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Estimation of alkaloid, saponin and flavonoid, content in various extracts of *Crocus sativa*

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Abstract

Crocus sativa is a well-known spice grown mostly in the Kashmir region of India. The flowers of the plant have a good aroma and are full of secondary metabolites due to which the flowers are being used as dyeing agents and the flavoring agents in almost all types of dishes. The flowers of the concerned plant have been analysed for the estimation of Alkaloid, Saponin and Flavonoid contents. Three solvents viz DCM, Methanol and Water have been used for the extraction of Alkaloid, Saponin and Flavonoid contents. Different solvent extracts extract different concentration of metabolites. The alkaloid content was found to be (6.4 and 2.4mg/g) in the methanol and water extract of the flowers, similarly the flavonoid content was found to be 1.8, 9.2 and 11.2mg/g in DCM, Methanol and water extract respectively. The Saponin content was found to be 1.2 and 3.4mg/g in the methanol and water extract of the *Crocus sativa*.

Keywords: Alkaloids, saponin, flavonoids, *Crocus sativa*, DCM, methanol and water

Introduction

The idea of using medicinal plants to treat live stock is not new. Many of the active constituents of drug or medicines were originally derived from plants. Also in many developing countries, medicinal plants are still being used in regular basis, there is a renewed, interest especially in developed countries, in using plants to treat livestock, pets and humans because many people believe that plants are more natural than manufactured medicines and can be made at home and are less expensive than artificially manufactured drugs. Medicinal plants are used also for healing purpose, and can be used intact to make tea or poultice; this is the pattern in herbal and traditional medicines. An advantage of using the intact plant is that several and different components in the plant may act synergistically (together) to produce the desired effect. Medicinal plants serve as source of drugs, after extracted and purified from the plant.

Saffron (*Crocus sativus* L.) is a light purple flower with reddish colored stigma. It is one of the most expensive spices with a range of quality in its color and aroma. A few key countries involved in saffron commercialization are Spain, Iran, Greece, Morocco, India and China. The main chemical constituents found in saffron are safranal, picrocrocin, crocetin and its glycoside, crocins. Bilal Ahmad Wani, *et al* ^[1] mentioned that Saffron (stigmas of *Crocus sativus* L.) has been used for medicinal purposes since decades. These constituents have been reported with pharmacological activities largely responsible for abundant of health benefits. Based on these research studies, saffron could be one of the significant natural products which needed much attention for its potential therapeutic roles. Gabriel Akyirem Akowuah *et al* ^[2] mentioned that Saffron (*Crocus sativus* L.) is a light purple flower with reddish colored stigma. It is one of the most expensive spices with a range of quality in its color and aroma. *Crocus sativus* is found to be effective as anti-proliferative, anti-oxidant, learning and memory enhancer, brain neurodegenerative disorder, sperm cryoconservation, biosurfactant, Alzheimer disorder as mentioned by Rajeev K Singla ^[3]. M. Malathi, *et al* ^[4] mentioned that the dried red stigma of *Crocus sativus* L. belonging to Iridaceae family is a variety of spice commercially named as Saffron. It consists of more than 150 volatile compounds chiefly the terpenes and their esters and it belongs to native of Greece and South west Asia. Saffron has the medicinally important activities such as anticancer, anti-inflammatory, anti-tissue, antioxidant, anxiolytic, aphrodisiac, antinociceptive, anticonvulsant, antihypertensive, antidepressant, antigenotoxic and relaxant activity.

Vijay *et al.* [5] mentioned that *Crocus sativus* Linn (Iridaceae) is used widely in tropical and subtropical countries for a variety of purposes in both household and for medicinal purposes.

Experiment Work

Study area and plant collection

The concerned "*Crocus sativa*" flowers were collected from the Kishtwar Region of J & K. The plant parts were segregated shade dried and powdered in mixture. The powder was used for experiment.

Extraction

30 gms of the plant powder were weighed separately and accurately and then extracted in a Soxhlet Apparatus using thimble in order to get the best extract. Various solvents were used depending upon their polarity index with increasing polarity (DCM, Methanol and Water).

Extraction A

The sample was extracted with a particular solvent (DCM) in a Soxhlet apparatus for a required period, till no extract was coming out of the sample, as being examined by taking a small amount of the extracted solvent from the main chamber of the Soxhlet apparatus over the watch glass for the appearance of precipitate. After the Extraction with DCM, the extract solution was subjected to filtration to remove the residue from extract. The filtrate was then collected and evaporated to remove the volatile solvent to its 1/4th volume on water bath at a suitable temperature. The whole filtrate was then made in solid form (powdered) after being kept in an oven. The residue was collected, and subjected to further extraction process.

Extraction B

The residue was then extracted with ethanol in a same manner as mentioned above, in extraction A.

Extraction C

The residue from extract B was subjected to water extraction by decoction technique. In this technique the extract was dissolved in 500 ml of water. The whole solution was heated over water bath to remove all the water from the extract. Finally additional 500 ml of water was added to the extract, the extracted solution was finally evaporated to remove nearly 250ml of water. The solution was then subjected to filtration. The filtrate was then evaporated to remove nearly 1/4th of its volume. Finally the extract was dried in an oven at a temperature range 30-50 °C.

Chemical Tests

Test for alkaloids

Mayer's test (Evans, 1997)

To a few ml of the filtrates, a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

Picric acid test

1g of picric acid was taken in 100ml of water. Add it to the plant extract with suitable amount. Creamy ppts show presence of alkaloids.

Test for flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973) [6]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminum solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for Tannins

About 2.5 g of the plant extract was dissolved in 5 ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins (Trease and Evans, 1989).

Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for saponins (Kokate, 1999)

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins.

Test for terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for carbohydrates

Benedict's test: To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 min. A characteristic red colored precipitate indicates the presence of sugar.

Test for steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for phytosterols (Finar, 1986)

Liebermann-Buchard's test the extract was mixed with 2 ml of acetic anhydride. To this 1 or 2 drop of concentrated sulphuric acid was added slowly along the sides of the test tubes. An array of color change shows the presence of phytosterols.

Qualitative Analysis

Table 1: Phytochemical Tests for *Crocus sativa* Flower

S. No	Phytochemical tests	Dichloromethane	Methanol	Water
1	Alkaloid Test(1)	-ve	-ve	-ve
2.	Alkaloid test(2)	-ve	-ve	-ve
3.	Flavonoids Test	+ve	-ve	-ve
4.	Tannins Test	-ve	+ve	-ve
5.	Saponin Test	-ve	-ve	+ve
6.	Terpenoids Test	+ve	-ve	+ve
7.	Carbohydrates	-ve	+ve	-ve
8.	Steroids	-ve	-ve	+ve
9.	Phytosterols	-ve	+ve	+ve

Quantitative Analysis

Alkaloid determination using Harborne (1973) [6] method 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoids determination by the method of Bohm and Kocipai- Abyazan (1994) [7]

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Saponin determination

20 g of plant sample was dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated in percentage (Nahapetian and Bassiri, 1974) [8].

Results and Discussion

The present study carried out on the "*Crocus sativa*" flower =stigma showed the presence of biologically and medicinally active constituents. The phytochemical active secondary metabolites have been carried out separately for flower petals and thalamus parts, using different solvents based upon their ionic potential. The qualitatively identified phytochemicals have been tabulated in table1. In the identification process alkaloids, tannins, saponins, flavonoids and terpenoids glycosides, phenols shows different types of results in different solvents. The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found

to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties. The steroids and saponins were responsible for central nervous system activities. Phytochemical screening of the various extracts of "*Crocus sativa*" flower were used to study the presence of contained alkaloids, flavonoids, steroids, saponins, tannins and triterpenoids and also have various medicinal values such as anti-inflammatory, anti-diabetic and analgesic activities and for central nervous system activity. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by (Kubmarawa, 2007) [9] reports alkaloids in 12 leafy vegetables studied. (Ayitey and Addae, 1977) [10] and earlier recorded that bitter leaf contains an alkaloid which is capable of reducing headaches associated with hypertension.

- The alkaloid content of "*Crocus sativa*" flower in methanol and water extracts were found to be (6.4 and 2.4) respectively.
- The flavonoid content of "*Crocus sativa*" in DCM, methanol and water extracts were found to be (1.8 and 9.2 and 11.2mg/g) respectively.
- The Saponin content of "*Crocus sativa*" was found only in methanol and water extracts and was found to be (1.2, 3.4mg/g) respectively.

Conclusion

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. The plant in reference showed that in addition to the ornamental purpose the gerbera species posses a good quality of phytochemicals which directly or indirectly help in the health maintenance of living beings.

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