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Market Sample Survey Of Crocus Sativus Linn.To Assess The Genuinity

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Abstract- Many of the drugs used in conventional medicine are dried from herbs. Despite the fluctuation in prices in international markets, saffron was still remains the most expensive spice. The main aim of this study is to examine the powdery microscopical and phytochemical analysis and adultration detection of saffron. Crocus sativus .Linn is a perennial stem less herb of the Iridaceae family. Saffron stigmas of sample1, sample2, sample3and sample4 are collected from different rates of market sample from Thrissur district, sample5 collected from the Oushadhi premises, and it is collected from Himachal Pradesh. The extracts were prepared by using ethanol as solvent. The preliminary phytochemical screening was carried out for the presence of alkaloids, flavonoids carbohydrates, glycosides, phenol, for different market samples of crocus sativus Linn. The standardized Genuinity tests were carried to detect the adulteration. The highest level of secondary metabolites reported in sample 5 collected from Himachal Pradesh. Sample 2 contains lowest levels of phytochemicals. Powdery microscopy enabled to identify the specific anatomical characters of each saffron stigma. The highest level of adultration detected in sample S3 and sample S2.Synthetic dyes, organic dyes,and various types of floral materials are also used in the adultraion. The quality of the samples depend on the price values. The qualitative specifications and anatomical characters of saffron of different market samples influenced by different factors such as the quality of saffron stigma, its price, and method of drying. 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 spices.
Key words: Crocus sativus, Phytochemicals, Powdery Microscopy, Adultration detection.

I. INTRODUCTION

Saffron consists of dried trilobed stigmas of Crocus sativus Linn. ,which is a bulbous perennial with a globular corms; the plant is only 15 - 25cm high , native of Southern Europe and cultivated in Mediterranean countries, particularly in Spain ,Austria, France ,Greece,England,Turkey and Persia,Iran,India(Jammu and Kashmir)and the orient. True saffron must not be confused with either meadow saffron (Colchicum autumnale Linn. family: Lilliaceae) or safflower or bastard saffron (Carthamus tinctorium, Family: Compositae), which are occasionally used as adulterants of true saffron. Saffron is one of the oldest and certainly among the world's most expensive species 1.
As saffron is the most expensive spices, quality control regulations have been proposed in an attempt to avoid these adulterations. The ISO (International Standards Organization) standards are the quality control regulations currently applied in the international business 2.
Its cultivation dates back to 550 A.D. About 3-4 centuries before it reported cultivation in Spain, Kashmir saffron, popularly known as Kesar, it is the princes of Indian Spices having a great potential for its export to other countries 3.
Different methods have been employed for the the assessment of quality and detection of adulteration in saffron 4.India has rich wealth of important medicinal flora and its varied climate is ideally suited for the cultivation of medicinal plants. Quality of the plant derived medicine is a matter of great concerns as the utilization of plant materials for cure of infections and chronic human diseases is increasing. One of the major drawbacks in popularization of plant based drugs is the inconsistent quality of the formulated preparations5 In addition to qualitative detection ,it provides semi quantitative information on the main active constituents of plant preparations.HPTLC method also provide provides a chromatographic fingerprint and is suitable for monitoring the identified purity of plants and for detecting adulterants and substitutions 6.

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection of samples

The Crocus sativus Linn, stigmas of sample1, sample2, sample3, sample4 of varying price were collected from market of Thrissur district. Sample 5 stigma collected from Oushadhi, which was collected from Himachal Pradesh on order. (Plate 2)
Sample details

S1-Market sample from Thrissur district (1gm=160RS)
S2 –Market sample from Thrissur district (1gm=100RS)
S3- Market sample from Thrissur district (1gm=55RS)
S4- Market sample from Thrissur district (1gm=300RS)
S5- Market sample from Himachal Pradesh (1gm=1500RS)
EXTRACTION: The extracts of samples were prepared by using the solvents water, ethanol and methanol.

2.2 Comparison Of Distinguishing Characters Of Sample
Macroscopic analysis7 The whole mount of Sample1, sample2, sample3, sample4 and sample5 saffron stigmas were prepared, and examined macroscopically to compare the external characteristic features of the samples.
Powder microscopy8 Characters of the saffron stigma powder were examined by the following method. Sufficient amount of sample was taken in 50%nitric acid solution on a slide and cover it with a coverslip, warmed over a low flame for a short time. Then examined under the Binocular microscope.

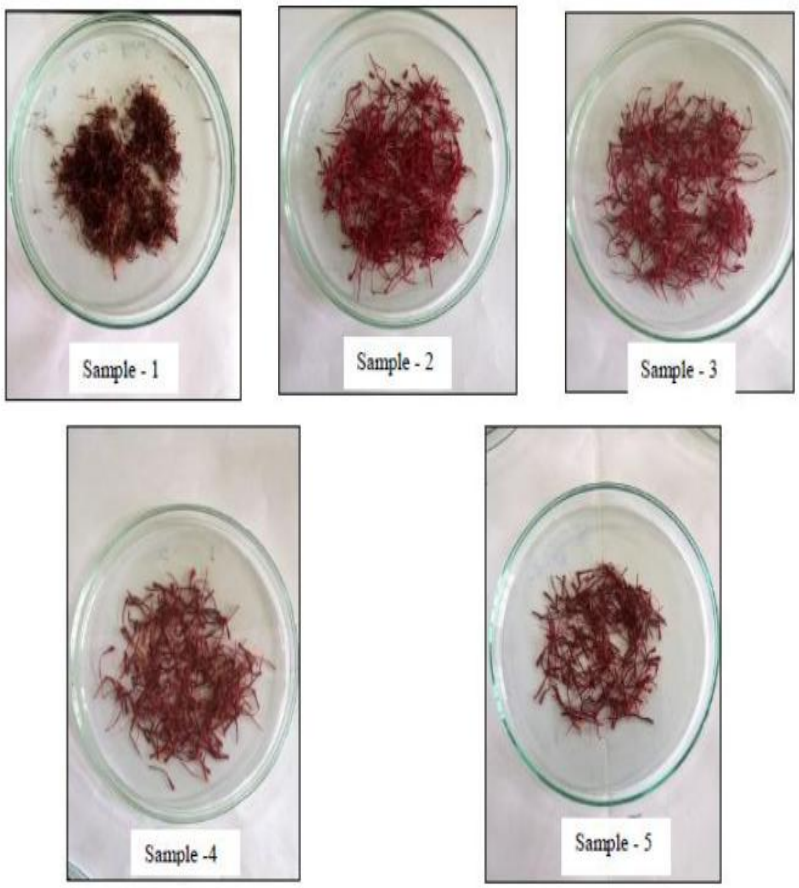
2.3 Phytochemical Analysis
Detection of alkaloids
Mayer's test 9,Wagner's test 9,Dragendorffs test 9

2.4 Detection Of Carbohydrates
Molish's test 9,Fehling's test 9,Benedicts test 9
DETECTION OF GLYCOSIDES AND STEROIDS Liebermann's test 9,Salkowski's test 9
DETECTION OF PROTEINS AND AMINO ACID Ninhydrin test 10
DETECTION OF PHENOLIC COMPOUNDS
Ferric chloride test 10,Gelatin test 10

2.5 Detection Of Flavanoids
Alkaline reagent test 10,Shinoda test 10,Lead acetate test 10

2.6 Adultration Detection
Chemical color tests 11Pure saffron gives yellow colouration in the solution of the water and methanol due to carotenoid pigments-crocin and crocetin, but not in benzene, xylene, ether, chloroform and toluene solution. Fake saffron imparts verities of color in different solvents.
Sulphuric acid test 11 The carotenoid pigments like crocin, crocetin and picrocrocin reacts with the H2So4to give bluish color immediately, which finally changes to violet to red. The reaction is due to hydrolysis of the carotenoid esters. Fake saffron produces yellow color only.
Water test 9 Saffron threads are adding 10 ml of water. Pure saffron imparts yellow orange brown color to water and stigma threads retain their dark red colouration .in fake saffron ,it shows yellow colouration of the water and the thread become white or light yellow color.
Organic dyes 8
1. Digest about .1g in 10ml of water for 15 minutes, with frequent shaking, filter and add 1g of decolorizing charcoal to the filtrate. Shake and allow standing for 10minuts; filter; the filtrate is colorless in pure saffron.
2. Macerate 10mg in 5ml of alcohol (95%) or methanol, a distinct greenish yellow color is imparted to the liquid; with corresponding quantities of kunkuma in eather or chloroform the solvents remain almost colourless.so also with xylene, Benzene or carbon tetrachloride.

Plate 2: Market samples of Crocus sativus Linn.



III. RESULTS

3.1 Comparison Of The Distinguishing Characters Of Samples

3.1.1Macroscopic analysis

The stigma of the s1 sample are very soft, yellowish style, broken or intact along with a trifid stigma, stigma is dark red, 2.7cm length and 4mm width, strongly aromatic and slightly bitter. The s2 sample showed the reddish brown stigma with 2.3cm length and 0.05mm width, aromatic and slightly bitter taste. The s3 sample showing dark red stigma with 1.7 cm length and .3mm width. It is slightly aromatic, bitter taste, broken styles are thin. Sample 4 showing yellowish style, broken or intact along with trifid stigma, stigma is dark red, imbricate margin about 2.7cm length and .5mm width, with a long cylindrical tube. Taste is mildly bitter .the sample s1 and sample s2 showed small variations in their morphological characters, and sample s3 and sample s4 showing small variations in their structures The stigma of the s5 sample became very soft, with yellowish style, broken or intact along with trifid stigma, stigma is reddish brown, cornucopia shaped with fimbricate margin. Broken styles are thin, strongly aromatic, taste slightly bitter, 3 stigmas attached to a very short or 1cm long cylindrical style and stigma with 2.5cm length and .4mm width and with a long cylindrical tube, narrow at the base getting slowly broader and flattened towards the upper extremities where it slits longitudinally on the inner side.

3.2 Powder microscopy

In sample s1 stigma powder is reddish in colour, aromatic and it shows the pollen grains and its fragments, spiral trachieds and vessels, inner papillose epidermal cells. Sample s2 and sample s3 powder is reddish in colour, and showing spiral trachieds and vessels, and pollen grains and its fragments .Sample s4 showing inner papillose epidermal cells, spiral trachieds and vessels, pollen grains and its fragments.sample s5 powder is pale reddish brown in colour, aromatic and showing inner papillose epidermal cells,pollengrains and its fragments,spiral trachieds and vessels,apical portion of stigma with papillose projections and transversly cut fragments of style.

3.3 Phytochemical analysis

Different solvents such as Ethanol, Aqueous and Methanol are used for the phytochemical analysis.Phytochemical screening of Methanolic extract of stigma, indicated the presence of secondary metabolites higher than the Ethanol and Aqueous extracts.Methanolic extract of stigma of sample S1 showed the presence of alkaloids,carbohydrates,glycosides and flavanoid .The Sample S2 showed the presence of alkaloids,carbohydrates,and flavanoids.Sample S3 showed only the presence of alkaloids..Ethanolic extract of sample S1 showed the presence of alkaloids, carbohydrates,glycosides and flavanoids. Sample S4 showed the presence of alkaloids, glycosides and flavanoids. Sample S5 showed the presence of alkaloids, carbohydrates, glycosides, amino acids, phenols, flavanoids and steroids.Aqueous extract of sample S1 showed the presence of alkaloids, carbohydrates,glycosides and flavanoids. S2 showed only the presence of alkaloids. Sample S4 showed the presence of glycosides, phenols and steroids. Alkaloids, carbohydrates, glycosides, aminoacids, phenols, flavanoids and steroids are present in sample S5.Methanolic extract Sample S5 stigma showed the presence of higher number of secondary metabolites

IV. ADULTERATION DETECTION

4.1 Chemical colour test

Sample s1 showing yellow colour in water and methanol. It imparts varieties of colour in chloroform and toluene. Sample s2 showing yellow colour in water ,methanol, and imparts varieties of colour in benzene,ether,chloroform and toluene sample s3 showing the yellow colour in water,methanol,and imparts varieties of colour in benzene,ether,chloroform and toluene. Sample s4 and sample s5 showing yellow colour only in water and methanol. They are colourless in benzene, ether, chloroform and toluene solution. Sample s4 and s5 showing the characters of pure saffron, they gives yellow colouration only in water and methanol

Sulphuric acid test Sample s1 and sample s4 stigma reacts with sulphuric acid and forming bluish colour immediately and then it changes to red colour. Sample s2 reacts with sulphuric acid, they immediately showing yellow colouration. Sample s3reacts with sulphuric acid to form red colour immediately. Sample s5 with sulphuric acid gave a bluish colour immediately. which finally change to violet to red colour. Sample s5 showed characters of pure saffron.

Water Test Sample s1 showing the light orange colour in water and stigma retaining the red colour.sample s2 and sample s3 showing yellow colouration in water and stigma turns light yellow or white colour. It indicates the characters of fake saffron.Sample s4 and sample s5 showing dark yellow colouration in water and retains the dark red colour in stigma.

V. ORGANIC DYES

A) Charcoal test Sample s1 sample s2 and sample s3 showed grey colouration in the filtrate.Sample s4 showing light grey colouration in the filtrate.it showed the presence of organic dyes.Sample s5 filtrate is colourles.So it cannot contain the organic dyes.

B) Maceration Sample s1,sample s3 and sample s4 showed the colouration in Methanol,Ether,Chloroform,Xylene,Benzene.sample s2 showing colouaration in Methanol,Chloroform,Xylene and Benzene.it showing the presence of organic dyes.sample s5 showing only greenish yellow colouration Methanol. It is colourless in Ether,Chloroform,Xylene and Benzene. It shows the absence of organic dyes.

VI. DISCUSSION

6.1 Comparison Of The Distinguishing Characters Of The Samples

6.1.1 Macroscopic analysis

The sample s1 and sample s2 showed variations in their morphological characters, and sample s3 and sample s4 showing small variations in their structures. The stigma of the s5 sample become very soft,with yellowish style ,broken or intact along with trifid stigma ,stigma is reddish brown,cornucopia shaped with fimbricate margin.broken styles are thin ,strongly aromatic ,taste slightly bitter,3 stigmas attached to a very short or 1cm long cylindrical style and stigma with 2.5cm length and .4mm width and with a long cylindrical tube ,narrow at the base getting slowly broader and flattened towards the upper extremities where it slits longitudinally on the inner side.s5 is slightly different from other samples s1,s2,s3,s4.s5 showing the papillose margin ,surface is longitudinally striated and occassionaly studded with pollengrains.Based on these differences in the macroscopical structures it is possible to differentiate between different samples of dried stigmas of saffron by macroscopiacal examinations .

Powder microscopy Sample s5 powder is pale reddish brown in colour,aromatic and showing inner papillose epidermal cells,pollengrains and its fragments,spiral trachieds and vessels,apical portion of stigma with papillose

projections and transversly cut fragments of style.anatomical characters are different in different samples.so it is possible to differentiate between different market samples of saffron by powdery microscopical examination.
Phytochemical analysis Phytochemical screening of different stigma extract of Crocus sativus linn ,indicated the presence of different secondary metabolites, such as alkaloids,carbohydrates,glycosides.proteins,aminoacids ,phenolic compounds,flavanoids and steroids.the highest level of secondary metabolite present in sample 5 collected from Himachal Pradesh. Phytochemicals are different in different market samples.The previous study 12reported that the preliminary phytochemical screening of different petal extracts of Crocus sativus 'Cashmerians' showed the presence of alkaloids,flavanoids,carbohydrate,glycosides,tannins,terpenoids,phenols,steroi ds,and saponins. Sample S3 showed only the presence of alkaloids.Sample S4showed the presence of alkaloids, carbohydrates, glycosides, phenolics, flavanoids and steroids . Sample S5 showed the presence of alkaloids, carbohydrates, glycosides, amino acids, phenols,flavanoids and steroids.Ethanolic extract of sample S1 showed the presence of alkaloids, carbohydrates,glycosides and flavanoids. Sample S4 showed the presence of alkaloids,glycosides and flavanoids. Sample S5showed the presence of alkaloids, carbohydrates, glycosides, amino acids, phenols, flavanoids and steroids. Aqueous extract of sample S1 showed the presence of alkaloids, carbohydrates, glycosides and flavanoids. S2 showedonly the presence of alkaloids. . Sample S4 showed the presence of alkaloids, carbohydrates, glycosides, amino acids,phenols and steroids. Sample S5 showed the presence of alkaloids, carbohydrates, glycosides, aminoacids, phenols, flavanoids and steroids.Methanolic extract Sample S5 stigma showed the presence of higher number of secondary metabolites. These secondary metabolite may be responsible for various pharmacological effects of the drugs and justify their use by herbal practitioners.

VII. ADULTERATION DETECTION ANALYSIS

7.1 Chemical color test

Sample s1 showing yellow colour in water and methanol. It imparts varieties of colour in chloroform and toluene. Sample s2 showing yellow colour in water ,methanol, and imparts varieties of colour in benzene,ether,chloroform and toluene.sample s3 showing the yellow colour in water,methanol,and imparts varieties of colour in benzene,ether,chloroform and toluene. Sample s4 and sample s5 showing yellow colour only in water and methanol. They are colourless in benzene, ether, chloroform and toluene solution.sample s4 ans s5 showing the characters of pure saffron, they gives yellow colouration only in water and methanol.The previous study11, reported that pure saffron gives yellow colouration in the solution of the water and m4ethanol due to carotenoid pigments-crocin and crocetin,but not in benzene ,xylene,ether,chlorophorm and toluene solution.fake saffron imparts varieties of colour in different solvent.

7.2 Sulphuric acid test

Sample s1 and sample s4 stigma reacts with sulphuric acid and forming bluish colour immediately and then it changes to red colour. Sample s2 reacts with sulphuric acid, they immediately showing yellow colouration. Sample s3reacts with sulphuric acid to form red colour immediately. Sample s5 reacts with sulphuric acid to gives a bluish colour immediately. Which finally changes to violet to red colour. Sample s5 showing characters of pure saffron. Hydrolysis of carotenoids by sulphuric acid yields blue colour immediately. These difference help to identifying the fake and pure saffron. The previous study 11 reported that the carotenoid pigments like crocin ,crocetin and picocrocin reacts with the sulphuric acid to give bluish colour immediately, which finally changes to violet to red.The reaction is due to the hydrolysis of the carotenoid esters. The fake saffron produces yellow colour only.

7.3 Water Test

Sample s1 showing the light orange colour in water and stigma retaining the red colour. Sample s2 and sample s3 showing yellow colouration in water and stigma turns light yellow or white colour. It indicates the characters of fake saffron. Sample s4 and sample s5 showing dark yellow colouration in water and retains the dark red colour in stigma. It is previously studied 9.This test is very helpful to identify the pure and fake saffron.

VIII. ORGANIC DYES

A) Charcoal test Sample s1 sample s2 and sample s3 showing grey colouration in the filtrate. Sample s4 showing light grey colouration in the filtrate.it showing the presence of organic dyes.sample s5 filtrate is colourles.So it cannot contain the organic dyes.Organic dyes are using in the form of adulterant .these method is detecting the presence of organic dyes8.
B) Maceration Samples1, samples 3 and samples 4 showing the colouration in Methanol, Ether, Chloroform, Xylene, Benzene. samples2 showing colouaration in Methanol, Chloroform,Xylene and Benzene.it showing the presence of organic dyes.sample s5 showing only greenish yellow colouration Methanol.s5 is colourless in

Ether,Chloroform,Xylene and Benzene. It shows the absence of organic dyes. Organic dyes are using in the form of adulterant .These method is detecting the presence of organic dyes.

IX. CONCLUSION

The highest level of secondary metabolite reported in sample 5 collected from Himachal Pradesh. Powder microscopy enabled to identify the specific anatomical characters of each saffron stigma and more distinctly seen in sample 5.The genuinity is higher in sample S5.These methods can be employed for assessing the genuinity of the market samples of Crocus sativus Linn. The authenticity of saffron is an extremely important matter for the industry and for the consumers in view of security and protection ,quality assurance , active properties and last but not least, economic impact. Despite the fluctuation in prices in international markets, saffron was and still remains the most expensive spice. The genuine saffron samples possess higher price value .The fake saffron available in market with lower price value. The quality of the saffron depends upon the price values

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