Quantitative indexation for determination of adulterant *Argemonemexicana* seeds in *Brassica nigra* seeds.

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ABSTRACT: Background: Adulteration is the most encountered barrier to reach the goals of safety and efficacy of foods and drugs derived from natural resources. *Brassica nigra* (mustard) is a useful source of edible oilseeds. Often this mustard seeds are found to be contaminated with its look alike *Argemone mexicana* seeds which have adverse health effects.The present study was aimed at generating a quantitative profile for the *Brassica nigra* seeds adulterated with *Argemone mexicana* seeds.

Methods: To this end, the changes in physical and chemical behavior of *B. nigra* seeds with variable percentages of adulteration by the seeds of *A. Mexicana* were studied. Physico-chemical and macroscopic characters as well as chromatographic profiling were evaluated to identify and quantify the amount of adulteration.

Results:Changes in physicochemical properties and physical properties are having a regular change with variation in quantity of adulteration. This finding was ascertained by the High Performance Thin Layer Chromatogram.

Conclusion:From the datasheet it was observed that with increasing percentage of adulteration, there were some gradual changes in different physico-chemical properties. The High Performance Thin Layer Chromatography fingerprinting indicated the presence and amount of adulteration. The fact sheet generated by the above experiments sets a scale for the measurement of adulterant by percentage and may serve as a quality control device for raw materials for the food and drug industry.

Key-words: Argemone, Brassica, Adulteration, Analysis, HPTLC.

INTRODUCTION:

With rapid industrialization and proliferation of human population, demand of raw materials is facing a serious challenge. To meet this ever increasing demand of raw materials, often the supply chains are being infiltrated with undesired materials, either by deliberation or by ignorance [1]. Accidental mixing and mixing due to ignorance is caused by the unaware cultivars or farmers [2], while deliberate mixing is done by the suppliers or vendors to scale up the profit margins. In both these above cases the quality of the authentic materials diminishes, efficacy decreases and causes the detrimental health effect. Spurious materials which are mixed with the genuine samples are called adulterants. The materials which are chosen to be mixed with generally look alike with the original materials. Most of the time simple naked eye observation is unable to break the disguise. To recognize or to identify such adulterants mixed in the authentic materials need to be investigated in laboratory with the help of analytical parameters. Adulteration of *Brassica nigra* seeds by the seeds of *Argemone mexicana* is an example of such situation. The alkaloid present in argemone seeds is sangunarine which has proven to have some detrimental health effect [3-6]. It is almost impossible to identify the presence of *A. mexicana* seeds as an adulterant in *B. nigra* seeds by normal observation and that is due to their identical physical appearances. In present study the seeds of *A. mexicana* were identified in seeds of *B. nigra*. Previously few researchers [7-10] worked on the issue of presence of argemone oil in mustard oil. Their study nicely detected the contamination or adulterant in oil. But the present work is different from all the previous study, because this study detected the presence of adulterant at very primary stage that is at raw materials from which oil is being extracted. Early detection is always desirable to avoid the rejection of products after processing which involves finance, energy and other supporting materials. Thus, detection of adulteration in raw materials is definitely a better option than to detect it after processing. Hence, the present work was undertaken to identify and quantify the amount of adulterant present in raw materials, through some analytical parameters and chromatographic profile.

MATERIALS AND METHODS:

Reagents and Chemicals

All solvents, chemicals and precoated silica gel TLC plates were purchased from E. Merck India P Ltd. Ethanol was purchased from Changshu Hongsheng Fine Chemical Co. Ltd., China. Standard sangunarine as sangunarine chloride was purchased from Sigma-Aldrich.

Collections of seeds and authentications

Brassica nigra seeds were collected in the mid of March, 2019 from the cultivated field in Burdwan district of West Bengal. Seeds of *Argemone Mexicana* were collected from wild bushy land in Burdwan district in the mid of April, 2019. Both the samples were authenticated in place of research, and samples were preserved therein for future reference.

Microscopical features of seeds

Seeds of *B. nigra* are nearly spherical, small, bright, smooth, dark reddish brown to greenish brown to black, 1-1.5 mm in diameter, under magnifying glass show very minute reticulation on surface. The seeds of *A. mexicana* are nearly spherical, minute, about 1mm in diameter, surface deeply netted, shinning, brownish black, covered in a fine network of veins. The macroscopic images of the *B. nigra* seeds and *A. mexicana* seeds and their missed adulterated samples are represented in Fig. 1.

Fig. 1: Morphological images of samples

Preparation of adulterated samples of *B. nigra* seeds

Adulterated samples of *B. nigra* were prepared by mixing the seeds of *A. mexicana* in different proportions. Mixing ratios and designation of resultant samples were noted (table 1). Each sample were prepared was 200 g. All experiments in the present work were carried out for these seven samples. Samples prepared here were kept in a closed mouth transparent glass container.

Table 1:Resultant samples of authentic and adulterated used in investigation

Sl. No.	Weight (g) of *B. nigra* seeds	Weight (g) of *A. Mexicana* seeds	Percentage of adulteration	Sample designation
1.	200	0	0	BN
2.	190	10	5	BN95
3.	180	20	10	BN90
4.	170	30	15	BN85
5.	160	40	20	BN80
6.	150	50	25	BN75
7.	0	200	-	AM

Determination of Physico-chemical Properties

All samples as designated in Table 1 are subjected to physico-chemical analysis like ash values, extractive values, pH values according to the standard guidelines [11].

Extraction of oil

Samples were individually subjected to soxhlet extraction with light petrol (Petroleum ether 40-60°C) for determining the fixed oil following the standard protocol [12].

Determination of Physical properties

All samples as designated (table 1)were subjected to determine different physical properties like refractive index, specific gravity, acid value, saponofication value, iodine value. Refractive index was measured using Abbe's refractometer. Specific gravity was determined using pycnometer. Acid value, saponification value and iodine value were determined by titrimetric method [11].

Preparation of standard solution of sanguinarine

A stock solution of sanguinarine (0.1 mg/ ml)was prepared by dissolving 10 mg accurately weighed sanguinarine chloride in methanol and diluting it to 100 ml with methanol. An aliquot (1.0 ml) of the stock solution was transferred to a 10 ml volumetric flask and the volume was adjusted to 100 ml with methanol to obtain the working standard solution containing 10 ng/µl.

Preparation of test solution for High Performance Thin Layer Chromatography (HPTLC)

For HPTLC, 10 g of each sample was separately extracted in methanol by soxhlet and extracts filtered through Whatman filter paper no. 41 and filtrates taken in volumetric flasks of 25 ml capacity, and diluted up to the mark with methanol.

Chromatographic profiling and Quantification

Aluminum supported precoated silica gel $60F_{254}$ plate of size 13x10 cm were used to apply 4 µl of each sample along with the standard sanguinarine solution at 15 mm height from the bottom edge of the plate. To get well separated bands, different mobile phases were tried and optimized mobile phase considered was Hexane: Ethyl acetate: Formic acid (6:4:0.5, v/v) for best resolution. Plates were developed up to 80 mm and dried for 5 min in ambient air. Images of the developed plates were captured under 366 nm UV light. Developed plate was subjected to densitometric scanning [13] at 366 nm to identify and quantify the sanguinarine by empirical calculations based on the area under the curve, for corresponding band of it.

Chromatographic Method validation

Method validation [14, 15]was performed to check the reliability of the method developed. Linearity ranges (10-160 ng per spot), limit of detection (5 ng) and precision (both intraday and interday precision) were studied to check the reproducibility (R= 0.999). The robustness or ruggedness of the method was studied by varying a few parameters like mobile phase volume, development distance, tank saturation time, time from application to development and time from development to scanning. Linearity curve and quantification curve are represented in Fig. 2 and Fig. 3 respectively.

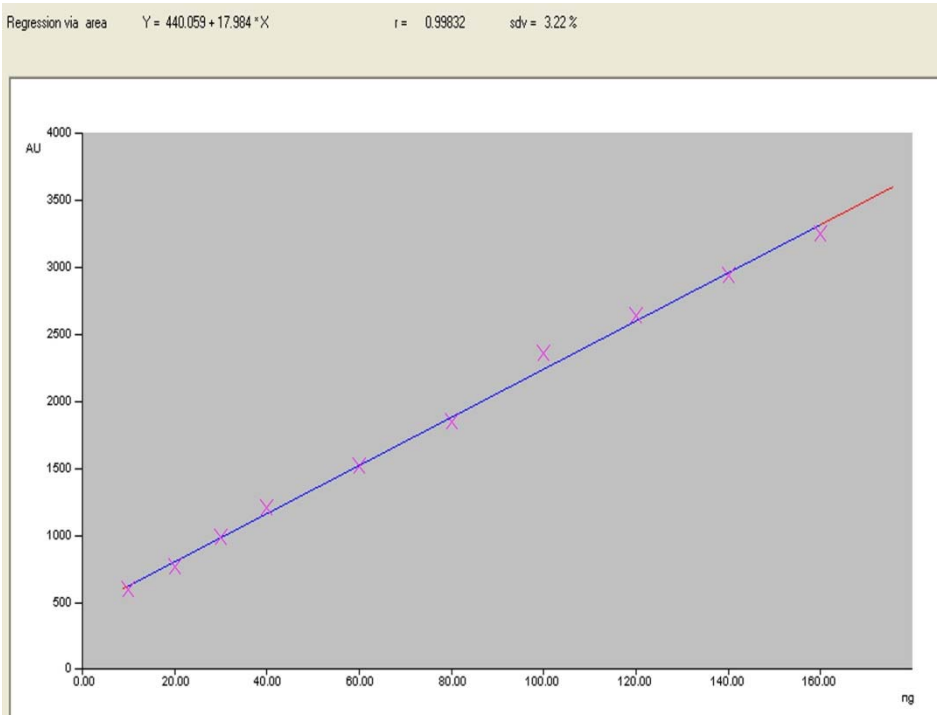

Fig. 2: Linearity curve

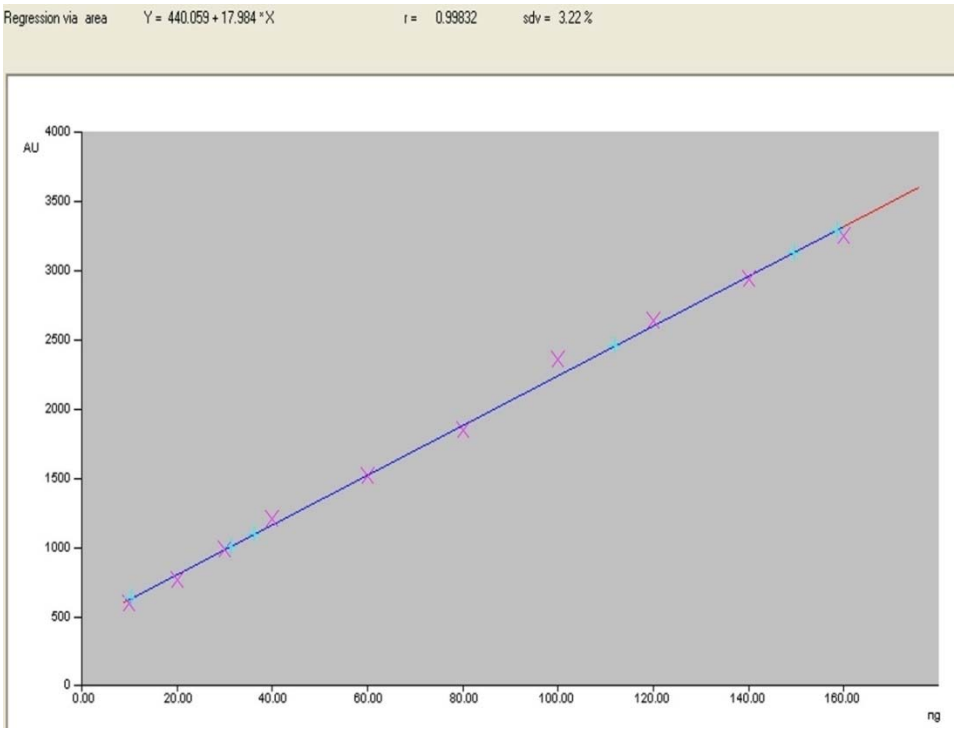

Fig. 3: Quantification curve

RESULTS AND DISCUSSION:

Physicochemical parameters are basic quality control parameters which determine the standard values of a sample. The physicochemical data (table 2), revealed that the losses on drying and total ash values are gradually increasing with the increase in *A. mexicana* percentage, while the pH values are diminishing gradually. It may also be noted that acid insoluble ash values are not having any particular trend of increasing or decreasing. Extractive values in different solvents are showing variable trends. Except methanol all other solvents are having gradual decrease in extractive values with increasing percentage of mexicana seeds.

Table 2: Experimental Physico-chemical data[a]

Parameters	BN	BN95	BN90	BN85	BN80	BN75	AM
Loss on Drying (at 105ºC)	5.91±0.01	6.66±0.04	6.47±0.04	6.67±0.07	6.65±0.01	7.02±0.02	6.49±0.07
Total ash values	4.32±0.02	4.51±0.03	4.73±0.02	4.73±0.03	5.19±0.05	5.55±0.04	9.55±0.05
Acid insoluble ash	0.19±0.01	0.39±0.01	0.37±0.02	0.34±0.02	0.29±0.03	0.22±0.03	0.44±0.03
pH values (10%) aq. suspension	5.34±0.01	5.26±0.01	5.21±0.01	5.15±0.01	5.09±0.01	4.69±0.02	4.34±0.03
Hexane Extract	34.28±0.13	34.262±0.11	34.251±0.09	34.291±0.14	34.349±0.13	33.692±0.09	24.183±0.08
Ethylacetate Extract	15.82±0.01	15.40±0.07	14.92±0.07	14.68±0.04	13.87±0.03	13.260.03±	9.39±0.05
Chloroform extract	17.83±0.11	16.47±0.09	16.30±0.05	16.13±0.08	15.02±0.05	14.39±0.04	13.23±0.11
Methanol extract	15.84±0.07	16.64±0.11	17.03±0.03	17.93±0.13	17.88±0.09	18.31±0.13	28.82±0.11

[a]Values are expressed as Mean ± S.D. from three sets of experiments

Oils extracted from the seed samples show appreciable changes in physical properties (table 3). Both the refractive index and specific gravity values are slowly diminishing as the mexicana percentages are going up. The results of titrimetric experiments like acid values, saponification values and iodine values are distinguishably higher with the higher percentage of mexicana seeds.

Table 3:Physical properties of oil extracted from samples[a]

Parameters	BN	BN95	BN90	BN85	BN80	BN75	AM
Refractive index	1.473±0.01	1.474±0.01	1.471±0.01	1.473±0.01	1.470±0.01	1.469±0.01	1.4612±0.01
Specific gravity	0.9086±0.01	0.9070±0.02	0.9072±0.02	0.9105±0.01	0.9083±0.01	0.9112±0.01	0.9211±0.01
Acid value	5.95±0.01	6.25±0.01	6.65±0.03	7.08±0.01	7.36±0.02	7.67±0.05	13.15±0.02
Saponification value	125.81±0.02	129.44±0.02	133.64±0.03	136.05±0.02	139.42±0.01	142.28±0.01	190.5±0.03
Iodine value	8.60±0.02	14.22±0.04	19.87±0.05	25.77±0.02	31.19±0.03	36.94±0.03	121.23±0.02

[a]Values are expressed as Mean ± S.D. from three sets of experiments

The photography of the TLC plate observed at 366 nm, given in Fig. 4 clearly indicates the absence of sanguinarine in *B. nigra* seeds, at R_f value of 0.72 as compared to the standard sanguinarine at track no. 4. Also the compounded 3D chromatogram represented in Fig. 5 indicates the absence of Sanguinarine peaks in B. nigra seeds. The naked eye observation of this plate gave an idea of increasing quantity of sanguinarine with the increasing amount of mexicana seeds. This fact was confirmed by the densitometric scanning at 366 nm. The area under the curve (AUC) of the corresponding spot (table 4) of sanguinarine obtained from scanning indicate the differences.

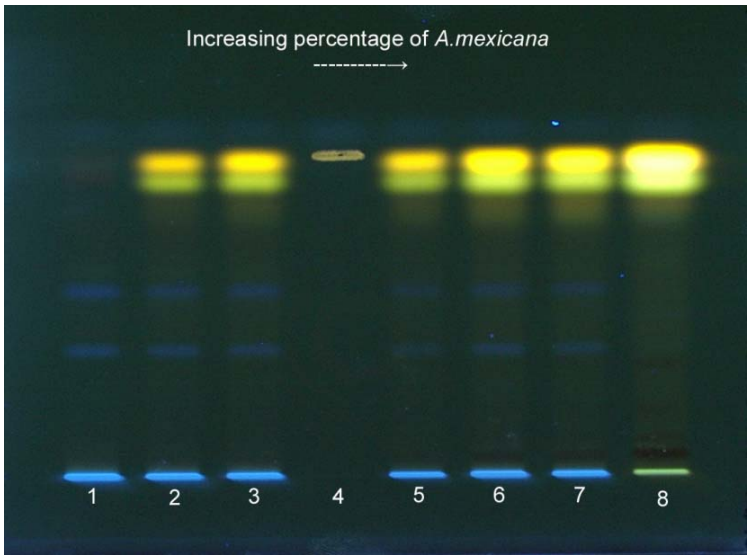

Fig. 4: Image of TLC plate visualized at 366 nm

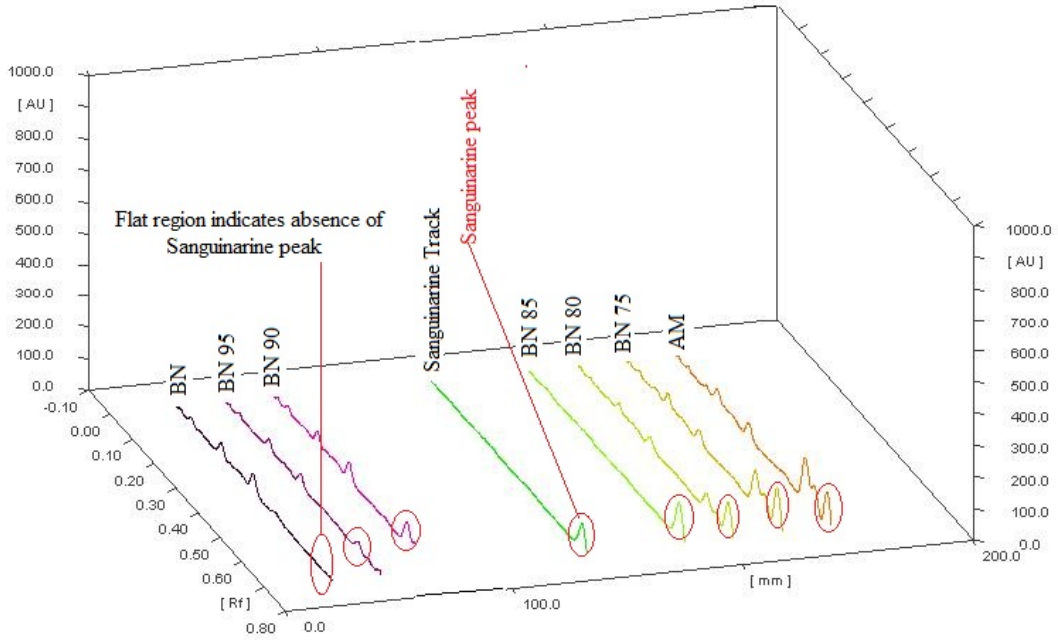

Figure 5: 3D chromatogram

Table 4: HPTLC data

Track No.	Sample Track details	Applied Vol. on TLC plate	Area Under the Curve (AUC) for Sanguinarine band
1.	BN	4µL	Nil
2.	BN95	4µL	607
3.	BN90	4µL	925
4.	Sangunarine Standard	1µL-16µL (applied as per linearity range)	550-3865
5.	BN85	4µL	1463
6.	BN80	4µL	1683
7.	BN75	4µL	1802
8.	AM	4µL	3410

CONCLUSION:

These sets of experiments have given a scale for measurements of *A. mexicana* seeds as an adulterant present in *B. nigra* seeds. Changes of different physical properties indicate the changes of percentage of adulteration. These experimental results cumulatively may be considered as a quality control check post for identification and determination of adulterant present in authentic sample.

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CONFLICT OF INTEREST:

Authors of this manuscript declare that there is no conflict of interest associated with this article.

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