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Detection of argemone oil in the adulterated mustard oil by using colour tests and Thin layer chromatography

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Introduction

Forensic Scientists are often confronted with the problem of detection of Argemone mexicana oil in adulterated mustard oil. The adulterated mustard oil, if used for edible purposes can cause a condition similar to epidemic dropsy which can result in fatalities. In the present study the sensitivity level of conventional chemical tests has been checked and a new Thin Layer Chromatographic (TLC) method has been developed to identify argemone oil in the adulterated mustard oil.



Figure 1: Mixture of Argemone seeds and Mustard seeds as seen by naked eye

Argemone mexicana grows abundantly all over the India mainly during the months of March to May. Its seeds are blackish brown, round and netted. These seeds apparently have close resemblance with mustard seeds. Due to this reason, mustard seeds are often adulterated with *Argemone mexicana* seeds either accidentally or intentionally.

The oil of *Argemone mexicana* seeds is pale yellow in colour and is almost tasteless. It has a specific gravity of 0.920 and remains clear at -8ºC. It contains toxic alkaloids namely Sanguinarine and Dihydro-sangunarine.[1]

Argemone mexicana is used as a medicinal plant in several countries. Its yellow juice is used in the treatment of Jaundice, cutaneous infections, eye diseases etc. In Nigeria, it is used as a stimulant. In Mexico, the seeds are considered as an antidote to snake venom. In India, the smoke of the seeds is used to relieve toothache.[2]

Argemone mexicana seeds are often mixed as an adulterant with mustard seeds, while extracting oil for edible purposes. Such oils are tremendous health hazard. When consumed, argemone oil produces a condition similar to epidemic dropsy. Several workers have described various instrumental methods for the detection of Argemone oil in the adulterated mustard oil[3,4] but colour tests and thin layer chromatography are the methods of choice, these being simple, sensitive, reliable and economic. Keeping these significant aspects in view, an attempt has been made in the

present study to check to the sensitivity of the conventional colour tests and to develop a new TLC method to sort out this problem.

Materials and Methods

Seeds of both *Argemone mexicana* and *Brassica compestris* (mustard) were observed visually and then under light microscope to study their morphology.



Figure 2: Seeds of Brassica campestris (mustard) when observed under magnifying lens

Argemone oil was expelled from the seeds of *Argemone mexicana* in HPLC grade methanol with the help of pestle and mortar. The extract was then filtered through whatmann filter paper no. 1 and collected in a beaker. The beaker was then heated over a water bath for some time to evaporate excess of methanol, so that pure argemone oil was left behind. Same method was used for the extraction of mustard oil from mustard seeds. Argemone oil and mustard oil were mixed in different ratios (vol./vol.) to make adulterated samples of mustard oil. The sensitivity of the following conventional colour tests has been checked with the help of these adulterated samples (as mentioned above).

Colour tests

The following colour tests were performed for the detection of argemone oil in adulterated mustard oil.

1. Nitric acid test

Took 5 ml. of adulterated mustard oil in a test tube. To this, we added 5 ml of concentrated nitric acid and the test tube was shaken. Orange yellow colour was developed in the acid layer confirming the presence of argemone oil in the sample.

2. Cupric acid test

Took 5 ml. of adulterated oil in a test tube. To this 1 ml. glacial acetic acid and 2 mg. of cupric acetate solution were added. Green colour confirmed the presence of argemone oil in the sample.

3. Ferric chloride test



Figure 3: Seeds of Argemone mexicana when observed under magnifying lens

Took 5 ml. of adulterated oil sample in a test tube. To this were added 2 ml. of concentrated HCl ml. of rectified spirit. It was shaken for one minute. The tube was then kept gently in a boiling water bath for 2 minutes. The acid and oil layers were separated clearly. Held the test tube in a slanting position and slowly added 1ml. of the Ferric chloride reagent allowing it to just tickle down the side of the tube. Mixed the solution by rolling the test tube gently between the palms. Placed the tube in the same water bath for 12 minutes. Then the test tube was examined. Reddish brown, needle shaped crystalline precipitate were obtained which confirmed the presence of argemone oil in the sample.

Thin layer chromatography

Extraction of standard sanguinarine sample

Standard sanguinarine sample was extracted from the argemone oil by using the method reported by Shenolikar et al. in 1977[5].

Method

Silica gel-G coated plates were taken and activated at 110°C for 30 minutes prior to spotting of the sample. Then using capillary tube spots of standard Sanguinarine alkaloid, adulterated mustard oil, pure argemone oil and pure mustard oil were spotted on the TLC plates. Then the plates were developed in ascending way in TLC chamber with 30 minutes prior saturation. The plates were run upto 10 cm. distance. 20 different solvent systems were used. After the completion of solvent run the plates removed and air dried.

Visualization of spots

The developed plates were visualized in ultra violet light. The spot of sanguinarine appeared as bright golden yellow fluorescent spot. The spots were also visualized with the help of iodine fuming.

Results and Discussion

The examination of mustard seeds and argemone seeds under low power of the microscope revealed that the mustard seeds were round, smooth and brown in colour whereas the *Argemone mexicana* seeds were darker in colour and had a netted appearance.



Figure 4: Visualization of spots with iodine fuming. 1. Pure Argemone oil sample 2. Pure Mustard oil sample 3. Adulterated sample 4. Standard Sanguinarine sample

The results of the colour tests are given in table no. 1 and 2. It is evident from the table no. 2 that the intensity of the colour decreases with the decrease in concentration of the Argemone oil in the adulterated mustard oil and it has also been found that colour is not produced when the concentration of Argemone oil is 1% or less in the sample. Results of the thin layer chromatography are shown in table no. 3. The diagram showing a developed TLC plate run with standard sanguinarine sample, pure argemone oil, adulterated mustard oil sample and pure mustard oil sample can be downloaded.

Table 1. Showing results of colour tests.

Sr. No.	Colour Tests Performed	Observations	Inference
1.	Nitric acid test	Crimson Orange colour	Argemone oil present
2.	Ferric chloride test	Reddish Brown precipitates having needle shaped crystals	Argemone oil present
3.	Cupric acetate test	Greenish coloration	Argemone oil present

Table 2. Showing Intensities of colour test at various concentrations of Argemone oil in adulterated oil

Colour Test	Concentration of Argemone oil in adulterated mustard oil			
	10%	5%	2%	1%
Nitric Acid test	+++	++	+	-
Ferric Chloride Test	+++	++	+	-
Cupric Acetate Test	+++	++	+	-

Table 3. Results obtained from unknown sample of mustard oil adulterated with argemone oil

Sr. No.	Solvent Systems used	No. of spots	Colour of the sports under UV light	Colour of spots with iodine fuming	hRf value
1.	Benzene : Acetone : Methanol 70 : 20 : 10	1	Golden yellow	Brown	99
2.	Benzene : Acetone : Methanol 80 : 10 : 10	1	Golden Yellow	Brown	99
3.	Benzene : Acetone : Methanol 60 : 20 : 20	1	Golden Yellow	Brown	99
4.	Benzene : Acetone : Ether : 10% NH3 40 : 60 : 10 : 3	1	Golden Yellow	Brown	98
5.	Benzene : Acetone : Ether : 10% NH3 50 : 50 : 10 : 3	1	Golden Yellow	Brown	98
6.	Benzene : Acetone : Ether : Isopropanol : 3% NH3 20 : 15 : 10 : 7.5 : 2.5	1	Golden Yellow	Brown	98
7.	Benzene : Acetone : Ether : Isopropanol : 3% NH3 25 : 20 : 15 : 10 : 5	1	Golden Yellow	Brown	99
8.	Butanol : Acetic acid : Water 60 : 15 : 25	2	(i) Blue (ii) Golden Yellow	Yellowish-brown Brown	41 98
9.	Toluene : Acetone : Methanol 70 : 20 : 10	1	Golden Yellow	Brown	98
10.	Hexane : Acetone : Methanol 70 : 20 : 10	1	Golden Yellow	Brown	70

Sr. No.	Solvent Systems used	No. of spots	Colour of the sports under UV light	Colour of spots with iodine fuming	hRf value
11.	Cyclohexane : Acetone : Methanol 60 : 20 : 20	1	Golden Yellow	Brown	92
12.	Benzene : Acetone : Ethanol 70 : 20 : 10	1	Golden Yellow	Brown	98
13.	Benzene : Acetone : Methanol 80 : 10 : 10	1	Golden Yellow	Brown	99
14.	Benzene : Acetone : Methanol : 10% NH3 40 : 60 : 10 : 3	1	Golden Yellow	Brown	99
15.	Benzene : Acetone : Ethanol : 10% NH3 40 : 60 : 10 : 3	1	Golden Yellow	Brown	98
16.	n-Butanol : Acetone : Ethanol : H2O 60 : 20 : 20 : 15	-	(i) Golden Yellow (ii) Green	(i) Light Brown (ii) Light Green	85 30
17.	Benzene : Acetone : Ethanol : Acetic acid 60 : 20 : 10 : 10	1	Golden Yellow	Brown	98
18.	Benzene : Acetone : Butanol : Acetic acid 60 : 20 : 10 : 10	1	Golden Yellow	Brown	98
19.	Benzene : Acetone : Methanol : Acetic acid 60 : 20 : 10 : 10	1	Golden Yellow	Brown	98
20.	Benzene : Acetone : Butanol : 10%NH3 40 : 60 : 10 : 3	1	Golden Yellow	Brown	99

Thin layer chromatography was found to be the most suitable method for separation and identification of argemone alkaloid in adulterated mustard oil. The solvent system comprising of n-Butanol : Acetone : Ethanol : H_2O (60 : 20 : 20 : 15) was found to be the best solvent system which results in the separation of the three spots (more or less heart shaped) at hRf values 85, 30 and 15 with pure argemone oil, 2 spots with adulterated mustard sample at hRf values 85 and 30, one spot with standard sanguinarine sample at hRf value 85 and no spot with pure mustard oil at temperature ranging from 34°C to 38°C. The one spot at hRf value 85 gave a bright Golden yellow fluorescence under long ultra violet light and a brown colored heart shaped spot, when fumed with the iodine. This spot is of alkaloid *Sanguinarine* is of same colour and it is at the same hRf value at which standard Sanguinarine sample produced the spot.

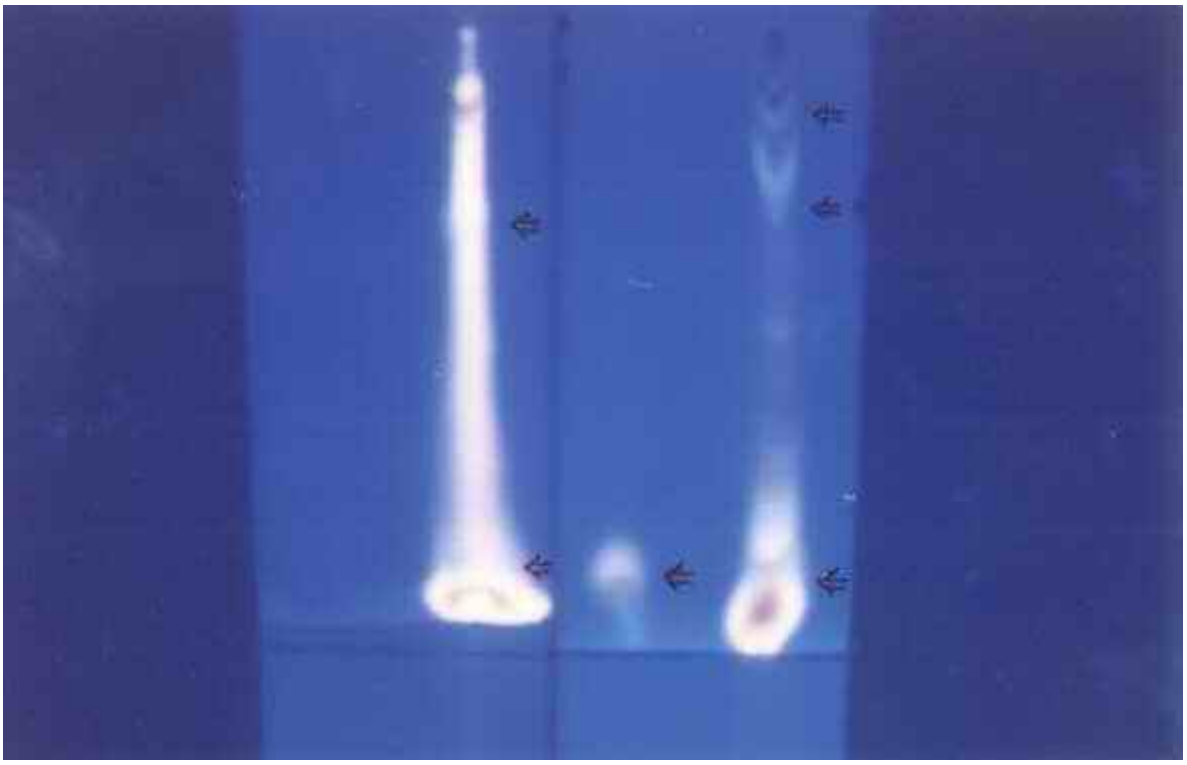


Figure 5: Visualization of spots under UV light 1. Pure Argemone oil sample 2. Standard Sanguinarine sample 3. Adulterated sample 4. Pure mustard oil sample

Shenolikar et al., 1981[6] reported a TLC method with solvent system n-Butanol, acetic acid and water (60 : 15 : 25) which is very slow, takes more than two hours to complete.

Thus, this new Thin Layer Chromatography solvent system comprising of n-Butanol : Acetone : Ethanol : H_2O (60 : 20 : 20 : 15) has been found to be the best system for the separation of the toxic alkaloid sanguinarine and its identification, that too, in a very quick time i.e. time of running of solvent is only 55 minutes.

It is expected that above findings made in the present study will be of immense value to the forensic scientists working in this field.

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