

# Physicochemical and Preliminary Phytochemical Studies on Petals of *Crocus sativus* 'Cashmerianus'

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## ABSTRACT

*Crocus sativus* 'Cashmerianus' is a plant cultivated in various parts of the world. The present communication attempts to evaluate the physicochemical and preliminary phytochemical studies on the petals of most expensive *Crocus* species found in Kashmir is *Crocus sativus* 'Cashmerianus' (Iridaceae). These observations would be of immense value in the botanical identification and standardization of the drug in crude form and would help to distinguish the drug from its other species. The percentage of total ash value, acid insoluble ash value, water soluble ash value and sulphated ash value were found as 18.36, 3.48, 11.36 and 9.01% respectively. Fluorescence characters of different extracts with various reagents were noted under UV (254 nm and 365 nm) and under normal ordinary light. Loss of weight on drying was found as 6.68% w/w. The percent yield of successive extract of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts were found as 6.18%, 7.79%, 6.28%, 30.63%, and 35.16% w/w respectively. The preliminary phytochemical screening was carried out for the presence of alkaloids, flavonoids, carbohydrate glycosides, tannins, terpenoids, phenol, steroids and saponins for different petal extracts of *Crocus sativus* 'Cashmerianus'. These studies provide referential information for correct identification and standardization of this plant material.

**Key words:** *Crocus sativus*, physicochemical, florescent behaviors. Pharmacognostical analysis, phytochemicals.

## INTRODUCTION

*Crocus sativus* var. cashmerian is a perennial stem less herb of the Iridaceae family commonly known as Kesar (Hindi), Saffron (English) and Zafran (Kashmiri). The word "saffron" is derived from the Arabic word za'faran, which translates to "yellow." Commercial saffron is made from the dried stigmas of the saffron flower, a triploid sterile plant and has a unique and distinctively pungent, honey-like flavor and aroma. Saffron is produced worldwide at an annual rate of 50 tons with a commercial cost of about 50 million dollars.<sup>[1]</sup> It comprises of the dried red stigma, purple petal with a small portion of the yellowish style attached. It is cultivated in Azerbaijan, France, Greece, Iran, Italy, Spain, China, Israel, Morocco, Turkey, Egypt, Mexico and Kashmir in India.<sup>[2]</sup> It is most expensive spice and used for cooking, staining, medicine, cosmetics<sup>[3]</sup> and is also used in folk medicine as antispasmodic, carminative, stomachic, expectorant, aphrodisiac, hypolipaeic effects<sup>[4]</sup> petals possesses antidepressant activity<sup>[5]</sup> cardio-tonic and stimulant.<sup>[6]</sup> In traditional medicine this plant is utilized as an exhilarant and

curative of anxiety.<sup>[7]</sup> 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 been isolated.<sup>[1]</sup> Saffron contains three main pharmacologically active metabolites: 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 -  $\alpha$ -crocins is the major component of saffron. Picrocrocins are the main substance responsible of the bitter taste in saffron. Safranal in the volatile oil is responsible for the characteristic saffron odor and aroma. Furthermore, saffron contains proteins, sugars, vitamins, flavonoids, amino acids, mineral matter, gums, and other chemical compounds.<sup>[1]</sup>

## Plant Material and Method

The petals of *Crocus sativus* 'Cashmerianus' were collected from Pampore area of Kashmir (J&K, India). *Crocus sativus* 'Cashmerianus' was properly identified (Voucher specimen-KUCS03) by Dr. A. R. Naqshi, Taxonomist, Department of Pharmaceutical Sciences, University of Kashmir, Srinagar. The collected petals were shade dried and coarsely powdered. The coarse powder was subjected to continuous extraction in a soxhlet apparatus separately using petroleum ether, chloroform, ethyl acetate, methanol and water as solvents.

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### Preparation of the extract

The petals of *Crocus sativus* 'Cashmerianus' were dried under shade, separated and crushed to form dry powder. 150 g of weighed petals were first defatted using petroleum ether (60-80 °C) in soxhlet apparatus. The marc was removed and dried after the completion of defatting process. Defatted petals were subjected to successively continuous extraction with 500 mL each of chloroform, ethyl acetate, methanol and distilled water to give petroleum ether extract (PE), chloroform extract (CE), ethyl acetate extract (EAE) methanol extract (ME) and aqueous extract(AE). After complete extraction the solvent was evaporated and concentrated to dry residue.<sup>[8]</sup> The percentage yield of successive extracts of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extract of petals of *Crocus sativus* 'Cashmerianus' were obtained as 6.18%, 7.79%, 6.28%, 30.63%, and 35.16% respectively. All the extractive values of the plant *Crocus sativus* 'Cashmerianus' with different solvents were calculated and are shown in the table 1.

### Preliminary phytochemical analyses

Successively obtained materials were filtered and concentrated using vacuum distillation. The different extracts were

subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure<sup>[9]</sup> and shown in the table no: 4.

### Physico-chemical parameters

Physicochemical constants were evaluated for the purpose of standardization such as the percentage of total ash, water soluble ash; acid insoluble ash, sulphated ash, loss on drying and total extract values were calculated according to standard procedures.<sup>[10]</sup> The values of physicochemical constants were calculated and are shown in table no: 2.

### Fluorescent behavior of powder

In fluorescence analysis<sup>[11]</sup> the powdered sample were treated with various chemical reagents like aqueous 10% Sodium hydroxide, alcoholic 1N sodium hydroxide, 1N hydrochloric acid, 50% sulphuric acid and concentrated nitric acid, picric acid, acetic acid, ferric chloride, conc. HNO<sub>3</sub> + NH<sub>3</sub> etc. and their extracts were subjected to fluorescence analysis in day light and UV light (254 nm and 365 nm). The observed values of fluorescence analysis are shown in Table 3.

**Table 1: Successive Extraction values of the petal extracts of *Crocus sativus* 'Cashmerianus' in different solvents**

Nature of extracts	%Yield (w/w)	Color of the extract
Petroleum ether (60-80°)	6.18	Golden yellow
Chloroform	7.79	Dark brown
Ethyl acetate	6.28	Dark brown
Methanol	30.63	Dark brown
Aqueous	35.16	Black

**Table 2: Physicochemical parameters of petal of *Crocus sativus* 'Cashmerianus'**

Parameters	% Content
Foreign organic matter	0.28
Methanol soluble extractive	39.63%
Water soluble extractive	46.10%
Total ash	18.76%
Acid-insoluble ash	03.48%
Water soluble ash	11.36%
Sulphated ash	09.01%
Loss on drying	06.68%

**Table 3: Fluorescence characteristics of the powdered sample of Petal of *Crocus sativus* 'Cashmerianus'**

Treatment	Visible/Day light	Short UV light (254 nm)	Long UV light (365 nm)
Powder as such	Violet	Purple	Black
Powder + 50% H <sub>2</sub> SO <sub>4</sub> + water	Reddish brown	Brown	Dark brown
Powder + Con. HCl	Golden brown	Light brown	Green
Powder + Con. HCl+ water	Pink	Purple	Light purple
Powder + Con. nitric acid	Golden brown	Green	Purple
Powder + Con. nitric acid+ water	Light green	Light green	Yellow
Powder + Acetic acid	Pink	Purple	Light pink
Powder + Methanol	Colorless	Intense light green	Light green
Powder + Chloroform	Purple	Colorless	Light green
Powder + Petroleum ether	Colorless	Colorless	Light green
Powder + Distilled water	Purple	Colorless	Light green
Powder + 10% NaOH (aqua.)	Yellow	Light green	Light yellow
Powder + 5% iodine	Light yellow	Light green	Light green
Powder + Picric acid	Light yellow	Intense green	Light green
Powder + Ferric chloride (5%w)	Muddy yellow	Intense green	Yellow green
Drug Powder + NH <sub>3</sub> solution	Light blue	Colorless	Light green
Powder + Ethyl acetate	Colorless	Colorless	Purple
Powder + 1N NaOH(alcoholic)	Light Yellow	Colorless	Light yellow
Powder + Conc. H <sub>2</sub> SO <sub>4</sub>	Greenish yellow	Green	Light green

**Table 4: Preliminary Phytochemicals Screening of Petal extracts of *Crocus sativus* 'Cashmerianus'**

Test	P.E.	C.E.	E.A.E.	M.E.	A.E.
Carbohydrates-					
Molish test	+	+	+	++	++
Fehling's test	+	+	+	+	+
Seliwnoffs test	-	-	-	+	+
Alkaloids					
Mayer's test	++	++	-	+	+
Hager's test	+	++	-	++	++
Wagner's test	+	+	-	+	-
Saponins					
Foam test	-	-	-	-	++
Steroids					
Salkovaski test	++	-	-	++	++
Fats and oils					
Filter paper test	-	-	-	-	-
Proteins					
Millon's test	-	-	-	-	++
Biuret test	++	-	++	-	++
Amino acid test					
Ninhydrine test	+	-	-	-	+
Glycosides					
Anthraquinone test	++	-	-	++	++
Keller kiliani test	-	-	-	+	+
Starch					
Iodine test	-	-	-	-	+
Tannins and Phenolic					
Ferric chloride test	-	-	-	++	++
Resins					
Ferric chloride test	+	-	-	++	++
Flavonoids					
Shinoda test	+	-	-	++	++
Lead acetate test	-	+	-	+	+

Symbol denoted: +: Positive, ++: Strong positive -: Negative (Symbol Based on color intensity)

## RESULT

The preliminary qualitative chemical tests performed shows that the petals are credited with different secondary metabolites such as alkaloids, glycosides, tannins, phenolics compounds, flavonoids, steroids, saponins, proteins, amino acids and carbohydrates. The percentage of total ash value, acid insoluble ash value, water soluble ash value and sulphated ash value were found to be 18.36, 3.48 11.36 and 9.01% respectively. Foreign organic matter in the petals was 0.28%. Loss of weight on drying was found as 6.68% w/w, which is not too high, hence could discourage bacterial, fungal, or yeast growth.<sup>[13]</sup> The percent yield of successive extract of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts were found to be 6.18%, 7.79%, 6.28%, 30.63%, and 35.16% w/w respectively. Extractive value was highest in water and alcohol indicating the possibility of considerable amount of polar compounds in crocus petals. Preliminary phytochemical screening of different petals extracts of *Crocus sativus* 'Cashmerianus'. Indicated the presence of alkaloids, flavonoids, carbohydrate, glycosides, tannins, terpenoids, phenol, steroids and saponins.

## CONCLUSION

Physicochemical constant values are useful in determining authenticity and purity of drug and also for quantitative standards as reference.<sup>[12]</sup> In this study total ash value is considerably low, which may be due to low content of carbonates, phosphates, silicates and silica. Study of crude fiber content could be useful in distinguishing between similar drugs or in the detection of adulteration. It will also help to remove the more resistant parts of plant organs which can be used for microscopic examination. Fluorescence behavior of drugs sample is an important character exhibited by various chemical constituents present in plant material. Some plant constituents have unique tendency to show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.<sup>[13]</sup> Preliminary phytochemical screening of different petals extracts have shown that the petals of *Crocus sativus* 'Cashmerianus' possess various secondary metabolites. These secondary metabolites may be responsible for various pharmacological effects of the drug and justify their use by herbal practitioners.

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