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Quantitative evaluation of sanguinarine as an index of argemone oil adulteration in edible mustard oil by high performance thin layer chromatography

Priyankar Ghosh [a], M.M. Krishna Reddy [a], R.B. Sashidhar [b,*]

[a] Central Forensic Science Laboratory, Directorate of Forensic Science, Ministry of Home Affairs, Government of India, Ramanthapur, Hyderabad 500 013, India
[b] Department of Biochemistry, University College of Science, Osmania University, Hyderabad – 500 007, India

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Abstract

A simple analytical procedure based on HPTLC method was developed for the detection of argemone oil adulteration in edible mustard oil using sanguinarine as an index of argemone oil adulteration (measured as % adulteration). The methodology required no elaborate extraction procedure. The quantitation was achieved by densitometric scanning of the chromatogram in fluorescence/reflectance mode. Dihydrosanguinarine concentration was determined after its conversion to sanguinarine by UV (366 nm) irradiation, for a period of 15 min. The limit of detection (LOD) of sanguinarine in argemone oil was estimated as 1 ng per 6 mm band with a signal-to-noise ratio of 3:1 and limit of quantitation in the samples was estimated as 3 ng per 6 mm band with a signal-to-noise ratio of 10:1. The total content of sanguinarine (sanguinarine + dihydrosanguinarine) in argemone oil samples were in the range of 4.84–5.79 mg/ml of oil. Standard sanguinarine was spiked to edible mustard oil at two levels (50 and 100 μg/ml) and recoveries were found to be 79% and 82%, respectively. A linear regression plot ($y = 40.44\ (\pm 0.014)x - 5.81\ (\pm 0.078)$) was generated between the percent adulteration of argemone oil in edible mustard oil and the concentration of sanguinarine in the edible mustard oil spiked with various levels of argemone oil (1–30%). Analyzing the adulterated edible mustard oil samples collected from the area, wherein an outbreak of epidemic dropsy was reported in the recent past, validated this linear regression plot. The percent adulteration in these samples was found to be in the range of 1.22–8.77%. Food and forensic laboratories can use this method routinely in order to assess the adulteration of edible oils by argemone oil and in poisoning cases due to argemone oil toxicity.

Keywords: HPTLC; Sanguinarine; Argemone oil; Adulteration; Edible mustard oil

1. Introduction

Unscrupulous traders for economic gain practice willful adulteration of edible oils by cheap non-edible oils. In the past century, more than 30 outbreaks of human poisoning due to consumption of adulterated edible oils have been reported from India. Majority of these outbreaks have been associated with the consumption of edible mustard (*Brassica compestris*) oil adulterated with argemone oil (Gomber, Daral, Sharma, & Faridi, 1994; Krishnamachari & Satyanarayan, 1972; Park & Park, 1986; Shanbhag, Jha, Kekre, & Rindani, 1968; Singh & Khanna, 1983; Singh et al., 2000; Tandon, Singh, Arora, Lal, & Tandon, 1975; Thatte & Dahanukar, 1999). The seeds of *Argemone mexicana* plant are a source of argemone oil, used for adulteration (Bui, 1974). This plant is a ubiquitous weed, found growing abundantly in wastelands, cultivated fields and on roadsides all over the country during the late winter season. Interestingly,

* Corresponding author. Fax: +91 4027 016868.
E-mail address: sashi_rao@yahoo.com (R.B. Sashidhar).

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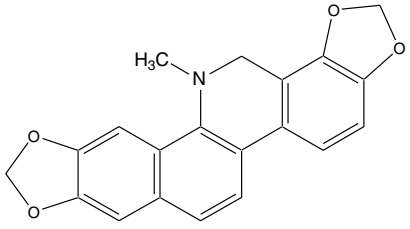
the argemone seeds closely resemble the edible mustard seeds and the colors of both the oils are also similar (Sanyal, 1950).

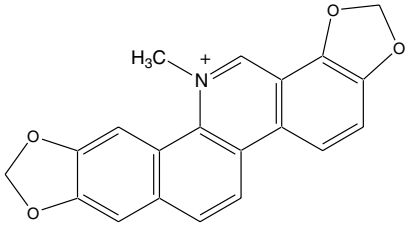
Ingestion of adulterated edible mustard oil with argemone oil results in a clinico-pathological condition that has been clinically referred to as *epidemic dropsy*. Epidemic dropsy has been reported not only from India but also from other regions of the world, such as Fiji Islands, Myanmar, South Africa, Mauritius and Madagascar etc (Manson-Bahr & Apte, 1982). Quaternary benzophenanthridine alkaloids (QBA's) have been implicated in the toxicity of argemone oil. The two major physiologically active QBA's that have been identified to mediate the toxic effects of argemone oil in the development of epidemic dropsy are Dihydrosanguinarine (1) and Sanguinarine (2) (Sarkar, 1948). Both are interconvertible by simple oxidation and reduction process.

Several analytical methods have been reported in the literature for the detection of sanguinarine in argemone oil and its occurrence in plants such as *Chelidonium majus* and *Sanguinarine canadensis*. These methods include paper chromatography (Hakim, 1957; Hakim, Mijovik, & Walker, 1961a, Hakim, Mijoric, & Walker, 1961b), TLC with UV-spectroscopy (Balderstone & Dyke, 1977; Shenolikar, Rukmini, Krishnamachari, & Satyanarayana, 1974), HPTLC (Sita Devi & Sastry, 2002), colorimetric analysis (Bose, 1972), HPLC (Hussain, Narsimha, & Rao, 1999) and Capillary Electrophoresis (Sevcik et al., 2000), with varying sensitivities and detection limits. Even though these analytical procedures are suitable for the detection of sanguinarine in samples originating from plants, they have limitations with respect to their applications in the determination of sanguinarine as an index of argemone oil adulteration in edible mustard oil samples. The paper chromatography reported is not completely reliable and limit of detection (LOD) are not known (Hakim, 1957; Hakim et al., 1961a, 1961b). The reported colorimetric method lacks sensitivity, tedious and time consuming. Even though, HPLC is a method of choice, it is limited by extensive sample clean up, requires expensive solvents and longer periods of column stabilization. In comparison to HPLC, HPTLC is a versatile analytical technique that requires less expensive instrumentation and expertise. The main advantages of HPTLC analysis are (i) simultaneous separation of several samples on a single TLC plate and (ii) in situ clean up and separation of analytes. Further, HPTLC analysis needs small amounts of developing solvent and minimal sample preparation, as compared to HPLC analysis.

Earlier Sita Devi and Sastry (2002) developed a method based on HPTLC wherein sanguinarine was detected in argemone and adulterated oil by using butanol:acetic acid:water (6:1.5:2.5, v/v) as a developing solvent system. The extraction procedure involved the conversion of sanguinarine to sanguinarine chloride. However, the method had certain limitations like tedious extraction procedures, longer time for development and delayed drying of the TLC plate due to high polarity of the solvents. In addition to that, the method is not suitable to resolve sanguinarine and dihydrosanguinarine and no detailed study on recovery of sanguinarine in argemone oil as well as edible mustard oil were reported.



(1)



(2)

The present communication reports a HPTLC based procedure for the detection of argemone oil adulteration in edible mustard oil using sanguinarine as an index of argemone oil adulteration (measured as % adulteration). By this method, a clear separation of sanguinarine and dihydrosanguinarine present in argemone oil as well as mustard oil adulterated with argemone oil is achieved. Further, the methodology developed was validated and successfully applied in the determination of percent argemone oil adulteration in edible mustard oil samples, collected from the various house holds (Shivpuri, Madhya Pradesh state, India), wherein, a recent outbreak of epidemic dropsy was reported during the year 2003.

2. Experimental

2.1. Chemicals and analytes

All solvents used were of analytical grade unless otherwise stated. Hexane, acetone, methanol and chloroform procured from E-Merck Ltd (Mumbai, India) were used throughout the study. The reference standard of sanguinarine (purity – 98% by HPLC) was procured from Sigma (St. Louis, MO, USA). Five batches of *Argemone mexicana* seeds were procured from the local

market, at different points of time. Edible mustard (*Brassica compestris*) oil was obtained from the local market of Kolkata, India. The edible mustard oil samples, suspected to be adulterated with argemone oil were collected from households of Shivpuri, Madhya Pradesh state of India (where a recent outbreak (2003) of epidemic dropsy was reported).

2.2. Standard solutions

Stock solution of standard sanguinarine was prepared by dissolving it in methanol; so as to obtain a concentration of 1 mg/ml. Stock solution was stored at −20 °C, until further use. The concentrations of sanguinarine used to generate a calibration plot were 1, 2, 3, 5, 10, 20 and 60 ng/μl in methanol. An aliquot of 5 μl of each concentration was spotted onto the HPTLC plate with the aid of an automated sample applicator.

2.3. Spiking studies

Various levels [1%, 3%, 5%, 7.5%, 10%, 12.5%, 15%, 20%, 25%, and 30% (v/v)] of pure argemone oil (Batch # 5, containing 5.37 mg of sanguinarine per ml of oil) were spiked to edible mustard oil in order to evaluate the percent adulteration of argemone oil in adulterated edible mustard oil samples. An aliquot of 100 μl of the spiked sample was dissolved in chloroform and the volume was made up to 1ml in a certified volumetric flask. Immediately, an aliquot of 5 μl from each spiked sample was spotted onto HPTLC plate.

Further, standard sanguinarine was spiked to edible mustard oil sample at a concentration of 50 and 100 μg per milliliter of oil in order to assess the recoveries of sanguinarine. An amount of 250 and 500 μl of standard sanguinarine (1 mg/ml) in methanol was taken in 5 ml certified volumetric flask and methanol was evaporated to dryness under nitrogen. 5 ml of edible mustard oil was added to the residue and vortexed thoroughly in order to dissolve the sanguinarine in edible mustard oil. An one ml aliquot of spiked edible mustard oil containing 50 or 100 μg/ml oil was used for analysis. Prior to the addition of sanguinarine to the edible mustard oil, the edible mustard oil was analyzed for the presence of sanguinarine, as an index of argemone oil contamination.

2.4. Chromatography

The HPTLC system (Camag, Muttenz, Switzerland) consisted of a Linomat IV sample applicator equipped with a 100 μl syringe and a Scanner III, operated using Camag software WinCATS Planar Chromatography Manager, loaded on a personal computer. Aluminum HPTLC plates (20 cm × 10 cm) pre-coated with silica gel 60, were purchased from Merck (Darmstadt, Germany).

2.5. Sample preparation

Argemone seeds (50 g, each) were powdered using a high-speed mechanical blender. Oil was extracted for 6 h by using soxhlet apparatus with *n*-Hexane as the extraction solvent. The oil was passed through anhydrous sodium sulfate to remove any traces of moisture.

An aliquot of 20 μl of argemone oil was dissolved directly in chloroform and made up to a final volume of 1 ml in a certified volumetric flask. An aliquot of 5 μl of each sample was spotted onto HPTLC plate.

Adulterated edible mustard oil samples (50 μl, each) were also dissolved directly in chloroform and made up to 1 ml in certified volumetric flasks. An aliquot of 5 μl of each sample was spotted onto HPTLC plate.

2.6. Chromatographic conditions

Prior to application of the standards and samples, the HPTLC plates were pre-developed with methanol and dried at 70 °C for one hour, in an air-circulating oven. Standard solutions of sanguinarine, argemone oil samples, edible mustard oil samples spiked with argemone oil and edible mustard oil samples adulterated with argemone oil were applied onto HPTLC plates with the help of sample applicator Linomat IV with a rate of 7 μl/s in the form of 6 mm band with the space of 4 mm between the bands. The plates were developed in a CAMAG Twin Trough Chamber (20 × 10 cm plates with stainless steel lid) previously saturated for 10 min with 10 ml of developing solvent system [hexane:acetone:methanol (80:15:5, v/v)]. The plates were developed up to a distance of about 6 cm. The plates were air-dried and later irradiated under long wave UV light (Peak $\lambda = 366$ nm) for 15 min to oxidize dihydrosanguinarine to sanguinarine (Balderstone & Dyke, 1977).

2.7. Scanning

The bands were visualized in an UV cabinet (Camag Universal UV cabinet, $\lambda = 366$ nm). The plates were scanned by the Camag scanner III in fluorescence/reflectance mode by using a Hg lamp at 366 nm, having K400 filter with a slit-width of 6 mm × 0.45 mm, at a scanning speed of 20 mm/s.

The plates were subsequently scanned to determine the in situ UV spectra of the bands between 200 and 400 nm. The sample band corresponding to dihydrosanguinarine after UV-irradiation ($R_F = 0.82$) and standard sanguinarine band ($R_F = 0.36$) were marked under UV light and scraped from the plate for further analysis. The standard sanguinarine and the analyte were extracted separately from the silica gel with methanol in a small elution column. The solvent extract was passed through a 0.45 μm syringe filter to remove any silica particles. Later the solvent was subsequently removed by

evaporation under nitrogen and the resulting mass was analyzed by MS Engine Mass Spectrometer (HP 5989A, Agilent Technologies, CA, USA) in chemical ionization (CI) mode through direct insertion probe (DIP).

3. Results and discussion

For the first time, a systematic approach has been made to develop a simple and rapid analytical procedure for the detection of argemone oil adulteration in edible mustard oil using sanguinarine as an index of percent argemone oil adulteration The HPTLC solvent system (hexane:acetone:methanol, 80:15:5, v/v) used for analysis of sanguinarine in argemone oil and in adulterated edible mustard oils was optimized in such a way that the bands corresponding to sanguinarine and dihydrosanguinarine are free from interferences of fatty acid components originating from both argemone and edible mustard oils. Further, the HPTLC system developed effectively resolved the sanguinarine and dihydrosanguinarine from other minor alkaloids present in the argemone oil. The present methodology involves no elaborate extraction procedures as reported by earlier workers, wherein oil samples were subjected to treatment with strong acids prior to extraction of sanguinarine (Shenolikar et al., 1974; Sita Devi & Sastry, 2002). Fig. 1 shows the separation of sanguinarine and dihydrosanguinarine from argemone oil, argemone oil spiked to edible mustard oil and edible mustard oil samples suspected to be adulterated with argemone oil (collected from the area of epidemic dropsy outbreak). Fig. 2, depicts the typical densitogram indicating the separation of sanguinarine and dihydrosanguinarine in argemone oil, argemone oil spiked to edible mustard oil and edible mustard oil samples suspected to be adulterated with argemone oil. The mean R_F of sanguinarine was found to be 0.36 ± 0.006 (mean ± SD), which was visualized as a bright golden yellow fluorescent band. The mean R_F of dihydrosanguinarine was found to be 0.82 ± 0.006 (mean ± SD), which was visualized as a bright bluish fluorescent band. The identification of sanguinarine in oil samples were done by comparing the R_F with that of corresponding reference standard. The identification of sanguinarine was further confirmed by comparing the in situ UV spectra of sanguinarine in argemone oil with that of standard sanguinarine (Fig. 3). The dihydrosanguinarine (bluish fluorescence) was found to convert readily to sanguinarine (bright golden yellow fluorescence) when irradiated under long wavelength UV light ($\lambda = 366$ nm) for 15 min. The conversion of dihydrosanguinarine to sanguinarine was confirmed by comparing the in situ UV spectra of dihydrosanguinarine after irradiation with standard sanguinarine (Fig. 3). Thus, there is no need to have a reference standard of dihydrosanguinarine for quantitation. The mass spectral analysis of band corresponding to dihydrosanguinarine after exposure to UV-radiation ($R_F = 0.82$) was carried out after scraping and extracting the analyte from the plates. The base peak (m/z 241) and the fragmentation pattern of analyte corresponding to $R_F = 0.82$ was found to be similar to that of band corresponding to standard sanguinarine, thus confirming unambiguously the conversion of dihydrosanguinarine to sanguinarine after UV irradiation (Fig. 4). Five argemone oil samples originating from five batches of seeds,

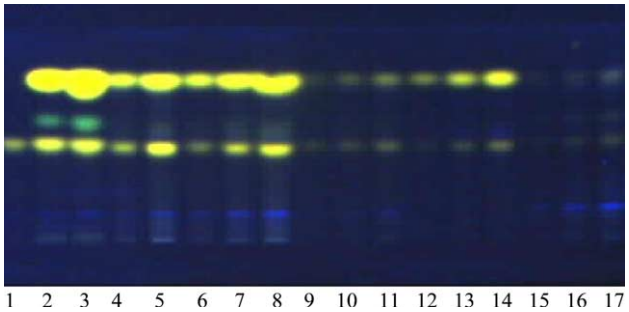


Fig. 1. TLC separation of: (i) standard sanguinarine (40 ng/2 μl) – Lane # 1; (ii) sanguinarine and dihydrosanguinarine in argemone oil – Lane # 2–8; (iii) argemone oil spiked to edible mustard oil – Lane # 9–11; (iv) adulterated edible mustard oil samples originating from the area of human epidemic dropsy outbreak – Lane # 12–17.

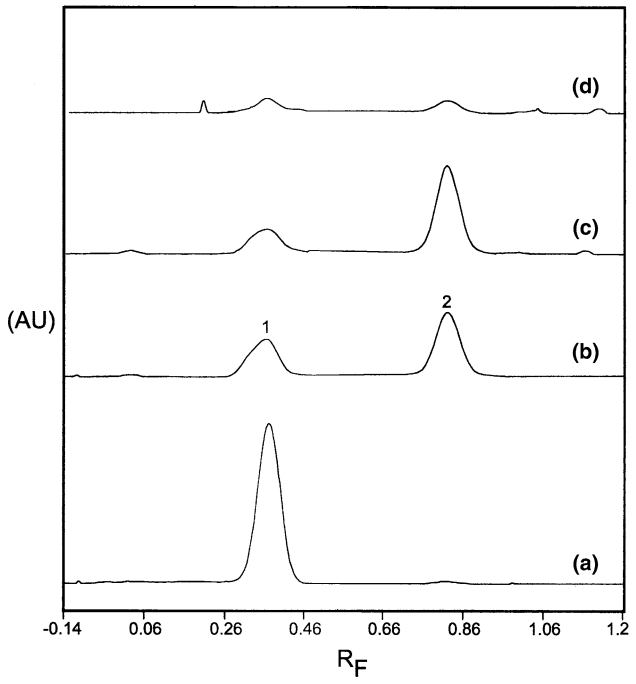


Fig. 2. Densitogram of: (a) standard sanguinarine (300 ng/5 μl); (b) argemone oil (537 ng/5 μl); (c) argemone oil spiked to edible mustard oil (615 ng/5 μl); (d) an adulterated edible mustard oil sample originating from the area of human epidemic dropsy outbreak (7.5 ng/μl). Peak **1**: sanguinarine; Peak **2**: dihydrosanguinarine.

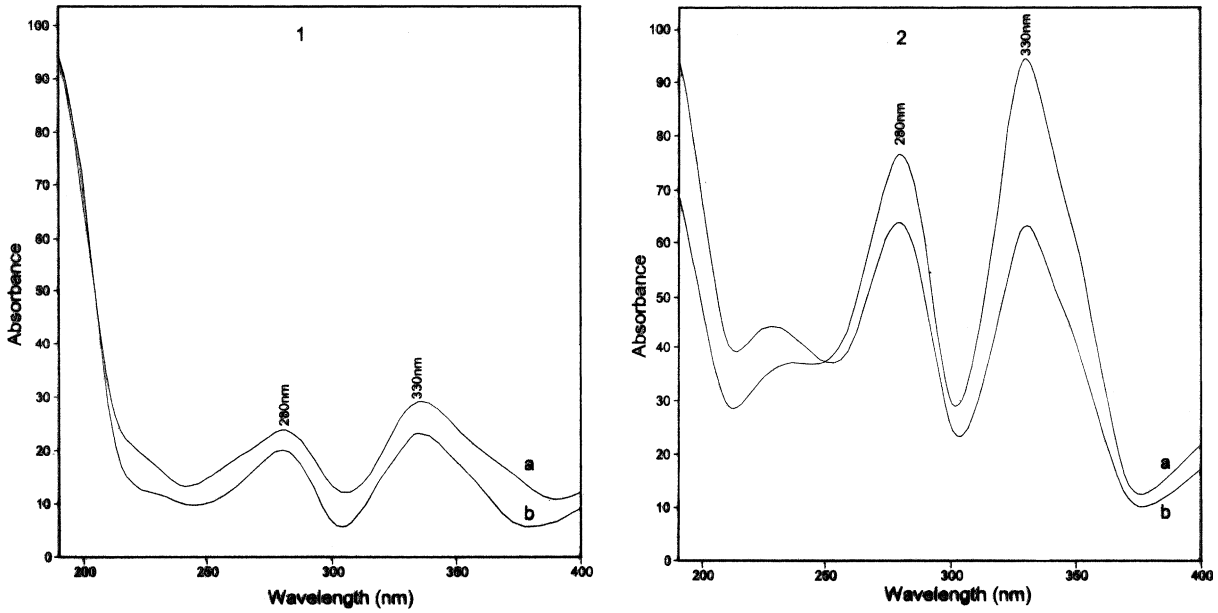


Fig. 3. In situ UV spectra of: (i) sanguinarine in argemone oil (172 ng/5 μl) (**1a**); (ii) standard sanguinarine (100 ng/5 μl) (**1b**); (iii) dihydrosanguinarine in argemone oil after UV irradiation (366 nm, for 15 min) (324 ng/5 μl) (**2a**); (iv) standard sanguinarine (300 ng/5 μl) (**2b**).

ten argemone oil spiked to edible mustard oil samples and fifteen edible mustard oil samples suspected to be adulterated with argemone oil, collected from Shivpuri, Madhya Pradesh, India, were subjected to the present HPTLC analysis.

Quantitative analysis of sanguinarine was done by external standard method on the basis of peak area in linear regression mode. The calibration range was set from 5–300 ng for 6 mm band, which showed acceptable linearity. The calibration curve was constructed in order to determine the concentration of sanguinarine in argemone oil, argemone oil spiked to edible mustard oil samples and edible mustard oil samples suspected to be adulterated with argemone oil. The calibration plot was based on linear regression analysis ($y = 82.17x - 259.45$). The standard curve for sanguinarine was found to be linear with a correlation coefficient (r^2) of 0.9998. The amount of sanguinarine and dihydrosanguinarine in argemone oil samples are shown in Table 1, which are similar to the value reported in the literature by the earlier workers (Das & Khanna, 1997; Shenolikar et al., 1974). The total content of sanguinarine (sanguinarine + dihydrosanguinarine) in argemone oil samples were in the range of 4.84–5.79 mg/ml of oil. The mean ratio of sanguinarine to dihydrosanguinarine in pure argemone oil sample was found to be 35:65.

The LOD of sanguinarine in argemone oil was found to be 1 ng per 6 mm band with a signal to noise ratio of 3:1. The limit of quantitation (LOQ) of sanguinarine in argemone oil was found to be 3 ng per 6 mm band with a signal to noise ratio of 10:1. The instrumental response for a series of five bands of sanguinarine ($R_F = 0.36$) in argemone oil gave a percent relative standard deviation (RSD) of 1.57% for R_F and 0.9% for peak area within the plate and 2.0% for R_F and 6.28% for area between the plates. The instrumental response for a series of five bands of dihydrosanguinarine ($R_F = 0.82$) in argemone oil gave a %RSD of 0.71% for R_F and 0.93% for peak area within the plate and 1.75% for R_F and 6.08% for peak area between the plates. The recovery studies carried out by spiking standard sanguinarine (50 and 100 μg/ml) directly to the edible mustard oil, showed the mean recoveries of sanguinarine to be 79% and 82%, respectively.

Known amounts of argemone oil was spiked to edible mustard oil in order to evaluate the percent adulteration of argemone oil in adulterated edible mustard oil samples based on the detection of sanguinarine and dihydrosanguinarine (as sanguinarine) as an index of argemone oil contamination. Batch # 5 of argemone oil sample was selected for spiking studies, because the total sanguinarine content (sanguinarine and dihydrosanguinarine) was found to 5.37 mg/ml, which was very close to the mean value of the five batches of the oil samples analyzed (5.32 mg/ml) (Table 1). The percent argemone oil spiked to edible mustard oil sample and the amounts of total sanguinarine detected are depicted in Table 2. A linear regression plot was generated between the percent adulteration and the concentration of sanguinarine in the argemone oil spiked to edible mustard oil. There was a positive correlation between the percent adulteration and concentration of sanguinarine ($y = 40.44 (\pm 0.014)x - 5.81 (\pm 0.078)$) with a correlation coefficient of (r^2) of 0.9993 at 95% confidence limit in the set range of 1–30%. Survey of published literature indicates that none of the earlier workers had undertaken a detailed

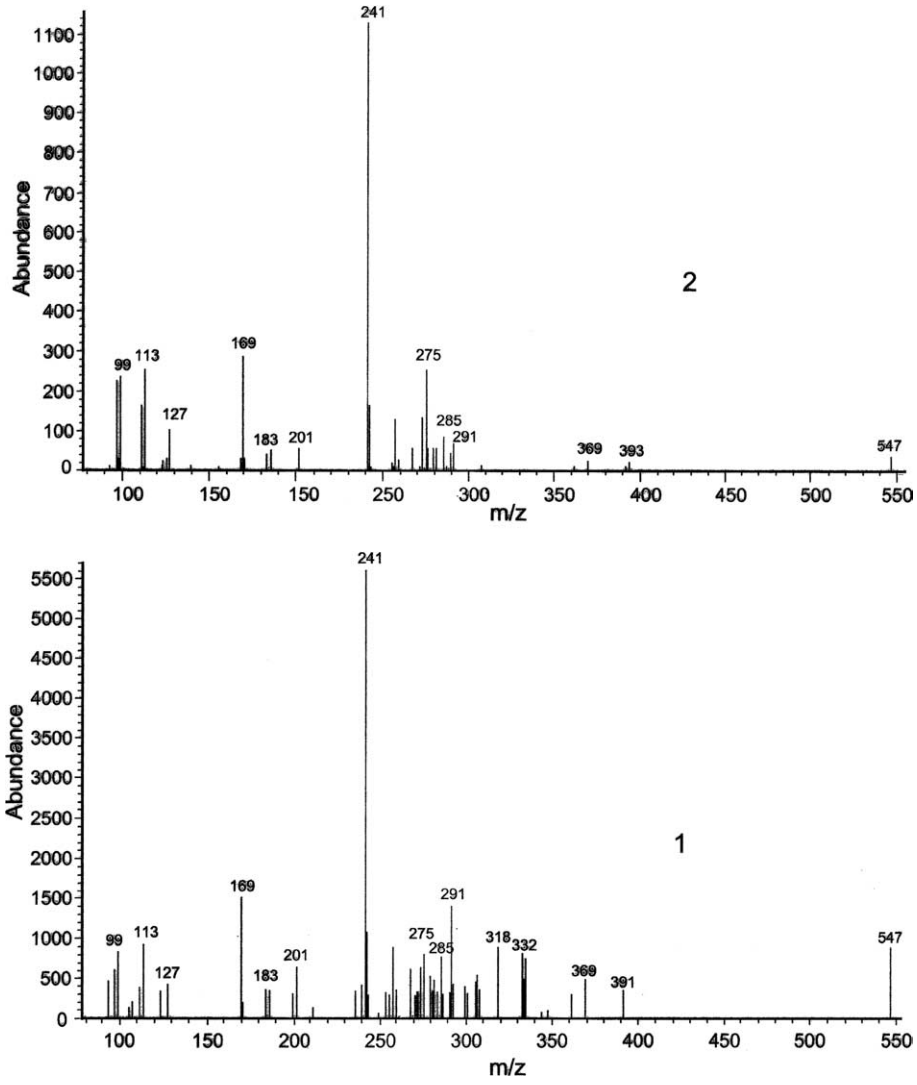


Fig. 4. Mass spectra of: (**1**) standard sanguinarine; (**2**) dihydrosanguinarine in argemone oil after UV irradiation (366 nm for 15 min).

Table 1
Distribution of sanguinarine and dihydrosanguinarine in argemone oil samples

Batch #	Oil content (g%)	Concentration of sanguinarine in argemone oil (mg/ml)[a]	Concentration of dihydrosanguinarine in argemone oil (mg/ml)[a]	Total content (sanguinarine and dihydrosanguinarine) (mg/ml)
1	33	1.82 ± 0.05	3.24 ± 0.10	5.06
2	29	2.22 ± 0.04	3.32 ± 0.09	5.54
3	29	1.75 ± 0.06	4.04 ± 0.17	5.79
4	22	1.71 ± 0.03	3.13 ± 0.08	4.84
5	28	1.90 ± 0.04	3.47 ± 0.14	5.37

[a] Values based on triplicate analysis; mean ± SD.

recovery studies based on spiking of standard sanguinarine or pure argemone oil to edible mustard oil, in order to evaluate the percent adulteration.

Edible mustard oil samples (15 samples in total) suspected to be adulterated with argemone oil were analyzed by the HPTLC method, in order to determine the percent argemone oil adulteration, based on the linear regression plot generated between the percent adulteration and the concentration of sanguinarine in the argemone oil spiked to edible mustard oil. The concentrations of sanguinarine detected in all these samples are shown in Table 3. Sanguinarine was not detected in four oil samples, out of fifteen samples analyzed. The percent argemone oil adulteration in the adulterated edible mustard oil samples was calculated based on the regression equation of spiked edible mustard oil samples. It was inferred from

Table 2
Total sanguinarine content as detected by HPTLC method, in argemone oil spiked to edible mustard oil samples

S.No.	Percent spiking of argemone oil in edible mustard oil (v/v)	Concentration of sanguinarine in edible mustard oil (μg/ml)[a]
1.	1	40 ± 2
2.	3	115 ± 2
3.	5	202 ± 2
4.	7.5	301 ± 5
5.	10	394 ± 4
6.	12.5	498 ± 4
7.	15	598 ± 7
8.	20	792 ± 11
9.	25	989 ± 10
10.	30	1230 ± 12

[a] Values based on triplicate analysis; mean ± SD.

Table 3
The concentration of total sanguinarine and percent argemone oil adulteration detected in edible mustard oil samples originating from the area of human epidemic dropsy outbreak[a], based on regression plot

Sample	Concentration of sanguinarine in adulterated edible mustard oil (μg/ml)[b]	% Adulteration
1	ND[c]	–
2	48.3 ± 2	1.3
3	45.5 ± 4	1.3
4	58 ± 2	1.6
5	ND	–
6	70 ± 2	1.9
7	60 ± 2	1.6
8	45 ± 3	1.3
9	46 ± 2	1.3
10	44 ± 3	1.2
11	ND	–
12	345 ± 11	8.7
13	300 ± 10	7.6
14	103 ± 17	2.7
15	ND	–

[a] The samples collected from the households of Shivpuri, Madhya Pradesh, India.
[b] Values based on triplicate analysis; mean ± SD.
[c] ND, not detected.

the plot that the percent argemone oil adulteration in the edible mustard oil samples collected from the area, wherein the outbreak of epidemic dropsy was reported, was in the range of 1.22–8.77% (Table 3). Earlier, reports on the outbreak of epidemic dropsy had indicated that more than 1% adulteration of argemone oil in edible mustard oil was enough to produce clinical symptoms (Sharma, Malhotra, Bhatia, & Rathee, 1999; Verma, Dev, Tyagi, Goomber, & Jain, 2001).

In the present method, use of developing solvents with low polarity decreases the developing (15 min) and drying period (3 min) of TLC plate. Further the method is superior compared to that of Sita Devi and Sastry (2002), as it involves no extraction, successfully resolved the sanguinarine and dihydrosanguinarine and for the first time, a detailed recovery study was carried out by spiking argemone oil in edible mustard oil in order to evaluate the percent adulteration of argemone oil in edible mustard oil.

4. Conclusion

The methodology developed for estimating sanguinarine as an index of argemone oil adulteration in edible mustard oil is simple, rapid and economical as compared to all other methods reported earlier. The method developed can also be used as an analytical tool in epidemiological studies involving outbreaks of human argemone oil poisoning through adulterated edible oils or for screening purpose and may also find wide application in food laboratories involved in ensuring food quality and in forensic investigations related to argemone oil poisoning.

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