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Comparative Study of Different Market Sample with Genuine Sample of Kesar (Crocus Sativa) W R T Phytochemical Analysis

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Abstract

Background: Kesar or saffron (Crocus sativus), is known as the most expensive spice in the world and as beneficial for human health due to three main bioactive compounds: crocetin, picrocrocin and safranal. The demand for saffron is increasing worldwide for its interesting role in cuisine, medicine and cosmetics. The therapeutic efficacy of medicines depends on the quality and purity of ingredients used in formulations.

Material and Method: The study samples is been collected and handpicked from Kashmir and other 3 samples were collected from three major markets i.e. Amritsar, Chennai and Mumbai to see the difference on preliminary test like Pharmacognostic screening, physio chemical, Phytochemical and TLC, which are important tools in detecting of adulteration and substitution.

Results and Discussion: The results showed slight difference in microscopic and macroscopic characters in which sample I and sample II (Chennai) showed better quality. In this study total ash value is considerably low in sample I i.e. 0.06gm and sample III i.e. 0.06gm, which may be due to low content of carbonates, phosphates, silicates and silica. Study reveals that alkaloids were not much appreciated in sample II and sample IV. Saponins were not present in sample II. Flavonoids, Tannins, resins and glycosides were absent in sample IV. High amount of flavonoids, tannins, steroids and resins were present in sample I and sample II. High amount of Glycosides were present in sample III. Samples III and IV were not showed good amount of phytochemical which indicates the poor quality of the sample. It might be because of old samples collected or over drying of the samples which loses down many constituents. It could be either collected not from good source. The number of TLC bands found in sample I were 10 and other sample II, III and IV showed 9, 8 and 18 respectively. Results showed sample II procured from Chennai market found to be equivalent with original and better than other two market samples.

Conclusion: Pharmacognostic, physiochemical and phytochemical methods are established techniques for the quality evaluation of highly valued medicinal plants. Present analytical study done to compare the original sample and market samples helpful in detecting the adulteration and authentication of drug. The preliminary data suggested sample collected from original source was superior in quality and possesses much phytochemicals with this sample II collected from Chennai market showed better quality as compared among other market sample.

Keywords: Kesar; Pharmacognosy; Physio chemical; Phytochemical; TLC.

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Introduction

Kesar or saffron (Crocus sativus), is known as the most expensive spice in the world and as beneficial for human health due to three main bioactive compounds: crocin, picrocrocin and safranal. The demand for saffron is increasing worldwide for its interesting role in cuisine, medicine and cosmetics.[1] Saffron is produced worldwide at an annual rate of 50 tons with a commercial cost of about 50 million dollars.[2] Due to the reduction

of its production, recent investigations have been conducted to study how to improve stigma yield, quality and antioxidant activity by selecting of corm geographical origin and climatic conditions, drying methods and storage processes etc.[3] In recent time where the demand is high and original drug is becomes rare then the adulteration takes place and thus on the name of the original drug we are getting other drug, thus we don't get the genuine drug. Adulteration is a practice of substituting original crude drug partially or whole with other similar looking substance but the latter is either free from or inferior in chemical and therapeutic properties. An adulterant must be some material which is both cheap and available in fairly large amounts. The Pharmacognostic screening, physic chemical, phytochemical and Thin Layer Chromatography is an important tool and preliminary techniques in detecting of adulteration and substitution and to resolve confusion and to find the exact source plant. Thus, this work is been done to study and evaluate the different market samples with original sample on the basis of pharmacognostic, physic chemical and phytochemical analysis of Kesar collected from natural source and market samples.

Aims & Objectives

- To collect the genuine sample of Kesar (Crocus sativus Linn.) from district Pulwama Kashmir and Market Samples from major three markets of Chennai, Amritsar, Mumbai.
- To study botanical authentication of collected genuine sample and market sample.
- To compare Pharmacognostic study, organoleptic study, Microscopic & macro scopic study, Physiochemical study & Phytochemical study the genuine and market samples.
- To compare the data and to find out whether there is any similarity or difference in their pharmacognostic and analytical profile among the samples.

Material and method

Plant Material

- Collection of Raw Drugs & Botanical Authentication of samples.

Drug were collected from Pulwama, Kashmir (Crocus sativus). Other three samples were procured from Amritsar, Chennai and Mumbai market. The samples were preserved, photographed and botanical authentication is done by Government Drug Testing Laboratory (DTL), Patiala, Punjab.

Pharmacognostic Study

- Microscopic, Macroscopic and Organoleptic study

Samples were separately evaluated for organoleptic characters like Taste, Odour, Colour and Touch. Photomicrographs of sections were taken by using Canon digital camera with the help of Pharmacognosy dept. Babe keayurvedic medical college and hospital, Daudhar, Moga, Punjab. Macroscopic, Microscopic, and organoleptic study were carried out as per Ayurvedic Pharmacopoeia of India.[4]

Physio chemical Study

Physio chemical analysis such as Moisture content, Total Ash, Acid insoluble ash, Water soluble extract, Alcohol soluble extract were carried out as per Ayurvedic Pharmacopoeia of India.[4]

Phytochemical study

The preliminary screening of phytochemical of samples such as Alkaloids, Carbohydrates, Lipids, Saponins, Flavonoids, Tannins and Phenolic compounds, Steroids & Terpen, Resins & Glycosides were carried out as per standard methods and procedure.[5]

Thin Layer Chromatography (TLC)

TLC studies of extracts of all samples were carried out by pre coated silica gel plates (Merck, Germany) which possess standardized adsorption layers, at room temperature. Mobile phase toluene: ethyl acetate: water (4:2:2) was selected for the present study. All The chromatograms were developed in twin through glass chambers on 10 X 10 cm plates till the mobile phase travelled up to a distance of maxium 10 cm from starting point in sample 1. After development, the plates were dried at room temperature for 5-10 min and observed under UV-366 mm wavelength, normal light and Rf values were recorded. All the plates were sprayed with Dragendorff's reagent spraying agent, dried at 105^0C in hot air oven, before recoding the Rf values.

Results and Discusison

Collection & Authentication details is listed in Table no 1. Figure 1, 2, 3 & 4 is showing various market samples.

Table 1: Collection &Authentification Details of different Samples

S No.	Sample-I	Sample-II	Sample-III	Sample-IV
Place of collection	Naturally handpicked from pulwama, Kashmir	Chennai	Mumbai	Amritsar
Botanical Authentication	Patiala	Branded sample procured from market	Branded sample procured from market	Branded sample procured from market
Total weight collected	24 gm	5gm	5 gm	5 gm)
Date of collection	8 november,2020	6-07-2021	27-6-21	29-07-2021
Bill no.	Self collected and picked	8495	Randomly buyed	987

There was a price difference observed among all the samples per 5gm which are listed in Table no. 2

Table 2: Market price rate difference among all samples.

S No.	Sample-I	Sample-II	Sample-III	Sample-IV
Place of collection	Naturally collected from Kashmir	Chennai	Mumbai	Amritsar
Rate/gm	2000/gm	1275/5gm	1195/5gm	500/5gm

Fig. 1: Sample 1 flower Bud of Kesar and Macroscopic study of stamens Collected from Pulwama, Kashmir.

Fig. 2: Sample 2 Stamens procured from Chennai Market.

Fig. 3: Sample 3 Stamens procured from Mumbai Market.

Fig. 4: Sample 4 Stamens procured from Amritsar Market.

Macroscopic Study, Microscopic Study & Organoleptic Study Results

Macroscopic Study

Sample 1 showed Yellowish style, broken or intact along with trifid stigma; stigma is dark red or reddish brown, cornucopia shaped, with fimbriate margin, and about 25 mm long; broken style are very thin, up to about 10 mm long and other samples were compared with original sample. The result of Macroscopic and Microscopic characteristics are listed in Table no. 3.

Table 3: Showing Macroscopic Characters & Microscopic Characters of all Samples.

Test	Samlple-I	Samlple-II	Samlple-III	Samlple-IV
Macroscopic	Yellowish style, broken or intact along with trifidstigma; stigma is dark red or reddish brown, cornucopia shaped, with fimbriate margin, and about 25 mm long; broken style are very thin, upto about 10 mm long broken style are very thin, upto about 10 mm long.	Yellowish dark brown style, broken or intact along with trifid stigma; stigma is light red or reddish brown, cornucopia shaped, with fimbriate margin, and about 25 mm long; broken style are very thin, upto about 8 mm long.	Slight Yellowish style, broken or intact along with trifid stigma; stigma is dark red or reddish brown, cornucopia shaped, with fimbriate margin, and about 22 mm long; broken style are very thin, upto about 10 mm long.	Yellowish style, broken or intact along with trifid stigma; stigma is dark red or reddish brown, cornucopia shaped, with fimbriate margin, and about 20 mm long; broken style are very thin, upto about 10 mm long.
Microscopic	Stigma composed mostly of elongated, thin walled, parenchyma cells containing colouring matter; at the upper end numerous cylindrical papillae or trichomes,; pollen grains, a few, spherical, nearly smooth, from 40 to 120 microns in dia; occasionally germinated and exhibiting pollen tubes.	Stigma composed mostly of elongated, thin-walled, parenchyma cells containing colouring matter; at the upper end numerous cylindrical papillae or trichomes,; pollen grains, a few, spherical, nearly smooth, from 40 to 120 microns in dia; occasionally germinated and exhibiting pollen tubes.	Stigma composed few elongated, very thin-walled, parenchyma cells containing colouring matter; at the upper end numerous cylindrical papillae or trichomes,; pollen grains, a few, spherical, from 40 to 120 microns in dia; few germinated and exhibiting pollen tubes.	Stigma composed mostly of slightly elongated, thin-walled, parenchyma cells containing colouring matter; at the upper end numerous cylindrical papillae or trichomes,; pollen grains, a few, spherical, nearly smooth, from 60 to 100 microns in dia; occasionally germinated and exhibiting pollen tubes.

Microscopic study of Stamen of Sample I, sample II, Sample III & Sample IV under Microscope having magnification (10x) is showed in figure No. 5.

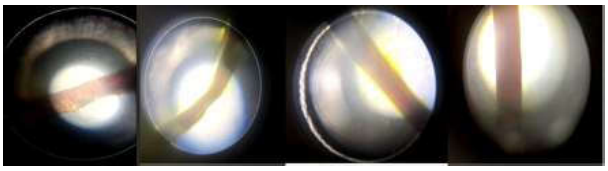

Fig. 5: Microscopic study of Stamen Sample I , sample II, Sample III & Sample IV under Microscope having magnification (10x).

Organoleptic ParametersResultsof all samples is listed in Table no. 4.

Table 4: Results of Organoleptic Parameters of all samples.

Sample	Parameters		
	Colour	Odour	Taste
Samlple I	Yellowish style, broken or intact along with trifid stigma; stigma is dark red or reddish-brown	Strongly aromatic	Slightly bitter
Samlple II	Yellowish style, broken or intact along with trifid stigma; stigma is dark reddish & shinning.	Slight aromatic	Bitter
Samlple III	Yellowish style, broken or intact along with trifid stigma; stigma is slight reddish-brown & rough	Strongly aromatic	Bitter
Samlple IV	Dark Yellowish style, broken or intact along with trifid stigma; stigma is moderately reddish	No aromatic odour	Slight sweat

Physio chemical study Results

- The ash value is indicates the presence of inorganic and salt materials in the sample. In this study total ash value is considerably low in sample I i.e., 0.06 gm and sample III i.e. 0.06 gm, which may be due to low content of carbonates, phosphates, silicates and silica. The sample II and sample IV having high Ash value 0.08 gm & 0.11 gm respectively. It indicates the inorganic material and salts were more in these samples.
- The loss on drying of any sample is directly related to its moisture content. If the moisture content is very high in any drug it may affect its preservation. Hence, the loss on drying of the sample was determined and it was found same in sample I and sample III i.e. 1.64 gm & 1.68 gm respectively. Whereas sample II & sample IV showed high moisture content 1.70 gm & 1.82 gm respectively. Which is not too high, hence could discourage bacterial, fungal, or yeast growth.[6] The results are showed in Table no. 5.

Table 5: Results of Physio Chemical Study of all samples.

Parameters	Sample I	Sample II	Sample III	Sample IV
Moisture content	1.64 gm	1.70 gm	1.68 gm	1.82 gm
Total Ash	0.06 gm	0.08 gm	0.06 gm	0.11 gm
Acid insoluble ash	0.01 gm	0.02gm	0.04 gm	0.09 gm
Water soluble extract	40%	25.86%	88.48%	20%
Alcohol soluble extract	78.12%	68.96%	61.6%	50%

Phytochemical Study Results

The qualitative phytochemical analysis of kesar (Crocus Sativus L.) showed the presence of various. Phytoconstituents in sample I i.e. hand picked from Kashmir like carbohydrates, proteins, Fats and lipids, steroids/Terpenes, saponins, alkaloids, flavonoids, phenolics/tannins, cardiac glycoside and tannins etc which showed the Good Quality of Kesar. The results are listed in Table no 6.

- Study reveals that alkaloids were not much appreciated in sample II and sample IV.
- Carbohydrates were present In all the samples & Lipids were not found in sample IV.
- Saponins were not present in sample II.
- Flavanoid, Tannins, resins and glycosides were absent in sample IV.
- High amount of flavonoids, tannins, steroids & resins were present in sample I and sample II.
- High amount of Glycosides were present in sample III.

Samples III & IV did not showed good amount of phytochemical which indicates the poor quality of the sample. It might be because of old samples collected or over drying of the samples which lose down many constituents.

Table 6: Results of Phytochemical Study of all Samples.

Test	Samlple I	Samlple II	Samlple III	Samlple IV
Alkaloids	+	-	+	_
Carbohydrates	++	+	++	+
Lipids	+	+	+	-
Saponins	+	+	-	+
Flavonoids	++	++	+	-
Tannins & Phenolic compounds	++	++	+	-
Steroids & terpen	++	++	-	+
Resins	++	++	++	-
Glycosides	+	+	++	-

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

TLC Study Results

According to previous study for maximum separation of bands toluene: ethyl acetate: water has been selected. The number of TLC bands found in sample I were 10 and other sample II, III and IV showed 9, 8 and 8 respectively. The respective Rf values of each sample extract are listed in Table and it is evident from the data that sample 1 showed highest Rf value i.e. 0.36 shows that it contains highest Phyto constituents with highest band. Whereas, sample II showed equivalent Rf value which explains that sample II found to be better sample as compared with other two market samples. The results are showed in Table no. 7.

Table 7: Results of TLC Observed Under Uv Light of all samples.

Sample	Solvent system	No. of spots	Observed under long UV light (366mm) Rf values
Sample I	Toloune: ethyl acetate: water (4:4:2)	10	0.36
Sample II	Toloune: ethyl acetate: water (4:4:2)	9	0.34
Sample III	Toloune: ethyl acetate: water (4:4:2)	8	0.32
Sample IV	Toloune: ethyl acetate: water (4:4:2)	8	0.32

It was observed that most of the samples received from different sources were admixed with both, the synthetic color and weight enhancers, mainly mineral compound. The most preferable and admissible synthetic colors were tartrazine yellow, erythrosine, sunset yellow, carmoisine, ponceau 4R and sometimes annatto water soluble liquid, norbixin. Previous data says Saffron is also mixed with materials such as beet, pomegranate fibers, and red dyed silk fibers are occasionally observed for decreasing the cost of saffron.[7] Sometimes the flowers of other plants, particularly Carthamustinctorius, or safflower, Calendula officinalis, or marigold, arnica and tinted grasses are fraudulently mixed with the genuine stigmas. It is reported that adulterants have been loaded with calcium sulfate and attached to saccharine or glucose which yielded on incineration 40% of ash. Turmeric, paprika, and other substances have still been combined with saffron powder.[7]

Physicochemical constant data are useful in determining authenticity and purity of drug and also for quantitative standards as reference.[8] The Pharmacognostic screening, physic chemical, phytochemical and TLC is an important tool and preliminary techniques in detecting of adulteration and substitution and to resolve confusion and to find the exact source plant. The work can serve as a valuable source of information to establish the plant in future study.

Conclusion

Pharmacognostic, physiochemical and Phyto-chemical methods are established techniques for the quality evaluation of highly valued medicinal plants. Present analytical study done to compare the original sample and market samples helpful in detecting the adulteration and authentication of drug. The Preyliminary data suggested sample collected from original source was superior in quality and possesses much phytochemicals & Sample II collected from Chennai market showed better quality as compared among other market sample.

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