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Extraction And Standardization Of Saffron Extract For Usage In Food, Beverage And Pharmacological Activities

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Abstract:
Saffron (Crocus sativus L.) has been widely used as a drug to promote health and fight disease from ancient times. In countries like India and other Asian countries, saffron is used very frequently in various alternative systems of medicine including Unani System of Medicine, Ayurveda and Traditional Chinese Medicine, as it is considered bitter, acrid, fragrant, stimulant, tonic, stomachic, aphrodisiac, anodyne, antispasmodic, emmenagogue, diuretic, laxative, galactagogue and is useful in bronchitis, pharyngoplasty, cephalgia, vomiting, fever, melancholia, hepatomegaly etc. Because of its wide range of medical uses in traditional systems of medicine, the saffron has undergone extensive phytochemical and biochemical studies and some of the studies have shown that number of constituents including Crocin, picrocrocin, Safranal are present, out of which crocetin is mainly responsible for pharmacological actions. This paper is an attempt to prepare saffron extract in liquid and powder form to enhance the usage of saffron ingredients at an affordable price in food , beverage, dairy , confectionery , nutraceuticals and pharmaceutical applications.Crocus sativus L. (Iridaceae), commonly known as saffron, is not only used as a spice in food, but also for its medicinal properties. The results obtained showed that the extracts of the solvent extractor and Propylene glycol extracts of saffron had significant advantages in terms of extraction efficiency and quality of extract; moreover, savings of time and therefore represented an important data in anticipation of the use of such extracts for the preparation of functional food and also phytodrugs.
Background : Saffron is utilized in traditional recipes for its flavor, color and aroma, despite the biological effects and chemical qualities of significant bioactive chemicals present in saffron. It is not only used as a spice, but it has long been renowned as a medicinal plant due to its therapeutic potential. The emergence of synthetic chemistry-based medications that impacted saffron's medical and pharmacological applications . All bioactive chemicals in saffron have anticancer, anti-oxidant, anti depression, and antitumor properties and reduce insomnia and anxiety.
Materials and Methods:
Materials : Saffron Stigma procured from Jummu and Kashmir region of India , Ethanol 95% ,water , Propylene Glycol , maltodextrin, cornstarch.
Methods:Saffron Stigma was collected from the Jammu & Kashmir region of India. As saffron is one of the costliest spices of India ,we have procured saffron stigma from the farmer from Jammu and Kashmir . Saffron Stigma has been subjected to solvent extraction and collected the extract for our experiment. From this solvent extract and Propylene Glycol extract , it has been converted to powder and liquids respectively.
Results: Saffron Stigma consists of Safranal ,Crocin, and Picrocrocin as active ingredients. In these ingredients Safranal provides aroma, crocin provides color and picrocrocin provides taste of the saffron. All these three ingredients were extracted and converted to different percentages in liquid and powder forms. These products were tested in Milk and Candy applications. The taste, aroma and color is consistent throughout the experiment.These extracts provide consistent results in the food and beverage applications, these can be replaced by using saffron stigma.
Conclusion: saffron extract has been standardized in liquid and powder forms which is suitable for various applications in food ,beverage and nutraceuticals industries. Instead of using saffron stigma these liquid and extract powder products can be used , as it is economical and consistent quality.

Keywords— *Saffron Stigma, Saffron Extract, Safranal, Crocin, Picrocrocin, Beverage, Food Ingredient, Cosmetics , saffron milk, saffron candy .*

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I. INTRODUCTION

Saffron is the dried stigmas and upper part or top of the styles of *Crocus sativus L*. It is the world's most expensive spice and genuine saffron is worth its weight in gold. This plant belongs to the Iridaceae family (1, 2, 3, 4)among the 85 species belonging to the Crocus genus, saffron is the most fascinating and intriguing spice 5. As a medicinal plant saffron has traditionally been considered as anodyne, antidepressant, respiratory, decongestant, antispasmodic, aphrodisiac, diaphoretic, emmenagogue, expectorant, sedative. Used in scarlet fever, smallpox, colds, asthma, heart diseases, tumor, cancer, flatulent colic and in menstrual disorders (6, 7)and because of all these actions and uses saffron is included in various Unani and Ayurvedic polyherbal formulations.

Modern pharmacological studies have demonstrated that saffron extracts have antinociceptive, anti-inflammatory, antitumor, radical scavenger, anticonvulsant, hypolipidemic, hypocholesterolemia activity. Studies have shown that a number of constituents including Crocin, Crocetin, Safranal are present, out of which crocetin is mainly responsible for pharmacological actions (8, 9). The word saffron is derived from the Arabic word za'faran, which translates to ancient Greek is Korikos, the Romans used the term Crocum; English, Saffron; Italian, In Sanskrit it bears the name of Kumkum and is described as charu (fair), vara (suitor), Agni shikha (having a crest of fire), saurabh (fragrant) (10)

Figure 1 : Saffron Stigma ,Safranal , Crocin ,Picrocrocin and Crocetin .

Phytochemical Studies

The stigmas of the saffron flower contain many chemical substances. Carbohydrates, minerals, mucilage, vitamins and pigments, amino acids, proteins, starch, gums, and other chemical compounds have also been described in saffron 6. Saffron contains more than 150 volatile and aroma-yielding compounds and most of the volatile oils are composed of esters, terpenes and terpene alcohol (11,12).

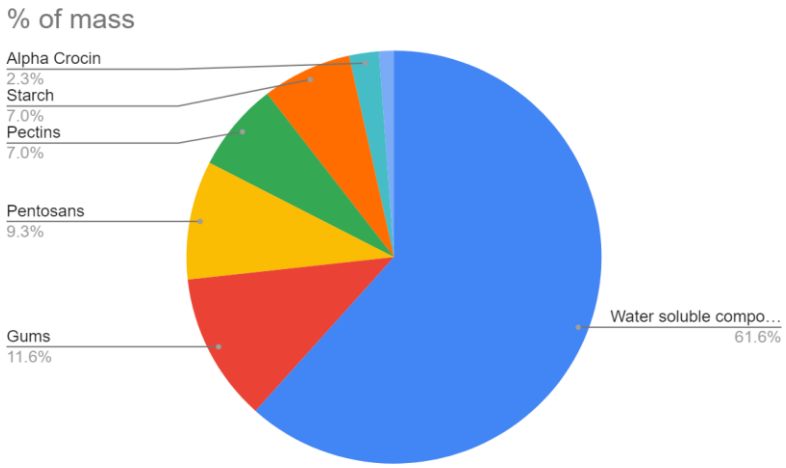
Carbohydrates: Glucose, fructose, gentibose and small quantity of xylose and rhamnose (13) Minerals: International standard organization reported that the total weight of different elements in 100 g of dried saffron as: calcium 111 mg, phosphorus 525 mg, potassium 1724 mg, sodium 148 mg, zinc and magnesium in small quantities (13)Vitamins: Thiamine and Riboflavin 13.Pigments: Crocin, anthocyanin, α-carotene and β-carotene, lycopene, zigzantin, flavonoids (6).

Table .1:Complete analysis of Saffron: (11)

Analysis of Saffron Stigma	
Components	% of mass
Water soluble components	53
Gums	10
Pentosans	8
Pectins	6
Starch	6
Alpha Crocin	2
Other Carotenoids	1
Crude fiber	5
Lipids	12

Non volatile oils	6
Volatile Oils	1
Proteins	12
Inorganic Matter (Ash)	6
HCl-Soluble ash	0.5
Water	10

Graph 1: Indicates complete ingredients present in saffron stigma



The saffron stigma, which is what basically forms commercial saffron, has a distinct and unique color, flavor and aroma and some of the groups of chemical compounds responsible for each of these properties have now been identified.

The color of saffron

Crocin is the most influential chemical in the coloring power of saffron. It is a rare carotenoid found in nature which can easily dissolve in water. In comparison to other carotenoids, crocin has a wider application as a colorant in food and medicine, mainly because of its high solubility. This substance was first discovered by Solomon and Carrar in crystal form (13).

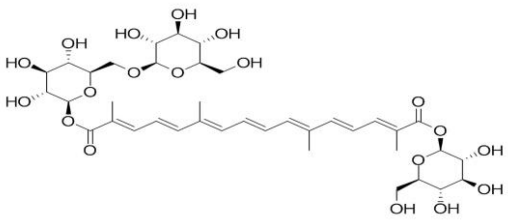


Figure 2 : Structure of Crocin

The taste of saffron

Glucose known as picrocrocin is the major factor for the bitter taste of saffron. This bitter substance can undergo crystallization, through acid hydrolysis, producing safranal (a glucose and aldehyde) (13).

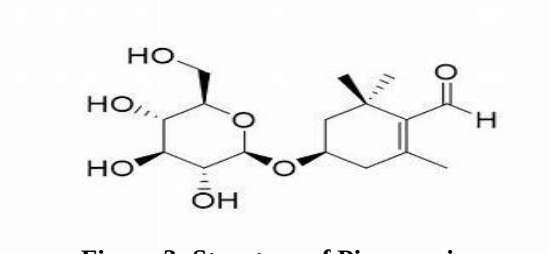


Figure 3: Structure of Picrocrocin

The aroma of saffron

The main aroma factor in saffron is safranal, which comprises about 60% of the volatile components of saffron. In fresh saffron, this substance exists as a stable picrocrocin but as a result of heat and with the passage of time, it decomposes releasing the volatile aldehyde, safranal (6).

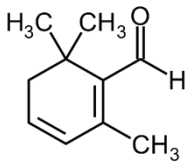


Figure 4: Structure of Safranal

Crocin inhibited an increase in serum triglycerides, total-, LDL-, cholesterol compared to the control group as seen before; however, the results also showed a significant increase in the fecal excretion of fat and cholesterol in the crocin group (100 mg/kg/day) (14). In another study, crocetin by virtue of its strong antioxidant activity prevented the cardiac hypertrophy induced by norepinephrine by increasing the levels of the antioxidant enzymes such as myocardial superoxide dismutase, catalase, glutathione peroxidase and also significantly improved the myocardial pathological histological changes induced by norepinephrine(15) .

Effect on Learning and Memory Behavior:

The extract of saffron and two of its main ingredients, crocin and crocetin, improved memory and learning skills in ethanol-induced learning behavior impairments in mice and rats. Oral administration of saffron may be useful in the treatment of neurodegenerative disorders and related memory impairment (5, 16) .

Effect on Premenstrual syndrome:

A study was conducted to investigate whether saffron (stigma of *Crocus sativus L.)* could relieve symptoms of premenstrual syndrome (PMS). Women aged 20–45 years with regular menstrual cycles and experience of PMS symptoms for at least 6 months was taken for the study. Women were randomly assigned to receive capsule saffron 30 mg/day (15 mg twice a day; morning and evening) (group A) or capsule placebo (twice a day) for two menstrual cycles (cycles 3 and 4). In this trial, saffron was found to be effective in relieving symptoms of PMS (17).

Effect on Respiratory system:

The relaxant effect of *Crocus sativus* on smooth muscle was evident as shown in guinea pig tracheal chain experiment. The relaxation produced with the aqueous-ethanolic extract and safranal in comparison with saline as negative control, and theophylline, was comparable to or even higher than that relaxation produced with theophylline suggesting its use in the treatment of various respiratory disorders like asthma etc (15.).

Effects on ocular blood flow and retinal function:

Crocin analogs isolated from saffron significantly increased the blood flow in the retina and choroid as well as facilitated retinal function recovery and it could be used to treat ischemic retinopathy and/or age-related macular degeneration (5, 14)

II. MATERIALS AND METHODS

Chemicals and apparatus

Distilled water and laboratory grade solvents were used in this study. The UV-visible spectra were recorded using UV-Vis spectrophotometer (Model: LabIndia) using quartz cells.

Materials and Methods

Saffron Stigma was procured from Jammu and Kashmir, Ethanol was of laboratory grade, Distilled water was used for the preparation of the powder and concentrated liquid extracts.

Preparation of Saffron extract:

Accurately weighed sample of saffron stigma was taken of a known required quantity, It was mixed with 1:1 Ethanol and water and kept for stirring. This was filtered and kept for second extraction by following the similar procedure described above. Saffron liquid extract was prepared using this extract and combining it with glycerine and safranal. Similarly saffron powder extract was prepared by combining this extract with Maltodextrin.

Evaluation of prepared saffron extract

Physical examination of saffron powder sample, the extracted and powdered saffron sample was evaluated for its organoleptic properties such as, color, odor and taste.

Determination of pH

Accurately around 1 gm of saffron powder sample was weighed and dissolved in 100 ml of distilled water. The pH was measured using a pH meter. Similarly a 0.5% sample was used for determination of pH of the liquid concentrate.

Solubility analysis

The Solubility of the saffron sample was determined by accurately weighing 0.1gm of powder sample and dissolved in 100 ml of water stirred on a magnetic stirrer and the solubility was recorded.

Tapped and Bulk density determination of sample

30 gm of sample was weighed on a weighing balance on which the TD BD cylinder was placed, and the volume was noted. This was mounted on the Density apparatus (Model: LabIndia) and set to 10, 500 and 1250 taps respectively. The volume at each of these individual points were noted, and thus TD, BD, Carr's Index and Hausner ratio values were obtained.

Figure 5: Different forms of Saffron

Content analysis by UV-Visible spectrophotometer

Standard Preparation

Take 500 mg of sample in a clean 100 ml of Standard Measuring Flask. Add water and keep for sonication for 10 mins and make up the volume with water. Pipette out 10 ml of this solution in a 100 ml volumetric flask and make up the volume with water. This sample is centrifuged for 5 mins. Determine the absorbance of the test solution at the wavelength of maximum absorbance at about. 257 nm of picrocrocin, 330nm safranal, 440nm crocin using water as the blank.

Test Solution Preparation

Take 500 mg of sample in a clean 100 ml of Standard Measuring Flask. Add water and keep for sonication for 10 mins and make up the volume with water. Pipette out 10 ml of this solution in 100ml of volumetric flask and make up the volume with water. This sample is centrifuge for 5 mins. Determine the absorbance of the test solution at the wavelength of maximum absorbance at about 257 nm of picrocrocin, 330 nm safranal, 440nm crocin using water as the blank.

Pharmacological properties of saffron

Table 2: Pharmaceutical properties of saffron.

Compounds	Invitro/Invivo	Activity
Safranal	Mice	Anticonvulsant (18)
Crocin and Crocetin	Rat	Neuroprotective (19)
Picrocrocin	Mice	Anticancer (20)

Results by UV: (Saffron powder Extract)

Table 3:Saffron Powder Extract Bio-active Ingredients

Saffron Extract	330 nm	440 nm	257 nm	% Content
Safranal	0.1835	-	-	19.7%
Crocin	-	0.4154	-	9.55%
Picrocrocin	-	-	0.1508	1.28%

Graph 2:Saffron powder extract Bio -active Ingredients

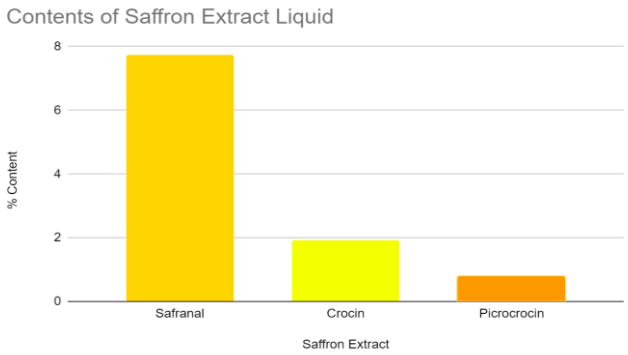


Table 4: **Saffron Liquid extract Bio -active Ingredients**

Saffron Extract	330 nm	440 nm	257 nm	% Content
Safranal	0.0713	-	-	7.74%
Crocin	-	0.0822	-	1.91%
Picrocrocin	-	-	0.0917	0.78%

Graph 3:Saffron Liquid extract Bio -active Ingredients

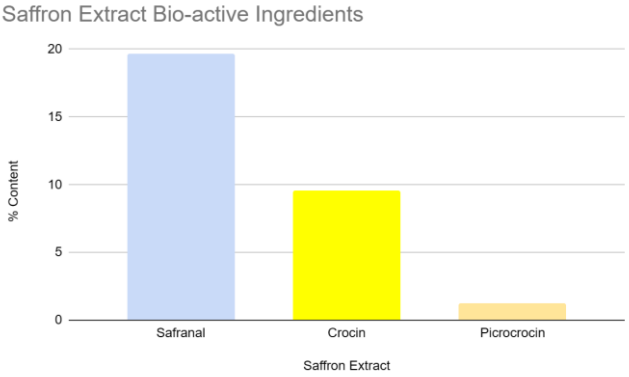


Table 5: Physical Parameters Analysis

Saffron Extract	COLOUR TASTE/ODOUR	TD, BD, Hausners Ratio, Carr's Index	pH	SOLUBILITY
POWDER	Orange to yellow powder, with Characteristic odour and Taste	TD - 0.7500 g/ml BD - 0.5085 g/ml Hausner's Ratio- 1.7714 Carr's Index - 43.5484	6.219	Soluble in water
LIQUID	Bright Red to orange colour Liquid concentrate, With Characteristic Odour and Taste	NA	6.6	NA

Food Application

Milk application

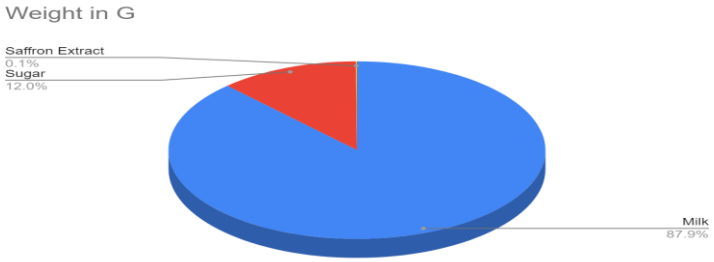
Saffron Extract is tested in milk application. 100 ml milk has been boiled and cooled at room temperature. Sugar 12 g has been added to 78 g of milk to provide a good taste. For this sugared milk 0.05% w/w of saffron extract has been added and mixed well to get uniform distribution of saffron extract. Milk has been tested by sensory evaluation with panel members. Its taste, aroma, and solubility were considered for sensory evaluation.

Figure 6 : Milk application

Table 6:Milk application

	Weight in %
Milk	88
Sugar	12
Saffron Extract	0.1

Graph 4:Milk application



Candy application

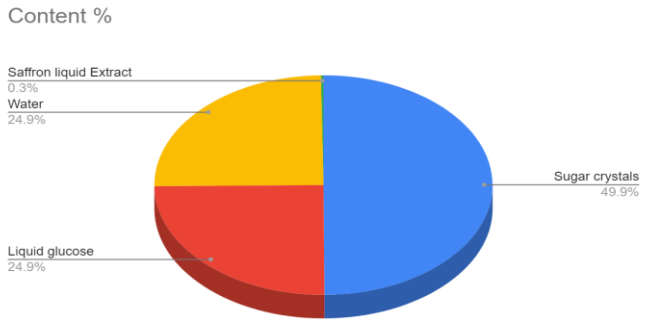
Saffron extract was tested in candies application also. Ingredients required : Sugar crystals, liquid glucose, water, saffron liquid extract and sugar powder. Dissolve 200 gm sugar crystals, 100gm liquid glucose and 100 gm water at 138°C to 140°C. Mix well and off the flame once caramelisation is achieved. Apply oil to the mold and add 1 gm saffron liquid extract in it. Cool it at room temperature. Carry out dusting with sugar powder on candies.

Figure 7:Saffron Candies

Table 7:Contents of Saffron Candy

Candy application	Weight in G
Sugar crystals	200
Liquid glucose	100
Water	100
Saffron liquid Extract	1
Sugar powder	50

Graph 5: Contents of Saffron Candy



Antinociceptive and anti-inflammatory Activity:

The antinociceptive activity of the *Crocus sativus* was evaluated by using the aqueous and ethanolic maceration extracts of stigma and petals. The effect of extracts against acute inflammation was studied using xylene induced ear edema in mice. The activity of the extracts against chronic inflammation was assessed by formalin-induced edema in the rat paw. In the hot plate tests, intraperitoneal injection of both extracts showed no significant antinociceptive activity in mice. The extracts exhibited antinociceptive activity against acetic acid induced writhing. Only the stigma extracts showed weak to moderate effect against acute inflammation. In chronic inflammation, both aqueous and ethanolic stigma extracts, as well as ethanolic petal extract, exerted anti-inflammatory effects (21).

Antioxidant Activity:

The methanolic extract of *Crocus sativus* and its components such as safranal, crocin etc. were reported to possess radical scavenging activity, suggesting its use as a cosmetic to treat age related disorders, as a food supplement etc (19).Fifty milligrams of saffron dissolved in 100 ml of milk was administered twice a day to human subjects and the significant decrease in lipoprotein oxidation susceptibility in patients with coronary artery disease (CAD) indicates the potential of saffron as an antioxidant (12).

Antitumor Activity:

The oral administration of the saffron ethanolic extract increased the life span of Swiss albino mice intraperitoneally transplanted with sarcoma-180 (S-180) cells, Ehrlich ascites carcinoma (EAC) or Dalton's lymphoma ascites (DLA) tumors. The authors did not identify the exact nature of the active compound from saffron stigmas, but suggested that this compound showed the presence of glycosidic linkage. Liposome encapsulation of saffron effectively enhanced its antitumor activity against S-180 and EAC solid tumors in mice, promoting significant inhibition in the growth of these tumors (6).Oral administration of saffron extract induced a dose-dependent inhibition of the growth in mice of ascite tumours (11).

Antitussive Activity:

The antitussive activity of C. sativus stigma and petal extracts and its components, safranal and crocin, was evaluated using the nebulizer solution of citric acid 20% in guinea pigs. The ethanolic extract of *C. sativus* (100-800 mg/kg) and safranal (0.25-0.75 ml/kg) reduced the number of coughs. The ethanolic and aqueous extracts of petal and crocin did not show antitussive activity (21).

Anxiolytic Activity:

The crocins were evaluated in rodents for the presence of anxiolytic properties. For this aim, the light\dark test was selected. Either crocins, at a dose which did not influence animals' motor activity (50 mg/kg), or diazepam (1.5 mg/kg), increased the latency to enter the dark compartment and prolonged the time spent in the lit chamber in the rats. Conversely, lower doses of crocins (15- 30 mg/kg) did not substantially modify animals'

behavior. The present results indicate that treatment with these active constituents of *C. sativus L.* induces anxiolytic-like effects in rat (16)(15).

Cardiovascular activity:

Crocin inhibited an increase in serum triglycerides, total-, LDL-, cholesterol compared to the control group as seen before; however, the results also showed a significant increase in the fecal excretion of fat and cholesterol in the crocin group (100 mg/kg/day) (14).

In another study, crocetin by virtue of its strong antioxidant activity prevented the cardiac hypertrophy induced by norepinephrine by increasing the levels of the antioxidant enzymes such as myocardial superoxide dismutase, catalase, glutathione peroxidase and also significantly improved the myocardial pathological histological changes induced by norepinephrine (10).

Antigenotoxic and Cytotoxic Activity:

The antimutagenic, co-mutagenic and cytotoxic effects of saffron and its main ingredients were assessed. When only using the TA98 strain in the Ames/Salmonella test system, saffron showed non-mutagenic, as well as non-antimutagenic activity against BP-induced mutagenicity, and demonstrated a dose-dependent co-mutagenic effect on 2-AA-induced mutagenicity. The saffron component responsible for this unusual co-mutagenic effect was safranal. In the in vitro colony formation test system, saffron displayed a dose-dependent inhibitory effect only against human malignant cells. All isolated carotenoid ingredients of saffron demonstrated cytotoxic activity against in vitro tumor cells. Overall, these results suggest that saffron itself, as well as its carotenoid components might be used as potential cancer chemopreventive agents (20). The cytotoxic effect of aqueous extract of saffron is evaluated on human transitional cell carcinoma (TCC) and mouse non-neoplastic fibroblast cell lines. The cell lines were cultivated and incubated with different concentrations of aqueous extract of saffron stigma (50 µg/mL to 4000 µg/mL). After 120 hours, a decrease in the percentage of survived cells at higher concentrations of saffron extract was seen in both cell lines. This shows that Saffron aqueous extract has inhibitory effects on the growth of both TCC 5637 and normal L929 cell lines (22)

Effect on Premenstrual syndrome:

A study was conducted to investigate whether saffron (stigma of *Crocus sativus L.*) could relieve symptoms of premenstrual syndrome (PMS). Women aged 20–45 years with regular menstrual cycles and those experiencing PMS symptoms for at least 6 months were taken for the study. Women were randomly assigned to receive capsule saffron 30 mg/day (15 mg twice a day; morning and evening) (group A) or capsule placebo (twice a day) for two menstrual cycles (cycles 3 and 4). In this trial, saffron was found to be effective in relieving symptoms of PMS (17).

Effects on ocular blood flow and retinal function:

Crocin analogs isolated from saffron significantly increased the blood flow in the retina and choroid as well as facilitated retinal function recovery and it could be used to treat ischemic retinopathy and/or age-related macular degeneration(5, 15).

Effects on Sexual Behaviour:

The effect of *Crocus sativus* (saffron) was studied on male erectile dysfunction (ED). Twenty male patients with ED were followed for ten days in which each morning they took a tablet containing 200 mg of saffron. After the ten days of taking saffron there was a statistically significant improvement in tip rigidity and tip tumescence as well as base rigidity and base tumescence 18. The aphrodisiac activities of *C. sativus* stigma aqueous extract and its constituents, safranal and crocin, were evaluated in male rats. Safranal did not show aphrodisiac effects. This study exhibited an aphrodisiac activity of saffron aqueous extract and its constituent crocin (16)

A clinical trial has been conducted in which 52 nonsmoker infertile men were enrolled. They were treated by saffron for 3 months. Saffron, 50 mg, was solved in drinking milk and administered 3 times a week during the study course. Semen analysis was done before and after the treatment and the results were compared. The mean percentage of sperm with normal morphology was 26.50 ± 6.44% before the treatment which increased to 33.90 ± 10.45%. The mean percentage of sperm with Class A motility was 5.32 ± 4.57% before and 11.77 ± 6.07% after the treatment. No significant increase was detected in sperm count; the mean sperm count was 43.45 ± 31.29 × 106/mL at baseline and 44.92 ± 28.36× 106/mL after the treatment period (P = .30). Hence it is concluded that Saffron, as an antioxidant, is positively effective on sperm morphology and motility in infertile men, while it does not increase sperm count (15).

Hepatoprotective activity:

Male Wistar rats (200-250g) were treated with saffron (40 or 80 mg/k/d) for 10 days and gentamicin 80 mg/kg/d for five days, starting from day 6. At the end of treatment, blood samples were taken for measurement of serum creatinine (SCr) and BUN. The left kidney was prepared for histological evaluation and the right kidney for Malondialdehyde (MDA) measurement. Gentamicin 80 (mg/k/d) increased SCr, BUN and renal tissue levels of MDA and induced severe histological changes. Saffron at 40 mg/k/d significantly reduced gentamicin-induced increases in BUN and histological scores ($p<0.05$). Gentamicin-induced increases in BUN, SCr and MDA and histological injury were significantly reduced by treatment with saffron 80 mg/k/d ($p<0.05$, $p<0.001$, $p<0.05$, and $p<0.001$ respectively) (18).

Radical Scavenging Activity:

The DPPH radical scavenging activity of extract of *Crocus sativus* L. (saffron), and some of its bioactive constituents (crocin, safranal) was studied. Crocin showed high radical scavenging activity (50% and 65% for 500 and 1000 ppm solution in methanol, respectively), followed by safranal (34% for 500 ppm solution). All the tested samples showed high radical scavenging activity, probably due to the ability to donate a hydrogen atom to the DPPH radical (19).

Relaxant Activity:

The effects of aqueous ethanolic extract of *Crocus sativus* were studied on the tracheal chains of guinea–pigs for its relaxant activity. The relaxant effect of the extract may be due to the safranal present in the *Crocus sativus.* The results indicated that safranal was, at least in part, responsible for the relaxant effect of Crocus sativus (11, 19).

Wound healing Activity:

The study was conducted to evaluate the efficacy of pollen of saffron extract cream in the treatment of thermal induced burn wounds and to compare its results with silver sulfadiazine (SSD) in rats. Animals were divided into four groups and administered a topical cream including control, base, saffron (20%) or SSD (1%) at 24 hours after a burn injury that was induced by hot water. On day 25, the average size of wound was 5.5, 4, 0.9 and 4.1 cm2 in control, base, saffron and silver groups. Histological comparison has shown that saffron significantly increased re- epithelialization in burn wounds (22).

III . RESULTS AND DISCUSSION

Saffron stigma is the costliest spice of Indian origin .Saffron stigma consists of safranal , crocin, and picrocrocin as active ingredients .Various research has been done throughout the world for the efficacy of bioactive ingredients of saffron. Biomed Ingredients Pvt.Ltd , has made an effort to convert saffron stigma into powder and liquid extract form which can be used in food , beverage, dairy ,bakery dietary supplements and nutraceuticals.

IV. CONCLUSION

This study describes the complete information about various aspects of *Crocus sativus*, these include description, habitat, botanical description, the Unani pharmacological as well as recently studied pharmacological actions and phytochemical studies. However, as saffron and its constituents show a wide spectrum of biological activities, it would be easier to develop new drugs.Saffron Extract standardized to liquid and powder which can be used in food , beverage, dairy, dietary supplements and nutraceuticals applications. Further to this more efficacy studies are required to claim actual pharmacological properties.

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