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Evaluation of edible oils for *Argemone mexicana* seed oil adulteration.

Maruti K Shelar*, Anand R Bafna, Atul M Wahile and Suresh V Tupkari.

Department of Pharmacognosy, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune. 411 018, Maharashtra, India.

ABSTRACT

Adulteration is important factor which may affect quality of food articles. Adulteration may be defined as substitution of genuine raw material with inferior or toxic materials. In number of cases, adulteration of Argemone mexicana (Family: Papaveraceae) seed oil in edible oils has been reported as cause of epidemic dropsy. Sanguinarine and dihydrosanguinarine are toxic principles (alkaloids) present in Argemone oil. Under present studies attempts have been made to evaluate Argemone oil adulteration in different edible oils. Adulterated and unadulterated edible oil samples were evaluated for primary quality control parameters and further subjected for instrumental analysis like FT-IR, GC-MS and HPTLC. Fatty acids present in edible oils are complex in nature and common in most of the oils, which causes interference in spectral analysis. In HPTLC analysis (hexane: acetone: methanol; 80:15:5), sanguinarine (Rf: 0.18) and dihydrosanguinarine (Rf: 0.33) were used as marker. HPTLC method was found to be well suited for separation of toxic principles like sanguinarine and dihydrosanguinarine in comparatively small proportion, present in adulterated samples.

Keywords: Adulteration; Edible oils; Argemone mexicana; Epidemic dropsy; Sanguinarine; Dihydrosanguinarine.

Corresponding author

INTRODUCTION

Nutritional and medicinal values of natural products are directly dependent upon quality and purity of raw materials. Chemotaxonomical variations due to geographical changes and adulteration (intentional or unintentional) are some of the factors which affect quality of food. Adulteration in practice is a substitution of genuine raw material partially or wholly with other similar looking substances but later is free from or inferior in chemical composition and may lead to toxic effects [01]. Adulteration in food items happens in many ways and may lead to toxic results. Epidemic dropsy is one of the several complications caused due to consumption of adulterated food. Epidemic dropsy is an acute problem caused due to consumption of edible mustard oil adulterated with Argemone mexicana (Mexican Poppy) seed oil [02, 03]. Numbers of toxicity cases have been reported for Argemone oil. 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 mustard (Brassica compestris) oil adulterated with Argemone oil. The seeds of Argemone mexicana plant are a source of Argemone oil, used for adulteration. This plant is a ubiquitous weed, found growing abundantly in wastelands, cultivated fields and on roadsides all over the country during late winter season. Interestingly, the Argemone seeds closely resemble the edible mustard seeds and the colors of both the oils are also similar which is considered as main cause of adulteration. 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. Quaternary benzophenanthridine alkaloids (QBAs) have been implicated in the toxicity of Argemone oil. The two major physiologically active QBAs that have been identified to mediate the toxic effects of Argemone oil in the development of epidemic dropsy are Dihydrosanguinarine and Sanguinarine. The outbreak of epidemic dropsy in the Indian capital, New Delhi, in 1998 was of one of the most severe forms and had repercussions in health services. Around 2552 toxicity cases were reported along with 65 deaths. Similar condition was first reported by Lyon in 1877 from Calcutta [04].

Literature survey revealed that several methods have been reported for detection of sanguinarine in Argemone oil. These methods include Paper chromatography, TLC with UV-spectroscopy, colorimetric analysis, color tests, HPLC and Capillary Electrophoresis, with varying sensitivities and detection limits. Even though these analytical methods are suitable for 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 oil samples [05, 06, 07]. Consumption of Argemone oil in form of adulteration, even at lower level for short duration, causes toxicity. Hence presence of Argemone oil even in trace quantities needs to be ascertained.

The edible oil samples were subjected for primary quality control parameters. Further the results were compared with standard values to assure the identity and purity of oil is used in experiment. Further adulterated oil samples (0.5% conc.) were subjected for FT-IR [08], GC-MS [09] and HPTLC analysis. Results highlighted the efficiency and applicability of HPTLC analysis even at lower concentration. HPTLC method showed separation of sanguinarine and dihydrosanguinarine as an index of Argemone oil adulteration.

MATERIALS AND METHODS

Argemone mexicana Linn. plant material (along with capsules containing seeds) was collected from the village Belvandi (Ahmednagar), Maharashtra, India and authenticated by Botanical Survey of India, Pune (Voucher number MKSP1).

Raw materials for different edible oils were purchased from local super market from Pune. All solvents used were of analytical grade unless otherwise stated. Hexane, acetone, methanol and chloroform procured from RANKEM.

Extraction of Argemone oil from Argemone mexicana seeds:

The seeds of Argemone mexicana were separated from capsules, crushed and subjected to solvent extraction using Soxhlet apparatus. The solvent used for extraction was petroleum ether (40^0-60^0). The extracted Argemone seeds gave pale greenish yellow oil with green fluorescence [10].

Extraction of edible oils:

The raw materials of edible oil (groundnut oil, sesame oil, mustard oil, and coconut oil; Arachis hypogoes, Sesamum indicum, Brassica species, Cocos nucifera) were supplied to Ramakrishna Oil Depot for extraction using traditional method of wooden churner. Further the oils were subjected for qualitative evaluation.

All the edible oil samples as well as Argemone oil were evaluated for parameter like moisture content, specific gravity, acid value, saponification value, unsaponifiable matter, iodine value, Reichert-Meissle value, Polenske value and refractive index [11, 12]. Adulterated samples of oils were prepared by incorporating of Argemone oil (0.5 v/v) in edible oil samples. Further these samples were subjected for analysis by using instrumental techniques like FT-IR, GC-MS, and HPTLC.

Instrumental analysis of oil samples

Fourier Transform -Infrared Analysis (FTIR):

The IR spectra of the Argemone oil, edible oils and adulterated oil samples were

recorded on JASCO, FTIR spectrophotometer, model No. FT/IR - 4100, JASCO Corporation, Tokyo, Japan, in KBR (anhydrous IR grade) pellets.

Gas Chromatography -Mass Spectroscopic Analysis (GC-MS):

The analysis of Argemone oil, edible oils and adulterated oil samples were carried out by GC-MS on a Shimadzu QP5050 (Shimadzu) system equipped with commercial mass spectral libraries. The temperature programmed, 250- 300^0 C at 2^{0}C/min.; Carrier gas Helium delivered at constant pressure of 400 kpa; injection volume was 1.0µl. The interface temperature was 280^{0}C and the ionization energy was 70ev.

High Performance Thin Layer Chromatographic Analysis (HPTLC):

Argemone oil, edible oils and adulterated oil samples were analysed by HPTLC. The results of adulterated oil samples were compared with Argemone oil and analysis were done.

Chromatography:

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Linomat V sample applicator equipped with a 100 µl syringe and a Scanner III, operated using Camag software WinCATS Planer Chromatography Manager, loaded on computer. Aluminum.

HPTLC plates (20 x 10 cm) pre-coated with silica gel 60, were purchased from Merck.

Sample preparation:

An aliquot of 20µl of Argemone oil was dissolved directly in chloroform and made up to final volume of 1 ml in a certified volumetric flask.

Adulterated edible oil samples (50µl, each) were also dissolved directly in chloroform and made up to 1 ml in certified volumetric flasks.

Procedure:

2µl of the working standard solution was applied by means of Linomat-V sample applicator over the plates about 1cm above the edge using a bandwidth of 2mm along with 2µl edible oil and adultrated edible oil samples. The chromatogram was developed under chamber saturation condition with solvent system Hexane: Acetone: Methanol (80:15:5) in Camag twin trough chamber. After development in earlier described solvent system, the plate was double eluted with petroleum ether for removal of fatty material. Further the plates were dried and observed under UV light (365nm) to confirm presence of sanguinarine and dihydrosanguinarine which appear with golden yellow fluorescence. The plate was scanned in

Camag TLC scanner at 365nm.

RESULTS AND DISCUSSION

Under the present study attempts have been made to evaluate adulteration of Argemone seed oil in few edible oils. Review of literature showed that 0.5 % adulteration of Argemone oil in food articles causes toxicity [13]. Thus the concentration was selected to adulterate the edible oils.

Argemone oil was extracted by solvent extraction as described earlier. The seeds of Argemone mexicana have been reported to yield 22 to 36 % of nauseous, bitter non-edible oil [03, 10]. The experimental yield of Argemone oil was found to be 35.3% w/v.

Further the oils were subjected for evaluation of preliminary quality control parameters prescribed for oils by Manual of methods of analysis of foods, Directorate general of health services ministry of health and family welfare Government of India [11], results of which have been tabulated in Table 1. Further the adulterated oil samples were subjected for instrumental and chromatographic analysis.

Table: 1. Evaluation parameters for oil samples

Evaluation Parameters	Groundnut oil		Mustard oil		Sesame oil		Coconut oil		Argemone Oil	
	Std.	**Expt.**	**Std.**	**Expt.**	**Std.**	**Expt.**	**Std.**	**Expt.**	**Std.**	**Expt.**
Moisture content	0.25	0.225	1.25	1.26	0.25	0.22	0.5	0.33	9.0	8.9
Specific gravity 0.909 - 0.9121	0.909-0.913	0.912	0.91-0.92	0.91	0.915-0.916	0.91	0.91-0.92	0.91	0.92	0.918
Refractive indices	1.462-1.464	1.463	1.464-1.466	1.465	1.464-1.466	1.450	1.448-1.449	1.448	1.460-1.461	1.460
Acid value	NMT6	3.89	NMT6	1.46	NMT6	2.92	NMT6	0.9745	10-12	11.3
Saponification Value	188-196	194.0	168-177	175.4	188-193	191.6	NLT 250	280.2	185-190	190.8
Iodine value	85-99	85	96-112	109.4	103-120	108.5	7.5-10	9.4	120-124	123.9
Unsaponifiable matter	NMT 1.05%	1.24%	NMT 1.2%	1.4%	NMT 1.5%	1.59%	NMT 1.0%	0.9887	2-4%	3.95%
Reichert-Meissle value	--	--	--	--	--	--	6-8.5	7.7	0.6	0.599
Polenske value	--	---	--	--	--	--			--	--

Std. - Standard, Expt. - Experimental, NMT-Not more than, NLT- Not less than
The above values obtained that of various oils were within the limits of standard values.
The values obtained were used for identification and standardization of oils.

FT-IR and GC-MS Analysis

Infrared spectroscopy is generally employed to determine functional groups and to identify the material. Each peak in the infrared spectrum is unique to functional group. The molecular structure of substances can be partially determined by analyzing the spectrum. When Argemone oil, edible oils and adulterated oil samples were analyzed by FTIR, fatty acids present both in Argemone oil and edible oil samples were found to be similar as they showed similar spectrum due to presence of common functional groups. Gas chromatography (GC) can separate the hydrocarbons and mass spectrum (MS) differentiates it. Mass spectrometer bombards molecules in the vapor phase with a high energy electron beam, records the results of electron impact as a spectrum of positive ions separated on basis of mass/charge (m/z). Fatty acids which are basic building blocks of fixed oils are complex in nature and literature review reveals that numbers of common fatty acids are present in Argemone oil and edible oils. Various fatty acids present in different oils have been tabulated in Table 2 [03, 10, 14]. Similar compositions (fatty acids) of Argemone oil and edible oil lead to interference in FT-IR and GC-MS analysis. Thus results obtained by FT-IR and GC-MS were not clearly distinguishable and conclusive.

TABLE 2. Common fatty acids present in the oils.

Common Name	Carbon Atoms	Double Bonds	Oil Sources
Butyric acid	4	0	Butterfat, coconut oil, argemone oil
Caproic Acid	6	0	Butterfat, coconut oil, argemone oil
Caprylic Acid	8	0	coconut oil
Capric Acid	10	0	coconut oil
Lauric Acid	12	0	Groundnut oil, coconut oil, argemone oil
Myristic Acid	14	0	Groundnut oil, coconut oil, argemone oil
Palmitic Acid	16	0	Sesame oil, coconut oil, argemone oil, mustard oil
Palmitoleic Acid	16	1	Sesame oil, coconut oil, argemone oil, Groundnut oil
Stearic Acid	18	0	Sesame oil, coconut oil, argemone oil, Groundnut oil, Mustard oil
Oleic Acid	18	1	Sesame oil, coconut oil, argemone oil, Groundnut oil, Mustard oil
Ricinoleic acid	18	1	Argemone oil
Linoleic Acid	18	2	Coconut oil, argemone oil, Mustard oil
Linolenic Acid	18	3	Sesame oil
Arachidic Acid	20	0	Sesame oil, Groundnut oil
Erucic acid	22	1	Mstard oil
Arachidonic Acid (AA)	20	4	Coconut oil

HPTLC:

When Argemone oil, adulterated and unadulterated edible oils samples were subjected for HPTLC analysis in chromatographic conditions described earlier, marker compounds

sanguinarine and dihydrosanguinarine were found to be sufficiently eluted and separated. The R_f values of sanguinarine and dihydrosanguinarine were found to be 0.18 and 0.33 respectively, which were visualized as bright golden yellow fluorescent band under UV (365 nm). In HPTLC chromatogram of adulterated sample similar peaks were observed at respective R_f. The identification of sanguinarine and dihydrosanguinarine in adulterated oil samples was done by comparing the R_f with that sanguinarine and dihydrosanguinarine. In unadulterated oil samples no peaks were observed at same R_f region. The method was found to be effective for detection of adulteration at lower concentration like 0.5%v/v. HPTLC chromatogram of Argemone oil and other adulterated edible oils have been shown in Fig. 1-5 and peak distribution data has been tabulated in table 3-7. Experimental results showed that chromatographic technique viz. HPTLC may be useful for detection of Argemone oil adulteration even at lower concentration. On the other hand an instrumental methods viz. FT-IR and GC-MS were not conclusive. Thus the method, if further developed and validated for specific applications may be adapted be industry and law makers as rapid technique to identify and quantify the degree of adulteration.

Table 3. Peak Distribution data of Argemone oil.

Peak	Start Rf	Start height	Max. Rf	Max. height	Max %	End Rf	End height	Area	Area %	Assigned
1	0.18	0.4	0.21	448.6	36.0	0.25	35.8	8986	27.31	Sangunarine
2	0.25	35.8	0.28	213.0	17.09	0.33	0.1	5239.9	15.92	Unknown
3	0.33	0.1	0.38	584.6	4.91	0.46	2.3	18683	56.77	Dihydro Sangunarine

Table 4. Peak Distribution data of Adulterated Groundnut oil.

Peak	Start Rf	Start height	Max. Rf	Max. height	Max %	End Rf	End height	Area	Area %	Assigned
1	0.17	0.9	0.21	353.1	31.73	0.24	66	7852	24.96	Sangunarine
2	0.24	66	0.28	181.0	16.27	0.32	5.6	4734.5	15.05	Unknown
3	0.32	0.56	0.37	578.5	52.0	0.46	2.06	18868.8	59.99	Dihydro Sangunarine

Table 5. Peak Distribution data of Adulterated Mustard oil.

Peak	Start Rf	Start height	Max. Rf	Max. height	Max %	End Rf	End height	Area	Area %	Assigned
1	0.17	5.2	0.20	370.6	40.15	0.24	55.8	7776.3	28.80	Sangunarine
2	0.24	55.8	0.26	159.1	17.24	0.31	0.0	4259	15.77	Unknown
3	0.31	0.4	0.37	393.3	42.61	0.45	0.3	14966	55.43	Dihydro Sangunarine

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Table 6. Peak Distribution data of Adulterated Coconut oil.

Peak	Start Rf	Start height	Max. Rf	Max. height	Max %	End Rf	End height	Area	Area %	Assigned
1	0.17	1.0	0.21	343.9	35.31	0.24	40.2	6955.1	25.44	Sangunarine
2	0.24	40.2	0.27	154.9	15.91	0.32	2.4	4310.1	15.76	Unknown
3	0.32	2.4	0.37	475.0	48.78	0.45	0.9	16078.1	58.80	Dihydro Sangunarine

Table 7. Peak Distribution data of Adulterated Sesame oil.

Peak	Start Rf	Start height	Max. Rf	Max. height	Max %	End Rf	End height	Area	Area %	Assigned
1	0.17	5.2	0.20	370.6	40.15	0.24	55.8	7776.3	28.80	Sangunarine
2	0.24	55.8	0.26	159.1	17.24	0.31	0.0	4259	15.77	Unknown
3	0.31	0.4	0.37	393.3	42.61	0.45	0.3	14966	55.43	Dihydro Sangunarine

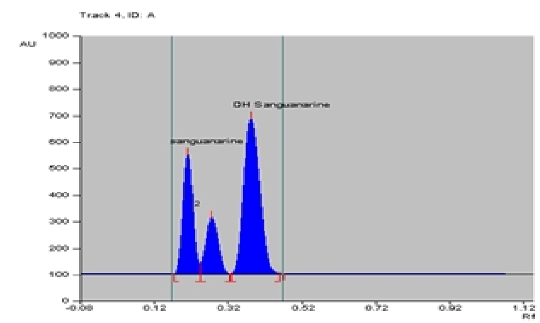

Fig.1. HPTLC chromatogram of argemone oil

(a) **(b)**

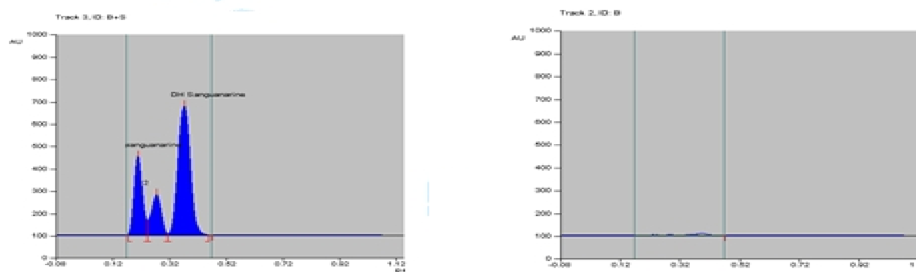

Fig.2 HPTLC chromatogram of adulterated ground nut oil (a) and ground nut oil (b)

(a) **(b)**

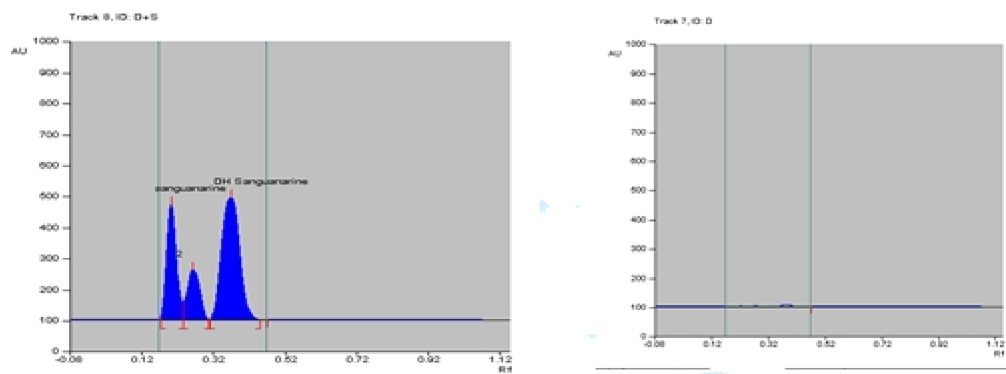

Fig. 3 HPTLC chromatogram of adulterated mustard oil (a) and Mustard oil (b).

(a) **(b)**

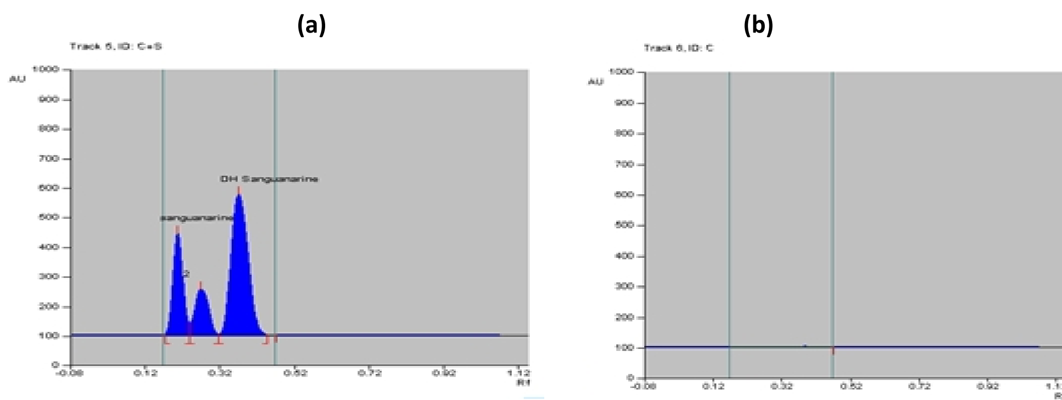

Fig. 4 HPTLC chromatogram of adulterated coconut oil (a) and coconut oil (b)

(a) **(b)**

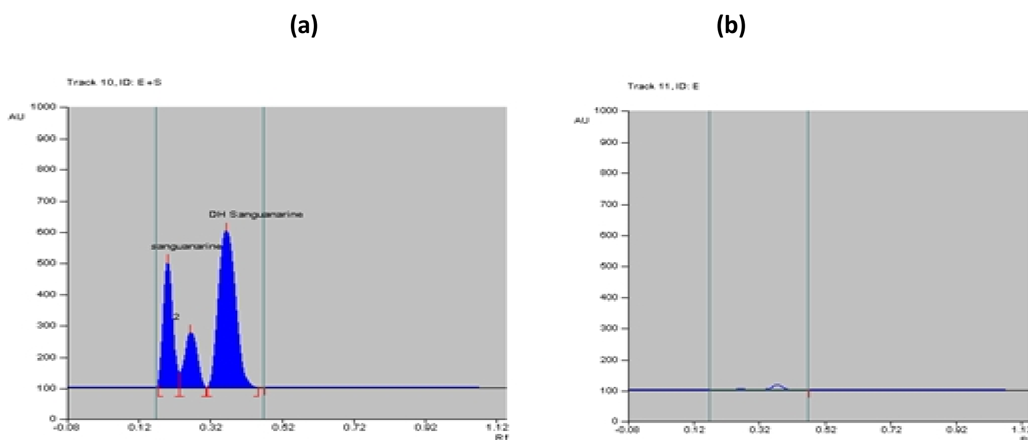

Fig. 5. HPTLC chromatogram of adulterated sesame oil (a) and Sesame oil (b).

CONCLUSION

It can be concluded that HPTLC was effective tool for determination of argemone oil adulteration up to lower concentration 0.5% v/v. Further quantitative evaluation or development of method may be used as quality control tool at manufacturer's level, public health laboratories

and custom laboratories.

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