MINIREVIEW

Cancer Chemopreventive and Tumoricidal Properties of Saffron (*Crocus sativus L.*)

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Since cancer is the most common cause of death in the world population, the possibility that readily available natural substances from plants, vegetables, herbs, and spices may be beneficial in the prevention of cancer warrants closer examination. Saffron in filaments is the dried, dark red stigmata of *Crocus sativus* L. flowers and it is used as a spice, food colorant, and a drug in medicine. A growing body of research has demonstrated that saffron extract itself and its main constituents, the carotenoids, possess chemopreventive properties against cancer. This review discusses recent literature data and our results on the cancer chemopreventive activities of saffron and its main ingredients. [Exp Biol Med Vol. 227(1):20–25, 2002]

Key words: saffron *Crocus sativus L.*; antitumor; anticarcinogenic; antimutagenic activities; cytotoxicity; chemoprevention

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives each year (1). An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or in combination) to block the development of cancer in human beings. Plants, vegetables, herbs, and spices used in folk and traditional medicine have been accepted currently as one of the main sources of cancer chemopreventive drug discovery and development (2). A large and increasing number of patients in the world use medicinal plants and herbs for health purposes. Therefore, scientific scrutiny of their therapeutic potential, biological properties, and safety will be useful in making wise decisions about their use. For example, one in three people in the United States has used at least one form of alternative medicine (3). From ancient times to the present, saffron has been used as a spice for flavoring and coloring food preparations, as a perfume, and also as a dye or ink. In folklore medicine, as well as in modern pharmacy, saffron has been reputed to be useful (Fig. 1) in the treatment of numerous human diseases (4–13).

Commercial saffron is produced from dried stigmas of *Crocus sativus* L., a member of the large family *Iridaceae*, and is cultivated in Azerbaijan, France, Greece, India, Iran, Italy, Spain, China, Israel, Morocco, Turkey, Egypt, and Mexico (14–16). Saffron is produced worldwide at an annual rate of 50 tons with a commercial cost of about 50 million dollars (16). The main reason for its great cost is that saffron is still cultivated and harvested as it has been for millennia—by hand.

The chemical composition of saffron has attracted the interest of several research groups during the last decades, and among the estimated more than 150 volatile and several nonvolatile compounds of saffron, approximately 40–50 constituents have already been identified (17–41). Based on these data, we can conclude that saffron contains three main pharmacologically active metabolites: 1.) Saffron-colored compounds are crocins, which are unusual water-soluble carotenoids (mono and diglycosyl esters of a polyene dicarboxylic acid, named crocetin). The digentiobiosyl ester of crocetin - α-crocin is the major component of saffron. 2.) Picrocrocin is the main substance responsible of the bitter taste in saffron. 3.) Safranal is the volatile oil responsible of the characteristic saffron odor and aroma. Furthermore, saffron contains proteins, sugars, vitamins, flavonoids, amino acids, mineral matter, gums, and other chemical compounds (4, 13, 17).

Animal studies indicate that the oral LD_{50} of saffron was 20.7 g/kg administered as a decoction (42). Our experi-

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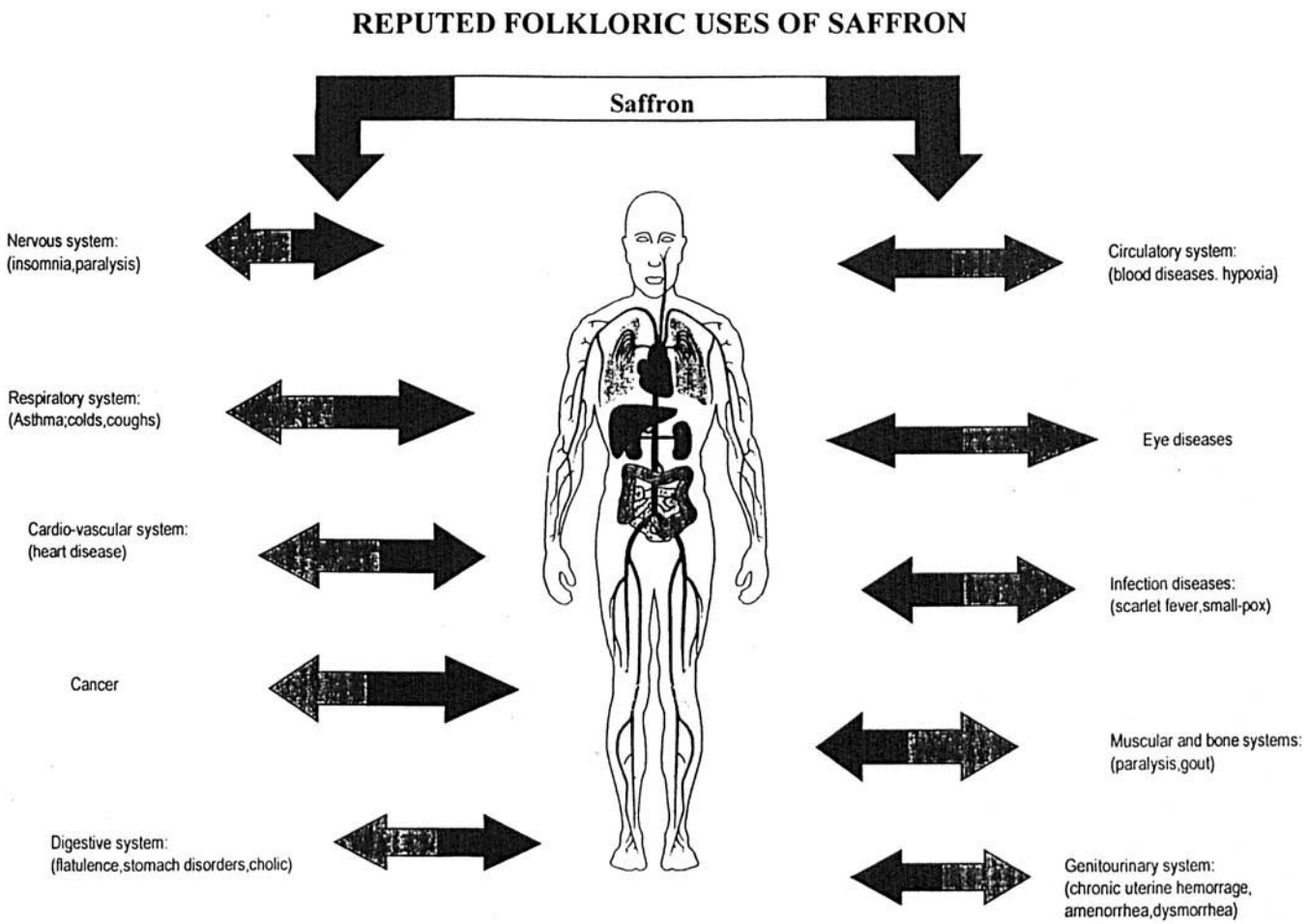

Figure 1. Reputed folkloric uses of saffron.

ments demonstrated that oral administration of saffron extract at concentrations from 0.1 to 5 g/kg was nontoxic in mice (Abdullaev F, *et al.*, unpublished data).

Some reports about the stability of saffron (13, 17, 36, 37) mention that two factors (temperature and humidity) exert a strong influence on the degradation of the main pharmacologically active ingredients of saffron under different storage conditions, but the developed HPLC assay can be utilized as a quality control method for saffron (39). On the other hand, when saffron is stored under –20°C, its pharmacological activities as a supplement remain unaltered for at least 2 years or even longer (43). Further studies to elucidate the structure and to characterize the biologically active ingredients of saffron are now in progress in different laboratories. The scientific evidence on the cancer chemopreventive effects of saffron extract and its main ingredients are outlined here, updating previous reviews on this topic (4, 44–46).

Cancer Preventive and Tumoricidal Effects of Saffron and Its Ingredients

Research into the effect of saffron on neoplastic cells has seen a renaissance in the last decade, and a growing body of evidence indicates that saffron and its characteristic components possess anticarcinogenic and antitumor activities *in vivo* and *in vitro* (47–71).

Saffron extract has been shown capable of inhibiting and/or retarding tumorigenesis in a variety of experimental models *in vivo* (47–56). Topical application of saffron extract (100 mg/kg body wt) inhibited two-stage initiation/promotion dimethylbenz[a]anthracene (DMBA)-induced skin carcinogenesis and oral administration of saffron extract in the same dose restricted 20-methylchloanthrene (MCA)-induced soft tissue sarcomas in mice (47–49). Later, it was demonstrated that saffron extract significantly prolonged (almost 3-fold) the life spans of cisplatin-treated (2 mg/kg body wt) mice and partially prevented the decrease in body weight, hemoglobin levels, and leukocyte counts (50).

Another study (51) examined the protective effect of concurrent administration of cysteine (20 mg/kg body wt) together with vitamin E (2 mg/kg body wt) and saffron extract (50 mg/kg body wt) against cisplatin-induced (3 mg/kg body wt) toxicity in rats. It was shown that treatment of animals with protective (saffron together with vitamin E and cysteine) agents significantly reduces blood urea nitrogen, serum creatinine levels, and blood glucose levels, as well as partially prevents many changes in the activities of different serum enzymes (51). Taken together, these studies indicated

that saffron may be a promising agent for reducing cisplatin-toxic side effects, including nephrotoxicity.

Oral administration of saffron extract (200 mg/kg body wt) induced a dose-dependent inhibition of the growth in mice of ascite tumors derived from sarcoma-180 (S-180), Ehrlich ascites carcinoma (EAC), Dalton's lymphoma ascites (DLA), and significantly increased (2- to 3-fold) life spans of treated tumor-bearing mice (52). Later, these authors reported that oral administration of saffron extract significantly suppressed the growth of DLA and S-180 tumor cells, but did not affect the growth of EAC tumor cells in mice (53). The authors suggested that increase in the levels of β-carotene and vitamin A in the serum of the experimental animals receiving saffron might be one explanation for this antitumor effect of saffron. Interestingly, when liposome-encapsulated saffron extract was injected i.p. into mice, the increasing of antitumor effect of this extract towards several solid cells was observed, including the EAC tumor cells, which were insensitive to orally administered extract (54). These authors suggested that enhancement in antitumor activity of saffron extract could be due to site-directed drug delivery or to carrier-mediated increased drug solubility. More recently, it was reported (55) that crocin, a carotenoid isolated from saffron, increased the survival time and decreased tumor (colon adenocarcinoma) growth in female rats without any significant effects in male animals. The authors suggested that the selective antitumor action of crocin in female rats compared with male might be related to hormonal factors.

In another study (56), crocetin at nontoxic doses inhibited genotoxic effect and neoplastic transformation in C3H1OT1/2 cells induced by benzo(a)pyrene (BP). Thus, studies *in vivo* showed that saffron extract and its purified constituent significantly increased the life span of animals with different types of tumor, but the mechanism of anticarcinogenic effect of saffron has not been elucidated.

A number of studies have demonstrated an antitumor effect of saffron and its constituents on different malignant cells *in vitro* (Table I). Observed differences in sensitivity to saffron and its ingredients between different cultured malignant cells (57–71) could be due to the existence of distinct cell surface receptors, intracellular retention transport, differences in the drug uptake, or differences in the methods of extraction and determination of cytotoxicity.

By using trypan-blue dye exclusion as a criterion of cell viability, the IC_{50} of saffron extract was found to range from 7 to 30 μg/ml, dependent upon the type of tumor cells, whereas there was no significant effect on normal mouse spleen cells (47, 52, 53). Utilizing the method of colony formation as a measure of cell viability, it was demonstrated that the IC_{50} of saffron extract ranged from 100 to 200 μg/ml upon the type of human malignant cells, but had no significant effect on normal human lung cells (57, 58). It was shown that the saffron extract inhibited cellular nucleic acid synthesis and had no effect on protein synthesis in tumor cells (54, 57, 58). Interestingly, there was a stimulatory or supporting effect of saffron extract on nonspecific proliferation of immature and mature lymphocytes *in vitro* and colony formation of normal human lung cells (54, 57, 58). It was also observed that saffron increased the intracellular levels of reduced glutathione and glutathione-related enzymes and suggested a possible antioxidant activity of saffron (54, 59). It was shown that saffron extract and its purified characteristic compounds crocin, safranal, picrocrocin, and β-carotene (Table I) inhibited different types of tumor cell growth (55, 59–61). Interestingly, in two stud-

Table I. Cytotoxic (IC_{50}) Effect of Saffron and Its Components on Tumor Cells *in vitro*

Agents	Cells	IC_{50}	References
Saffron extract	S-180; EAC; DLA; P388 osteosarcoma; and ovarian sarcoma	7–30 μg/ml	44, 48, 52, 54
Saffron extract	HeLa; A549; WI-38VA	100–250 μg/ml	57–58
Saffron extract	A-204; HEPG-2; SW-480	150–200 μg/ml	Abdullaev F.I. *et al.*, unpublished data
Saffron extract	HeLa	2.3 mg/ml	62
Crocetin	HL-60; K562	2 μM	60, 61
Dimethyl crocetin	HL-60; K562	0.8 μM	60, 61
Crocin	HL-60; K562; HeLa; and HT-29 DHD/K12-PROb	2 μM, 3 mM, 0.4 mM, and 1 mM, respectively	55, 60–62
-Carotene	K562	3 μM	60
Safranal	HeLa	0.8 mM	62
Picrocrocin	HeLa	3 mM	62
All-trans retinoic acid	HL-60	0.12 μM	61
Saffron corm callus extract	HeLa	100–150 μg/ml	64–68
Saffron proteoglycan	HeLa, fibrosarcoma, and breast carcinoma	7 μg/ml, 9 μg/ml, and 22 μg/ml, respectively	64, 66
Glucoconjugate from saffron corms	Tobacco BY-2 cells, protoplasts	0.5 μg/ml and 2 μg/ml, respectively	68

ies (60, 61), crocetin isolated from saffron had a cytotoxic activity on tumor cells, but in another study, it was shown that crocetin did not show any cytotoxic effect (62). Our study (63) demonstrated that crocetin had no cytotoxic effect on colony formation of different tumor cells, but had a dose-dependent inhibitory effect on DNA, RNA, and protein synthesis in these human malignant cells. We also reported that treatment of tumor cells with saffron extract in combination with well-known antitumor agents such as selenium compounds caused a more effective inhibition of colony formation and nucleic acid synthesis relative to the effects of these agents alone (59).

It was reported that a novel glucoconjugate isolated from corms and callus of saffron possessed cytotoxic activity against different tumor cells (64–68). These authors demonstrated that glucoconjucate from corms of *C. sativus L.* possessed cytotoxic activity on human tumor cells derived from fibrosarcoma, cervical epithelioid carcinoma, and breast carcinoma (Table I). This compound was about eight times more cytotoxic for malignant cells than for their normal counterparts and it caused plasma membrane damage in these cells. Interestingly, that analysis of DNA fragmentation indicated that cell death was not mediated by apoptosis. Thus, extracts of saffron stigmas, corm, and callus and its ingredients possessed both anticarcinogenic and antitumor activities *in vivo* and *in vitro* (64–68).

Only one study (48) using the Ames assay had indicated that crocin and dimethyl-crocetin from saffron were nonmutagenic and nonantimutagenic. In our laboratory, it has been demonstrated that saffron extract was nontoxic, nonmutagenic, and nonantimutagenic (69).

Thus, saffron and its constituents are suggested as alternative anticancer agents, which alone and in combination with other synthetic substances may have the potential for the prevention and the treatment of certain forms of cancer.

Proposed Mechanisms for Cancer Preventive and Tumoricidal Effects of Saffron

Different hypotheses for the modes of anticarcinogenic and antitumor actions of saffron and its components have been proposed. One of the mechanisms for the antitumor or anticarcinogenic action of saffron and its components is the inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis (44, 52, 57–59, 63). A second suggested mechanism for the antitumor action of saffron and its constituents is the inhibitory effect on free radical chain reactions, because most carotenoids are lipid-soluble and might act as membrane-associated high-efficiency free-radical scavengers, which is connected with their antioxidant properties (44, 46, 53, 70–75). A third proposed mechanism by which the saffron extract exerts its antitumor effect is the metabolic conversion of naturally occurring carotenoids to retinoids (61, 71), but recently, it was reported that conversion carotenoids to vitamin A is not a prerequisite for anticancer activity (76). A fourth suggested mechanism is that the cytotoxic effect of saffron is connected with interaction of carotenoids with topoisomerase II, an enzyme involved in cellular DNA-protein interaction (44, 50, 76).

Recently, several other mechanisms for the antitumor effect of saffron and its constituents have also been proposed. It was demonstrated that a novel glucoconjugate, isolated from corm and callus extract of saffron, caused swelling and local plasma membrane evagination, and it was suggested that cytotoxicity is mediated via extracellular fluid uptake (64–66). It was also reported that saffron contains lectins (77, 78), and it might also be suggested that antitumor activity of saffron is mediated via lectins (45, 79). The literature also contains reports that saffron extract and/or its components inhibited activities of different cellular enzymes, and it was suggested that the antitumor effect of these agents might be associated with the effect on enzyme functions (45, 51, 54, 80). Treatment of tumor cells with saffron resulted in an increase in the level of intracellular sulphydryl compounds (51, 59), and this could be one explanation for the potentiation of saffron cytotoxicity. Another suggested mechanism is that cytotoxic effect of carotenoids from saffron is mediated via apoptosis (60).

Interesting studies (54, 81) indicate that encapsulation in amorphous polymer matrices of saffron extracts or saffron carotenoid greatly improves their stabilities and enhances their antitumor effects. More recently, it was shown that γ-irradiation, necessary for microbial decontamination, did not produce significant qualitative changes of volatile essential oil constituents of saffron, but induced a slight decrease in glycosides and an increase in aglycon content in carotene constituents of saffron (82). This relative stability of saffron to irradiation should also be taken to account in the search for an explanation of the chemopreventive potential of this spice.

Thus, although several hypotheses have been put forward, the exact mechanism(s) of anticarcinogenic and antitumor effects of saffron and its main constituents are not clear at present.

Conclusion

Chemoprevention involves pharmacological intervention with naturally occurring and synthetic agents alone or in combination to reverse, suppress, or prevent the cancer in human beings, and today it plays a key role in the fight against this terrible disease. Considerable scientific evidence has suggested that plant-based dietary agents can inhibit the process of carcinogenesis effectively (2).

In the last decade, much attention has been focused on the biological and medical properties of an ancient spice—saffron and its ingredients. Recent scientific findings have been encouraging, uniformly showing that saffron and its components can affect carcinogenesis and currently have been studied extensively as the most promising cancer chemopreventive agents.

Because the relationship between saffron and cancer is an important concern, comprehensive, in-depth studies need

to be conducted further along the following lines: 1.) Define the mechanism(s) involved in the therapeutic properties of saffron; 2.) Investigate the mechanism(s) involved in saffron cancer chemoprevention; 3.) Determine the biologically active components of saffron; and 4.) Perform human studies to define efficacy of saffron in cancer treatment and prevention.

The scarcity and expense in obtaining large quantities of saffron may provide impediments to human chemoprevention and cancer treatment using this agent; however, an indoor cultivation method is advantageous in achieving the highest quality of saffron and for decreasing its price.

The results of each of these researches provide parts of the scaffolding to construct a logical platform for the appearing of a new scientific discipline to be called saffronology.

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1. Abdullaev FI, Rivera Luna R, Roitenburd Belacortu V, Espinosa Aguirre J. Pattern of childhood cancer mortality in Mexico. Arch Med Res **31(5):**526–531, 2000.
2. Abdullaev FI. Plant-derived agents against cancer. In: Gupta SK, Ed. Pharmacology and Therapeutics in the New Millennium. New Delhi: Narosa Publishing House, pp345–354, 2001.
3. Eisenberg D, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL. Unconventional medicine in the United States: prevalence, cost and patterns of use. N Engl J Med **328:**246–252, 1993.
4. Abdullaev FI. Biological effects of saffron. BioFactors **4(2):**83–86, 1993.
5. Gainer JI, Jones JR. The use of crocetin in experimental atherosclerosis. Experienta **31:**548–549, 1975.
6. Grisolia S. Letter: hypoxia, saffron, and cardiovascular disease. Lancet **2(7871):**41–42, 1974.
7. Wuthrich B, Schmid-Grendelmeyer P, Lunberg M. Anaphylaxis to saffron. Allergy **52(4):**476–477, 1997.
8. Nadkarni KM. *Crocus sativus, Nigella sativa.* In: Nadkarni KM, Ed. Indian Materia Medica, Bombay: Popular Prakashan, pp386–411, 1976.
9. Suzhou New Medical College. Dictionary of Traditional Chinese Medicine (Zhong Da Zi Dian). Shanghai: Shanghai People's Publication House, Vol. **2:**pp2622–2623, 1977.
10. Zhou Q, Sun Y, Zhang X. Saffron, *Crocus sativus* L. J Tradition Chinese Med **28:**59–61,1978.
11. Basker D, Negbi M. The use of saffron. Econ Bot **37:**228–236, 1983.
12. Oberdieck R. Ein beitrag zur kenntnis und analytik von safran (*Crocus sativus* L.). Deutshe Lebensmittel Rundschau **87(8):**246–252, 1991.
13. Rios JL, Recio MC, Giner RM, Mañez S. An update review of saffron and its active compounds. Phytother Res **10(3):**189–193, 1996.
14. Xue XH. Cultivation of *Crocus sativus.* Chung Yao Tung Pao **7(4):**3–4, 1982.
15. Xuabin N. Research progresses on the saffron crocus (*Crocus sativus*). Zhongcaoyao **23(2):**100–107, 1992.
16. Negbi M. Saffron cultivation: past, present and future prospects. In: Negbi M, Ed. Saffron *Crocus sativus* L. Amsterdam: Harwood Academic Publishers, pp1–19, 1999.
17. Winterhalter P, Straubinger M. Saffron-renewed interest in an ancient spice. Food Rev Int **16(1):**39–59, 2000.
18. Zargani NS, Heinz DE. The volatile constituents of saffron. Lebensm Wiss Technol **4(2):**43–45, 1971.
19. Curró C, Micgelli G. Determinazione specttrofotometrica-del potere colorante, Americane ed odoroso dello zafferano. Boll Chim Farm **118:**553–562, 1979.
20. Pfander H, Schurtenberge H. Biosynthesis of C20-carotenoids in *Crocus sativus.* Phytochemistry **21:**1039–1042, 1982.
21. Himeno H, Sano K. Synthesis of crocin, picrocrocin and safranal by saffron stigma-like structures proliferated *in vitro.* Agric Biol Chem **51(9):**2395–2400, 1987.
22. Rödel W, Petrzika M. Analysis of the volatile components of saffron. J High Res Chromatogr **14:**771–774, 1991.
23. Iborra JL, Castellar MR, Cánovas M, Manjón A. TLC preparative purification of picrocrocin, HTCC and crocin from saffron. J Food Sci **3:**714–716, 1992.
24. Iborra JL, Castellar MR., Cánovas M, Manjón A. Picrocrocin hydrolysis by immobilized β-glucosidase. Biotechnol Lett **14(6):**475–480, 1992.
25. Narasimhan H, Chand H, Rajalakshmi D. Saffron, quality evaluation by sensory profile and gas chromatography. J Food Qual **15:**303–314, 1992.
26. Sujata V, Ravishankar GA, Venkataraman LV. Methods for the analysis of the saffron metabolites crocin, crocetind, picrocrocin and safranal for the determination of the quality of spice using thin-layer chromatography, HPLC and GC. J Chromatogr **624(1–2):**497–502, 1992.
27. Iborra JL, Castellar MR, Cánovas M, Manjón A. Analysis of a packed-bed reactor for hydrolysis of picrocrocin by immobilized β-glucosidase. Enzyme Microb Technol **15:**780–784, 1993.
28. Castellar MR, Montijano H, Manjón A, Iborra JL. Preparative high-performance liquid chromatographic purification of saffron secondary metabolites. J Chromatogr **648:**187–190, 1993.
29. Tarantilis PA, Polissiou M, Mentzafos D, Terzis A, Manfait M. The structure of dimethylcrocetin. J Chem Crystallogr **24(11):**739–742, 1994.
30. Tarantilis PA, Polissiou M., Manfait M. Separation of picrocrocin, *cis-trans*-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection. J Chromatogr A **664:**55–61, 1994.
31. Tarantilis PA, Tsoupras G, Polissiou M. Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. J Chromatogr A **699(1–2):**107–118, 1995.
32. Corti P, Mazzei E, Ferri S, Franchi GG, Dreassi E. High-performance thin layer chromatographic quantitative analysis of picrocrocin and crocetin, active principles of saffron (*Crocus sativus* L.-*Iridaceae*): a new method. Phytochem Anal **7:**201–203, 1996.
33. Saito K, Utsumi Y. Enhancing effect of UV light on accumulation of carthamine in dyer's saffron florets. Z Naturfosch [C] **51(9–10):**667–670, 1996.
34. Straubinger M, Jezussek M, Waibel R, Winterhalter P. Novel glucosidic constituents from saffron. J Agric Food Chem **45(5):**1678–1681, 1997.
35. Straubinger M, Bau B. Eckstein S, Fink M, Winterhalter P. Identification of novel glycosidic aroma precursors in saffron (*Crocus sativus* L.). J Agric Food Chem **46(8):**3238–3243, 1998.
36. Alonso GL, Salinas MR, Esteban-Infantes FJ, Sánchez-Fernández MA. Determination of safranal from saffron (*Crocus sativus* L.) by thermal desorption-gas chromatography. J Agric Food Chem **44:**185–188, 1996.
37. Alonso GL, Salinas MR, Garijo J. Method to determine the authenticity of aroma of saffron (*Crocus sativus* L.). J Food Prot **61(11):**1525–1528, 1998.
38. Tarantilis PA, Polissiou M. Isolation and identification of the aroma components from saffron (*Crocus sativus* L.). J Agric Food Chem **45:**459–462, 1997.
39. Li N, Lin G, Kwan YW, Min D. Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. J Chromatogr A **849(2):**349–355, 1999.
40. Lozano P, Castellar MJ, Simancas MJ, Iborra JL. Quantitative high-performance liquid chromatographic method to analyze commercial

saffron (*Crocus sativus* L.) products. J Chromatogr A **830**:477–483, 1999.
41. Lozano P, Delgado D, Gomez D, Rubio M, Iborra JL. A non-destructive method to determine the safranal content of saffron (Crocus sativus L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography. J Biochem Biophys Methods **4328513-N (1–3)**:367–378, 2000.
42. Chang PY, Wang CK, Liang CT, Kuo W. The pharmacological action of *Zang Hong Hua (Crocus sativus* L.): effect on the uterus and/or strous cycle. Yao Hsueh Hsueh Pao **11**:94–100, 1964.
43. Marimoto Y, Umezaki Y, Shoyama Y, Saito H, Nishi K, Irino N. Post-harvest degradation of carotenoid glucose esters in saffron. Plant Med **60**:438–440, 1994.
44. Nair SC, Kurumboor SK, Hasegawa JH. Saffron chemoprevention in biology and medicine: a review. Cancer Biother **10(4)**:257–264, 1995.
45. Abdullaev FI, Gonzalez de Mejia E. Actividad antitumoral de compuestos naturales: lectinas y azafran. Arch Latinoam Nutr **47(3)**:195–202, 1997.
46. Abdullaev FI, Frenkel GD. Saffron in biological and medical research. In: Negbi M, Ed. Saffron *Crocus sativus* L. Amsterdam: Harwood Academic Publishers, pp103–113, 1999.
47. Salomi MJ, Nair SC, Panikkar PR. Inhibitory effects of *Nigela sativa* and saffron (*Crocus sativus*) on chemical carcinogenesis in mice and its non-mutagenic activity. Proc Ker Sci Congr **3**:125–126, 1990.
48. Salomi MJ, Nair SC, Panikkar PR. Cytotoxicity and non-mutagenicity of *Nigela sativa* and saffron (*Crocus sativus*) *in vitro*. Proc Ker Sci Congr **5**:244, 1991.
49. Salomi MJ, Nair SC, Panikkar PR. Inhibitory effects of *Nigela sativa* and saffron (*Crocus sativus*) on chemical carcinogenesis in mice. Nutr Cancer **16(1)**:67–72, 1991.
50. Nair SC, Salomi MJ, Pannikar. B, Pannikar KR. Modulatory effects of the extracts of saffron and *Nigela sativa* against cisplatinum induced toxicity in mice. J Ethnopharmacol **31**:75–83, 1991.
51. el Daly ES. Protective effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicity in rats. J Pharm Belg **53(2)**:93–95, 1998.
52. Nair SC, Pannikar B, Pannikar KR. Antitumour activity of saffron (*Crocus sativus*). Cancer Lett **57(2)**:109–114, 1991.
53. Nair SC, Varghese CD, Pannikar KR, Kurumboor SK, Parathod RK. Effects of saffron on vitamin A levels and its antitumor activity on the growth of solid tumors in mice. Int J Pharmacog **32(2)**:105–114, 1994.
54. Nair SC, Salomi MJ, Varghese CD, Pannikar B, Pannikar KR. Effect of saffron on thymocyte proliferation, intracellular gluthathione levels and its antitumor activity. BioFactors **4(1)**:51–54, 1992.
55. Garcia-Olmo DC, Riese HH, Escribano J, Ontañon J, Fernandez JA, Atienzar M, Garcia-Olmo D. Effects of long-term treatment of colon adenocarcinoma with crocin, a carotenoid from saffron (*Crocus sativus* L.): an experimental study in the rats. Nutr Cancer **35(2)**:120–126, 1999.
56. Chang VC, Lin YL, Lee MJ, Show SJ, Wang CJ. Inhibitory effect of crocetin on benzo(a)pyrece genotoxicity and neoplastic transformation in C3H1OT1/2 cells. Anticancer Res **765**:3603–3608, 1996.
57. Abdullaev FI, Frenkel GD. Effect of saffron on cell colony formation and cellular nucleic acid and protein synthesis. BioFactors **3(3)**:201–204, 1992.
58. Abdullaev FI, Frenkel GD. The effect of saffron on intracellular DNA, RNA and protein synthesis in malignant and non-malignant human cells. BioFactors **4(1)**:43–45, 1992.
59. Abdullaev FI, Gonzalez de Mejia E. Inhibition of colony formation of Hela cells by naturally occurring and synthetic agents. BioFactors **5(3)**:133–138, 1995/1996.
60. Morjani H, Tarantilis P, Polissiou M, Manfait M. Growth inhibition and induction of crythroid differentiation activity by crocin, dimethylcrocetine and β-carotene on K562 tumor cells. Anticancer Res **10**:1398–1406, 1990.
61. Tarantilis PA, Morjani H, Polissiou M, Manfait M. Inhibition of growth and induction of differentiation promyelocytic leukemia (HL-60) by carotenoids from *Crocus sativus* L. Anticancer Res **14(5A)**:1913–1918, 1994.
62. Escribano J, Alonso GL, Coca-Prados M, Fernandez A. Crocin, safranal and picocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. Cancer Lett **100(1–2)**:23–30, 1996.
63. Abdullaev FI. Inhibitory effect of crocetin on intracellular nucleic acid and protein synthesis in malignant cells. Toxicol Lett **40**:243–251, 1994.
64. Escribano J, Rios J, Fernandez JA. Isolation and cytotoxic properties of novel glycoconjugate from corms of saffron plant (*Crocus sativus* L.). Biochim Biophys Acta **1426(1)**:217–222, 1999.
65. Escribano J, Diaz-Guerra MJ, Riese HH, Ontañon J, Garcia-Olmo D, Garcia-Olmo DC, Rubio A, Fernandez JA. In vitro activation of macrophages from corms of *Crocus sativus* L. Cancer Lett **144(1)**:107–114, 1999.
66. Escribano J, Piqueras A, Medina J, Rubio A, Alvarez-Orti M, Fernandez JA. Production of a cytotoxic proteoglycan using callus culture of saffron corms (*Crocus sativus* L.). J Biotechnol **73(1)**:53–59, 1999.
67. Escribano J, Diaz-Guerra MJ, Riese HH, Alvarez A, Proenza R, Fernandez JA. The cytotoxic effect of glucoconjugate extracted from corms of saffron plant (*Crocus sativus*) on human cell lines in culture. Planta Med **66(2)**:157–162, 2000.
68. Fernandez JA, Escribano J, Piqueras A, Medina J. A glycoconjugate from corms of saffron plant (*Crocus sativus* L.) inhibits root growth and affects in vitro cell viability. J Exp Bot **51(345)**:731–737, 2000.
69. Abdullaev FI, Riveron Negrette L, Rotenburd Belacortu V, Kasumov FJ, Perez Lopez I, Hernandes JM, Espinosa Aguirre JJ. Saffron as chemopreventive agent. In: Wenyi T, Ed. Food of 21st Century: Food and Resource Technology Environment. China: Ligth Industry Press, pp185–195, 2000.
70. Molnar J, Szabo D, Pusztai R, Mucsi I, Berek L, Ocsovski I, Kawata E, Shoyama Y. Membrane associated antitumor effects of crocine-, ginseniside- and cannabinoid derivates. Anticancer Res **20(2a)**:861–867, 2000.
71. Dufresne C, Cormier F, Dorion S. In vitro formation of crocetin glucosyl esters by Crocus sativus callus extract. Planta Med **63(2)**:150–153, 1997.
72. Takashi I. Antioxidative property of the anthraquinone-pigment from the cultured cells of saffron, and enzymatic comparison between some cultured cells. Shokubutsu Soshiki Baiyo **9(1)**:51–53, 1992.
73. Verma SK, Bordia A. Antioxidant property of saffron in man. Indian J Med Sci **52(5)**:205–207, 1998.
74. Tseng TH, Chu CY, Huang JM, Shiow SJ, Wang CJ. Crocetin protects against oxidative damage in rat primary hepatocytes. Cancer Lett **97(1)**:61–67, 1995.
75. Palozza P, Krinsky NI. Antioxidant effects of carotenoids *in vivo* and *in vitro*: an overview. Methods Enzymol **213**:403–420, 1992.
76. Smith TAD. Carotenoids and cancer: prevention and potential therapy. Br J Biomed Sci **55(4)**:268–275, 1998.
77. Oda Y, Tatsumi Y. New lectins from bulbs of *Crocus sativus*. Biol Pharm Bull **16(10)**:978–981, 1993.
78. Escribano J, Rubio A, Alvarez-Orti M, Molina A, Fernandez JA. Purification and characterization of mannan-binding lectin specifically expressed in corms of saffron plant (*Crocus sativus* L.). J Agric Food Chem **48(2)**:457–463, 2000.
79. Abdullaev FI, Gonzalez de Mejia E. Antitumor effect of plant lectins. Natural Toxins **5**:157–163, 1997.
80. Kubo I, Kinst-Hori I. Flavonols from saffron flower: tyrosinase inhibitory activity and inhibition mechanism. J Agric Food Chem **47(10)**:4121–4125, 1999.
81. Selim K, Tsimidou M, Biliaderis CG. Kinetic studies of degradation of saffron carotenoids encapsulated in amorphous polymer matrices. Food Chem **71(2)**:199–206, 2000.
82. Zareena AV, Variar PS, Gholar AS, Bongirwar DF. Chemical investigation of γ-irradiated saffron (*Crocus sativus* L.) . J Agric Food Chem **49(2)**:687–691, 2001.