Stigma Croci

Definition: Stigma Croci consists of the dried stigmas of *Crocus sativus* L. (Iridaceae)**.**

Synonyms: *Crocus officinalis Martyn*.

Selected vernacular names:
Accfrao, azaferan, azafran, crocus, crocus hispanicus, crocus orientalis, dye saffron, Echter Safran, fan-hung-hua, Gewurzsafran, hay saffron, kamkana, kesar, keshara, koema-koema, kumkum, Safran, saffraon, saffron, saffron crocus, safrany, sapran, Spanish saffron, true saffron, szafran, szafrana, z'afaran, za afran l-hor, zaafaran, zafaran, zafarfon, zafferano, zang hong hua, zafrane hor.

Geographical distribution
Indigenous to southern Europe and south-western Asia. Cultivated in the Eastern Mediterranean and in China, France, India, Italy and Spain.

Description
A perennial, low growing (8–30 cm high), bulbous herb with an underground globular corm, producing six to nine sessile leaves, surrounded in its lower part by four or fi ve broad membranous scales. Flowers borne on the terminal region of a scape, each fl ower consisting of a pale reddishpurple perianth showing a cylindrical tube about 10 cm long and six oblong oval segments, an androecium of three stamens and a gynoecium of three syncarpous carpels. Ovary inferior, three-locular. Style slender, elongated and pale yellow in the perianth tube, divided in its upper part into three drooping, deep-red stigmas.

Plant material of interest: dried stigmas

General appearance
Thin cord-like stigmas, dark yellow-red to red-brown, 1.5–3.5 cm long, tripartite or separate, the upper part broader and slightly fl attened, the distal end split longitudinally and rolled into a slender funnel with a crenate edge. Margin of the apex irregularly dentate, with a short slit at the inner side, sometimes with a small piece of style remaining at the lower end. Texture light, lax and soft, without oily lustre.

Organoleptic properties
Odour: characteristic, aromatic, slightly irritant; taste: pungent, slightly bitter.

Microscopic characteristics
When softened by immersion in water, upper ends of the stigmas show numerous tubular protrusions about 150 µm long, with a small number of pollen grains, which are spherical, smooth and without spines.

Powdered plant material
Orange-red. Epidermal cells long, thin-walled, slightly sinuous, stripeshaped in the surface view; outer walls sometimes protrude, showing papillae, with indistinct fi ne striations. Terminal epidermal cells of stigma are papillose, 26–56 µm in diameter, with sparse striations on the surface. Parenchymatous cells are crowded with round-fascicle, fusiform or subsquare granular crystals of calcium oxalate, 2–14 µm in diameter.

General identity tests

Macroscopic and microscopic examinations, microchemical and spectrophotometric tests, and thin-layer chromatography.

Purity tests

Microbiological

Tests for specifi c microorganisms and microbial contamination limits are as described in the WHO guidelines on quality control methods for medicinal plants.

Total ash

Not more than 7.5%.

Loss on drying

Not more than 12.0%.

Pesticide residues

The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg. For other pesticides, see the *European pharmacopoeia* and the WHO guidelines on quality control methods for medicinal plants and pesticide residues.

Heavy metals

For maximum limits and analysis of heavy metals, consult the WHO guidelines on quality control methods for medicinal plants.

Radioactive residues

Where applicable, consult the WHO guidelines on quality control methods for medicinal plants for the analysis of radioactive isotopes.

Other purity tests

Chemical, foreign organic matter, acid-insoluble ash, water-soluble extractive and alcohol-soluble extractive tests to be established in accordance with national requirements.

Chemical assays

Colorimetric and spectrophotometric assays are used. Qualitative and quantitative high-performance liquid chromatography methods are available for picrocrocin, safranal and crocins.

Major chemical constituents

The major constituents include essential oils (0.4–1.3%) with and pinene, 1,8-cineole (eucalyptol), a monoterpene glucoside, picrocrocin (4%), safranal, which can be obtained by hydrolysis of picrocrocin, and a series of carotenoid glucosides known as crocins (2%), dimethylcrocetin and their aglycone crocetin. Representative structures are presented below.

Medicinal uses

Uses supported by clinical data

None. Although Stigma Croci showed antioxidant effects in human studies, data from controlled clinical trials are lacking.

Uses described in pharmacopoeias and well established documents

As a tonic and antiarteriosclerotic, and as a sedative and emmenagogue.

Uses described in traditional medicine

As an emmenagogue and for treatment of ammenorrhoea, abdominal pain, coughs, depression, digestive ailments, fever and pain due to wounds. Also as an aphrodisiac, appetite stimulant, diaphoretic, contraceptive, antispasmodic and nerve sedative.

Pharmacology

Experimental pharmacology

Antiarteriosclerotic effects

Administration of a monthly intramuscular injection of crocetin (dose not specifi ed) to rabbits fed an atherosclerosis-inducing diet reduced serum cholesterol concentrations by 50%, and reduced the severity of atherosclerosis by ~30%.

Anticoagulant activity

A hot aqueous extract of Stigma Croci, 10–100.0 mg/ml, prolonged partial thromboplastin and prothrombin times, and inhibited platelet aggregation in human platelets induced by adenosine diphosphate and collagen in vitro.

Cell proliferation inhibition

Treatment of cervical epitheloid carcinoma (HeLa) cells with a concentrated extract (undefi ned) of the stigmas, 50.0–150.0 µg/ml, for 3 hours inhibited colony formation by 25% and decreased the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) by 50% in vitro.

Crocin and crocetin, 0.8–2.0 µmol/l, isolated from an extract of the stigmas, inhibited the growth of human acute promyelocytic leukaemia cells in vitro. Crocetin, 35–55.0 µg/ml, inhibited the synthesis of nucleic acids and protein in cervical epitheloid carcinoma, lung carcinoma and transformed fetal fi broblast malignant human cell lines. Incubation of cervical epitheloid carcinoma cells (HeLa), lung adenocarcinoma cells (A549) and SV-40 transformed fetal lung fi broblast cells with varying concentrations of crocetin for 3 hours resulted in a dose-dependent reduction in DNA and RNA synthesis, and suppression of RNA polymerase II activity.

Central nervous system effects

Intragastric administration of 125–250.0 mg/kg body weight (bw) of a 50% ethanol extract of the stigmas had a tranquillizing effect in mice, and potentiated the sedative effects of barbiturates.

Chemical carcinogenesis inhibition

Topical application of 100 mg/kg bw of a 95% ethanol extract of the stigmas inhibited two-stage initiation and promotion of skin carcinogenesis in mice, delaying the onset of papilloma formation and reducing the mean number of papillomas per mouse. Intragastric administration of 100.0 mg/kg bw of the same extract per day for 30 days reduced the incidence of soft tissue sarcomas induced by 20-methylcholanthrene by 10% in mice. Intragastric administration of 100.0 mg/kg bw of an ethanol extract of the stigmas to mice inhibited the growth of solid Dalton lymphoma ascites and sarcoma 180 tumours by 87% and 41%, respectively. Subcutaneous administration of 400.0 mg/kg bw of crocin weekly for 13 weeks, slowed the growth of colon adenocarcinoma and increased the lifespan of female but not male mice. Intraperitoneal administration of 50 mg/kg bw of a 95% ethanol extract of the stigmas to mice partially prevented the decreases in body weight, haemoglobin levels and leukocyte counts caused by cisplatin treatments.

Circulation effects

External application of a 1% aqueous solution containing crocin analogues isolated from *Crocus sativus* signifi cantly ($P < 0.05$) increased blood fl ow to the retina and choroid in rabbits with ocular hypertension. Intraperitoneal administration of 10.0 mg/kg bw of crocin analogues to rats facilitated the recovery of retinal function after induction of retinal ischaemia by occlusion of the central retinal and posterior ciliary arteries.

Cytotoxicity

In vitro, crocin had potent cytotoxic effects on human and animal adenocarcinoma cells, with median lethal doses (LD50) of 0.4 mmol/l and 1.0 mmol/l, respectively. An aqueous extract of the stigmas (LD50 2.3 mg/ml), crocin (LD50 3 mmol/l), picrocrocin (LD50 3 mmol/l) and safranal (LD50 0.8 mmol/l) inhibited the growth of HeLa cells in vitro. The cells treated with crocin exhibited wide cytoplasmic vacuole-like areas, reduced cytoplasm and cell shrinkage, indicating the induction of apoptosis.

Nootropic effects

An unspecifi ed alcohol extract of the stigmas enhanced learning and memory in learning-impaired mice. Intragastric administration of 125.0–500.0 mg/kg bw of the extract did not affect learning behaviours in normal mice, but prevented ethanol-induced learning impairment, and prevented ethanol-induced inhibition of hippocampal long-term potentiation (a form of activity-dependent synaptic plasticity that may support learning and memory) in anaesthetized rats. Intragastric administration of a single dose of 250.0 mg/kg bw of the same extract prevented acetaldehyde-induced inhibition of long-term potentiation in the dentate gyrus of anaesthetized rats. In a follow-up study, treatment of mice with an ethanol extract of 250.0 mg/kg bw of the stigmas improved ethanol-induced impairments of learning behaviours in mice and prevented ethanol-induced inhibition of hippocampal long-term potentiation. The effect was attributed to crocin, but not crocetin.

Toxicity

The LD50 for Stigma Croci was reported to be 20.7 g/kg bw in rodents. The LD50 of a 95% ethanol extract of the stigmas was > 600 mg/kg bw in mice. Mice treated with dimethylcrocetin isolated from the stigmas did not exhibit haematological or biochemical toxic effects after intragastric administration of up to 50.0 mg/kg bw.

Clinical pharmacology

The antioxidant effects of the stigmas were assessed in a clinical trial involving 30 subjects in three groups: 10 healthy volunteers, 10 patients with coronary artery disease and 10 healthy controls. The two test groups received 50 mg of Stigma Croci in 100.0 ml of milk twice daily for 6 weeks, the controls received milk only. Lipoprotein oxidation in blood samples decreased by 42.3% in healthy volunteers ($P < 0.001$) and 37.9% ($P < 0.01$) in patients with coronary artery disease compared with controls.

Adverse reactions

The lethal dose of Stigma Croci is reported to be 20.0 g; however, smaller doses may cause vomiting, uterine bleeding, bloody diarrhoea, haematuria, bleeding from the nose, lips and eyelids, vertigo, numbness and yellowing of the skin and mucous membranes. Oral administration of 5.0 g resulted in localized skin haemorrhages, marked thrombocytopenia, and abnormalities of blood clotting in one patient.

Contraindications

Stigma Croci may induce uterine contractions and is therefore contraindicated during pregnancy. Owing to a lack of safety data, use of the stigmas in children and nursing mothers should be restricted to normal food use. Stigma Croci is contraindicated in bleeding disorders.

Warnings

At doses of 5.0 g or more, Stigma Croci may cause serious adverse reactions (see Adverse reactions). Overdose of Stigma Croci (12.0–20.0 g/day) may be fatal.

Precautions

Drug interactions

Stigma Croci inhibits platelet aggregation and should therefore be used with caution in patients taking anticoagulant or antiplatelet drugs.

Carcinogenesis, mutagenesis, impairment of fertility

Ethyl acetate, methanol and aqueous extracts of Stigma Croci (concentrations not specifi ed) were not mutagenic in the *Salmonella*/microsome assay using *S. typhimurium* strains TA98 and TA100 with or without metabolic activation. Crocin and dimethylcrocetin,1.0 mg/plate, 2.0 mg/plate and 4.0 mg/plate, were not mutagenic in the *Salmonella*/ microsome assay using *S. typhimurium* strain TA 1535. A chloroform methanol extract (2:1) of the stigmas, 100.0 mg/plate, was not mutagenic in pig kidney cells or in trophoblastic placenta cells.

Pregnancy: non-teratogenic effects

See Contraindications.

Nursing mothers

See Contraindications.

Paediatric use

See Contraindications.

Other precautions

No information available on general precautions or on precautions concerning drug and laboratory test interactions; or teratogenic effects in pregnancy.

Dosage forms

Dried stigmas; extracts of dried stigmas. Store the dried stigmas in a tightly sealed metal or glass container, protected from light and moisture.

Posology

There is insuffi cient information available to give an accurate assessment of dose range. No risk is associated with consumption in standard food use quantities. The recommended therapeutic daily dose is 3.0–9.0 g (*2*). However, owing to a report of toxicity at 5.0 g, doses below 5.0 g/day are recommended.

References

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