Int J Biol Med Res. 2025; 16(2): 7997-8013

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original Article

Assessing the Authenticity of Honey and Saffron: Unveiling Adulteration through Analytical Detection Techniques

Dr. S. P. Patil[a*], Ms. A. B. Patil[a], Ms. R. S. Bhore[a], Ms. K. D. Shelake[a], Dr. S. P. Shah[b]

[a] Department of Pharmaceutical Chemistry, Dr. Shivajirao Kadam College of Pharmacy, Kasabe Digraj, Sangli, Maharashtra, 416 305, India.

[b] Assistant Professor (Dept.Practice of Medicine) Late. HousabaiHomoeopathic MedicalCollege, Nimshirgoan, Kolhapur, Maharashtra, 416 401, India

Keywords:
Adulteration Detection;
Saffron Purity Tests;
Honey Authenticity;
Food Quality Analysis;
Sulfuric Acid Test

This research aimed to assess the authenticity and detect adulteration in various commercial brands of honey and saffron using a combination of advanced chemical and analytical techniques. Three brands of each product were procured from local markets and subjected to a series of rigorous tests. Adulteration detection and purity assessments were performed using Thin Layer Chromatography TLC), UV-visible spectrophotometry, and a variety of chemical assays. Additionally, microscopic analysis and paper chromatography were employed to further elucidate the structural and compositional integrity of the samples. The results demonstrated that while certain samples maintained their purity, others exhibited clear signs of adulteration, indicating the presence of foreign substances or dilution. The integration of multiple analytical methodologies provided a comprehensive evaluation of the samples, offering a reliable approach for the detection of adulteration in food products. These findings emphasize the critical need for routine quality control and stricter regulatory measures to ensure the authenticity of commercially available honey and saffron, which is essential for consumer safety and maintaining product standards in the food industry.

© Copyright 2025 BioMedSciDirect Publications IJBMR -ISSN: 0976:6685.

Introduction

Adulteration, a widespread issue in food and medicinal products, involves the replacement of original ingredients with inferior, chemically distinct substances. This practice poses significant health risks and reduces the quality and safety of products. Adulterants are often introduced to enhance product appearance or increase profitability, but at the expense of nutritional value or consumer safety. Food adulteration occurs when substances that lower the quality or safety of food are added, or when essential components are removed. This misleads consumers and can lead to serious health concerns due to the consumption of substandard or hazardous products [1]. For instance, artificial colours or chemicals added to food may result in toxic reactions or chronic health issues.

The most commonly adulterated foods include staples like dairy products, edible oils, cereals, spices, and condiments [2]. These items are essential for daily nutrition, making their adulteration particularly concerning, as it impacts the health of a large population. Financial motives drive adulteration, where suppliers use cheaper alternatives or manipulate the product's appearance to maximize profit.

Corresponding author:

***Dr. Sarika Pranam Patil**
Department of Pharmaceutical Chemistry,
Dr. Shivajirao Kadam College of Pharmacy,
Kasabe Digraj, Sangli, Maharashtra, 416 305, India.

Email: naradesarika@gmail.com

© Copyright 2025 BioMedSciDirect Publications IJBMR -ISSN: 0976:6685.

Types of Adulteration

Adulteration is classified into three main types: unintentional, incidental, and metallic. Unintentional adulteration often occurs due to confusion in identifying raw materials, particularly in traditional medicine practices such as Ayurveda and Siddha [3]. Misidentification of plants with similar appearances leads to the use of inferior substitutes. Incidental adulteration involves contamination by pesticide residues, industrial chemicals, or improper handling during food processing, unintentionally introducing harmful substances [4]. Metallic adulteration, involving toxic metals like lead, occurs due to environmental pollution or the use of contaminated water in food production, posing severe long-term health risks [5].

Causes of Adulteration

One of the leading causes of unintentional adulteration is confusion between local and vernacular plant names [6]. This is prevalent in traditional medicine, where similar names are often used for distinct species, leading to the use of incorrect plants. Morphological similarities also result in unintentional substitutions, such as the confusion between Mucuna pruriens and related species due to their similar appearance [7]. Additionally, collectors lacking proper training may inadvertently mix inferior species with authentic ones, further contributing to adulteration, as seen in the collection of Parmeliaperlata used in Ayurveda [8].

Adulteration of Honey and Saffron

High-value products like honey and saffron are frequently adulterated due to their market price. Honey, a natural health product, is commonly adulterated with molasses, cane sugar syrup, high-fructose corn syrup HFCS), and artificial invert sugar [9]. These substances degrade honey's medicinal qualities and reduce its nutritional value. The use of HFCS in commercial honey production is widespread, reducing the natural benefits of pure honey [10]. Similarly, saffron, one of the world's most expensive spices, is adulterated with other plant materials, such as Calendula or Carthamus tinctorius, or even with exhausted saffron mixed with oil or glycerin to increase weight and profitability [11]. These adulteration practices diminish saffron's potency and deceive consumers [12].

Homeopathic Pharmaceutical Preparations

Homeopathy involves preparing remedies through potentization, a process of serial dilutions and vigorous shaking succussion). This method is believed to remove toxicity while amplifying the therapeutic potential of the original substance. Remedies are labeled with numbers and letters e.g., 6X or 30C), indicating the number of dilutions and dilution ratios X for 1:10, C for 1:100) [13]. The law of infinitesimals, a cornerstone of homeopathy, asserts that more diluted remedies have greater healing potential. For example, a 30X solution one part in 10^{29} parts of water) is considered more potent than less diluted forms [14]. Despite skepticism from the scientific community, homeopathy advocates argue that the preparation method enhances the efficacy of these remedies [15].

Use of Potentized Saffron and Honeysuckle in Homeopathy

1. Crocus Sativus Saffron

Saffron Crocus Sativus), known for its medicinal uses, is potentized in homeopathy to retain its therapeutic effects while eliminating any toxic properties. It is commonly used to treat hemorrhages, especially those involving dark, stringy blood [16]. Additionally, it addresses emotional conditions like sudden mood swings and hysteria, and physical symptoms such as headaches, uterine hemorrhages, and wheezy coughs [17]. The holistic approach of homeopathy emphasizes treating both emotional and physical ailments through remedies like saffron [18].

2. Lonicera Caprifolium Honey suckle

Lonicera Caprifolium honey suckle is a homeopathic remedy used to help individuals overcome emotional distress, such as grief, homesickness, or trauma from life changes like divorce or bereavement [19]. By fostering emotional resilience, honeysuckle helps patients focus on the present and move past painful experiences [20]. This remedy exemplifies homeopathy's potential to support emotional well-being alongside physical health.

The adulteration of food and medicinal products, particularly honey and saffron, is a significant health concern, reducing product quality and exposing consumers to harmful substances. In contrast, homeopathy offers a unique approach to healing, utilizing potentized remedies such as saffron and honeysuckle to address both emotional and physical conditions. This study highlights the need for stringent quality control to combat adulteration and underscores the potential of homeopathy in providing holistic healthcare solutions.

This paper explores the pressing issue of adulteration in food and medicinal products, with a focus on frequently adulterated items like honey and saffron. Adulteration, primarily driven by profit motives, compromises product quality and poses health risks. Various forms of adulteration—unintentional, incidental, and metallic—are examined, along with alternative healing methods such as homeopathy. The use of potentized remedies like saffron and honeysuckle in homeopathy is discussed for their ability to treat both physical and emotional ailments. The paper emphasizes the need for strict quality control measures and the value of homeopathy in holistic health care.

MATERIALS AND METHODS
Materials

The research experiment was carried out using deionized double distilled water. All other chemicals and solvents were of analytical grade.

Sample of Honey:

For this study, three different brands of honey were collected from the local market: Brand A honey, Brand B honey, and Brand C honey. Brand A honey costs 20 Rs for a 30g pack, Brand B honey is priced at 50 Rs for a 25g pack, and Brand C honey is available for 60 Rs for a 100g pack.

Sample of Saffron:

Additionally, two brands of saffron, Brand X and Brand Y, were collected from the local market, while Brand Z saffron was sourced directly from Kashmir. Brand X is priced at 300 Rs per gram, Brand Y costs 100 Rs per gram, and Brand Z saffron is the most expensive at 400 Rs per gram.

Methods
Methods for detection of adulteration in honey

a) Solubility Test

The solubility test is performed to assess the purity of honey. A sample of honey is introduced into a glass of water. If the honey settles at the bottom without dissolving, it is generally indicated that the honey is pure. In cases of adulteration, where added sugars or syrups may be present, the honey tends to dissolve more readily [21].

b) Blot Test or Flowing Test

The blot test, also known as the flowing test, is conducted to evaluate the purity of honey qualitatively. A few drops of honey are placed on blotting paper or cloth. If the honey flows without saturating the paper, it is considered pure. Conversely, if the honey wets or is absorbed by the paper, this suggests that adulteration has occurred from added water or syrups [22].

c) Fiehe's Test

Fiehe's test is employed to detect commercial invert sugars in honey by measuring hydroxymethyl furfural HMF) levels, which are found to be higher in adulterated honey. The procedure is carried out as follows:

1. Five milliliters of honey is combined with ten milliliters of solvent ether in a mortar and mixed thoroughly.

2. The supernatant is transferred to a Petri dish and allowed to dry.

3. In a test tube, five milliliters of hydrochloric acid is mixed with one gram of resorcinol crystals until full dissolution is achieved.

4. Once the ether has evaporated, a few drops of the resorcinol-HCl solution are added to the residue in the Petri dish, and any colour change is observed, indicating the presence of HMF [23].

d) Aniline Chloride Test

The aniline chloride test is utilized to identify common sugars, particularly sucrose, in honey. The following steps are performed:
1. Five milliliters of honey is placed in a porcelain dish.
2. An aniline chloride reagent is prepared by mixing three milliliters of aniline with seven milliliters of hydrochloric acid in a test tube.
3. A few drops of the aniline chloride solution are added to the honey sample, and any colour changes are observed, indicating the presence of sucrose [24].

e) Vinegar Test

The vinegar test is conducted as a simple method to assess honey purity. A few drops of honey are mixed with vinegar water. If the mixture produces foam or bubbles, it is indicated that the honey may be adulterated [25].

f) Thin Layer Chromatography TLC)

Thin layer chromatography TLC) is employed as an effective analytical method for separating components in a mixture. In TLC, a silica gel or aluminum oxide-coated plate serves as the stationary phase. The mobile phase consists of a solvent mixture of chloroform, acetic acid, and water in a ratio of 3.3:5:0.5. The retention factor Rf) is calculated by dividing the distance traveled by the compound by the distance traveled by the solvent front. TLC is particularly useful for isolating and identifying potential adulterants in honey samples [26].

Methods for detection of adulteration in honey

a) Chemical Colour Test

The chemical colour test is a preliminary method for assessing saffron purity. Clean test tubes are prepared, and small aliquots of various solvents, including water, methanol, benzene, ether, chloroform, xylene, toluene, and 1-butanol, are added to each respective tube. Pure saffron is expected to yield a distinct yellow colorationwhen mixed with water and

methanol, attributable to carotenoid pigments such as crocin and crocetin. Conversely, no colorationis anticipated in the presence of non-polar solvents, indicating the absence of these carotenoids and thus potential adulteration [27, 28].

b) Cotton Colour Test

In the cotton colour test, cotton is soaked in polar solvents like water or methanol, and the resultant colour of the cotton is examined. The intensity and hue of the colorationcan indicate the presence of colourants or adulterants, offering a qualitative measure of saffron's purity [29].

c) Whatman Paper Test

The Whatman paper test involves rubbing saffron threads against paper that has been moistened with polar solvents. This technique allows for the visual assessment of colour transfer, with any unexpected colorationsuggesting the possibility of adulteration [28].

d) Flotation Test

The flotation test serves as a qualitative assessment for saffron purity. A small quantity of saffron is placed on the surface of water. Pure saffron is expected to float due to its low density and high buoyancy. In contrast, adulterated saffron, which may contain heavier foreign materials, is likely to sink, thereby indicating potential contamination [30].

e) Sulfuric Acid Test

The sulfuric acid test is utilized to assess the presence of carotenoid pigments in saffron. Upon addition of concentrated sulfuric acid, carotenoid compounds react to produce an initial bluish colour, which then transitions to violet or red as a result of the hydrolysis of carotenoid esters. This colour change serves as an indicator of saffron purity and the integrity of its chemical constituents [27, 31].

f) Nitric Acid Test

The nitric acid test is a straightforward method for evaluating saffron authenticity. When pure saffron is exposed to nitric acid, a light blue colorationis generated. The persistence and shade of this coloration provide insights into the saffron's quality [31].

g) Pigment Extraction

Pigment extraction involves using water to solubilize the carotenoids present in saffron, followed by evaporation in a watch glass to obtain dried pigment. The dried pigment undergoes two key reactions:

h) Reaction with Nitric Acid

Two to three drops of nitric acid are introduced to the dried pigment, and the resulting colour change is noted.

i)Reaction with Ammonia

Similarly, the addition of two to three drops of ammonia is made to the dried pigment, with observations recorded regarding any resulting colour changes [32].

j) Diphenylamine Test

The diphenylamine test is conducted by preparing a mixture of 0.1 grams of diphenylamine, 20 milliliters of sulfuric acid, and 4 milliliters of water in a shallow porcelain dish. A small amount of the saffron sample is added to this mixture. A yellow colour that rapidly transforms to brown-red is indicative of potential adulteration. The presence of nitrates can result in a persistent blue colouration. After one minute, the mixture is covered with a slip, and excess liquid is removed with absorbent paper. Microscopic examination is performed at magnifications of 50X and then 100X to observe any blue-green colouration, along with any increase in pollen grain diameter [33].

k) Synthetic Dyes

A. Basic Dyes:

A sample weighing 0.2 grams is combined with 20 milliliters of a 1% sodium hydroxide solution, followed by thorough mixing. The solution is then extracted with 6 milliliters of ether, which is subsequently treated with dilute acetic acid. The absence of colour in the dilute acetic acid layer indicates the lack of synthetic dyes [32].

B. Other Acidic Dyes:

For the detection of other acidic dyes, one gram of saffron sample is mixed with 0.3 milliliters of ammonia test solution and 2.4 milliliters of water. The mixture is agitated, and the resulting oily layer is discarded. The remaining solution is analyzed using ascending paper chromatography. Two microliters of the solution serve as the sample, with a developing solvent composed of a pyridine and ammonia mixture (2:1). The chromatography is halted once the solvent front has moved an adequate distance. No spots should be observed at the solvent front after drying in daylight; if spots appear, they will be decolourized upon treatment with a stannous chloride solution in hydrochloric acid (2:5) [32, 34].

l) Thin Layer Chromatography (TLC)

Thin layer chromatography is employed to differentiate between pure and adulterated saffron. A methanol extract is prepared from equal amounts of saffron (two stigma threads in 3 milliliters of methanol). The stationary phase utilized is silica gel G60, and the mobile phases are defined as follows:

For Brand X and Brand Y saffron, a mobile phase of 1-butanol, acetic acid, and water in a ratio of 4:1:5 is utilized.For Brand Z saffron, a mobile phase of pyridine and ammonia in a 2:1 ratio is applied [34].

m) UV Spectroscopy

UV spectroscopy is conducted by preparing a solution of 0.1 grams of saffron in 100 milliliters of water. The absorbance of the solution is measured at a wavelength of 440 nm to evaluate the concentration of carotenoid pigments and assess saffron purity [32, 35].

Results And Discussion

The results and discussion highlight the effectiveness of various methods for detecting adulteration in saffron. Chemical colour tests using polar solvents, such as water and methanol, revealed saffron's carotenoid pigments, while non-polar solvents showed no colour change, indicating purity. The cotton colour and Whatman paper tests provided quick qualitative assessments of saffron's authenticity based on colour transfer. The flotation test distinguished pure saffron, which floated on water, from adulterated samples that sank. Sulfuric and nitric acid tests confirmed the presence of carotenoids by producing specific colour changes. Additionally, thin layer chromatography and UV spectroscopy effectively identified saffron's carotenoid content, while the diphenylamine and synthetic dye tests ruled out chemical adulterants. These methods collectively demonstrate reliable, accessible approaches to identifying saffron adulteration, ensuring product quality and consumer safety.

Detection of adulteration in honey

a) Solubility Test

The result of solubility test for three different brands of honey are shown in **table1** and **figure 1.**

Sr.no.	Brand name	Observation	Inference
1	Brand A Honey	Settled at the bottom of the beaker; clear solution obtained after heating	Pure
2	Brand B Honey	Settled at the bottom of the beaker; clear solution obtained after heating	Pure
3	Brand C Honey	Settled at the bottom of the beaker but instantly dissolved in water; clear solution obtained after heating	Adulterated

Brand A and Brand B honey samples settled at the bottom and produced clear solutions upon heating, confirming their purity. In contrast, Brand C honey dissolved gradually in water before yielding a clear solution when heated, indicating adulteration.



Figure 1: Results of solubility test

b. Blot Test or Flowing Test:

The result of blotting testf or three different brands of honey are shown in table2 and figure 2.

Table 2: Blot Test or Flowing Test for honey

Sr. No.	Brand name	Observation	Inference
1	Brand A Honey	It flowed smoothly on cloth or paper	Pure
2	Brand B Honey	It flowed smoothly on cloth or paper	Pure
3	Brand C Honey	It flowed smoothly on cloth or paper	Pure

The honey samples from all three brands Brand A, Brand B, and Brand C) were tested using the blot or flow test. In this test, the honey was poured onto cloth or paper, and all samples flowed smoothly without wetting or absorbing into the material. This observation indicates that the honey samples from all three brands were pure, as pure honey does not get absorbed into the paper or cloth.



Figure 2: Results of Blot Test or Flowing Test

c. Fiehe's test

The result of blotting test for three different brands of honey are shown in **table 3 and figure 3.**

Table 3: Fiehe's test for honey

Sr.no	Brand name	Observation	Inference
1	Brand A Honey	Nochangeincolour	Pure
2	Brand B Honey	Nochangeincolour	Pure
3	Brand C Honey	Colour changed from yellow to cherry red	Adulterated

Brand A Honey and Brand B Honey showed no colour change, confirming their purity. Brand C Honey displayed a change in colour from yellow to cherry red, indicating adulteration.



Brand A Honey



Brand B Honey



Brand C Honey

Figure 3: Fiehe's test for honey

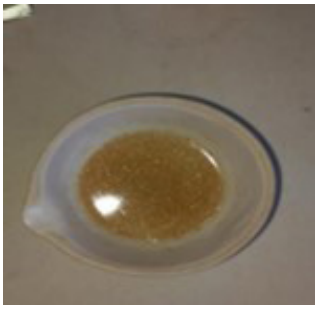
d. Aniline chloride Test:

The result of an ilinetest for three different brandsof honey are shown in **table 4 and Figure 4.**

Table 4: Aniline chloride test for honey

Sr.no	Brand name	Observation	Inference
1	Brand A Honey	Nochangeincolour	Pure
2	Brand B Honey	Nochangeincolour	Pure
3	Brand C Honey	Colour changed from yellow to orange red	Adulterated

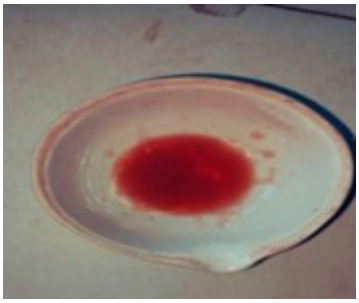
Brand A Honey and Brand B Honey showed no change in colour, indicating purity.Brand C Honey exhibited a colour shift from yellow to orange-red, which suggests adulteration.



Brand A Honey



Brand B Honey



Brand C Honey

Figure 4: Aniline chloride Test for honey

e. Vinegar Test:

The result of vinegar test for three different brands of honey are shown in table and Figure5. Brand A Honey and Brand B Honey did not produce foam, indicating they are pure. Brand C Honey produced foam, indicating it is adulterated.

Table5: Vinegar Test for honey

Sr.No	Brandname	Observation	Inference
1	Brand A Honey	No foam produced	Pure
2	Brand B Honey	No foam produced	Pure
3	Brand C Honey	foam produced	Adulterated

Figure 5: Vinegar Test for honey



Brand A Honey



Brand B Honey



Brand C Honey

f. Thin layer chromatography

The Rf for three different brands of honey are shown in table 6.

Table6: RF for honey

Sr. No	Brand Name	RF
1	Brand A Honey	0.85
2	Brand B Honey	0.9
3	Brand C Honey	0.05

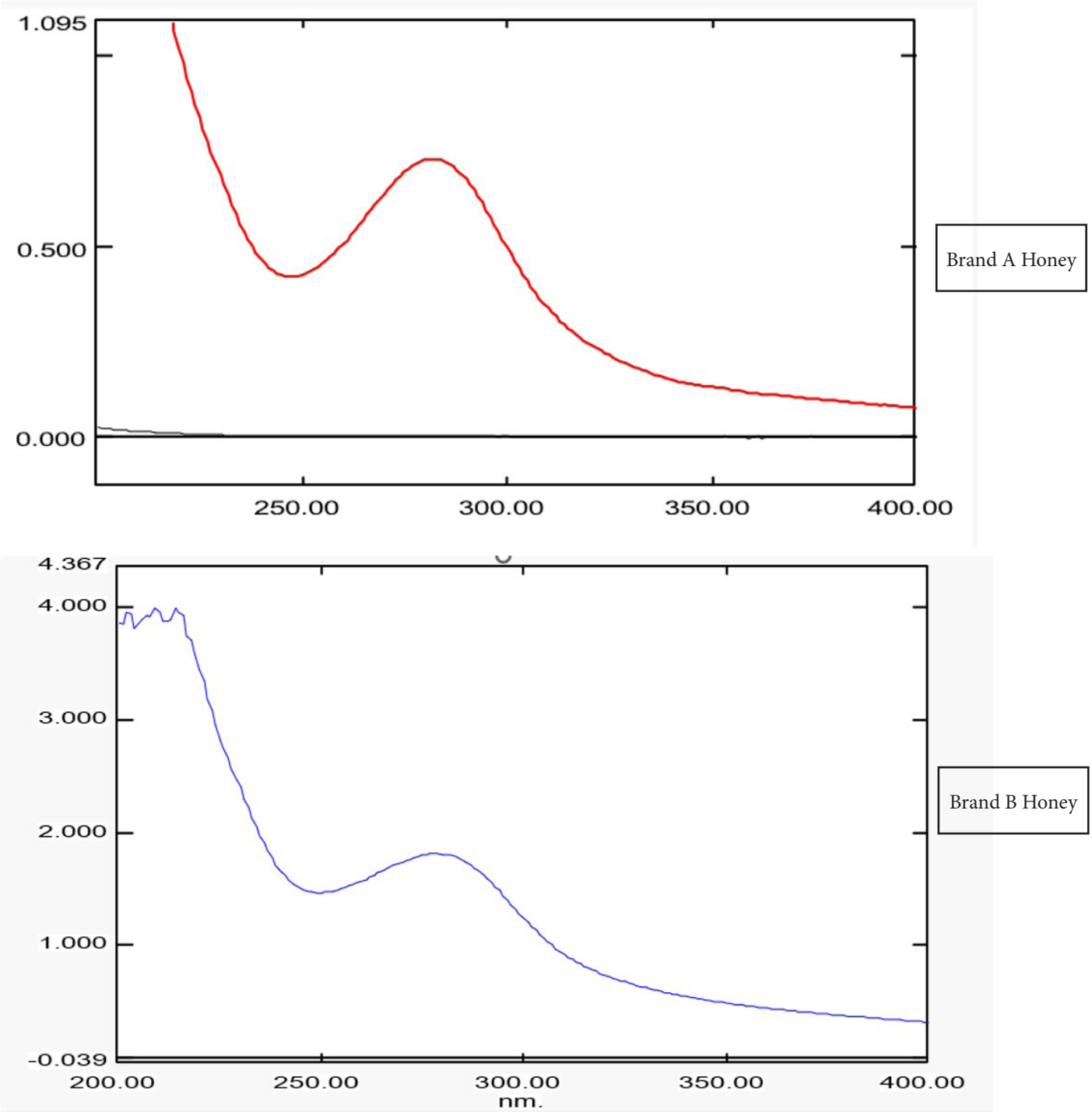
g) UV visible spectroscopy

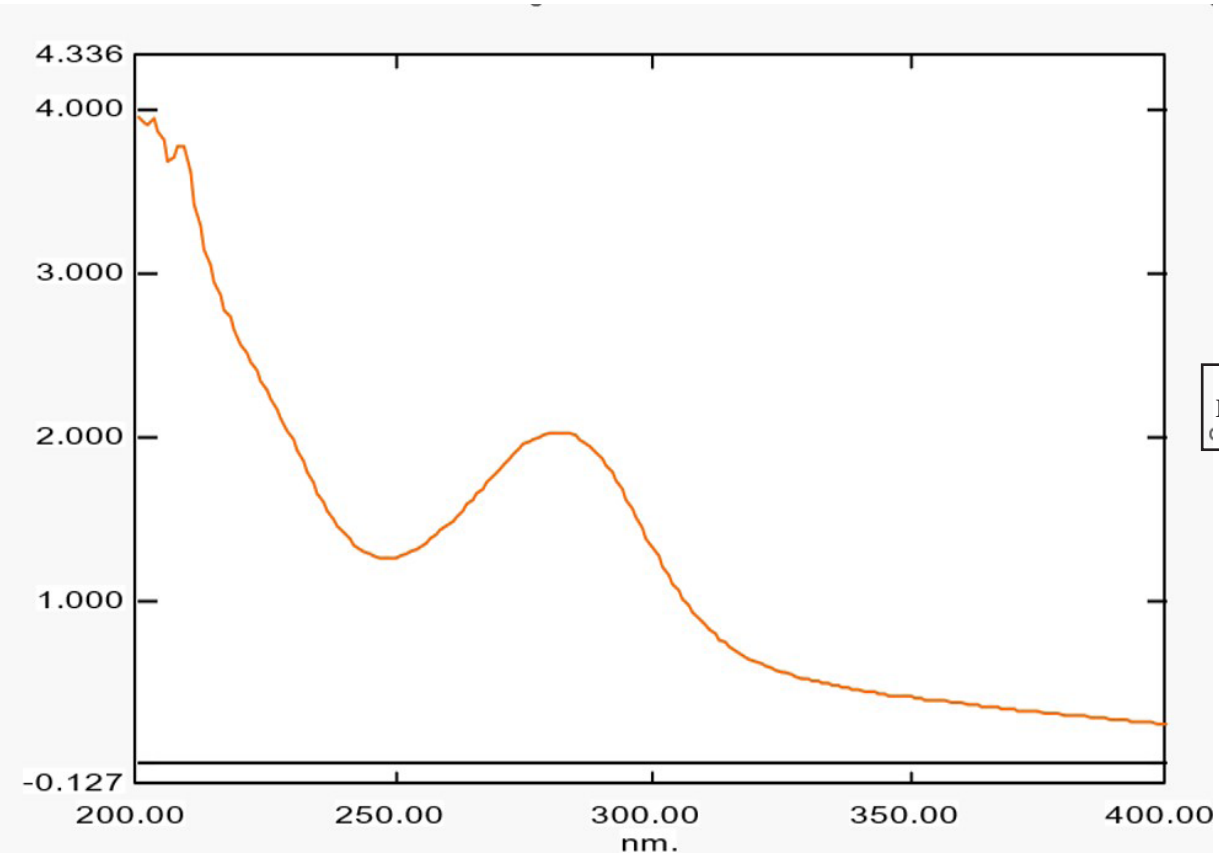
The results of UV Visible Spectroscopy, wavelength of maximum absorbance for three different brands of honey are shown in table 7 and Figure 6

Table 7:Wavelength, maximumAbsorbancefor honey

Sr.No	Brandname	Wavelength nm)	Absorbance
1	Brand A Honey	282	0.731
2	Brand B Honey	277	1.821

Figure 6: Wavelength of maximum absorbance for honey





Detection of adulteration in Saffron

a. Chemical colour test

The results of chemical colour test for three different brands of saffron adulteration are shown in table 8 and figure7.

Table 8: Chemical colour test for three different brands of saffron adulteration

Sr No	Brand Name	1 Water	2 Methanol	3 Benzene	4 Ether	5 Chloroform	6 Xylene	7 1- Butanol	Inference
1	Brand A Saffron	Pale Yellow	Pale Yellow	No Colour	No Colour	No Colour	No Colour	No Colour	Pure (based on expected results)
2	Brand B Saffron	Pale Yellow	Faint red	Dark red	No Colour	No Colour	No Colour	No Colour	Adulterated (abnormal colouration)
3	Brand C Saffron	Pale Yellow	Pale Yellow	Pale Yel-low	No Colour	No Colour	No Colour	No Colour	Pure (based on expected results)

b. Cotton colour test:

The result of cotton colour test for three different brands of Saffron adulteration are shown in Table 9 and Figure 8. All three saffron samples displayed similar results, with yellow colorationon cotton confirming their authenticity and purity.

Table 9: Cotton colour test for saffron

Brandname	Observation	Inference
Brand X Saffron	Yellowcolour	Pure
Brand Y Saffron	Yellowcolour	Pure
Brand Z Saffron	Yellowcolour	Pure

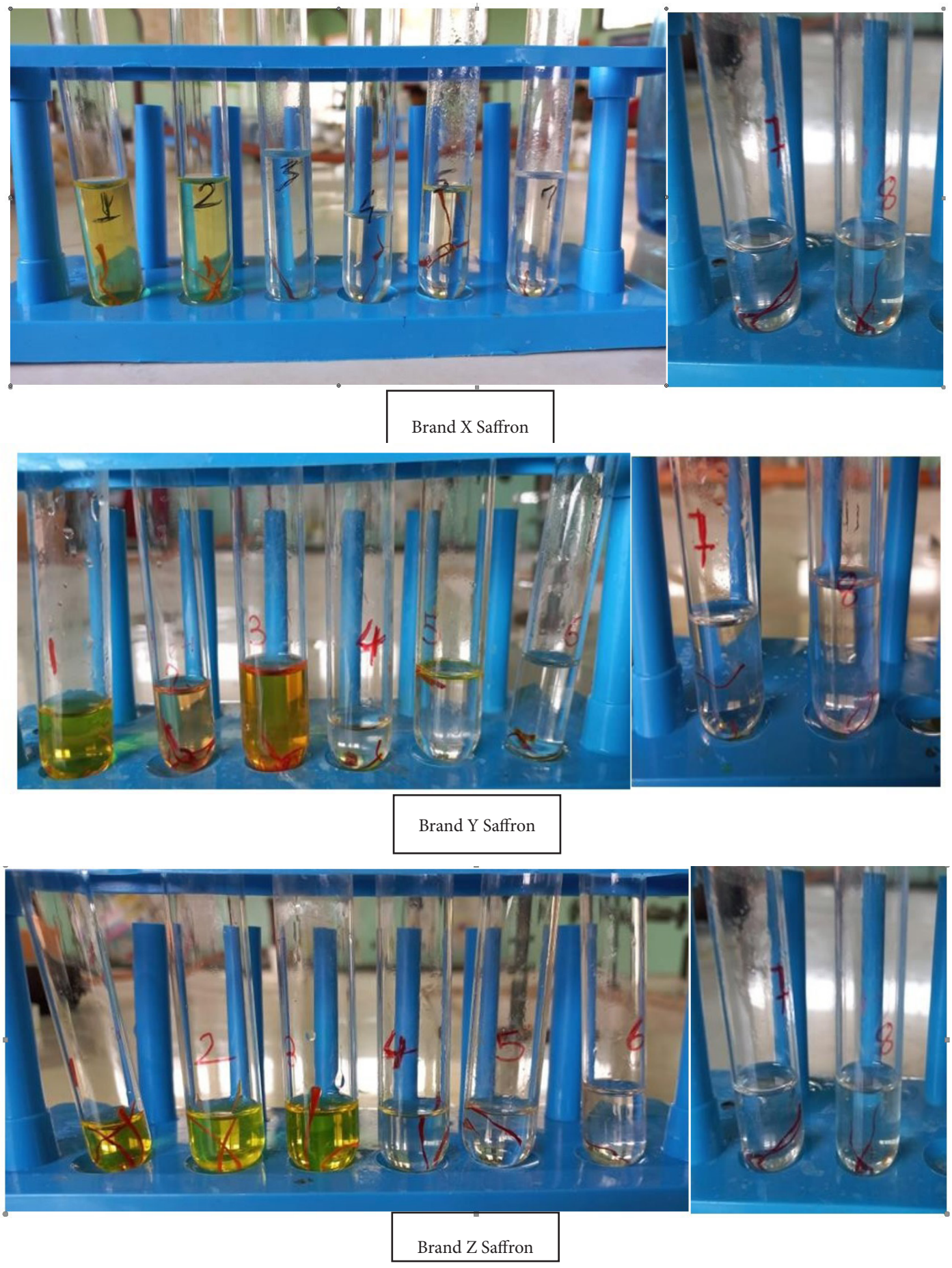


Figure 7: Chemical colour test for three different brands of saffron adulteration

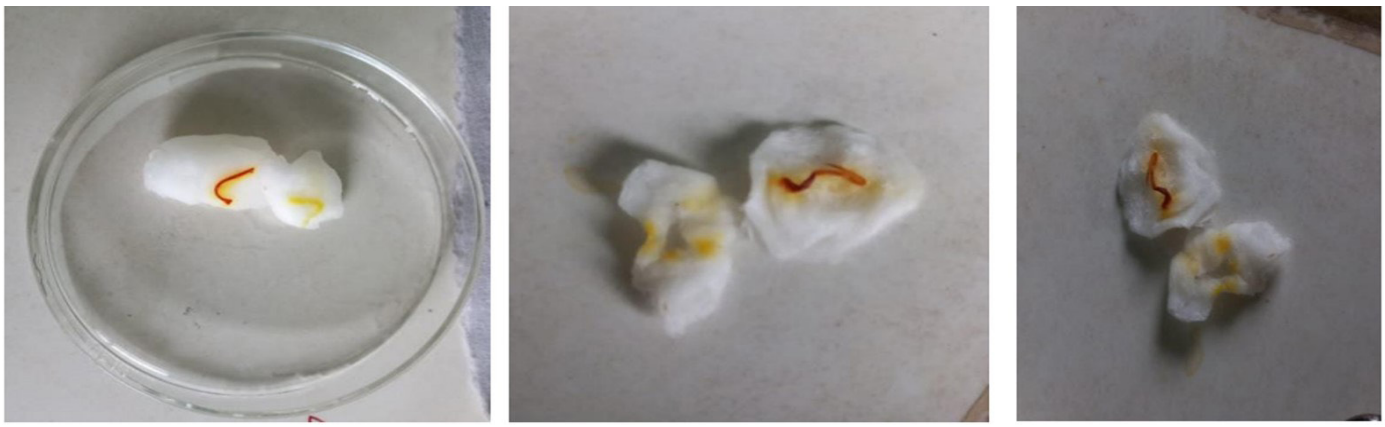


Figure 8: Chemical Colour Test for saffron

Brand X Saffron | Brand Y Saffron | Brand Z Saffron

c. Whatman paper test

The result of Whatman paper test for three different brands of saffron adulteration are shown in figure 9.



Brand X Saffron | Brand Y Saffron | Brand Z Saffron

Figure 9: Whatman paper test for saffron

Brand X and Brand Z saffron samples showed a delayed yellow coloration, confirming their purity. However, Brand Y saffron displayed a rapid yellow color change, suggesting adulteration. These observations suggest that Brand X and Brand Z saffron are pure, while Brand Y shows signs of adulteration due to the rapid colour change.

d. Flotationtest

• The resul tof Flotation test for three different brands of saffron adulteration are shown in figure 10. Brand X and Brand Y saffron samples floated in water, confirming their purity, while Brand Z saffron sank, indicating adulteration. These flotation results indicate that Brands X and Y are pure, while Brand Z is likely adulterated based on its sinking behavior.

e. Sulphuric Acid test

The result of Sulphuric Acid test for three different brands of Saffron adulteration are shown in Table 10 and Figure 11. Brand X and Brand Y saffron samples produced an immediate blue color with sulfuric acid, which then transitioned to violet and red, confirming their purity. In contrast, Brand Z saffron turned yellow upon reaction, indicating adulteration.

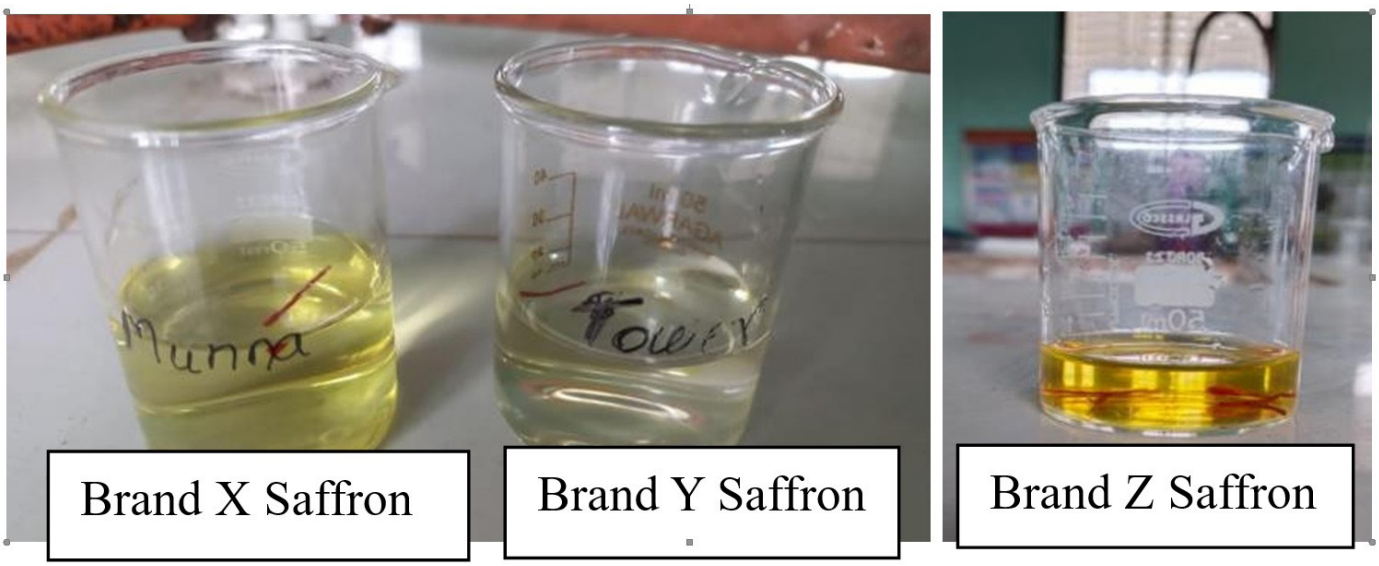


Figure 10: Flotation test

Table 10: Result of sulphuric acid test for saffron

Sr. no.	Brandname	Observation	Inference
1	Brand X Saffron	Bluish colour that changes to violet and then red	Pure
2	Brand Y Saffron	Redcolour	Pure
3	Brand Z Saffron	Yellowcolouronly	Adulterated



Figure 11: Sulphuric Acid test for saffron

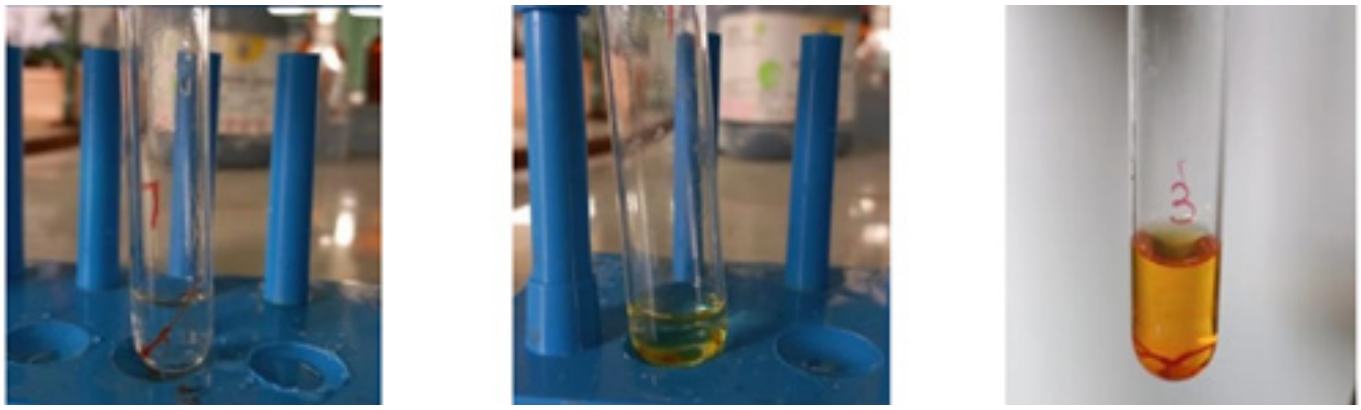
f. Nitric Acid Test:

The resul to f.Nitric Acid test for three different brands of Saffron adulteration are shown inTable 11 and Figure 12. The X saffron exhibited no colour, indicating that it is pure. The Y saffron imparted a yellow colour, suggesting that it is adulterated. The Z saffron also imparted a yellow colour, confirming that it is adulterated.

Table11: Nitric Acid Test for saffron

Brandname	Observation	Inference
Brand X Saffron	Nocoloration occurs	Pure
Brand Y Saffron	Yellow	Adulterated
Brand Z Saffron	Yellow	Adulterated

Figure 12: Nitric Acid Test for saffron



Brand X Saffron | Brand Y Saffron | Brand Z Saffron

g. Pigment Extraction

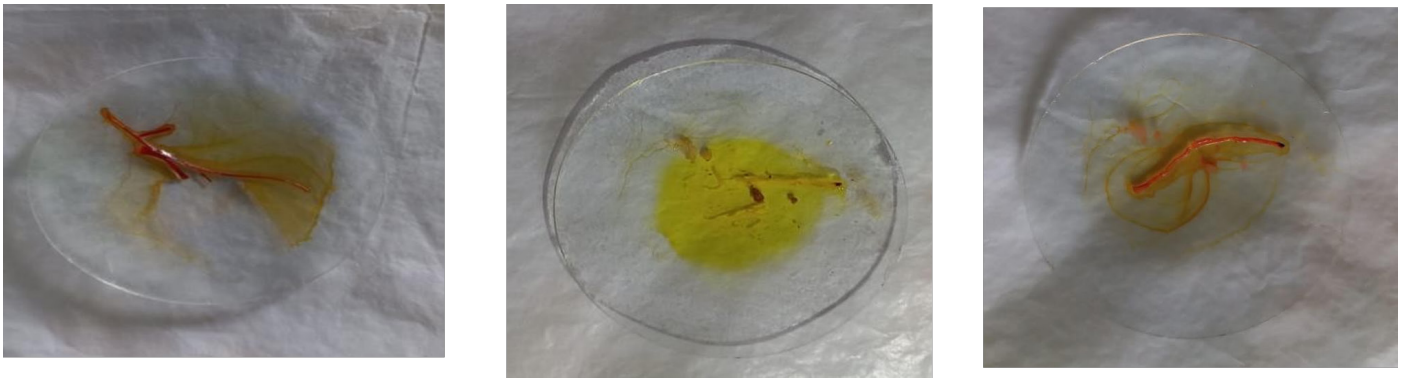
A. Reaction with nitric acid

The result of Pigment Extraction reaction with Nitric Acid test for three different brands of Saffron adulteration are shown in Table 12 and Fig 13. The pigment of X saffron turned light blue with nitric acid, indicating purity. Y and Z saffron pigments turned yellow, indicating adulteration.

Table12: Pigment extraction Reaction with nitric acid

Brandname	Observation	Inference
Brand X Saffron	Light blue colour	Pure
Brand Y Saffron	Yellow colour	Adulterated
Brand Z Saffron	Yellow colour	Adulterated

Figure13: Pigment extraction Reaction with nitric acid



Brand X Saffron | Brand Y Saffron | Brand Z Saffron

B. Reaction with Ammonia

The result of Pigment Extraction reaction with Ammonia test for Saffron adulteration are shown in Table 13 and Figure 14. The pigment of X saffron turned pale yellow with ammonia, indicating purity. Y saffron also turned yellow, indicating purity. Z saffron turned orange, indicating adulteration.

Table13: Pigmente xtraction Reaction with Ammonia

Brandname	Observation	Inference
Brand X Saffron	Paleyellowcolour	Pure
Brand Y Saffron	Yellow	Pure
Brand Z Saffron	Orange	Adulterated



Figure 14: Pigment extraction Reaction with Ammonia

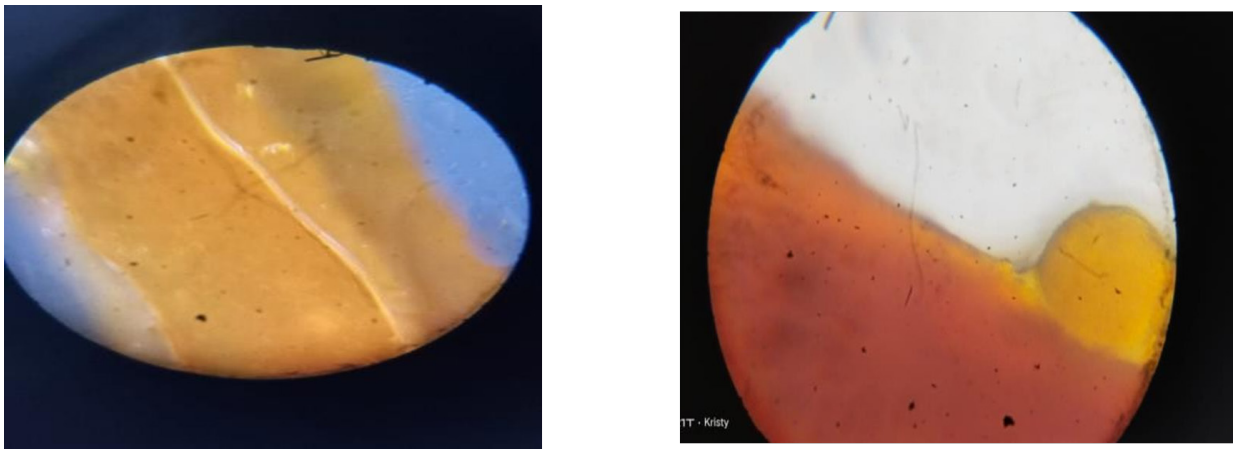
h. Diphenylamine Microscopic test

The result of Diphenylamine Microscopic test for three different brands of Saffron adulteration are shown in Table 14 and Figure 15.

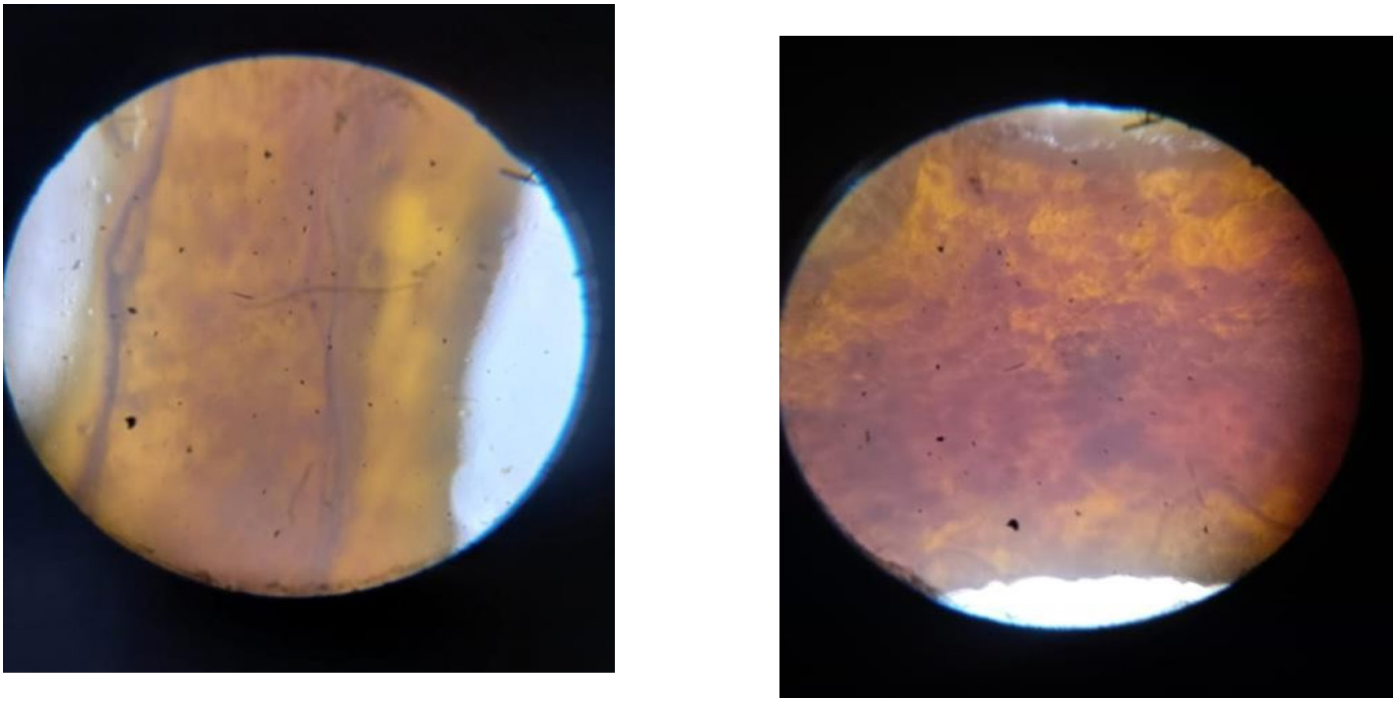
Table14: Diphenylamine Microscopic test of saffron

Brandname	Observation	inference
Brand X Saffron	Thepollen graindoes nottakeablue tint.	Pure
Brand Y Saffron	Thepollen graintakes a blue tint.	Adulterated
Brand Z Saffron	Thepollen graintakes a blue tint.	Adulterated

Brand X of saffron(a)and(b)microscopic structure respectively 45X and 100X



Brand Y of saffron(a)and(b)microscopic structure respectively 45X and 100X



Brand Z of saffron(a)and (b)microscopic structure respectively 45 X and 100 X

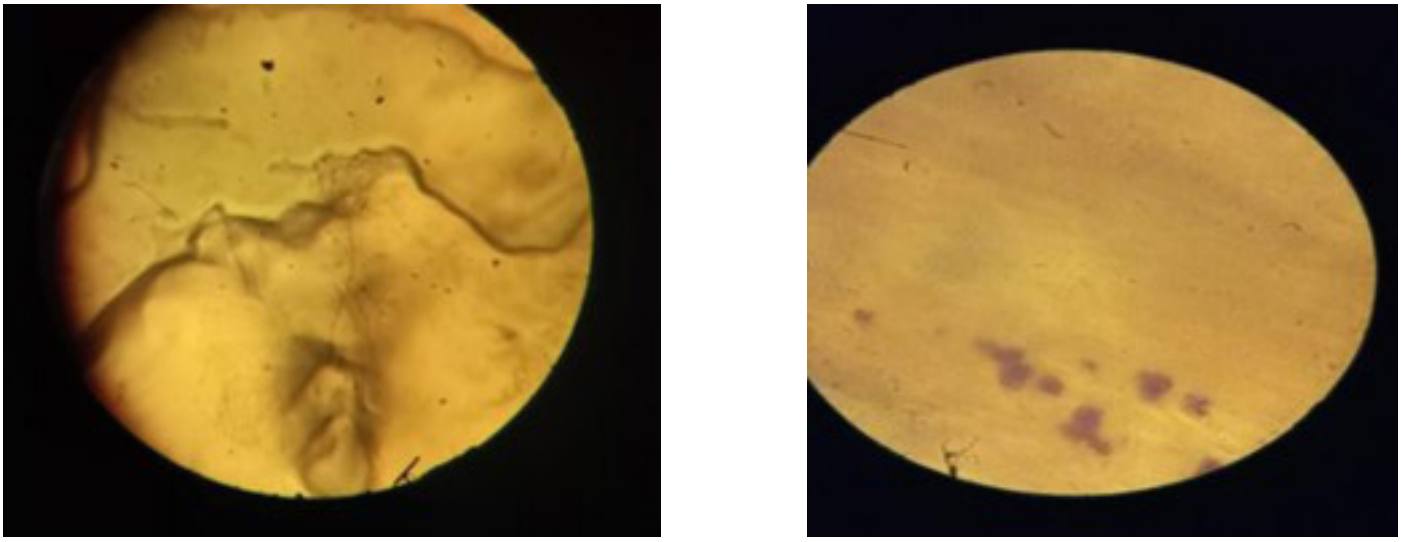


Figure 15: Diphenylamine Microscopic test of saffron

In X saffron, the pollen grains did not take a blue tint, indicating the absence of nitrate and purity. In Y saffron, the pollen grains turned blue, showing the presence of nitrate and adulteration. In Z saffron, the pollen grains also turned blue, indicating the presence of nitrate and adulteration.

i. Synthetic Dye

A. Basic dyes

The result of Basic dyes test for three different brands ofSaffron adulteration are shown in Table 15 and Figure 16. In Brand X saffron, no colour appears in the acetic acid layer, indicating purity. In Brand Y and Brand Z saffron, the layer turns yellow, indicating adulteration.

Table15:Basic dyestest of saffron

Brandname	Observation	Inference
Brand X Saffron	Colourlessaceticacidlayer	Pure
Brand Y Saffron	aceticacidlayercontainscolour	Adulterated
Brand Z Saffron	Aceticacidlayercontainscolour	Adulterated

Figure 16: Basic Dyes Test of saffron



Brand X Saffron



Brand Y Saffron



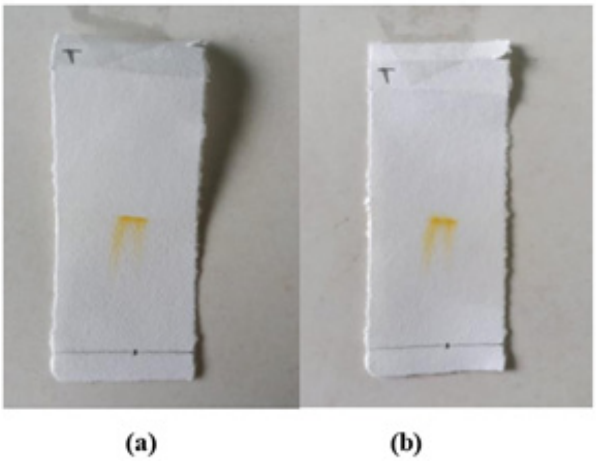
Brand Z Saffron

B. Acidic dyes

The result of basic dye test of paper chromatography for Three different brands of saffron adulteration shown in Table 16 and Figure 17

Table16: Acidic dyes test for saffron

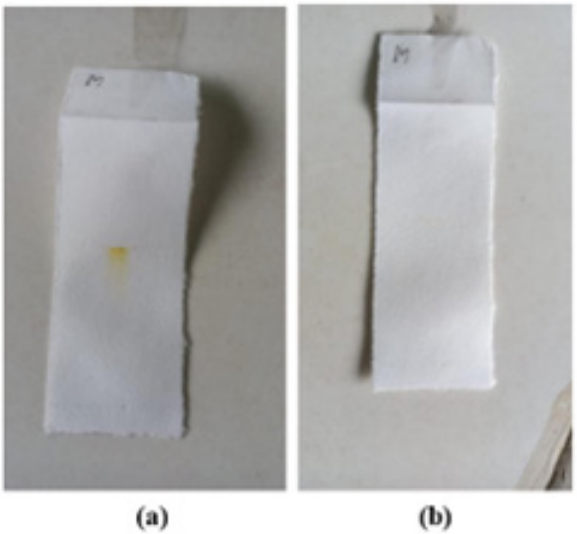
Brandname	Observation	Inference
Brand X Saffron	Spot observed after spraying stannous chloride	pure
Brand Y Saffron	No spot observed after spraying stannous chloride	adulterated
Brand Z Saffron	No spot observed after spraying stannous chloride	adulterated



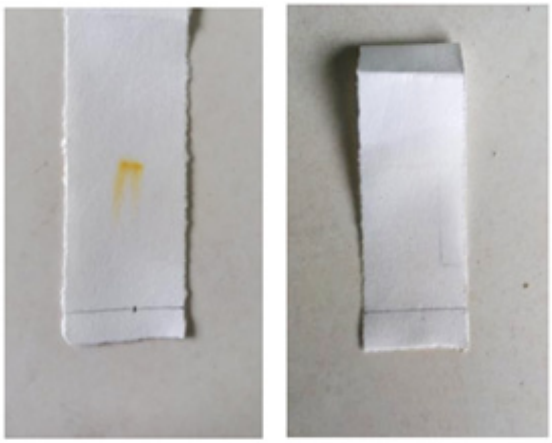
(a) (b)

Brand Y saffron: (a)and(b)respectively before and after spreading Stannous Chloride

Brand X saffron: (a) and(b)respectively before and after spreading Stannous Chloride



(a) (b)



Brand Z saffron: (a) and (b)respectively before and after spreading Stannous Chloride

Figure 17: Acidic dyes Test for Saffron

(a) (b)

j. Thin Layer Chromatography

The results of TLC, RF for three different brands of saffron are shown in table 17 and Figure 18

Table 17: RF for Saffron

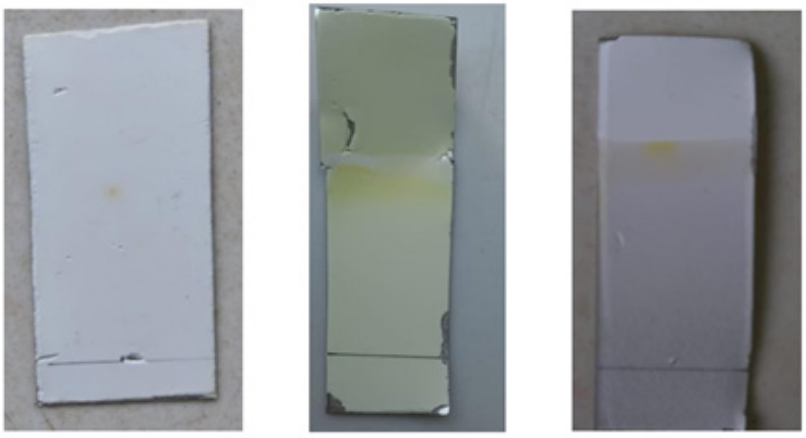
Sr.No.	Brand Name	RF
1	Brand X Saffron	0.6
2	Brand Y Saffron	0.91
3	Brand Z Saffron	1

k. UV SPECTROSCOPY

The results of UV Visible Spectroscopy, wavelength of maximum absorbance for three different brands of Saffron are shown in table 18 and Figure 19

Table 18: Wavelength and Maximum absorbance of saffron

Brandname	**Wavelength (nm)**	**Absorbance**
Brand X Saffron	440 nm	0.942
Brand Y Saffron	426 nm	0.797
Brand Z Saffron	340 nm	0.565



Brand X Saffron Brand Y Saffron Brand Z Saffron

Figure 18: TLC Test of saffron

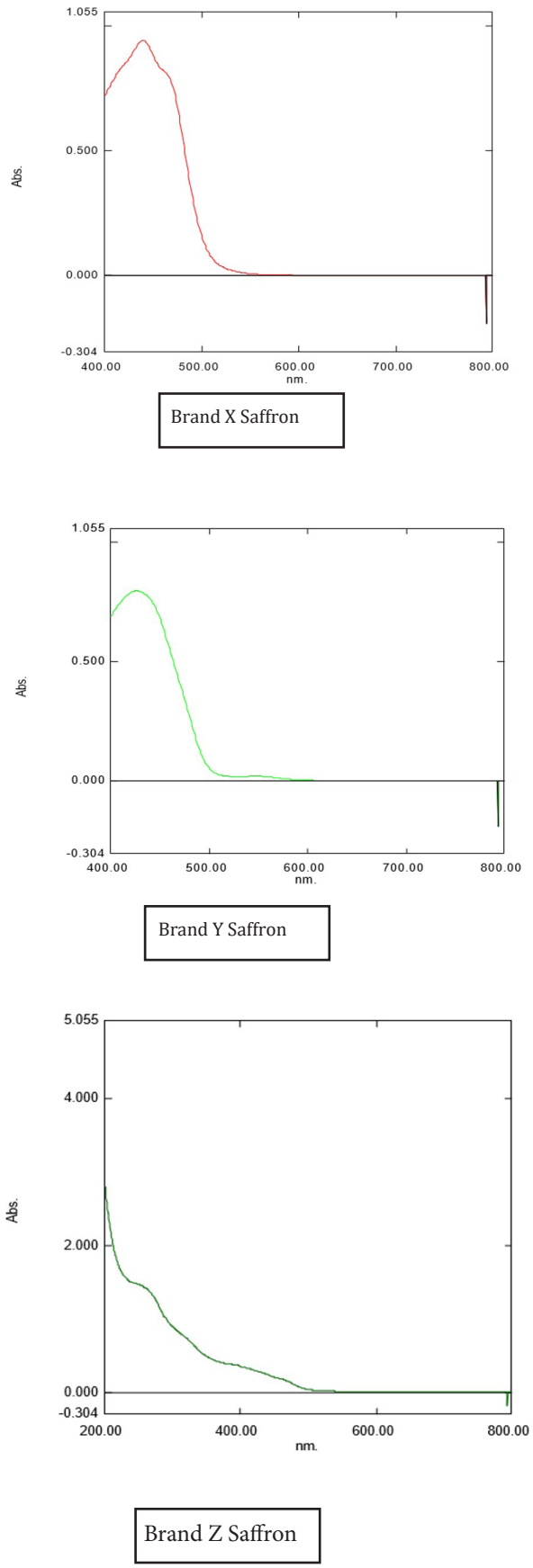


Figure 19: Wavelength and Maximum absorbance of saffron

CONCLUSION

This study demonstrated the effectiveness of various analytical methodologies in detecting adulteration in honey and saffron. The assessment of honey utilized tests such as solubility, blot, Fiehe's, and Aniline chloride tests, revealing that Brand X and Brand Y were pure, while Brand Z was conclusively identified as adulterated. These findings underscore the reliability of both physical and chemical assays in ensuring the authenticity of honey products.

Similarly, saffron samples underwent rigorous evaluation through chemical colour tests, flotation tests, and pigment extraction methods. The results indicated that Brand X and Brand Y were free from adulteration, whereas Brand Z showed significant signs of compromised quality. This highlights the importance of employing robust analytical techniques to safeguard the integrity of saffron, given its economic value and vulnerability to fraud.

Overall, this research emphasizes the necessity for improved quality control measures within the honey and saffron industries to protect consumer interests. The methodologies established in this study can serve as a foundational framework for future investigations into food adulteration and assist regulatory bodies in implementing effective monitoring strategies.

ACKNOWLEDGEMENTS

Authors are thankful to Principal, Dr. Shivajirao Kadam College of Pharmacy, Kasabe Digraj, Sangli, Maharashtra, India for providing laboratory facilities and constant encouragement.

CONFLICT OF INTEREST

The author(s) declare(s) that they have no declaration of interests to disclose.

REFERENCES

[1]Food and Drug Administration (FDA). Adulteration of food products. Washington, DC: FDA; 2020.

[2]Kumar A, Yadav R, Yadav N, Sharma S. Food adulteration: A review. Int J Pharm Bio Sci. 2015;64:331–7.

[3]Sharma S, Bansal S. Adulteration in traditional medicine: A systematic review. J Ethnopharmacol. 2019;245:112–6.

[4]Scherer R, Costa D, Rios A. Pesticide residues in food: The hidden dangers of unintentional adulteration. Food Chem Toxicol. 2018;118:110–7.

[5]Sharma S, Yadav R. Heavy metal contamination in food and its health risks. J Environ Health Sci Eng. 2017;151:43–51.

[6]Muliya VS, Nandkumar D, Joshi H. Confusion in vernacular names leading to adulteration of medicinal plants. J Herbal Med. 2018;11:1–5.

[7]Khan F, Shamsher A, Zubair A. Morphological similarity leading to adulteration in herbal medicines. J Adv Pharm Educ Res. 2020;102:18–24.

[8]Wadhwa S, Mehta A. Adulteration in herbal medicines: Causes and consequences. J Nat Remedies. 2019;192:78–82.

[9]US Department of Agriculture (USDA). Honey adulteration: Economic impacts and health risks. USDA; 2020.

[10]Carpio R, Zepeda C. Commercial honey production and the risk of adulteration with high fructose corn syrup. Food Chem. 2017;214:177–82.

[11]Mazhar M, Rahman M, Khatun M. Adulteration in saffron: A review. Food Control. 2016;66:242–8.

[12]Alavi M, Ahmadi M, Khatami M. Economic analysis of saffron adulteration and its effects on consumer safety. Food Policy. 2020;95:101108.

[13]Kirtman B, Suh DH, Koster RD. Homeopathy: An overview of potentization and its significance. Homeopathy. 2015;104:21–6.

[14]Linde K, Clausius N, Ramirez G. Are the clinical effects of homeopathy placebo effects? A meta-analysis of randomized controlled trials. Lancet. 1997;350:834–43.

[15]Ernst E. Homeopathy: A systematic review of the clinical evidence. Eur J Clin Pharmacol. 2002;58:1–8.

[16]Choudhury B, Halder N, Bhattacharya M. Therapeutic potential of Crocus sativus in homeopathy. J Homeopathy. 2019;82:123–9.

[17]Kaur R, Kumar R. Medicinal properties of saffron: A review. Int J Res Ayurveda Pharm. 2016;7:88–92.

[18]Singh A, Bansal A. Homeopathic treatment of emotional disturbances: A review. Homeopathy. 2018;107:165–70.

[19]Thakur A, Dutta S. Emotional Healing with Lonicera Caprifolium. Homeopathy Today. 2019;36(4):16–9.

[20]Gupta R, Sharma P. Potentized Honeysuckle in Homeopathy: Uses and Effects. J Altern Complement Med. 2022;28(5):451–7.

[21]Hossain MA, et al. Honey adulteration and its determination: A review. J Food Sci Technol. 2019;56:3539–51.

[22]Eteraf-Oskouei T, et al. Honey and health: A review. J Med Food. 2016;19:230–43.

[23]Fiehe A, et al. Method for determining hydroxymethyl furfural in honey. J Sci Food Agric. 2021;101:1030–6.

[24]Georgiev V, et al. Aniline chloride test in sugar analysis. J Anal Chem. 2017;72:1120–5.

[25]Chowdhury S, et al. Evaluation of honey purity using vinegar test. Int J Food Sci Technol. 2019;54:231–5.

[26]Kumar S, et al. Application of TLC in food analysis: A review. Crit Rev Food Sci Nutr. 2020;60:2482–98.

[27]Fathollahi A, Rasooli I, Karamian R. Detection of saffron adulteration using chemical colour tests. Food Chem. 2021;345:128897.

[28]Kamal B, Shahrani S. Evaluation of saffron purity using colour tests with polar solvents. J Food Sci Technol. 2020;57:5076–84.

[29]Ghasemzadeh H, Rahmanzadeh S, Ghafari F. The efficacy of Whatman paper test for saffron authenticity assessment. Int J Food Sci Technol. 2019;54:908–15.

[30]Vafaei S, Ranjbar A, Kiani A. Flotation test as a rapid assessment method for saffron purity. J Food Qual. 2019;2019:1–7.

[31]Zarei M, Ghaffari A. Evaluating carotenoid pigments in saffron using sulfuric acid tests. Food Chem. 2020;320:126647.

[32]Kharazmi M, Zamanian A, Hashemi M. The nitric acid test as an indicator of saffron quality. J Agric Food Chem. 2020;68:12905–12.

[33]Mahdavi S, Amiri M. Methods for the extraction and analysis of pigments in saffron. Food Sci Nutr. 2020;8:2276–82.

[34]Jamshidi S, Adibi N. Application of diphenylamine test for detecting saffron adulteration. J Appl Res Med Aromat Plants. 2021;20:100272.

[35]Zarei M, Moradkhani S. UV spectroscopy in evaluating the quality of saffron and its carotenoid content. Food Control. 2021;123:107773.

© Copyright 2025 BioMedSciDirect Publications IJBMR -ISSN: 0976:6685.