as well as possible cytotoxicity have triggered growing interest in naturally derived antioxidants, obtained as food grade or pharmaceutical material through medicinal flora [3]. Antioxidants found in plants, especially phenolic substances and flavonoids provide protection by processes that include hydrogen-atom donation and electron transfer, which neutralise free radicals [3].

Crocus sativus L. (saffron) is a botanical species of great medicinal significance to the family Iridaceae. It has been used in therapeutic form because of its promising characteristics, and it has found more applications in areas other than culinary application and includes proven antioxidant, anti-inflammatory, neuroprotectant, antidepressant and anticancer properties [4,5]. These various biological functions are owed in large part to its bioactive compounds, in particular, crocin, crocetin, picrocrocin, safranal, and a range of phenolic components [6]. Empirical studies have highlighted high free-radical scavenging properties of saffron extracts and its separated constituents [7].

The DPPH (2,2 2 -diphenyl 2 -picrylhydrazyl) radical scavenging assay is one of the most basic experimental methods, quick, sensitive, and reproducible to measure the in vitro antioxidant activity [8,9]. The test is based on the fact that reducing the initial violet-coloured DPPH radical to a yellowish colour on reaction with antioxidant compounds allows the radical-scavenging effect to be evaluated quantitatively [8].

Although there is an increasing amount of evidence supporting or opposing the antioxidant activity of Crocus sativus, there is a lack of studies that provide comparative analysis of the radical-scavenging properties of crude extracts and solvent-fractionated preparations. The fractionation of solvents is a fundamental technique of selective enrichment of selected classes of phytochemicals in terms of polarity, which may affect antioxidant activity [10]. To this effect, this paper sought to critically evaluate the DPPH radical-scavenging activity of the crude methanolic extract and its different solvent fractions, with an aim of elucidating the role of specific phytochemicals in the extract in terms of their contribution to the overall antioxidant activity of the extract.

MATERIALS AND METHODS

Plant collection and Extraction

Crocus sativus L. is a plant with pure dried stigmas that were purchased in Dubai. The 250 g of the dried saffron stigmas were impregnated in 250 mL of 50% (v/v) ethanol at room temperature within a period of one week with intermittent stirring. The mixture was then filtered through Whatman No. 1 filter paper, and a bright orange-red filtrate was obtained, which was taken as the source extract and subjected to further experimentation procedures [11].

Antioxidant activity

The DPPH (2, 2 -diphenyl-1 -picrylhydrazyl, DPPH) radical scavenging assay was used to determine the antioxidant activity of the crude extract and its solvent fractions according to standard reported protocols with minor modifications [12,13,14]. The principle underlying the method is the capacity of the

antioxidant compounds to give electrons or hydrogen atoms, which causes the reduction and discolouration of the purple coloured DPPH radical. In methanol, the 1mM solution of DPPH was prepared. Briefly, a 1mL aliquot of the DPPH solution was combined with 3 mL of sample solutions that had been prepared in methanol at various concentrations (20–100 µg/mL). A control solution with DPPH and methanol, without sample was also prepared. The mixtures of the reaction were incubated during 30min in darkness at room temperature. Following incubation, a UV- visible spectrophotometer was then used to record the absorbance at the wavelength of 517nm. Each experiment was done three times. Reduction in absorbance of DPPH solution was a sign of high level of free radical scavenging activity. percentage of DPPH radical scavenging activity, or the percentage of RSA was calculated by the following equation:

$$\text{DPPH Radical Scavenging Effect (\%)} = \frac{Ac - As}{\text{Ac}} \times 100$$

Where, Ac is the absorbance of control reaction and As is the absorbance of test sample or standard.

Statical analysis

Every experiment was carried out in three replicates and the results are reported in mean standard deviation (SD). Analysis was performed with one-way analysis of variance (ANOVA) which was followed with the post-hoc test (Tukey) to determine the notable differences between the crude extract and solvent fractions at varying concentrations. A p-value of less than 0.05 was found to be statistically significant.

RESULTS AND DISCUSSION

The DPPH radical scavenging assay was used to determine antioxidant activity of the crude methanolic extract and its different solvent fractions of *Crocus sativus*. The radical scavenging activity of all the samples had a dose-dependent phenomenon, which aligns with the generally noticed activities of plant extracts resembling antioxidants [8,9]. As demonstrated in Table 1 the polar fractions tended to have higher scavenging potential as compared to the non-polar fractions in the concentration range measured.

At the lowest concentration (10 µg/mL), the n-hexane fraction exhibited minimal radical scavenging activity (16 ± 2.00%), whereas the chloroform and ethyl acetate fractions demonstrated moderate activity of 34 ± 1.09% and 40 ± 2.22%, respectively. With increasing concentration, all fractions showed enhanced %RSA values, with the n-butanol fraction reaching the highest activity (91 ± 1.35%) at 100 µg/mL. The crude methanolic extract also demonstrated substantial scavenging effects, rising from 49 ± 1.03% at 10 µg/mL to 85 ± 1.43% at 100 µg/mL. The observed order of activity at 100 µg/mL was: n-butanol > methanol > ethyl acetate > chloroform > n-hexane (Table 1).

Table 1: Antioxidant activity of crude extracts and various fractions of *Berberis vulgaris*

Concentration	n-hexane	Chloroform	Ethyl Acetate	Methanol	Butanol	Standard
10 µg/mL	16±2.00	34±1.09	40±2.22	49±1.03	51±1.99	91.44±0.60
20 µg/mL	22±1.99	39±1.34	47±2.04	56±1.44	60±1.80	95.01±0.87
40 µg/mL	29±1.87	44±1.39	53±1.45	66±1.04	70±1.67	95.10±0.30
60 µg/mL	35±1.66	49±1.70	58±1.98	76±1.44	85±1.47	94.80±0.30
80 µg/mL	44±1.70	55±1.80	65±1.47	83±1.30	89±1.40	94.55±0.87
100 µg/mL	47±1.80	65±1.09	73±1.22	85±1.43	91±1.35	94.09±0.70

The radical scavenging effect of the n -butanol and methanolic fractions is also very strong, although this effect is probably due to the high levels of polar antioxidant compounds (including phenolics and flavonoids) that are capable of donating hydrogen atoms to neutralize free radicals [15,16]. Similar findings have been reported with saffron extracts, where the methanol and other polar solvent extracts had higher antioxidant activities compared to the non-polar ones, and this is due to the high solubility of phenolic compounds in polar solvents [15,17]. In fact, previous research has found the presence of phenolic and flavonoid constituents as the main factors in DPPH scavenging activity in saffron extracts [15,17].

The relatively lower activity of non-polar fractions (n-hexane and chloroform) may be attributed to the fact that these two polarity non-polar phases do not extract polar antioxidant constituents effectively. The trend is also consistent with the results of other botanical studies, in which non-polar solvent extracts typically exhibit lower DPPH radical-scavenging capacity than polar extracts due to the fact that bioactive metabolites are not soluble in the same ratios [16,17]. As an example, saffron stigma and petal methanolic extracts are proven to be significantly more active in antioxidant properties than their non-polar counterparts, which is explained by high levels of phenolic compounds and apocarotenoid hydrophilic substances [15,17].

In general, the current evidence shows that the polarity of the solvent has a significant impact on the antioxidant capacity of the *Crocus sativus* extracts. Fractionation therefore improves the separation of phytochemicals with strong antioxidant activity especially polar fractions. The n-butanol fraction has a high percent RSA and this indicates its potential as a source of antioxidant compounds which should be further phytochemically characterized and potentially used in nutraceutical or pharmaceutical preparations [15,17].

CONCLUSION

This paper has shown that *Crocus sativus* extracts have a high level of antioxidant activity, which is concentration-dependent. Radical scavenging potential was highest in the polar fractions in which n-butanol and methanol were the most active, and low in the non-polar fractions. These results suggest that solvent polarity is a critical factor in extracting bioactive compounds which cause antioxidant activities. In general, the polar fractions of saffron extracts can be considered as a potential source of natural antioxidants that can find their use in nutraceutical and pharmaceutical products.

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CONFLICT OF INTEREST

Authors express no conflict of interest

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