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ORIGINAL ARTICLE

Antiproliferative Effect of Saffron and Its Constituents on Different Cancerous Cell Lines

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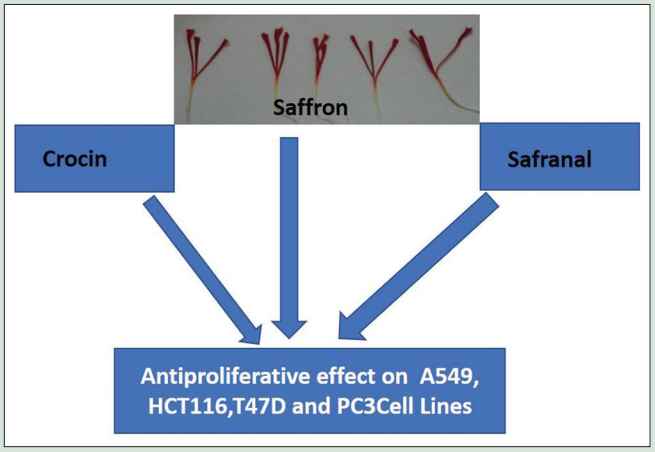
ABSTRACT

Background: Use of herbs as medicinal plants to treat various clinical ailments has grown for the last two decades. Cancer is one of the most dreadful disease and extracts of various medicinal herbs have been subjected for the analysis to elucidate their possible role in the prevention of proliferation of cancer cells. Crocus sativus is an autumnally flowering plant rich in active ingredients apocarotenoids such as crocin, picrocrocin, and safranal which have been reported to have antiproliferative potential due to their strong antioxidant potential. **Objective:** To elucidate the antiproliferative potential of *C. sativus* extract (CSE) and its major constituents crocin and safranal on four different malignant cell lines (Alveolar lung epithelial cancerous cell line [A549], breast epithelial cancerous cell line [T47D], colon colorectal cell line [HCT-116], and prostrate cancerous cell line [PC3]) and nonmalignant cell line (L929). **Materials and Methods:** High-performance liquid chromatography was used to measure the content of crocin and safranal in saffron extract and antiproliferative effects of CSE, crocin, and safranal were evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. All cells were incubated with different concentrations of CSE, crocin, and safranal for 48 h. In a concentration-dependent manner, both safranal and crocin reduced cell proliferation in all malignant cell lines. **Results:** The IC50 values ranged between 0.32 and 0.42 mM for safranal, 0.31 and 0.92 mM for crocin, and 0.58 and 0.98 mg/ml for saffron extract. **Conclusion:** Based on these findings, it can be concluded that saffron and its components can inhibit cell proliferation in cancerous cells. Consequently, these agents could potentially be used as a chemopreventive agent for cancer management in the near future.

Keywords: Antiproliferative effect, crocin, *Crocus sativus*, high-performance liquid chromatography, safranal

SUMMARY

- 1. HPLC profiling of saffron extract was performed.
- 2. Antiproliferative effect of CSE and Its components on four different cancer Cell lines was evaluated.
- 3. Results showed significant antiproliferative role of CSE crocin and safranal on cancer cell lines.
- 4. However, the effects of CSE and its components varied from one cell lines to another.



Abbreviations Used: CSE: *Crocus sativus* extract; A549: Alveolar lung epithelial cancerous cell line, HCT-116: Colon colorectal cell line, T47d cell line: Breast epithelial cancerous cell line, PC-3 cell line: Prostrate cancerous cell line; HPLC: High-performance liquid chromatography, MTT: 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide.

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INTRODUCTION

Plants possess economically and therapeutically valuable metabolites which are considered to be medicinally important.[1] For the last two decades, the importance of natural products/secondary metabolites of plants has increased and has been used in various clinical ailments, for example, diabetes,[2] physiological conditions,[3] autoimmune disease,[4] lung cancer and cardiovascular disease,[5,6] atherosclerosis,[7] and antiasthmatic and in allergic responses.[8-12]

Chemoprevention of cancer by natural compounds is considered to be promising strategy against cancer initiation. Natural compounds with strong antioxidative and anti-inflammatory potential are interesting candidates to evaluate their ability to influence the initiation and growth of tumors. Carotenoids, an important class of natural compounds, are considered valuable candidates for further investigation in the study of their antiproliferative role.[13,14]

Crocus sativus is one such important medicinal herb belonging to Iridaceae family and contains more than 150 volatile and nonvolatile constituents that have been identified including anthocyanin, carotenoids, aldehydes, and flavonoids.[15-17] Saffron, the dried stigmas of *C. sativus*, is rich in apocarotenoids (a class of carotenoids) that include crocins (the main saffron-colored compounds with their unusual water-soluble properties), safranal (the volatile oil responsible for the characteristic saffron smell and aroma), and picrocrocin (the main substance responsible for the bitter taste) in saffron [Figure 1].[18]

Saffron and its components have been used in traditional medicine

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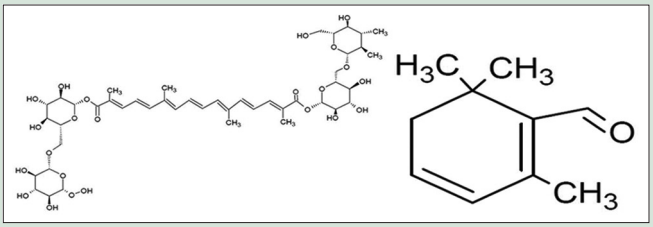

Figure 1: Components of saffron (a) Crocin (b) Safranal

as an anti-aphrodisiac, antiasthmatic, antispasmodic, expectorant, stomach ailments, and for smoothing menstruation.[11,15,19,20] Recent studies have revealed anti-carcinogenic, anti-mutagenic, anti-tumor and cytotoxic effects of saffron and its components. These effects are mediated via various mechanisms including inhibition of cell growth. These properties of saffron and its components could be of great significance in the prevention/treatment of cancer.[16,21-24] Although various studies have shown antiproliferative and potent cytotoxic effects of saffron and its components against various cancer cell lines (HeLa, HL60, K562, P388, and S-180);[24,25] however, there is a need of further evaluation of antiproliferative role of saffron extract along with its components on other cancer cell lines. Such studies could be an important step toward understanding the antiproliferative effect, hence, application of saffron in the development of safe and efficacious anticancer therapies. Moreover, administration of saffron along with other anticancer agents could prove to be more effective in the inhibition of colony formation and nucleic acid synthesis compared to the anticancer agent alone.[26]

The aim of this study was to investigate the effect of saffron and its components, i.e., safranal and crocin on cancer cell lines alveolar lung epithelial cancerous cell line (A549), colon colorectal cell line (HCT-116), breast epithelial cancerous cell line (T47D), and prostrate cancerous cell line (PC-3). In addition, effect of *C. sativus* on nonmalignant cells (L929 cells) was also analyzed.

The content of *C. sativus* extract CSE and its constituents, crocin and safranal was measured by high-performance liquid chromatography (HPLC) and were used in the present study for further evaluation.

MATERIALS AND METHODS

Plant extract and high-performance liquid chromatography analysis

Saffron stigmas collected from plants cultivated at Pampore, Kashmir, were selected for HPLC analysis. Extraction of saffron stigmas was performed according to Lozano *et al.*[27] with some modifications. For the estimation of crocin and safranal, 50 mg of saffron stigmas were suspended in 10 ml methanol–water (50:50, v/v) and stirred for 24 h at 4°C in the dark. After extraction, samples were centrifuged at 20,000 g for 45 min to eliminate plant residues and the supernatant was collected and filtered through a 0.45-µm nylon membrane (Millipore, USA). The samples were stored in the dark till analyzed. HPLC analysis was performed with a Waters (USA) HPLC system equipped with 515 quaternary gradient pumps, 717 rheodyne injector, 2996 PDA detector, and Empower software (Version 3.0). The column used for separation was RP-18 (4.6 mm × 250 mm, 5 µm) (Merck) column. The mobile phase consisted of methanol–water (50:50) (v/v) delivered at a flow rate of 0.8 ml/min. Safranal and crocin (crocetin digentiobiose ester) used in this study were purchased from Sigma-Aldrich, USA.

Cell viability

Cell culture and treatments

Different human cancer cell lines were purchased from American Type Culture Collection, USA. Malignant cell lines selected in this study were A549, PC3, HCT116, and T47D cell line and nonmalignant cell line L929. All the cell lines were grown in Dulbecco's Modified Eagle's medium growth medium. PC3 cells were grown in RPMI growth medium. Media for both the cell lines was supplemented with 10% fetal calf serum, 100U penicillin G, and 100 µg/ml of streptomycin. Cells were grown in 5% CO_2 at 37°C with 95% humidity. All the test compounds were dissolved in dimethyl sulfoxide (DMSO) for treatment of either different cells, while the untreated control cultures received only the vehicle (DMSO < 0.2%).

Antiproliferation assay using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method

The anticancer activity *in vitro* was measured using the MTT assay 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), a yellow tetrazole). The assay was carried out according to known protocol.[28] The cell proliferation assay was performed on different human cancer cell lines, e.g., A549, HCT116, PC3, T47D, and nonmalignant cell line L929 cell lines to assess the dose-dependent effect of CSE, crocin, and safranal on their proliferation. Cells (1×10^4 cells/well) were seeded into each well of a 96 plate for 48 h. Medium was changed and cells were treated with different concentrations of crocin, safranal, and their crude extracts for 48 h. MTT dye (10 µl) was added to 100 µl of medium in each well 4 h before the termination of the experiment. Formazan crystals were dissolved in DMSO before taking absorbance at 570 nm. Cell viability of the untreated control samples was considered to be 100%, while viability of other samples was calculated using the following formulae.

$$\%\,\text{cell viability} = \frac{\text{OD(test)}}{\text{OD(control)}} \times 100$$

RESULTS AND DISCUSSION

HPLC profiling of CSE was performed and the effect of crude extract and its components, i.e., crocin and safranal on different cancerous cell lines was evaluated. CSE used in this study was prepared from stigmas of *C. sativus*. CSE contains several pharmacologically active constituents and among these compounds, crocin and safranal are considered to be important because of their significant biological activities.[15] Crocin is a colored compound that is unusually water-soluble carotenoids containing mono- and di-glycosyl esters of a polyene dicarboxylic acid, and safranal is the volatile oil responsible for odor and aroma. Therefore, quantification analysis of crocin and safranal in CSE extracts is an important step for the effective evaluation of their content, thus for the future investigations such as anticancer or antiproliferative activity.

In this study, we used HPLC method for the quantification of crocin and safranal content. HPLC profiling study revealed that the saffron used in this study is of good quality with quantity of crocin and safranal to be 68.23 mg/g [Figure 2a] and 0.92 mg/g, respectively [Figure 2b]. The percentage of crocin and safranal found in the extract was 43%. To determine the antiproliferative effect of saffron, i.e., crude extract, the HPLC-analyzed samples were used.

Antiproliferative potential of saffron and its components was measured using MTT assay. MTT is reduced to purple formazan in living cells, and using this property, proliferation of cells after drug administration can be measured. To determine the antiviability effect

of saffron, all the four malignant cell lines and nonmalignant cell lines were treated with various concentrations of saffron for 48 h. Cell lines selected in this study were A549, PC3, HCT116 and T47D, and L929. In this study different concentrations of CSE i.e. 0.2, 0.4, 0.6, 0.8, and 1 mg/ml were used and the concentrations of safranal and crocin used ranged between 0.1 mM/ml to 1 mM/ml. MTT assay was used to determine the cell viability after the treatment of CSE, safranal, and crocin. Figure 3a-c shows the effect of different concentrations of CSE, crocin, and safranal used on four cancer cell lines, respectively. Table 1 enlists the IC50 values of (a) Crocin (b) Safranal and (c) Crocus sativus extract A549, HCT 116, T47D, and PC3 cell lines IC50 value.

Crude extract of saffron (CSE) showed IC_{50} values of 0.58 mM in A549, 0.98 mM in HCT116, and 0.86 mM in T47D cell lines. The effect of CSE in PC3 cell line was not observed at the concentration used in the present study, indicating that the IC_{50} value may be beyond 1 mg/ml concentration [Figure 4a].

IC_{50} value of safranal in A549, PC3, HCT116, and T47D cell lines were reported to be 0.33 mM, 0.17 mM, 0.42 mM, and 0.36 mM, respectively, [Figure 4b] and the IC_{50} values of crocin observed during the investigation were 0.35 mM in T47D, 0.78 mM in A549, 0.68 mM in HCT116, and 0.92 in PC3 cell lines [Figure 4c]. In nonmalignant cell line L929, CSE, crocin, and safranal did not show any effect hence not mentioned further.

Our reported results suggest that the response of different cancerous cell lines to CSE and its components is not the same and have varying inhibitory effect on the proliferation of these cell lines [Figures 3 and 4]. CSE attenuated the proliferation of HCT 116, T47D, and A549 cell lines. However, the effect on PC3 cell lines at the used concentrations was nonsignificant. The results show that A549 show significantly higher sensitivity to CSE cell line and PC3 cell line showed the lowest response at the used concentration (IC_{50} > 1 mg/ml).

The effect of crocin was significant on T47D cell lines (IC_{50} = 0.35 mM/ml) and comparatively less effective in PC3 cell line with IC_{50} value of 0.921 mM/ml. Among CSE, safranal, and crocin, safranal with its IC_{50} values ranging between 0.17 mM (PC3 cell line) and 0.42 mM/ml (HCT116 cell line) was found to be more effective on all the cell lines. Crocin and safranal were able to suppress the proliferation of A549, HCT116, T47D, and PC3 cell lines, but the effect varied in different cell lines as depicted by its IC_{50} values. Figure 3 depicts the effect of CSE, crocin, and safranal on different cancerous cell line.

The antiproliferative evaluation study showed that CSE, crocin, and safranal have different effect on the cell lines used in the study. The observation of the study show that a particular cell line does not respond in same manner to the different components of the extract or the extract itself that were used during the investigation, e.g., in A549 cell line, safranal is more effective than crocin (IC_{50} = 0.78 mM), and in PC3 cell lines, again safranal (IC_{50} = 0.173 mM) is more effective

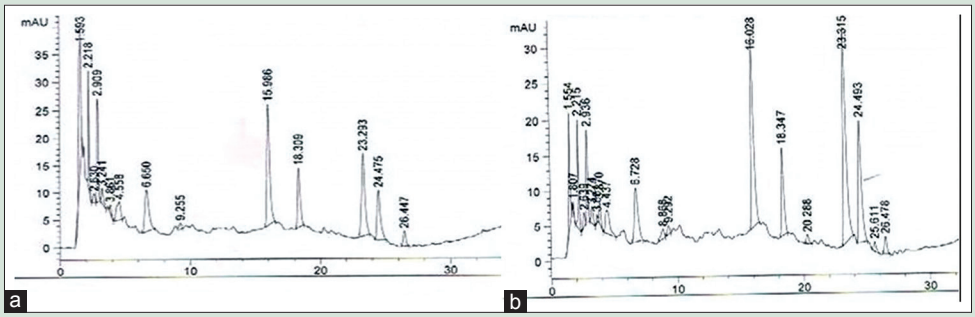

Figure 2: High- performance liquid chromatogram of saffron extract (a) Crocin , (b) Safranal

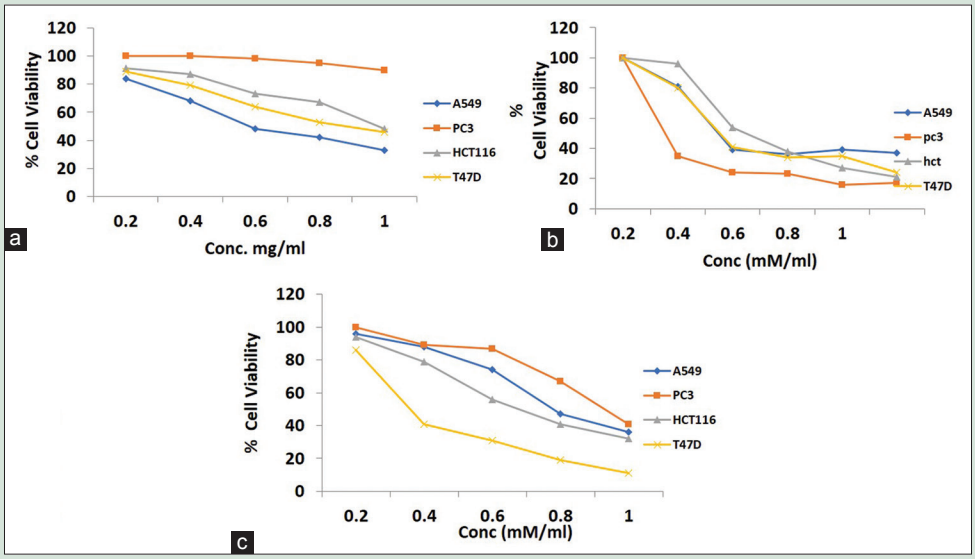

Figure 3: Dose-dependent effects of (a) crocin (b) safranal and (c) saffron extract on A549, HCT116, T47D, and PC3 cell lines

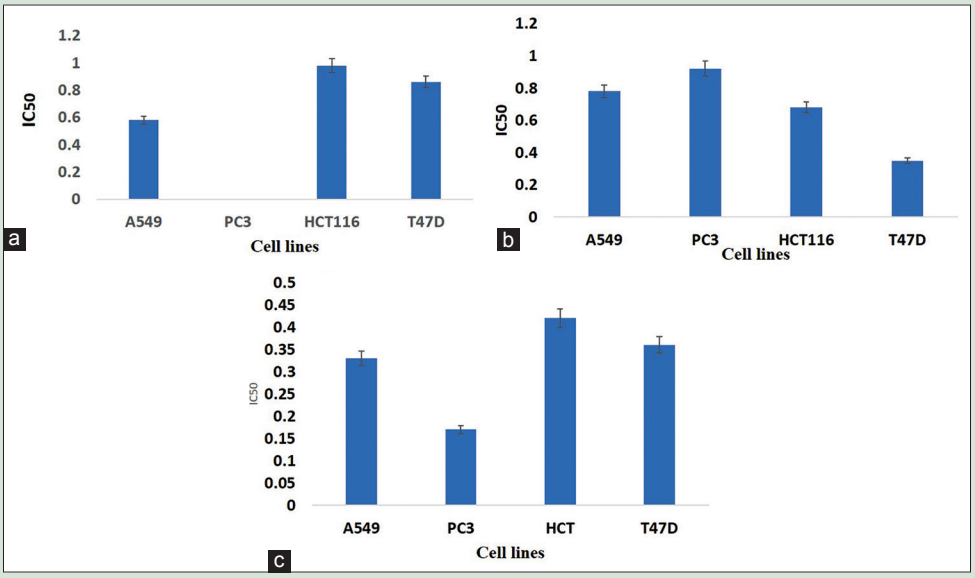

Figure 4: IC^{50} value of (a) CSE (b) Crocin (c) Safranal on A549, HCT116, T47D, and PC3 cell lines

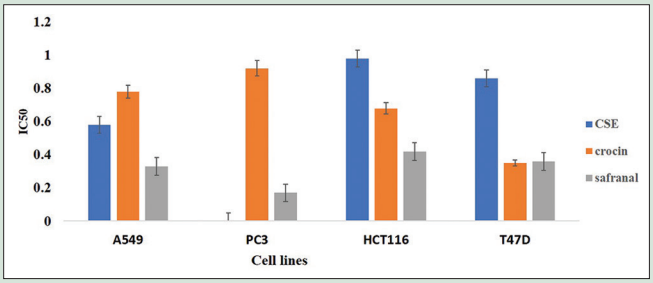

Figure 5: Comparative analysis (in terms of IC50 value) of the CSE, crocin, and safranal on the individual cell line

than crocin (IC_{50} = 0.921 mM). In addition, CSE showed varied effect on two different cell lines, wherein in case of A549, IC_{50} values was 0.58 mg/ml, and in case of PC3 cell line, the value lied beyond the concentration used in the study. Considering the values reflected in the present study, it is evident that the cell lines used in the study show different response to the components/extract used and also when a single component/extract was used on different cell lines, it showed varied results, e.g., the IC_{50} of 0.35 mM in T47D and 0.92 mM in PC3 [Figures 3 and 4]. Figure 5 shows comparative analysis (in terms of IC_{50} value) of the CSE, crocin, and safranal on the individual cell line and it can be observed that safranal is the most effective among the three almost on all the cell lines used followed by crocin except in the case of T47d, wherein both crocin and safranal were almost equally active. No effect was observed in nonmalignant L929 cells on the concentration used during the study.

As crocin and safranal are the main constituents of CSE, the antiproliferative effect shown by CSE in this study could be suggested to be mainly because of these bioactive components. However, the results show that safranal and crocin are more potent antiproliferative agents against cancerous cell lines compared to CSE. The differential response of the studied cancerous cell line to CSE, crocin, and safranal could be possibly because of the difference in the expression of receptors and due to the existence of distinct cell surface receptors, intracellular retention transport, and differences in the uptake of certain drugs in

Table 1: IC_{50} values of (a) crocin (b) safranal and (c) Crocus sativus extract A549, HCT 116, T47D, and PC3 cell lines IC_{50} value

Cell lines	IC_{50} values		
	CSE	Crocin	Safranal
A549	0.58	0.78	0.33
PC3	0	0.92	0.17
HCT116	0.98	0.68	0.42
T47D	0.86	0.36	0.36

conclusion in these cell lines.[29-32] In addition to the above, the other possible mechanisms via which saffron and its components impart its antiproliferative effect involves interaction with nucleic acids to protect from harmful damages,[33] through inhibition of DNA adduct formation[34] and through exerting inhibitory effect on cellular DNA and RNA synthesis. Other possible mechanisms include apoptotic modulation by selectively promoting pro-apoptotic effect in tumoural cells[35,36] and inhibition of free-radical chain reactions that could lead to oxidative damage and DNA alterations and changes in the level of enzymes such as glutathione S-transferase, protein kinase C, and reduction in the expression of proto-oncogenes.[37]

Higher concentrations exerted pro-apoptotic effect, suppressing pulmonary tumor promotion and induction of apoptotic effect in a lung cancer-derived cell line. Saffron and its constituents inhibit the growth of malignant cells both *in vivo* and *in vitro* and inhibition of intracellular nucleic acid synthesis, and free radical chain reactions are considered to be the possible molecular mechanisms involved in antitumor effect of saffron. However, the precise molecular mechanism of antitumor activity of these natural agents is not well understood at present. Although these findings are of great significance indicating saffron as a chemopreventive agent, however, elaborate clinical trials are required to warrant the use of saffron and/or its main ingredients as natural agents for prevention and treatment of different human cancers.

CONCLUSION

Cancer is a growing health problem around the world. Natural products have long been used to prevent and treat many diseases, including cancer and are considered to be good candidates for the development

of anticancer drugs.[38] The biological and medical properties of saffron and its ingredients have been reported earlier. Recent scientific findings and present study have been encouraging, uniformly showing that saffron and its components can affect carcinogenesis and currently are being studied as one of the cancer chemopreventive agents. Saffron and its active compounds can prove to be promising anticancer candidates. However, saffron extract along with its components need extensive therapeutical evaluation at higher concentrations and the mechanistic studies are warranted for the same.

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Conflicts of interest

There are no conflicts of interest.

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